**UNIVERSITY OF DAR ES SALAAM**

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**SCHOOL OF AQUATIC SCIENCES AND FISHERIES TECHNOLOGY (SoAF)**

**DEGREE PROGRAM: BACHELOR OF SCIENCE IN AQUATIC SCIENCES AND FISHERIES TECHNOLOGY**

**AQ: 399 RESEARCH PROJECT**

**TITLE: Quantitative and qualitative analysis of Microbiological quality of pond water used for fish culture in relation to physico-chemical parameters at Kunduchi**

**AQ 399: RESEARCH PROJECT**

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# 1.0 INTRODUCTION

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## 1.1 General introduction

Water consist essential requirement such dissolved oxygen Temperature microorganism nutrients for fish metabolism growth and reproduction fish perform all bodily functions within water as culture environment also fish are totally depend water to breathe, feed and grow, excrete wastes, osmoregulation , and reproduce (Hansson et al 2007) Microorganism have major function in pond culture through nutrients cycling necessary for water productivity but some of fish farmers fertilize pond to accelerate growth of essential microbes such algae ,bacteria on other hand undesirable species of algae ,bacteria and other microorganism occur in pond as investigated by aquatic biologist these affect growth of cultured fish result mortality of fish occur Microbiological. Water quality focuses on determination of various kinds of pathogenic and non-pathogenic microbes in relation to physicochemical parameters of water and Each of these parameters has a standard value for fish culture (Hassall 2014). A guiding principle of fish culture is that water quality Microbial community found natural in pond water are Archae Helminths Protoctists Coliform Salmonella sp and cyanobacteria most of these are beneficial and vital in pond ecosystem Health of pond become unbalanced when environment favoure types of microbes that are less desirable or pathogenic bacteria that stunt fish growth and cause mortality hence efficient production are a direct consequence of good water chemistry. The majority of fish culture throughout the world is conducted in ponds. maintenance of good water quality is essential for healthy fish culture. pond A 1998 survey of 557 owners in Pennsylvania show that about 10% were faced with problems of water quality result to fish mortality Unfortunately, many of the pond owners had never tested their ponds water quality before they detected after problem to occur (Sabatino et al 2018). some common water quality parameters that may cause problems in ponds were assessed Water quality conditions in a pond are depend on both natural causes and human influences. originate from land uses or other activities near or within the pond site also Natural factors such as the source of the pond water, the kinds of soil in the pond, watershed change water quality properties The effects of these activities can often be minimized through proper management and early detection of problems through repeated testing

Different parameter used for water quality analysis such physico chemical parameter include temperature, transparency, turbidity, water colour, carbon dioxide, pH, alkalinity, hardness, ammonia, nitrite, nitrate, biochemical oxygen demand (BOD), primary (Bhatnagar, Devi 2013) most of the culturists are not so aware of importance of water quality management in aquaculture production. If they are properly guided and make aware about water quality management practices, they can get maximum fish yield in their ponds to a greater extent through applying water quality management practice The maximum fish production is totally dependent on the physical, chemical and biological qualities of water to most of the extent. Hence, successful pond management requires an understanding of water quality management The guidelines are given to a fish farmer to know when pond water is deteriorating in quality and therefore not suitable for fish growth by looking Clearance of water that indicates low rate of biological production Deep green water indicates over-production that serve as food for fish due to enough fertilizers, manure or nutrient rich feeds to a pond or high rate of algae Also another technique is microbiological quality analysis of water for fish culture The presence of coliform bacteria *Enterococcus* and *Staphylococcus*, bacteria in water is indicative of contamination by faecal material and considered indicative of health risk. Significance of coliform group density is established as an indication of the degree of pollution and the sanitary quality of water microbes were measured as a concentration, usually expressed as an estimate of the number of individual organisms per ml of water sample measurement of water quality involve analysis concentration of single samples, and in the ‘average’ concentration of a series of samples taken over a period of time This performed by plating microbes on highly selective culture media that is Agar plates that used for incubation at laboratory, Colonies formed after the necessary incubation period were counted directly, and the logarithm of the arithmetic means were used in presentations Microbiological assessment are very essential for water quality analysis due to fact that enable aquaculture list to prevent effect of harmful microbes to cultured fish and human being as consumer many aquaculture list in society are faced with with challenge of pathogenic microbes in pond water that transfer in series from water to fish finally to consumers as the cultured fish send to market

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## 1.2 Problem statement of the research

