Microbiological Assessment of *Tenualosailisha* In Different Preservation Treatments

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To

my beloved

parents

TO WHOM IT MAY CONCERN

This is to certify that **Md. HashibulHaque**is a student of M.S. Program of 2013-2014 Session under Department of Fisheries of University of Dhaka bearing Examination Roll No.: Curzon-4202, Registration no. HA -2610 (2009-20010). As part of her curriculum he has carried out a thesis under our supervision the title of which is "**Microbiological Assessment of*Tenualosailisha* In Different Preservation Treatments".**

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

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The author

Abstract

The present study was conducted for comparative preservation assessment of*Tenualosailisha by using olive oil to observe their microbial load before and after preservation*. For microbiological assessment this species of fish was selected because of it high demand in export market and popularity in our country. This study was conducted because the microbiological status of this fish could be a major public health issue.Fish samples was collected from local fish market. The present study was designed to assess Standard Plate Counts (SPC), Total *Coliform* Counts (TCC) and qualitative analysis of *Salmonella* spp. and *Vibrio cholerae* and to preserve the fish in the olive oil to observe whether it is suitable or not. To carry out this study three treatments were used to observe which on show less microbial contamination after preservation with olive oil. The study was conducted by using four media EMB, TCBS, SS and PYG for enumerating total coliform ,vibriospp, salmonella and total heterotrophic bacteria respectively. The study also conducted with three preservative treatment: cloves, turmeric and boiled fish sample with olive oil. Again this study was conducted with two subsequent sub-samples with 15 days interval to observe the microbial load. From this observation it was found that in the treatment 1(boiled fish and olive oil) highest coliform bacteria was found in (6.1×10^5 cfu/g) and lowest coliformbacteria was found in (5×10^4cfu/g).The highest *vibrio* spp. bacteria was found in (1.23×10^5cfu/g) and lowest *Vibrio* spp. bacteria was found in (5.26×10^4cfu/g).In the experiment highest *Salmonella* bacteria was found in (1.9×10^5 cfu/g) and lowest *Salmonella* bacteria was found in (1.54×10^4cfu/g).And the highest heterotrophic bacteria was found in (9.25×10^5cfu/g) and lowest heterotrophicbacteria was found in (4.0×10^3cfu/g).In the treatment 2 (turmeric with olive oil) highest coliform bacteria was found in (1.8×10^5 cfu/g) and lowest coliform bacteria was found in (2.08×10^4Cfu/g). The highest *Vibrio* spp. bacteria was found in (1.7×10^4cfu/g) and lowest *Vibrio* spp bacteria was found in (3.8×10^3cfu/g). In the experiment highest Salmonella bacteria was found in (7×10^4 cfu/g) and lowest Salmonella bacteria was found in (1.1×10^4cfu/g). And the highest heterotrophic bacteria was found in (1.5×10^5cfu/g) and lowest heterotrophicbacteria was found in (2.7×10^4cfu/g). And In the treatment 3 highest coliformbacteria was found in (2×10^5 cfu/g) and lowest coliformbacteria was found in (8.1×10^3cfu/g).The highest *Vibrio* spp. bacteria was found in (1.2×10^5 cfu/g) and lowest *Vibrio* spp.bacteria was found in (5.3×10^3cfu/g). In the experiment highest *Salmonella* bacteria was found in (2.2×10^5 cfu/g) and lowest *Salmonella* bacteria was found in (5.0×10^3cfu/g). And the highest heterotrophic bacteria was found in (1.2×10^5 cfu/g) and

lowest heterotrophicbacteria was found in (1.62×10^4 cfu/g). In the 2nd observation, it was found that the highest coliform bacteria was found in (1×10^6 Cfu/g) and lowest coliformbacteria was found in (4.1×10^4cfu/g) the treatment 1. The highest Vibrio spp. bacteria was found in (1.6×10^5 cfu/g) and lowest Vibrio spp bacteria was found in (1.61×10^3cfu/g).In the experiment highest *Salmonella* bacteria was found in (2.3×10^5 cfu/g) and lowest *Salmonella* bacteria was found in (1.5×10^3cfu/g).And the highest heterotrophic bacteria was found in (2.8×10^5 cfu/g) and lowest heterotrophic bacteria was found in (2.82×10^4 cfu/g). In the 2nd observation, it was found that the highest coliform bacteria was found in (3×10^4 cfu/g) and lowest coliform bacteria was found in (3.3×10^3cfu/g).the treatment 2.The highest *Vibrio* spp. bacteria was found in (2×10^5 cfu/g) and lowest *Vibrio* spp. bacteria was found in (8.7×10^3cfu/g).In the experiment highest *Salmonella* bacteria was found in (4.0×10^4 cfu/g) and lowest *Salmonella* bacteria was found in (1.1×10^3cfu/g).And the highest heterotrophic bacteria was found in (2.0×10^4 cfu/g) and lowest heterotrophic bacteria was found in (1.08×10^4 cfu/g). In the 2nd observation, it was found that the highest coliform bacteria was found in (1.3×10^5 cfu/g) and lowest coliform bacteria was found in (8.5×10^5cfu/g).the treatment 3. The highest *Vibrio* spp. bacteria was found in (1.3×10^5 cfu/g) and lowest *Vibrio* spp.bacteria was found in (5.7×10^3cfu/g).In the experiment highest *Salmonella* bacteria was found in (2.5×10^5 cfu/g) and lowest *Salmonella* bacteria was found in (6×10^3cfu/g).And the highest heterotrophic bacteria was found in (1.4×10^4 cfu/g) and lowest heterotrophic bacteria was found in (1.68×10^4 cfu/g). This study revealed that Preservation with Turmeric and Olive oil shows better results than preservation with Cloves and boil fish with Olive oil. The findings of this study indicate that the fish samples preserve Turmeric with Olive oil could be a good preservative in respect of short term preservation. Although this practice is not applied in our country so further research should be needed in this field.

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CHAPTER 1: INTRODUCTION

Introduction:

Fish is one of the important sources of quality animal proteins and availability and affordability is better for fish in comparison to other animal protein sources. Fish serves as a health-food for the affluent world owing to the fish oils which are rich in polyunsaturated fatty acids (PUFAs), especially ω-3 PUFAs and at the same time, it is a health-food for the people in the other extreme of the nutritional scale owing to its proteins, oils, vitamins and minerals and the benefits associated with the consumption of small indigenous fishes (Mohanty, 2011). Fish, especially saltwater fish, is high in ω-3 fatty acids, which are heart-friendly, and a regular diet of fish is highly recommended by the nutritionists. This is conjectured to be one of the major causes of reduced risk of cardiovascular diseases in Eskimos (Bang *et al*. 1976). It has been suggested that the longer lifespan of Japanese and Nordic populations may be partially due to their higher consumption of fish and seafood. Oily fish is claimed to help prevent a range of other health problems from mental illness to blindness. Thus fish has medicinal and therapeutic value. *Tenualosailisha*(Hamilton, 1822) of the subfamily Alosinae, family Clupeidae, order Clupeiformes, is one of the most important tropical fishes of the Indo-Pacific region and has occupied a top position among the edible fishes owing to its taste, flavor and culinary properties. Popularly known as hilsa, it is a fast swimming euryhaline known for its cosmopolitan distribution in brackish water estuaries and marine environment in the Indo-pacific faunistic region and in the riverine environments where it migrates for breeding. Major catch of hilsa, about 95%, comes from Bangladesh, India and Myanmar. Naturally hilsa is in great demand globally, specifically in the oriental world and enjoys high consumer preference. Its high commercial demand makes it a good forex earner. Five varieties of *Tenualosasp*(*T. toli*from Malaysia, *T. macrura*from Indonesia, *T. thibaudeaui*from Mekong, *T. reevesii*from Southern China and *T. ilisha*from India, Bangladesh and Myanmar) are found in tropical Asian region (Blaber et al. 1997) out of which *T. ilisha*and to some extent *T. toli*and *T. kelee*are prevalent in the Indian waters. The normal habitat, age and growth and trend of migratory habit differ from species to species. Among the five species, *T. ilisha*is the major component of fishery in the Ganga-Brahmaputra-Padma river system. In Hooghly estuary, hilsa, the state fish of West Bengal, accounts for 15-20% of the total fish landing (Bhaumik 2010). Hilsa, the national fish of Bangladesh, contributes 12-13% of the total fish production and about 1% to the GDP.

Among the various types of fish river shad (*Tenualosailisha)* plays a very important role. It is one of the members of the genus *Tenualosa*of the family Clupeidae, order Clupeiformes. Locally known as Ilish, it has been designated as the national fish of Bangladesh. This fish is highly tasty and very much well known to the people of Bangladesh. It is rich in protein and poly unsaturated fatty acids. Its liver contains considerable amount of vitamin A, while its body oil contains vitamin C (Bhuiyan 1984). It also contains calcium, phosphorus and other mineral salts. It is estimated that one pound of *Tenualosailisha*fish has an average 300-1100 calories energy (Rahman 1976).

But fish is considered as one of the most perishable of all food stuffs. As soon as a captured fish dies, it begins to deteriorate. The deterioration of the flesh of the fish is caused by the action of enzymes,by micro-organism and by chemical action. Bacteria on the surface of fish skin, gills and in the guts are generally harmless in the living fish, but they start their destructive activities as soon as this fish dies. They grow and multiply rapidly at ordinary temperature, invade the flesh through the skin and breakdown the complex chemical construction of the flesh, producing the stale and later the putrid smells and tastes which are usually associated with spoilage of fish (Jadhev and Magar 1970). The activity of enzymes, bacteria and chemicals could be minimized by standards of cleanliness, careful handling technique, preservation, quality control and temperature reduction. The quality of frozen fish is determined mainly by the total number of bacteria present and by the individual count of bacteria of public health significance such as *Escherichia coli, Fecal coli*, coagulate positive staphylococcus, Vibrio cholera, Salmonella *etc.* The quality of fish and fishery products depend on various factors i.e. the freshness of the raw fish, method of handling and processing factories, pre-and post- process temperature etc. Strict control of every stage of processing is necessary to prevent bacterial multiplication and the various chemical changes. A regular assessment of the quality of raw material is essential especially in view of the variation in the freshness of raw materials like *T. ilisha*, where the rate of spoilage may be high and will depend on the size and species (Rahman 1976). Unfortunately, a huge amount of fish spoils every year in Bangladesh due to the growth and activity of pathogenic bacteria and fungi. A variety of fishes consumed regularly are prone to pathogenic spoilage especially by *Vibrio* spp., *Shigella*spp, *Salmonella* spp., streptococci, staphylococci, coliforms, *Listeria* spp., *Clostridium* spp. (Rahman*et al*., 2012) which may get entry into the fish from theirhabitat or during the fish transportation and storage (Frazier and Westhoff, 1995; Eze*et al*., 2010). A number of reports suggested that the consumption of the microbiologically

spoiled seafoods might be responsible for food-borne diseases like Diarrhea, Salmonellosis, shigellosis, cholera and even some neurological diseases by an array of viruses, bacteria, fungi and parasites (Snowdon *et al.*, 1989; Starutch, 1991; Karunasagar*et al*., 1994; Cray and Moon, 1995; Wallace *et al*., 1999 WHO, 2012). Thus, with the growing importance of shrimp and prawn as the major export items from Bangladesh, it is worth to maintain the microbiological quality of these products. Therefore, it is crucial to estimate the rate of microbial spoilage and to establish the preventive strategy to ensure the general food safety.

Along with consumption of microbiologically spoiled fish, the off-odor and off-taste of the products caused by oxidation of lipids and some other metabolites may largely affect the consumer acceptability (Moini*et al* 2009 and Rostamzad*et al*., 2010). For maintenance better quality of fish preservation is very necessary. Good preservation retains the nutritional quality of fish. Storage time and temperature are the major factors affecting the rate of loss of quality and shelf life of fish (Whittle, 1997).

Fishes are mainly contaminated by Coliform, *Pseudomonas, Staphylococci*. Coliforms include all aerobic and facultitively anaerobic gram negative non spore forming bacilli which ferment lactose with gas formation within 48 hours at 35 °C. Coliforms include psychotropic type microbes capable of multiplying at 3-10 °C. Thus they can multiply in foods even when refrigerated. *Staphylococcus* are non-motile cocci that are catalase positive and facultatively anaerobic, having both an oxidative and fermentative type of metabolism. In the lab, *S. aureus*produces white to golden colored colonies and is positive for the coagulase test. *Pseudomonas* is a genus of gammaproteobacteria, belonging to the family pseudomonadaceae containing 191 validly described species (Euzeby, 1997). The best studied species include *P. aeruginosa*in its role as an opportunistic human pathogen, the plant pathogen *P. syringae*, the soil bacterium *P. putida*, and the plant growth promoting *P. fluorescens*(Matthijs*et. al.* 2007).

Fishery products, which are of great importance for human nutrition and provide clear health benefits, can also act as a source of various food borne diseases(Darlington and Stone, 2001). Export market of Bangladesh is threatened with low quality processed foods which may be contaminated with pathogenic bacteria such as coliform, faecal coliform, *Pseudomonas, Staphylococcus* etc. (Noor *et al*., 2013). In the recent time, modern biotechnology has introduced new techniques that can detect early fish contamination, improve the taste, modify

the quality of fish and prolong the shelf life of fish (William and Michael, 2009). Research on fish contamination assessment and the way of preservation is also increasing to maintain quality of fish products (Okoro*et al*. 2010; Begum *et al*. 2010; Prabakaran*et al*. 2011; Anbudhasan*et al*. 2012).

Fisheries sector plays an important role in the national economy as well as in socio-economic development of Bangladesh. It contributes 3.74 % in national GDP, 2.7 % in export earnings, and 22.23 % of the agriculture sector and supplying about 58 % of the domestic animal protein consumption (DoF, 2011). But every year huge fish and fish product rejected from the foreign country because of spoilage by microorganism.

In the study, bacteriological status was assessed for standard plate count (SPC), total coliform count (TCC) and total faecal coliform count (TFC) and occurrence of *Salmonella* spp. and *Vibrio cholerae* was examined. Coliforms are gram negative bacteria which ferment lactose and produce gas and acid. Faecal coliforms are generally found in gastrointestinal tract of human and animals. So, if Faecal coliforms are found in fish or fish products, then it can be said that these are contaminated by man or animal excreta. There are three genera of Faecal coliforms, e.g. *Escherichia*, *Klebsiella* and *Enterobacter*.

Salmonella are motile rod and gram negative bacteria. *Salmonella* occurs commonly in domestic animals and birds. Contamination of fish with *Salmonella* is due to growth in polluted waters and poor handling, hygiene and sanitation standards after harvesting.

Vibrio cholerae is gram negative, comma shaped bacteria. *Vibrio* frequently occurs in pollutedwater. After transmission these organisms multiply rapidly in the intestines of the victims.

Antony *et al.* (2002) assessed that total bacterial load of raw shrimps collected from three seafood processing were almost uniform with 10^4 to 10^5cfu/g. Raw shrimps from first two plants had lower counts of total bacteria and coliforms than the third one. The pathogens like *Vibrio cholerae, Salmonella* and *Listeria monocytogenes* were totally absent in raw shrimps.

Oramadike*et al.* (2010) studied microbiological qualities of some frozen fishes available in some reputable supermarkets in Lagos State and reported that total bacterial count ranged

between 2.0 $\times 10^3$ to 7.4$\times 10^3$cfu/g, total coliforms per gram ranged between 0 and 53 MPN/g and did not exceed acceptable total coliforms limit per gram for frozen fish. The sanitary, storage and hygienic conditions of the supermarkets were relatively the same.

Rahman*et al.* (2010) studied the variation of bacterial loads among the fishes of various feeding habits. The TBC, TC, FC, FS and total Vibrio counts ranged from 1.72 ± 0.68 × 108 to 7.00 ± 3.39 × 108, 2.49 ± 1.72 × 106 to 6.55 ± 3.00 × 106, 1.58 ± 1.29 × 106 to 2.76 ± 1.42 × 106, 4.83 ± 2.09 × 104 to 1.19 ± 0.46 × 105 and 2.06 ± 0.67 × 103 to 3.68 ± 2.02 × 105, respectively among various feeding groups.

Ali *et al.* (2012) assessed the microbial load of frozen shrimps processed for exporting to different countries of the world and reported that the mean total coliforms was <3 ±0.00 MPN/g in Cooked IQF shrimp, while it was 23.50 ± 13.72 MPN/g in raw block frozen shrimp. Fecal coliforms for both raw block frozen and cooked IQF shrimp were <3 MPN/g.

Noor *et al.* (2013) studied the prevalence of pathogenic microflora along the two major sea fish samples, Rupchanda (*Pampuschinensis*) and Surmai (*Scomberomorusguttatus*) and reported that the total bacterial count was 2.5×10**6** cfu./g in fish blend samples and the samples were highly contaminated with *Shigella*spp., *Listeria* spp., *Staphylococcus aureus.*

Rahman*et al.* (2012) assessed the health hazard microbes in raw and finished product of coral (*Latescalcarifer*) and reported that total coliform was between 15 MPN/g and 20 MPN/g in the finished product of coral, faecal coliform in raw and finished product of coral was found <3 MPN/g and *Salmonella* spp. and *Vibrio cholerae*, both were absent.

Geldreich and Clarke (1966) made a study of the occurrence, distribution and persistence of coliform, fecal coliform and faecal streptococci in the intestinal tract of freshwater fish. Fecal coliform densities were lowest in blue gills (less than 20/g) and highest in catfish. The occurrence of fecal coliform in fish caught in little Miami River reflected the warm-blooded animal pollution level of the water.

Ahmed *et al.* (1997) reported that in Hilsa fish, bacterial load in muscle of 4 days ice stored fish was 2.5×10^2cfu/g after 20 days when the fishes were organoleptically in unacceptable condition.

Das *et al.* (2007) studied the quantitative count of microorganism (bacteria, fungi, yeast) in marketed (local market) indigenous fish species in Bangladeh. The study showed that

sampled indigenous fish species contained not only high load of microbial flora but also certain pathogenic bacteria such as *E. coli* and *Salmonella*spp..

Joarder and Khatun (1995) studied both quantitatively and qualitatively the bacterial contents of scale, skin, muscle or flesh, gills and intestines of four batches of Hilsa fish. Maximum quantities of bacteria were found in the gills (average 6.3×10^6/g). The flesh contained the least amount of bacteria (average 16×10^3/g).

Sen*et al.* (1966) studied the bacteria from variety of freshwater fish; *Cyprinuscarpio var. communis* and reported the presence of micrococci, gram positive and gram-negative rods.

In the present study, Hilsha fish species was selected for microbiological assessment and preservation by olive oil method with several ingredients and procedures because this species is not only highly demandable in export market but also popular in our country. So, fish samples was collected from local fish market. Because local fish markets generally collect fish from wholesale markets and bring this to the sites, store with ice and sell them to consumers. Several studies have been done to analyze microbial quality of fresh fishes from local markets but only a few studies have done to preserve the fish with olive oil. Preservation is done by several method like smoking, canning, salting ,freezing ,icing etc. This study was designed to compare the microbial assessment of export oriented fishes and fishes from local fish markets. Thus a comparative study of microbial status of export oriented fishes from local fish market may give a clear idea about health concern. In this study some preservative methods are used observe whether it is effective or not.

1.1 Objectives:

The specific objectives of this study include:

- Assessment of microbiological status of raw fish.
- To observe in microbial load in different preservation treatments.

CHAPTER 2

MATERIALS AND METHODS

MATERIALS AND METHODS

2.1 Laboratory of Investigation

This study was carried out in Microbiology Laboratory, Department of Botany, University of Dhaka.The duration of this study was from November, 2015 to January, 2016.

2.2 Selection of Fish Samples

For the assessment of microbial status, *Tenualosailisha* a commercially important fish species was selected because this species have high demand in foreign market, not only that, this is also popular in our country.

2.3 Collection of Fish Samples

Fish sample was collected from our local fish market.

2.4 Microbiological Methods and Analysis

2.4.1 Microbiological Isolation

Bacterial status was assessed from fish sample of fish, local fish markets. Bacteriological parameters for examination of fish samples was qualitative analysis of *Salmonella* spp., *Vibrio cholera, E.coli, Enterobacteraerogens.*

2.4.2 Culture Media Used for Bacteriological Assessment

- Sterilized saline water (85%) used for serial dilution.
- Peptone Yeast extract Glucose (PYG) used for counting total heterotrophic.
- Thiosulfate Ctrate Bile salt Sucrose) (TCBS) : Used for counting of *Vibrio* spp.
- EosineMethyline Blue Agar.(EMB): used for differentiate E.coliand *Aerobaacteraerogens*
- SS: used for isolating *salmonella* and *shigella.*

(Media composition and preparation, Appendix)

2.4.3 Enumeration of Standard Plate Count (SPC):

SPC was enumerated according to ISO 4833:2003.

2.4.3.1 Media Preparation

For enumeration of SPC, Bacteriological peptone plate count agar (PCA) media was used. To dilute the fish sample Salaine water was prepared by mixing 1g of media with 1000 ml distilled water (according to the manufacturer's instructions).

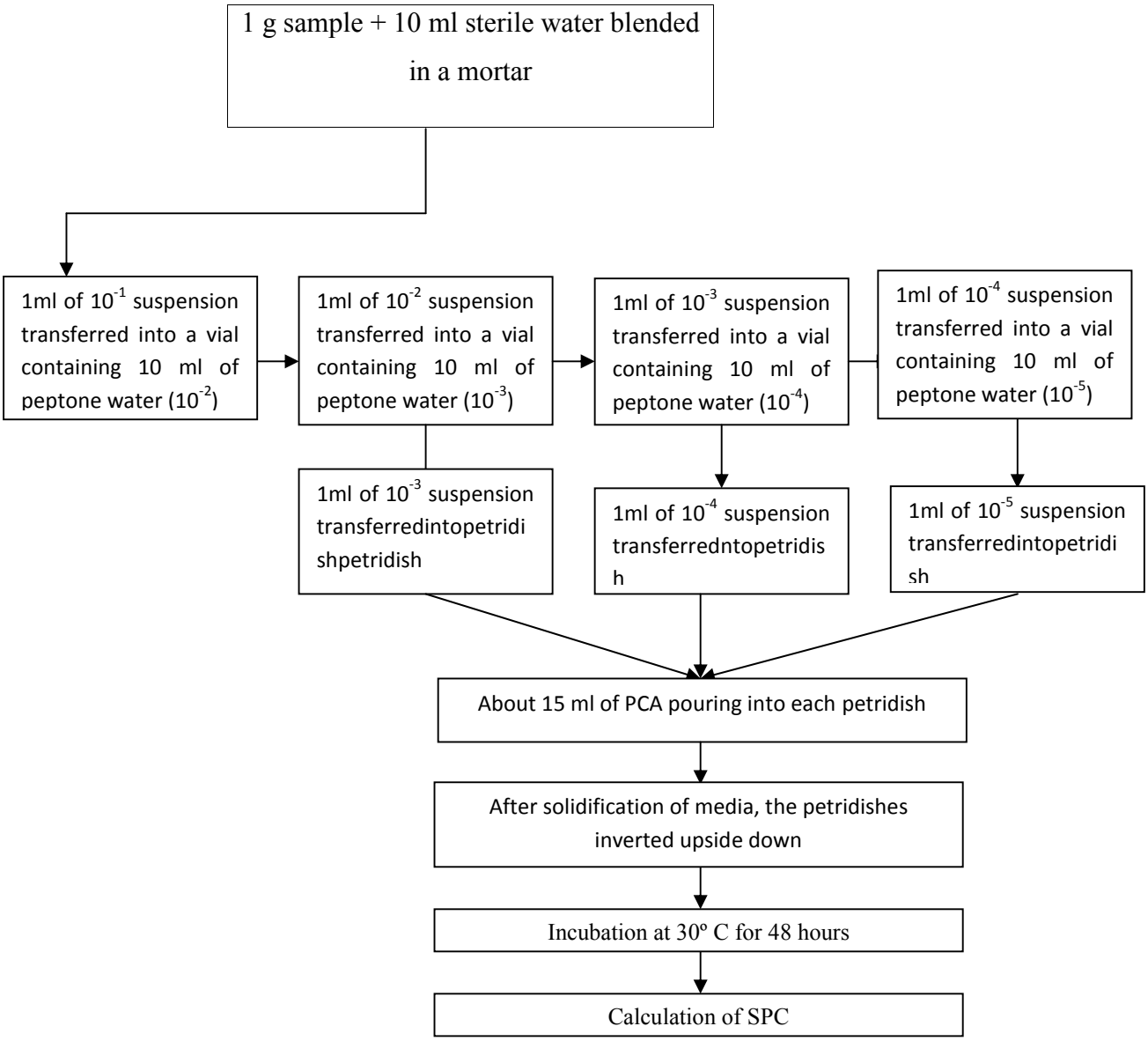
2.4.3.2 Processing of Fish Samples

Fish samples were cut & 1 g of each sample was blended with 10 ml of sterile water in a stomacher blender (Stomacher 400). Then 1 ml of this 10^{-1} dilution was transferred to a screw cap vial containing 10 ml of sterile dilute of salaine to make a dilution of 10^{-2}. Then the vial was shaken gently. This process was repeated progressively to prepare of 10^{-3}, 10^{-4}&10^{-5}.