Most of fish farms are faced with problem of fish diseases result to stagnant growth and fish mortality The problem rising depend on performance of water quality analysis some of study revealed that deterioration of water quality in fish production it contribute poor fish growth ,increasing of disease (Yukgehnaish et al 2020) also revealed that the quality of water it depend on factors such Technology used both quantity and quality food supplied to fish and quality of water supplied to farm hence those factors it tend to change both qualitative and quantitative of water quality parameter include physical, chemical and microbiological parameter The solution to the problem is to assess quality and quantity of water quality parameter for fish farm particularly microbiological parameter in relation to physical chemical parameters the study will help fish farmers to manage or control water quality against fish mortality and stagnant growth so as to allow proper growth rate ,reproduction of cultured fish for maximum yield therefore This study will conducted for the aimed of address this gap in knowledge The study will assess microbiological quality of pond water used for fish culture in relation to physico chemical parameter at kunduchi

## 1.3 OBJECTIVES

## 1.3.1 Main Objective

To assess Microbiological quality of pond water used for fish culture at Kunduchi

### 1.3.2 Specific objectives

1. To determine bacterial load of fish ponds at kunduchi (Total plate count)
2. To identify pathogenic bacterial in fish ponds at kunduchi
3. To examine relationship between bacterial growth and physico chemical parameter of ponds water particularly Temperature, PH and Dissolved oxygen

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## 1.4 Hypotheses

1. There is significance difference in bacterial load at kunduchi fish ponds
2. Concentration of pathogenic bacteria will be greater than non-pathogenic bacteria in kunduchi ponds
3. Bacterial growth in fish pond are influenced by physicochemical parameter changing

## 1.5 Significance of the study

1. Study it helps fish farmer to improve fish health and their ecology because fish health can affected by biotic and abiotic factor this ensure high growth and reproduction performance for economic efficiency.
2. also the study will help fish farmers in choosing the optimal way of water quality management The results from this scientific study will be used to give out the best option on solving the problem of water quality management to control fish reproduction and growth
3. Stud will help to know relationship between microbes growth and physico chemical parameter of water this is due to that changing of physical chemical parameter it contributes stagnant growth and slow reproduction rate as well as mortality rate similar to microbes effect

## 1.6 Literature review

### 1.6.1 Preventing Water Quality Problems for fish culture

Water quality problems in ponds can be stopped by consider or follow some precaution for proper management of water quality techniques pond water should be test periodically so as to know bacteria or general microbes levels To avoid growth of aquatic plants and algae stop treat more than half of the pond site with aquatic herbicides and follow instruction when apply aquatic herbicides Strictly limit polluting activities near the pond or in areas that drain into the pond .presence of vegetated buffer strip around the pond . For gentle slopes around a pond, a buffer four to ten feet wide of unmoved grass will suffice. A wider buffer would be needed if the land slopes more steeply around the pond Use ditches and grading to divert polluted surface water away from the pond. (Hlordzi et al 2020). The purpose of this work is to study microbiological water quality changes resulting from increasing both anthropogenic pollution and human intervention on the fish pond water microbiological composition are being measured by means of a water quality and quantity model.so as to give over view of tasted water if is suitable for fish culture or not.

### 1.6.2 Water Quality indicators

Coliform bacteria used as indicator of water pollution due to serious effect to fish and human being such as gastroenteritis and diarrhea legal standard for the total and fecal coliforms in water has measure water quality factor but. Total coliforms group, which includes microorganisms from vegetation, soil, and water. Fecal coliforms are the members of the total coliform group that originate in the intestinal gut of warm-blooded animals the study used to determine the relationship between coliform bacteria and water quality if concentration of coliform is less than 10 fecal coliform bacteria per 100 mL water can be suitable The isolation of *Salmonella,* and *E. coli* indicate faecal and environmental pollution (Yagoub et al 2009). Coliforms such as *E. coli* are usually present where there has been faecal contamination from warm blooded animals (Chao *et al.,* 2007). The organism *E. coli* is recognized as the reliable indicator of faecal contamination in small numbers and in large numbers it is an indicator of mishandling (Eze *et al.,* 2011). *E. coli* is the only species in the coliform group that is found in the human intestinal tract and in the other warm blooded animals as a commensal and is subsequently excreted in large quantities in faeces (Geldreich, 1989). poor water quality, allow opportunistic bacterial infections to prevail Pathogenic and potentially pathogenic bacteria associated with water, fish and shellfish include *Mycobacterium,* the microbial association with fish from water compromises safety and the quality for human consumption; particularly critical is when the micro-organisms are opportunistic and / or pathogenic in nature (Mango *et al.,* 2010). There may be a potential risk of infection from food borne diseases to the residents from the surrounding communities from consuming the fish from the cultured water. Also Dissolved oxygen (DO) is a measure of the amount of oxygen in water and is an alternative indicator of water contamination. Low DO means high bacterial concentrations which use most of the oxygen for respiration and decomposition. Biochemical Oxygen Demand (BOD) is related to the concentration of the bacterial facilitated decomposable organic material in the water). The higher the BOD level the greater the need for oxygen due to a high bacterial population