2.4.3.3 Test Procedures

(i) Each of 1 ml of solution from 10^{-3}, 10^{-4}&10^{-5} dilutions was plated by pipette into sterile plates.
(ii) About 15 ml of sterile PCA was poured into the plates.
(iii) After solidification of the media, the plates were inverted & incubated in incubator (Shel Lab) at 30°C for 72 hours.
(iv) The total number of bacteria per gram of sample was obtained by multiplying the average number of colonies on Petri dishes by the respective dilution factor.

The total numbers of bacteria found from each Petridis for each dilution were averaged to find a reliable standard plate count (SPC).



2.4.4 Enumeration of Total*Vibrio* spp.:

2.4.4.1 Media Preparation:

For enumeration of total *vibrio* spp. TCBS (Thiosulfate citrate bile salt sucrose) was prepared in Suspended in 40.05 grams of 450 ml distilled water.Then the solution was heated to boiling to dissolve the medium completely.

Cool to 50°C and pour into sterile Petri plates.

2.4.4.2 Test Procedures

i. From processed fish samples 1 ml of each of the 10^{-1}, 10^{-2} , 10^{-3} ,-10^{-4} , 10^{-5} dilutions was transferred into the five separate sterile petri plates.

ii. Then the plates were poured with the TCBS media at the volume of 15 ml.
iii. Then the plates were shook carefully to get mixed the media thoroughly.
iv. The plates were incubated in an incubator (Binder BD 115) at 37˚C for 48 hours.
v. After incubation the bacteria was counted by digital colony counter.

2.4.5 Enumeration of Total Coliforms

TFC was enumerated according to ISO 7251:1993

2.4.5.1 Media Preparation:

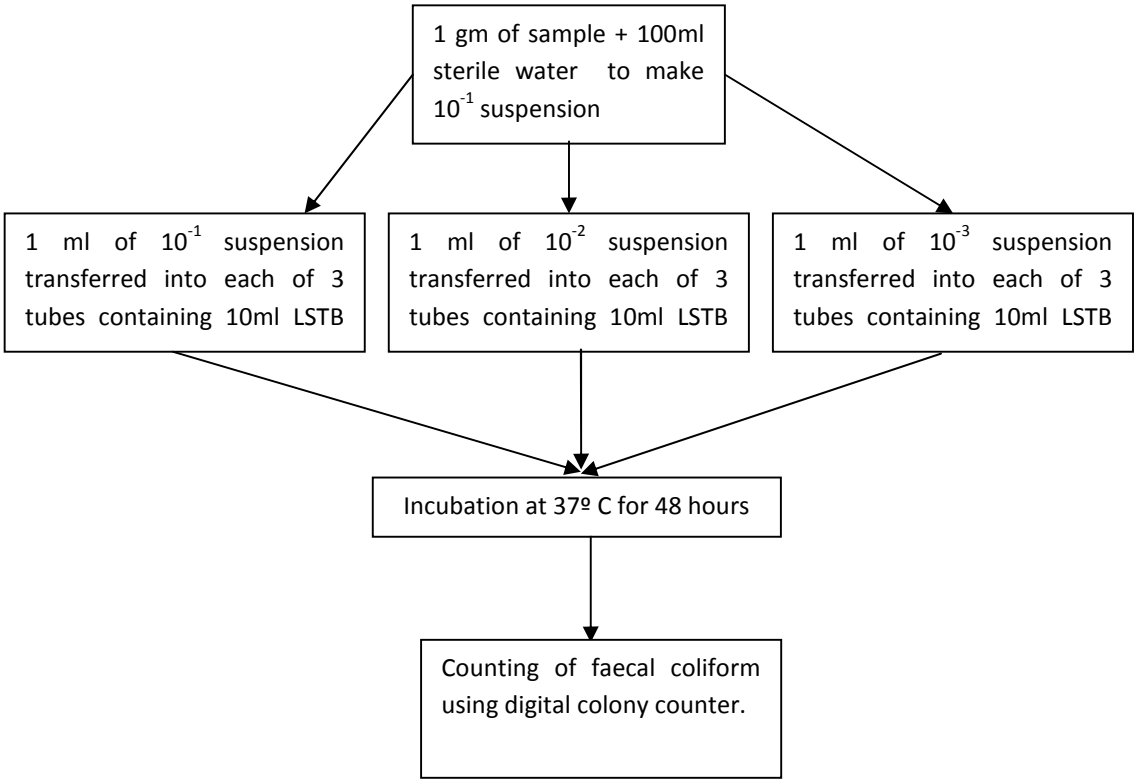
For enumeration of totalColiforms EMB (EosineMethyline Blue Agar) was prepared.Suspended in 16.182 grams in 450 ml distilled water. Then it was heated to boiling to dissolve the medium completely.

Cool to 50°C and pour into sterile Petri plates.

2.4.5.1 Test Procedure

i. From processed fish samples 1 ml of each of the 10^{-1}, 10^{-2} , 10^{-3} ,-10^{-4} , 10^{-5} dilutions was transferred into the five separate sterile petri plates.
ii. Then the plates were poured with the EMB media at the volume of 15 ml.
iii. Then the plates were shook carefully to get mixed the media thoroughly.
iv. The plates were incubated in an incubator (Binder BD 115) at 37˚C for 48 hours.
v. After incubation the bacteria was counted by digital colony counter.

Diagram 2: Enumeration of Total Coliform



2.4.6 Enumeration of *Salmonella spp.*

Presence of *Salmonella* spp was detected according to ISO 6579:2002.

2.4.6.1 Media Preparation

For enumeration of *Salmonella spp.* SS agar was prepared. Suspended 25.659 grams in 450 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE.

Cool to 50°C and pour into sterile Petri plates

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2.4.6.2 Test Procedures

i. From processed fish samples 1 ml of each of the 10^{-1}, 10^{-2} , 10^{-3} ,-10^{-4} , 10^{-5} dilutions was transferred into the five separate sterile petri plates.
ii. Then the plates were poured with the SS media at the volume of 15 ml.

iii. Then the plates were shook carefully to get mixed the media thoroughly.
iv. The plates were incubated in an incubator (Binder BD 115) at 37˚C for 48 hours.
v. After incubation the bacteria was counted by digital colony counter.

2.4.7.Enumeration of total heterotrophic bacteria:

2.4.7.1 Media Preparation

For enumeration of *Total* Heterotrophic bacteria spp. PYG media was prepared. Suspended in 6.75gm agar powder ,yeast extract 2.25 gm , glucose 4.5 gm in 450 ml distilled water at the p^H 7. Then it was heated to boiling to dissolve the medium completely..

Cool to 50°C and pour into sterile Petri plates

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2.4.7.2 Test Procedures

i. From processed fish samples 1 ml of each of the 10^{-1}, 10^{-2} , 10^{-3} ,-10^{-4} , 10^{-5} dilutions was transferred into the five separate sterile petri plates.
ii. Then the plates were poured with the PYG media at the volume of 15 ml.
iii. Then the plates were shook carefully to get mixed the media thoroughly.
iv. The plates were incubated in an incubator (Binder BD 115) at 37˚C for 48 hours.
v. After incubation the bacteria was counted by digital colony counter.

2.5 Preparation of Homogenized Fish Sample

To prepare fish sample, 1 g (raw fish) was taken and homogenized in mortar and pastel from each sample. It was then transferred to 100 ml distilled water contained in a conical flask and the whole volume was made uniform by shaking. Then 1 ml sample was diluted stepwise through of test tube containing 9 ml of distilled water. Test tubes were shacked with vortex mixture for uniform solution.

2.6.1 Plating Procedure

1 ml of homogenized samples was poured into Petri dishes with micropipette. Sterilization media of conical flask was then poured in Petri dishes and shaken horizontally to spread out the sample uniformly over the media. The lids of the Petri dishes kept few minutes partially closed for solidification of the media. At that time only media was poured in 2 Petri dishes for checking Laminar flow (SLEE, TechlinkGMbH D-55070 Mainj) and purity of media. The Petri dish which containing Laminar flow control was fully opened and the Petri dish which was containing media controller was partially opened for solidification of the media, then their lids covered the Petri dishes when the media got cooled. The Petri dishes were placed in inverted position at 37^0C in an incubator. All the operations were carried out aseptically in a laminar air cabinet.

2.6.2 Counting Method

Colonies that developed on the plates after incubation for 24 and 48 hours were counted with the help of colony counter (Stuart Scientific Counter- S.S Co. Ltd.). The number of bacterial colony per Gram of the sample was obtained by multiplying the number of colonies on the dish dilution factor. The count was expressed as colony forming unit (cfu) per gram.

2.7 FISH PRESERSVTION:

Fish were preserved by using three treatment method.

2.7.1: Treatment:

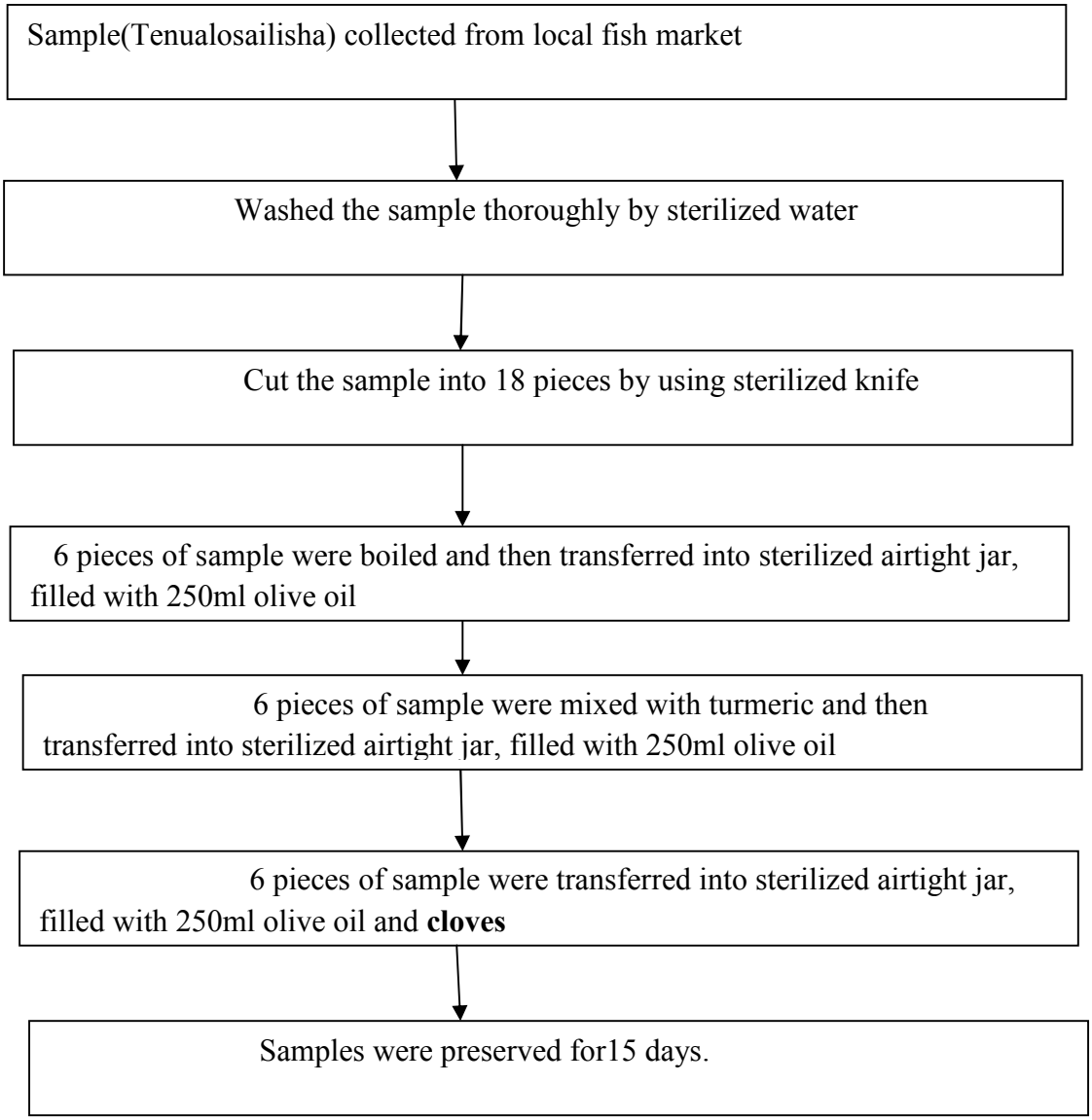
Three treatments method were used to preserve the fish sample

1 .Boiled fish piece+ olive oil(250 ml)

2. Mixed Turmeric (20g) with fish piece+ Olive oil(250 ml).

3. Add Dry Cloves (10g) with fish piece+ olive oil(250 ml).

2.7.2 PROCEDURE:



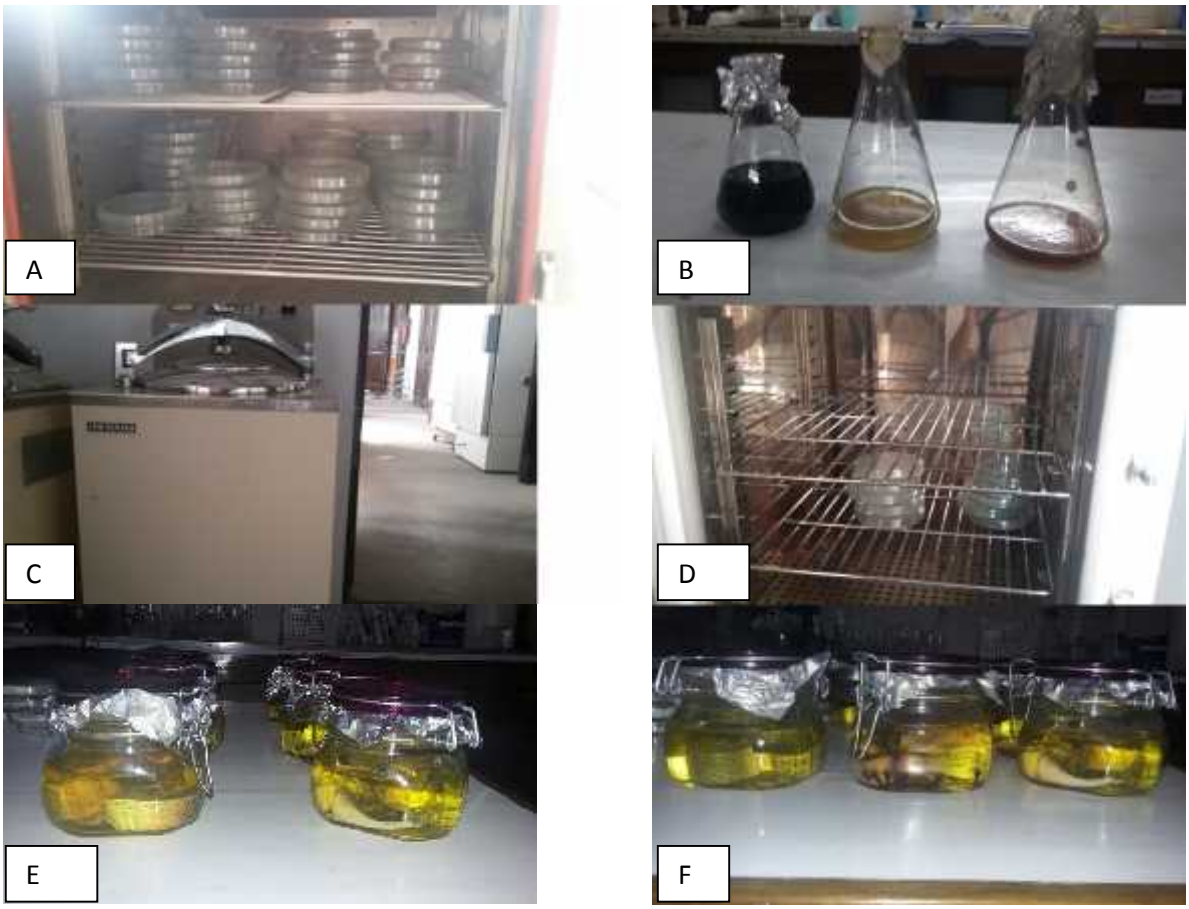


Fig:1 A: Sterilized plate B: Media Preparation C: Autoclave

D: Incubator. E and F: Fish Preservation

Chapter 3: Results

Results

3.1 Microbial count before treatment:

3.1.1 Total bacterial count

The term total bacterial count refers to all organisms living and dead. The total count also gives an estimate of the total number of microorganisms to which a substance has been exposed .In the sample Hilsha (*T. ilisha*) the average highest bacterial count was found at the EMB media(6.5×10^4cfu/g) and the average lowest bacterial count was found at the SS media (1.74×10^3 CFU/g).Where EMB media and SS media was used to identify total coliform bacteria and salmonella respectively. A significant number of average total bacterial counts was found in the Hilsha (*T. ilisha*, 2.9×10^4cfu/g) at the TCBS media which was used as identifying vibrio spp.in the sample shown in Table 1,2 and 3 respectively.

Table 1: Occurrence of Salmonella in raw fish sample by SS medium.

Plate	Form	Elevation	Margin	Surface	Colour	Transparency	Cell Abundance cfu/gm
1	irregular	Flat	curled	Smooth	black	Opaque	1.74×10^3
2	irregular	Convex	entire	Smooth	Rose red	Opaque	1.61×10^4
3	circular	Raised	entire	Smooth	black	Opaque	1.65×10^4
4	circular	Raised	curled	Smooth	Rose red	Opaque	1×10^4

`

Table 2: Occurrence of *coli form* in raw fish sample by EMB :

Plate	Form	Elevation	Margin	Surface	Colour	Transparency	Cell abundance cfu/gm
1	irregular	Convex	entire	Smooth	Dark violet with black centre	opaque	1.91×10^{3}
2	irregular	Convex	circular	Smooth	Dark violet	opaque	1×10^{4}
3	circular	Raised	circular	Smooth	Pink with blue centre	opaque	2.5×10^{4}

Table: 3: Occurrence of *Vibrio spp* in raw fish sample by TCBS medium:

Plate	Form	Elevation	Margin	Surface	colour	Transparency	Cell abundance cfu/g
1	irregular	Convex	entire	Smooth	green	opaque	1.8×10^{3}
2	circular	Raised	curled	Smooth	yellow	opaque	9.6×10^{3}
3	circular	Raised	entire	Smooth	yellow	opaque	2.9×10^{4}
4	circular	Raised	entire	Smooth	Greenish yellow	opaque	1.5×10^{5}

3.1.2 Microbial count after treatment:

In this experiment three preservation treatment or methods were used to determine which method was effective for reducing microbial load.

Treatment: 1(BOIL FISH +OLIVE OIL)

Table: 4: Total *coliform* count (TCC) cfu/g of fish sample by EMB medium

Plate	**Colour**	**Cell abundance(cfu /g)**
2	Dark violet with black centre.	5×10^4
3	Violet.	1.98×10^5
4	dark violet, greenish yellow	6.1×10^5

In the treatment 1 highest coliformbacteria was found in (6.1×10^5 Cfu/g) and lowest coliform bacteria was found in (5×10^4cfu/g).

Table: 5: Total *Vibrio* counting(TVC) in sample 1(Boil+ olive oil) by TCBS medium.

Plate	**Colour**	**Cell abundance(cfu /g)**
2	Green	5.26×10^4
3	Green	1.23×10^5
4	Yellowish green	1.1×10^5

The highest *vibrio* spp. bacteria was found in (1.23×10^5 cfu/g) and lowest *vibrio* spp. bacteria was found in (5.26×10^4cfu/g).

Table: 6: Total heterotrophic bacteria counting in sample 1(boil fish +olive oil) by PYG medium:

Plate	**Colour**	**Cell abundance(cfu /g)**
2	White	4×10^3
3	White, yellow	6.7×10^4
4	White	9.25×10^5

And the highest heterotrophic bacteria were found in (9.25×10^5Cfu/g) and lowest heterotrophic bacteria were found in (4×10^3cfu/g).

Table: 7: Total *Salmonella* counting in sample 1 (boil fish+ olive oil) by SS medium:

Plate	**Colour**	**Cell abundance(cfu /g)**
2	Black	1.54×10^4
3	White	4.1×10^4
4	Black	1.9×10^5

In the experiment highest *Salmonella* bacteria was found in (1.9×10^5 cfu/g) and lowest *Salmonella* bacteria was found in (1.54×10^4cfu/g).

TREATMENT: 2 (TURMERIC+ OLIVE OIL)

Table: 8: Total coliformcount (TCC) Cfu/g of fish sample by EMB medium:

Plate	**Colour**	**Cell abundance cfu/gm**
2	Dark violet with black centre	2.08×10^4
3	Dark violet with black centre	9.4×10^4
4	Violet	1.8×10^5

In the treatment 2 highest coliformbacteria was found in (1.8×10^5 cfu/g) and lowest coli formbacteria was found in (2.08×10^4cfu/g).

Table: 9 Total *Vibrio* counting (TVC) in sample 2(turmeric+ olive oil) by TCBS medium.

Plate	**Colour**	**Cell abundance** **Cfu /gm**
2	Green	3.8×10^3
3	Greenish yellow	1.7×10^4
4	Green	4×10^4

The highest *vibrio* spp. bacteria was found in (1.7×10^4 cfu/g) and lowest *vibrio*spp bacteria was found in (3.8×10^3cfu/g).

Table: 10: Total heterotrophic bacteria counting in sample 2(Turmeric +olive oil) by PYG medium:

Plate	**Colour**	**Cell abundance Cfu/g**
2	Yellowish white	2.7×10^4
3	White	1.2×10^5
4	White	1.5×10^5

And the highest heterotrophic bacteria was found in (1.5×10^5 cfu/g) and lowest heterotrophic bacteria was found in (2.7×10^4cfu/g

Table: 11: Total *Salmonella* counting in sample 2 (turmeric+ olive oil) by SS medium:

Plate	**Colour**	**Cell abundance cfu/g**
2	White	1.1×10^4
3	Black	4.2×10^4
4	White	7×10^4

.

In the experiment highest *Salmonella* bacteria was found in (7×10^4 cfu/g) and lowest *Salmonella* bacteria was found in (1.1×10^4cfu/g).

TREATMENT: 3 :(CLOVES+ OLIVE OIL)

Table: 12: Total coliformcount (TCC) cfu/g of fish sample by EMB medium:

Plate	**Colour**	**Cell abundance cfu/g**
2	Dark violet with black centre	8.1×10^3
3	Dark pink with black centre	3.6×10^4
4	Pink	2×10^5

In the treatment 3 highest coliform bacteria was found in (2×10^5 cfu/g) and lowest coliform bacteria was found in (8.1×10^3cfu/g).

Table: 13: Total *Vibrio* counting(TVC) in sample 3(cloves+ olive oil) by TCBS medium.

Plate	Colour	Cell abundance cfu/g
2	Green	5.3×10^3
3	Greenish yellow	2.1×10^4
4	Green	1.2×10^5

The highest *vibrio* spp. bacteria was found in (1.2×10^5Cfu/g) and lowest *vibrio* spp.bacteria was found in (5.3×10^3cfu/g).

Table: 14: Total heterotrophic bacteria counting in sample 3(cloves +olive oil) by PYG medium:

Plate	Colour	Cell abundance cfu/g
2	Yellowish white	1.62×10^4
3	White	7.8×10^4
4	White	1.2×10^5

And the highest heterotrophic bacteria was found in (1.2×10^5 cfu/g) and lowest heterotrophic bacteria was found in (1.62×10^4 cfu/g).

Table : 15: Total *Salmonella* counting in sample 3 (cloves+ olive oil) by SS medium:

Plate	Colour	Cell abundance cfu/g
2	White	5×10^3
3	White	2.9×10^4
4	White	2.2×10^5

In the experiment highest *Salmonella* bacteria was found in (2.2×10^5 cfu/g) and lowest *Salmonella* bacteria was found in (5×10^3cfu/g).

OBSERVATION: 2

TREATMENT 1 (Boil + OLIVE OIL)

Table: 16 Total coliform count (TCC) cfu/g of fish sample 1 by EMB medium:

Plate	**Colour**	**Cell abundance cfu/g**
2	Dark violet with black centre	4.1×10^4
3	Violet	3.2×10^5
4	Orange	1×10^6

In the 2nd observation, it was found that the highest coliform bacteria was found in (1×10^6 cfu/g) and lowest coliform bacteria was found in (4.1×10^4cfu/g). the treatment 1.

Table: 17 Total Vibrio counting(TVC) in sample 1(boil+ olive oil) by TCBS medium:

Plate	**Colour**	**Cell abundance** **cfu /gm**
2	Green	1.61×10^3
3	Greenish yellow	4.45×10^4
4	Green	1.6×10^5

The highest *vibrio spp*. bacteria was found in (1.6×10^5 cfu/g) and lowest v*ibrio spp* bacteria was found in (1.61×10^3cfu/g).

Table: 18 Total heterotrophic bacteria counting in sample 1(boil +olive oil) by PYG medium:

Plate	Colour	Cell abundance cfu/g
2	White	2.82×10^4
3	White	2.8×10^5
4	White	6.1×10^4

And the highest heterotrophic bacteria were found in (2.8×10^5 cfu/g) and lowest Salmonella bacteria was found in (2.82×10^4 cfu/g).

Table: 19 Total Salmonella counting in sample 1 (boil+ olive oil) by SS medium:

Plate	Colour	Cell abundance cfu/g
2	White	1.5×10^4
3	White	4.5×10^4
4	White	2.3×10^5

In the experiment highest *Salmonella* bacteria was found in (2.3×10^5 cfu/g) and lowest Salmonella bacteria was found in (1.5×10^3cfu/g).

TREATMENT 2 (TURMERIC+ OLIVE OIL):

Table: 20 Total *coliform* count (TCC) cfu/g of fish sample 1 by EMB medium:

Plate	Colour	Cell abundance cfu/g
2	Violet	3.3×10^3
3	Violet	1.35×10^4
4	Violet	3×10^4

In the 2^{nd}observation,it was found that the highest coliform bacteria was found in (3×10^4Cfu/g) and lowest coliform bacteria was found in (3.3×10^3cfu/g). the treatment 2.

Table: 21 Total *Vibrio* counting (TVC) in sample 2(turmeric+ olive oil) by TCBS medium.

Plate	Colour	Cell abundance cfu/g
2	Green	8.7×10^3
3	Greenish Yellowish.	1.9×10^4
4	Green	2×10^5

The highest *vibrio* spp. bacteria was found in (2×10^5 cfu/g) and lowest *vibrio* sppbacteria was found in (8.7×10^3cfu/g).

Table: 22Total heterotrophic bacteria counting in sample 2(Turmeric +olive oil) by PYG medium:

Plate	Colour	Cell abundance(cfu/g)
2	White	1.08×10^4
3	White	1.1×10^4
4	White	2×10^4

And the highest heterotrophic bacteria were found in (2×10^4 cfu/g) and lowest heterotrophic bacteria were found in (1.08×10^4 cfu/g).

Table: 23 Total *Salmonella* counting in sample 2 (turmeric+ olive oil) by SS medium:

Plate	Colour	Cell abundance cfu/g
2	White	1.1×10^3
3	Black	1.4×10^4
4	White	4×10^4

In the experiment highest *Salmonella* bacteria was found in (4×10^4 cfu/g) and lowest *Salmonella* bacteria was found in (1.1×10^3cfu/g).

TREATMENT 3 (CLOVES+ OLIVE OIL)

Table: 24 Total coliform count (TCC) Cfu/g of fish sample3 by EMB medium:

Plate	**Colour**	**Cell abundance cfu/g**
2	Dark violet with black centre	8.5×10^3
3	Violet	3.9×10^4
4	Violet	1.3×10^5

In the 2nd observation,it was found that the highest coliform bacteria was found in (1.3×10^5 cfu/g) and lowest coliform bacteria was found in (8.5×10^5cfu/g). the treatment 3.

Table 25: Total *Vibrio* counting(TVC) in sample 3(cloves+ olive oil) by TCBS medium.

Plate	**Colour**	**Cell abundance cfu/g**
2	Green	5.7×10^3
3	Greenish yellow	2.8×10^4
4	Green	1.3×10^5

The highest *vibrio* spp. bacteria was found in (1.3×10^5 cfu/g) and lowest *vibrio spp*. bacteria was found in (5.7×10^3cfu/g).

Table 26: Total heterotrophic bacteria counting in sample 3(cloves +olive oil) by PYG medium:

Plate	**Colour**	**Cell abundance cfu/g**
2	White	1.68×10^4
3	White	3.7×10^4
4	White	1.4×10^5

And the highest heterotrophic bacteria was found in (1.4×10^4 cfu/g) and lowest heterotrophic bacteria was found in (1.68×10^4 cfu/g).

Table: 27 Total Salmonella counting in sample 3 (cloves+ olive oil) by SS medium:

Plate	Colour	Cell abundance cfu/g
2	White	6×10^3
3	Black	3.1×10^4
4	White	2.5×10^5

.

In the experiment highest *Salmonella* bacteria was found in (2.5×10^5 cfu/g) and lowest *Salmonella* bacteria was found in (6×10^3cfu/g)..

3.1.3 Total coliformcount*:*

After preserving with boil fish with olive oil the amount of total coliform count decreased in a very little amount. Sample collected from local market; coliform count of Hilsha fish before preserving was 6.5×10^5cfu/g and after preserving with boil fish with olive oil coliform count is 6.1×10^5cfu/g(Table). After preserving with turmeric with olive oil, amount of this coliform decrease into 1.8×10^5cfu/g (Table). When the preservation was done with cloves and olive oil the coliform count decreased to 2×10^5cfu/g

3.1.4 Total *Vibrio* count

After preserving with boil fish with olive oil the amount of total vibrio count decreased in a very little amount. Sample collected from local market; vibrio count of Hilsha fish before preserving was 1.5×10^5cfu/g and after preserving with boil fish with olive oil vibrio count is 1.1×10^5cfu/g(Table). After preserving with turmeric with olive oil, amount of this vibrio decrease into 4×10^4cfu/g (Table). When the preservation was done with cloves and olive oil the vibrio count decreased to 1.2×10^5cfu/g .

3.1.5 Salmonella count

After preserving with boil fish with olive oil the amount of total*shalmonella* count decreased in a very little amount. Sample collected from local market; *shalmonella* count of Hilsha fish before preserving was 1×10^5cfu/g and after preserving with boil fish with olive oil *shalmonella* count is increased 1.9×10^5cfu/g(Table). After preserving with turmeric with olive oil, amount of this *shalmonella* decrease into 7×10^4cfu/g (Table). When the preservation was done with cloves and olive oil the vibrio count increased to 2.2×10^5cfu/g .

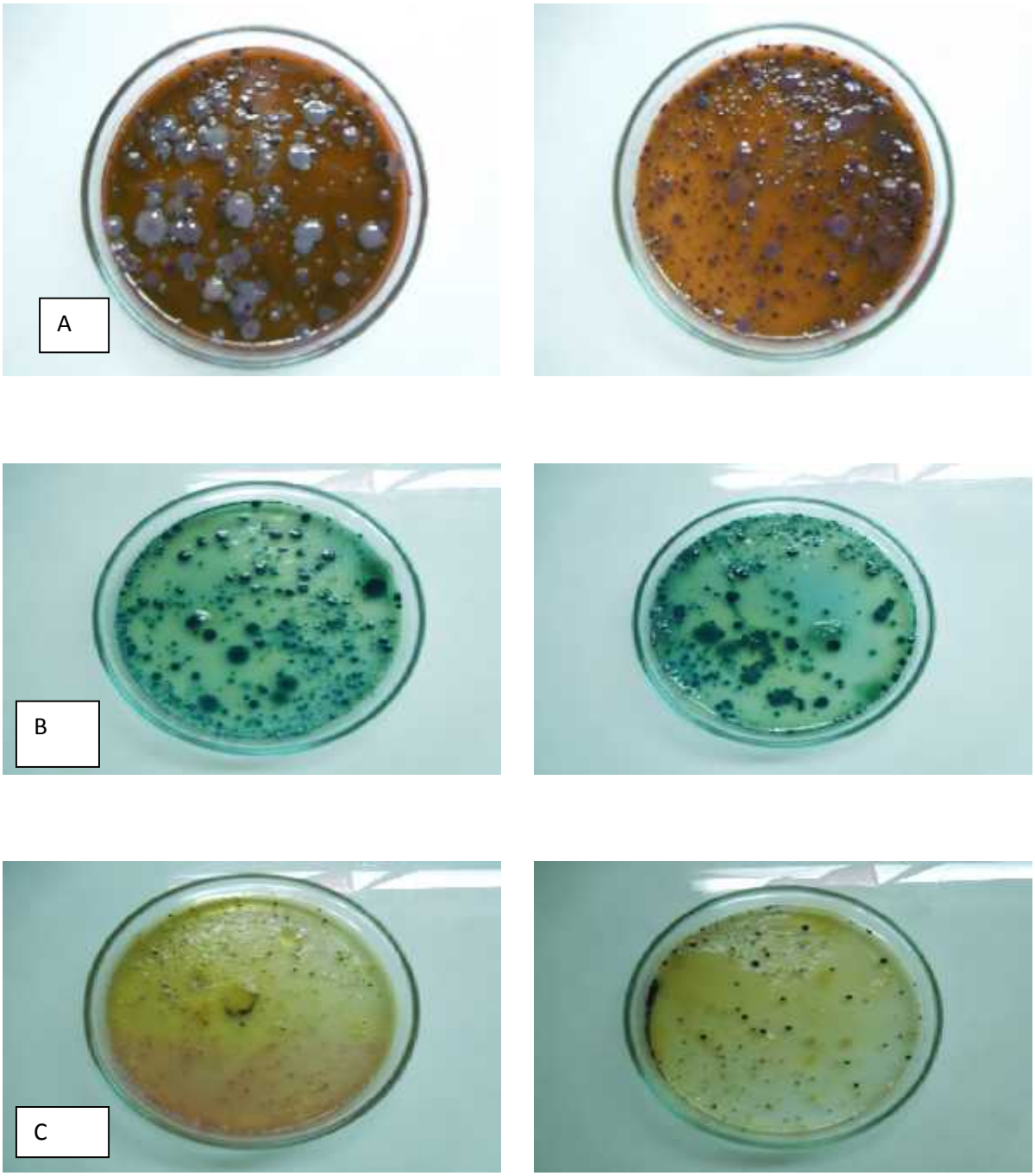


Figure: 2 Bacterial abundance in raw fish (*T. ilisha*)

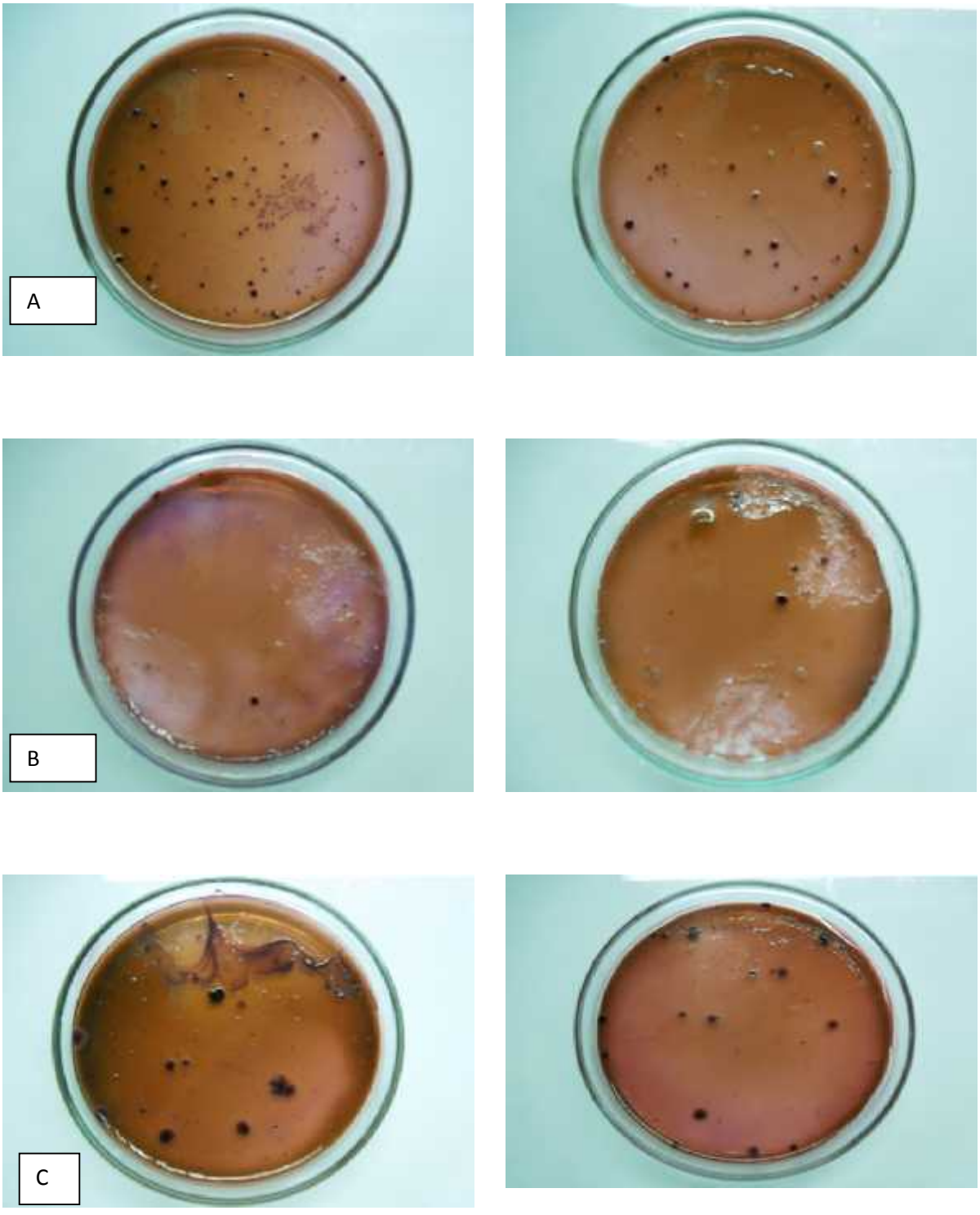


Fig:3 Microbial abundance in EMB medium after preservation the fish sample in three treatment A.(boiled fish+ olive oil), B. (Turmeric+ Olive oil), C. (Cloves+ Olive oil)

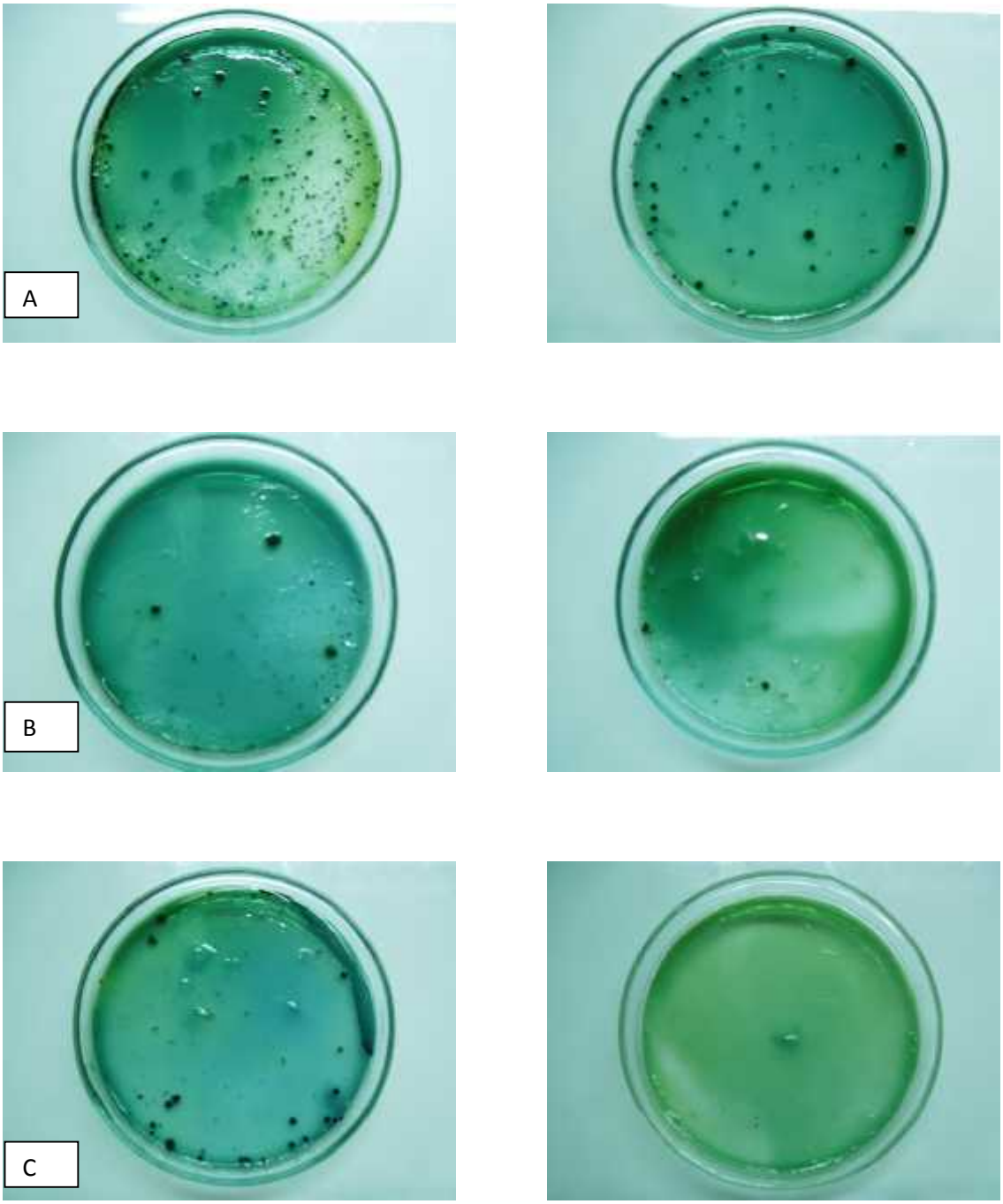


Fig: 4 Microbial abundance in TCBS medium after preservation the fish sample in three treatment A.(boiled fish+ Olive oil), B. (Turmeric+ olive oil), C. (Cloves+ Olive oil)

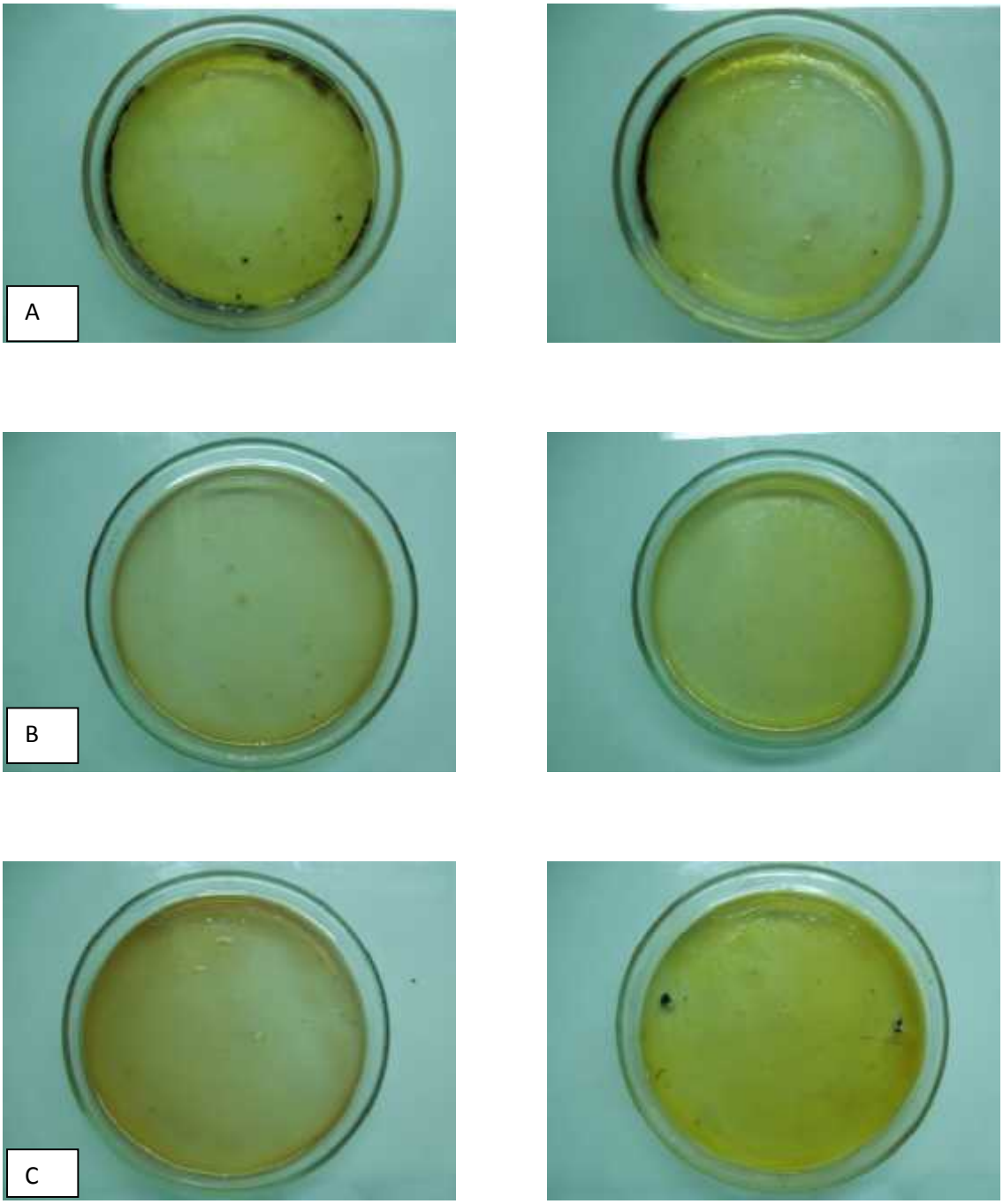


Fig:5 Microbial abundance in SS media after preservation the fish sample in three treatment A.(boiled fish+ Olive oil), B. (Turmeric+ olive oil), C. (Cloves+ Olive oil)

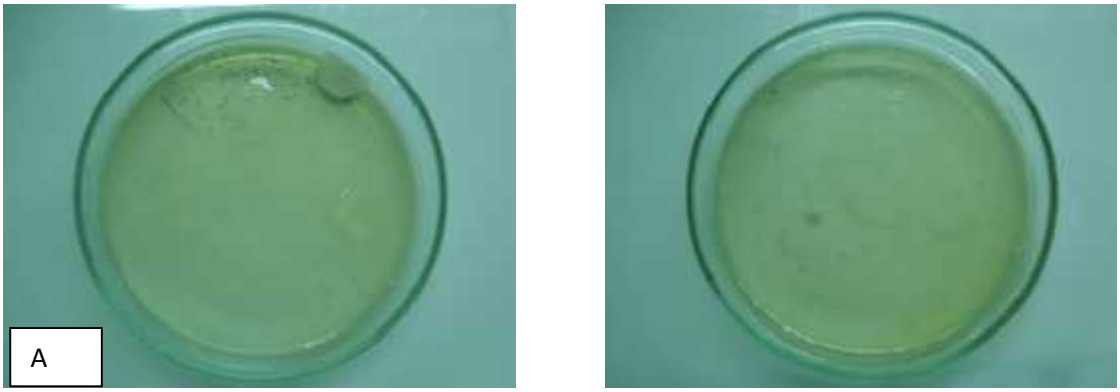


Fig: A

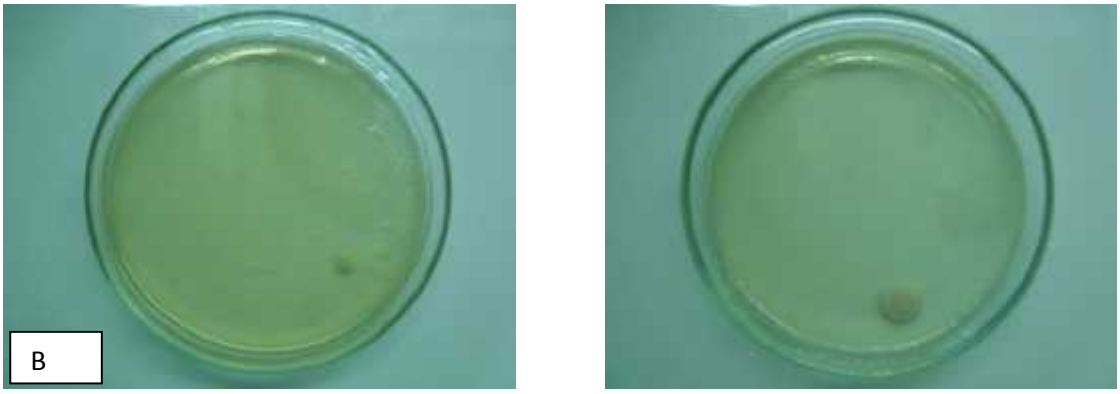


Fig: B

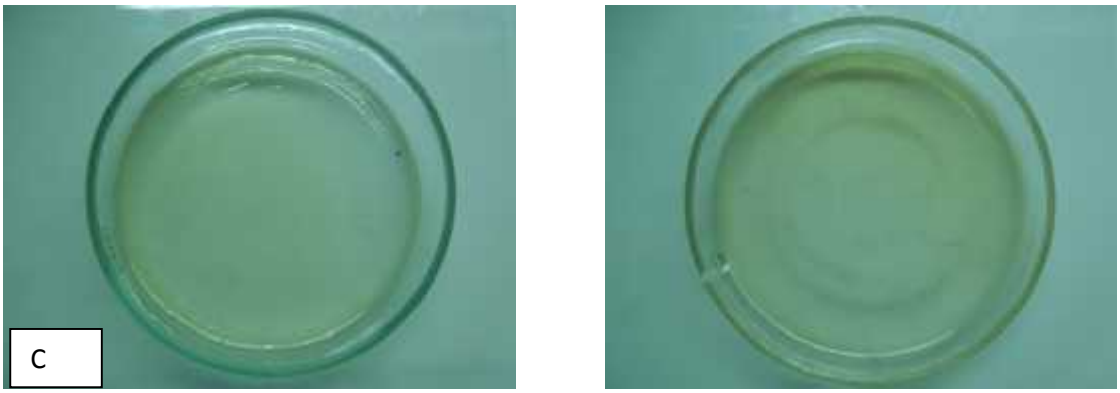


Fig:6 Microbial abundance in SS media after preservation the fish sample in three treatment A.(boiled fish+ Olive oil), B. (Turmeric+ olive oil), C. (Cloves+ Olive oil)

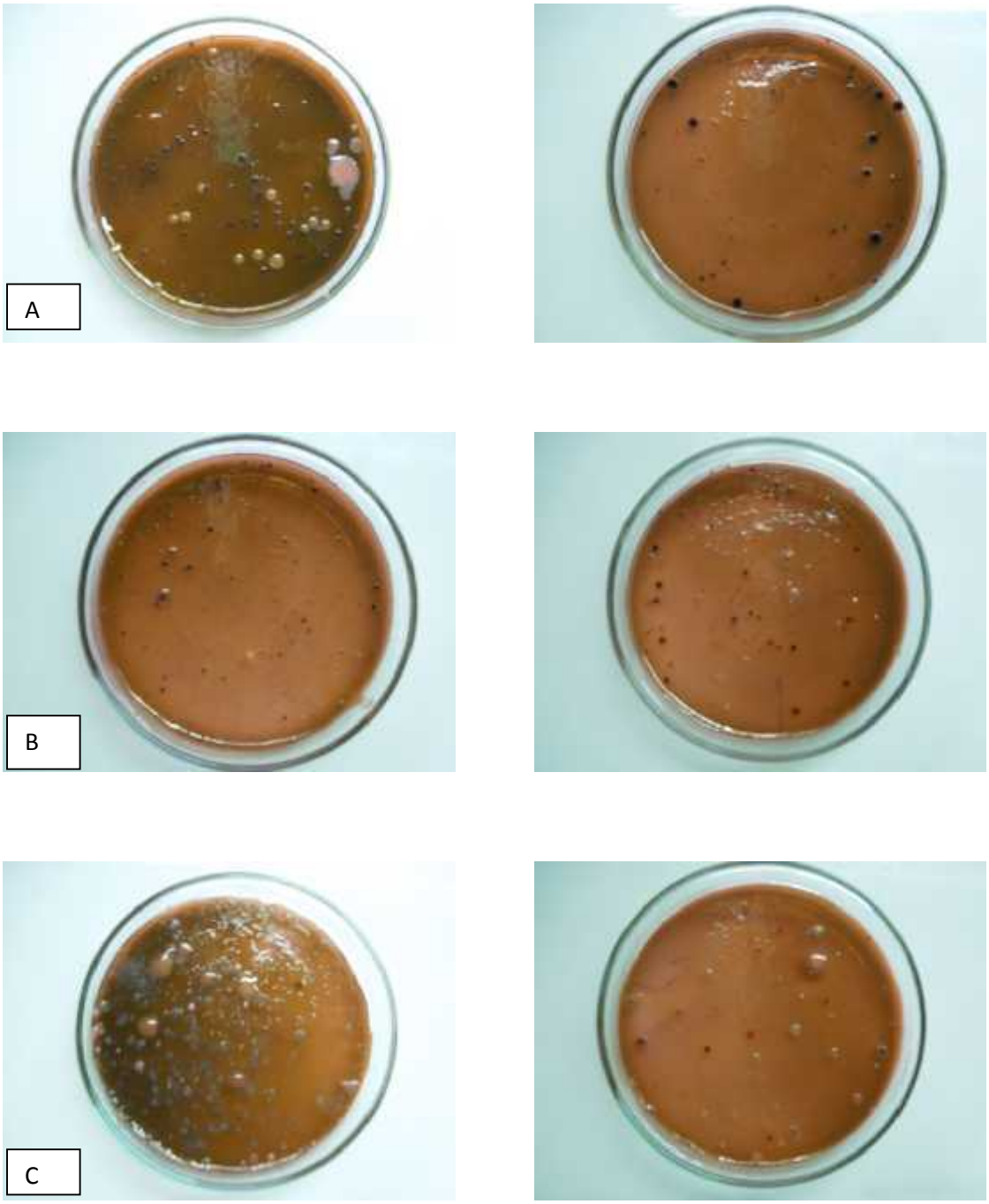


Fig: 7 Microbial abundance in EMB media after preservation the fish sample in three treatment A.(boiled fish+ Olive oil), B. (Turmeric+ olive oil), C. (Cloves+ Olive oil)

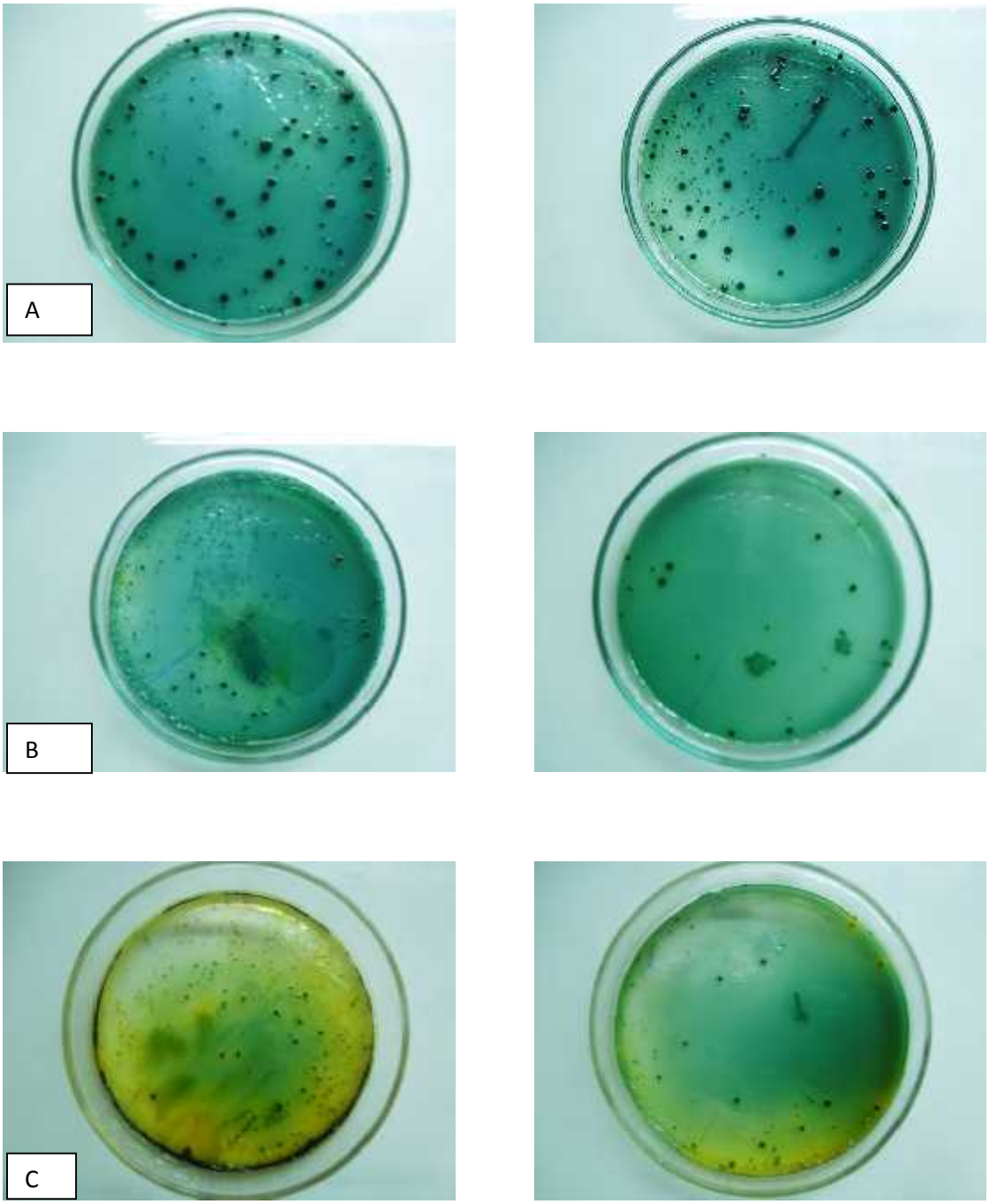


Fig:8 Microbial abundance in TCBS media after preservation the fish sample in three treatment A.(boiled fish+ Olive oil), B. (Turmeric+ olive oil), C. (Cloves+ Olive oil)

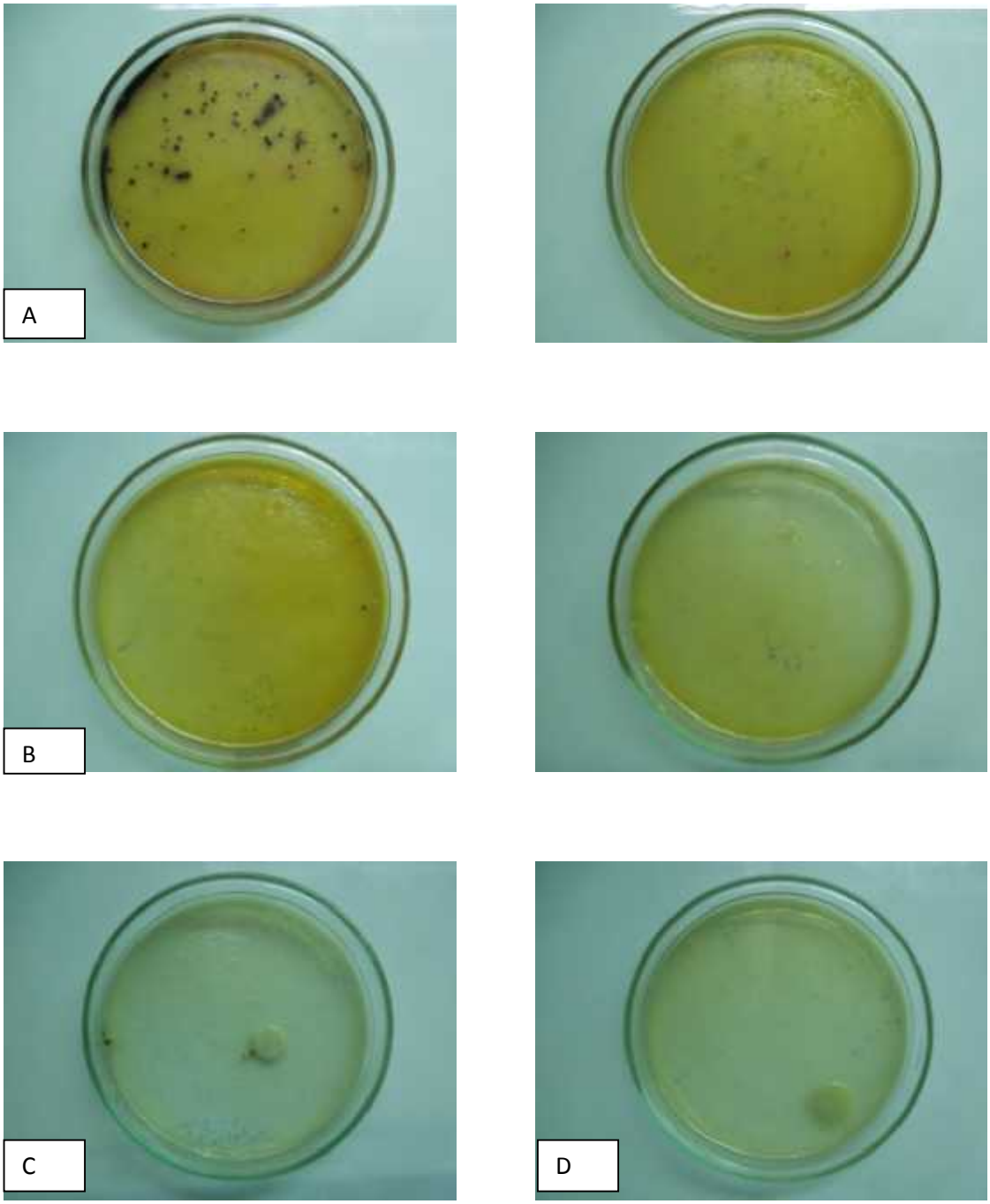


Fig: 9 Microbial abundance in SS and PYG media after preservation the fish sample in three treatment A.(boiled fish+ Olive oil), B. (Turmeric+ olive oil), C. (Cloves+ Olive oil)

Chapter 4. DISCUSSION

Discussion

In order to assess the bacteriological load of fish and their changes in different treatment were tested. This test includes the total viable bacterial count, total no. of coliform, and detection of *Pseudomonas*, and*Staphylococcus sp*.. The pathogens that are normally associated with the contamination of processed sea foods are *Escherichia coli* (Candrian*et. al*., 1991)*, Staphylococcus aureus*(Yang *et. al*., 1993), and human pathogens such as *Salmonella sp.* (Bej*et. al.*, 1994). It is well known that the spoilage of any food product is attributed to microbial growth due to improper handling, long gap between harvesting and processing and poor storage conditions (Gram and Huss, 2000). *S. aureus*contamination up to 25% has been reported in marine fishes like frozen grouper and mackerels (Adebayo- Tayo*et. al.,* 2012).

Different types of preservation methods such as drying, smoking, freezing, chilling, brining, fermentation and canning are reported to extend the self-life of seafoods and meat products. However, low temperature storage and chemical techniques for controlling water activity, enzymatic, oxidative and microbial spoilage are the most common in the industry today (Akinola*et al*., 2006; Berkel*et al*., 2004).

Since mid of 19th century, the low temperature storage method have been used for the preservation of wide varieties of seafood's which retard the growth of microorganisms. This method of preservation does not kill the microorganisms but reduces microbial metabolism which is responsible for spoilage (Ashie*et al*., 1996). Johnston *et al*. (1994) stated that freezing and cold storage are efficient methods of fish preservation but they do not improve product quality. It is necessary to preserve the fish at 0°C after catch as its spoilage is very rapid (FAO, 1973). Berkel*et al*. (2004) reported two possibilities for storing fresh fish at low temperatures: (a) cooling at -1° to +4°C, which inhibits the growth of microorganisms and (b) freezing at -18 to -30°C, which completely stops bacteria from growing. However, both enzymatic and non enzymatic changes continue but at a much slower rate. The use of ice or other methods of chilling is recommended to keep the fish all times in a cool condition before freezing (Johnston *et al*., 1994).

Arannilewa*et al*. (2005) investigated the effect of duration of freeze storage on the chemical, microbiological and sensory profile of tilapia fish (*Sarotherodungaliaenus*). They reported decreases in the values of protein and fat by 27.9 and 25.92%, respectively. The total coliform count was increased from 3.0´103-7.5´106 during storage.

Fish spoilage can be prevented by controlling water activity. For the growth of every microorganism there are minimum, optimum and maximum water activity same like pH and temperature. Therefore, lowering water activity (aw) can minimize putrefaction and improve preservation of fish (Abbas *et al*., 2009).

In order to remove the strong proteases from the digestive track, gutting of the fish immediately after catch is essential. This procedure needs to be performed properly in order to avoid contact of digestive enzymes into tissue (Pedrosa-Menabrito and Regenstein, 1988).

Raw fish are highly perishable protein source that contain normal bacterial flora from their environments in addition to the contaminants occurred during harvesting and handling of the products. The living fishes carry populations of predominantly Gram-negative psychotropic bacteria on their external skin. Coliforms could be absent or present in very low density and *Salmonella, Shigella, Vibrio* and other enteric pathogens are usually not found as these organisms are not the normal flora of the fishes or of their environment (FAO 1979).

The bacterial load on newly caught fish depends on the environment in which it is caught rather that on the fish species (Shewan 1961).The presence of coliform group (*E. coli*) in higher range suggests contamination of the samples before or during handling, processing and marketing. Higher load of TCC and TFC in samples indicates low range of contamination than local fish markets. FC is present highly in diarrheal stools of infected persons. So, the unwashed hands of infected food handlers forgetting to wash hands with soap after using the bathroom may also contaminate food (CDCP 2010).

Salmonella is highly pathogenic and this is the major reason for isolation of such bacteria from fish samples. Most of the samples fish markets was contaminated by *Salmonella* spp. After preserving with turmeric with olive oil, amount of this *shalmonella* decrease into **7×10^4cfu/g.** *So the results indicate that if*the turmeric with olive oil is used to preserve the fish the microbial attack could be reduced. The environment acts as main source of this organism in aquaculture products rather than poor standards of hygiene and sanitation. But external contamination may also may also be the source of the occurrence of these bacteria in fish (Huss 1994).

Presence of *V. cholerae* can be a cause of infection to the consumer. This organism can cause cholera or diarrheal diseases. In the present study *V. cholerae* was controlled by using

preservative turmeric with olive oil. So, proper care should be taken to avoid contamination of fish products with *V. cholerae* during handling, processing and preservation.

In most of the treatment the total microbial load of *coliform*, *Salmonella,Vibrio* and heterotrophic bacteria were significantly different among fish preservation method that was applied. Preservation with turmeric and olive oil shows better results than preservation with cloves and boil fish with olive oil.

This study gives a clear perspective on the variation of bacterial load and occurrence of *Salmonella* and *v.cholerae* comparatively in three types of preservation method applied in Hilsha fish preservation. This study also reveals that the bacteriological state of fish preservation with turmeric and olive oil was better than the two types preservative that were used.

This preservation method have not yet been studied in our country. But fish preservation with olive oil specially commercially important fish like Tuna is being preserved in the North American countries and as well as many countries of the world. This study reveals that this method of preservation has a great prospect in preserving fish. As this is for the first time this method applies so I think further research is needed in this field.

Chapter 5. CONCLUSION AND RECOMMENDATION:

Conclusion and Recommendation

From the present investigation, it has been found that microbial load in local market was high as they showed higher counts in most of microbial parameters. The investigation that was conducted also indicates that the fish of local market was in high risk to transmit various types of pathogens to the consumers. From the result, it can be concluded that the fish are sold in the local retail markets are not standard to consume since the microbial levels were always higher than the recommended levels because they don't maintain the hygienic condition during preservation and lower cold storage facilities. To overcome this situation, proper hygienic condition should be maintained at every step of catching, landing and transportation, processing and marketing following HACCP steps for good quality of fish and fishery products. This study also reveals that, pathogenic microorganism from fish can be prevented by using turmeric with olive oil. This preservation method have not yet applied in our country. But fish preservation with olive oil specially commercially important fish like Tuna is being preserved in the North American countries and as well as many countries of the world. This study reveals that this method of preservation has a great prospect in preserving fish. As this is for the first time this method applies so I think further research is needed in this field.

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Appendices

Appendix

Physiological saline:

Sodium chloride was weighted 8.5 g/l and transferred to a leak-proof bottle pre-marked to hold 1 litre. Distilled water was added to the 1 litre mark, and mixed well until the salt was fully dissolved. The mixture was sterilized by autoclaving at 121º C for 15 minutes. The bottle was stored at room temperature.

Thiosulfate citrate bile and sucrose (TCBS) agar

Composition

Peptone	10.0 g
Yeast extract	5.0 g
Sodium citrate	10 g
Sodium thiosulfate	10 g
Iron (III) citrate	1.0 g
Sodium chloride (NaCl)	10 g
Dried bovine bile	8.0 g
Sucrose	20.0 g
Bromothymol blue	0.04 g
Thymol blue	0.04 g
Agar	8.0 g to 18.0 g
Water	1000 ml

Preparation

Dissolve the components or complete dehydrated medium in the water by bringing to the boil. Adjust the pH if necessary, so that after sterilization it is 8.6±0.2 at 25 ºC. No autoclave is required.

Peotone Yeast extract Glucose (PYG) agar

composition

Glucose	10g
Peptone	5g
Yeast extract	5g
Agar	15
pH	8.5
H2O	1000ml

Preparation

Dissolve the components or complete dehydrated medium in the water by bringing to the boil. Adjust the pH if necessary, so that after sterilization it is 8.6±0.2 at 25 ºC. autoclave is required.

EosineMethyline blue Agar (EMB)

composition

Peptic digest of animal tissue	10g

Dipotassium phosphate	2g
Yeast extract	5g
lactose	5g
Sucrose	5g
Eosin-Y	0.4g
Methylene blue	0.065g
Agar	13.5g

Preparation

Dissolve the components or complete dehydrated medium in the water by bringing to the boil. Adjust the pH if necessary, so that after sterilization it is 7.2±0.2 at 25 ºC. Autoclave is required.

Salmonella and Shigella agar (SS)

composition

Beef extract	5g
Enzymatic Digest of casein	2.5g
Enzymatic Digest of animal tissue	2.5g
Lactose	10g
Bile Salts	8.5g
Sodium Citrate	8.5g
Sodium Thiosulfate	8.5g
Ferric citrate	1g

Brilliant Green	0.00033g
Neutral Red	0.025g
Agar	13.5g

Preparation

Dissolve the components or complete dehydrated medium in the water by bringing to the boil. Adjust the pH if necessary, so that after sterilization it is 7.±0.2 at 25 ºC. No Autoclave is required.

Nutrient agar

Composition:

Meat extract	3.0 g
Peptone	5.0 g
Agar	9 g to 18 g
Water	1000 ml

Preparation:

Dissolved the dehydrated components or dehydrated complete medium in the water, by heating if necessary. Adjusted the pH if necessary, so that after sterilization it is 7.2±0.2 at 25 ºC.