### 1.6.3 Factors affecting distribution of microbes in fish pond

There are varieties factors affecting the distribution of bacteria in fish pond (Lin et al 2021) includes predatory protozoa present in water. This has great impact in decreasing the number of bacteria. Protozoa need living or dead bacteria as source of food this made easily for protozoa to engulf large number of these organisms, provided the water contains sufficient dissolved oxygen. Also in water quality the toxicity of ultraviolet rays is inversely proportional to turbidity. Similarly, high level of temperature supports harmful effect up on the survival of some microbes in water, especially those capable of producing disease. Also PH level influence microbial growth On the other side, multiplication of certain soil and intestinal forms occur when the temperature of the water is rise, e.g. *E. coli* is capable of multiplying when inoculated at 37° Excessive of food supply in pond cause increase in bacterial number. Hence, toxic substances include acids and bases it lead reduction in the number of viable organisms in this study, we will determine different types of microorganisms present in fish pond including coliforms Salmonella sp Vibrio cholera as well as ecological conditions for the survival, multiplication, and growth this helps to understand management of microbiological quality maintenance of pond water sources

### 1.6.4**.** Determination of Total Coliform Load

This will performed based on colony-forming unit counted on the agar plate After incubation, the colony-forming units (CFUs) of bacteria that developed on the culture plates used to counting and estimate total number of bacterial found in given sample (Adinortey et al 2020)The total coliform load per milliliter (mL) or per gram  will estimated as the original cell density (OCD) of the sample and can be obtained by calculation Hence V is the volume of inoculum plate, OCD is the original cell density, D is the dilution of inoculum plated, CFU is the colony forming unit in agar plate after incubation Estimation of original cell density(OCD) =CFU/DXV also the mean of total bacterial load obtained will be average value of coliform total from each samples microbiological analysis procedure it based on assumption that microbial cells will form visible and separate colony by mixing with Agar or other solid media and allowed to grow as viable colony

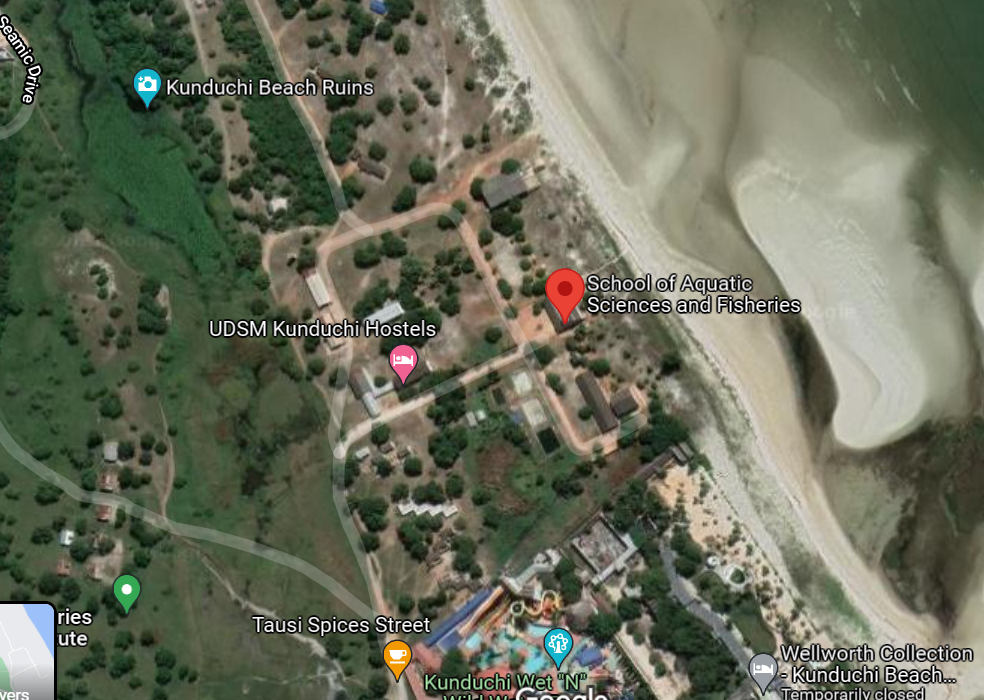
# 2.0 MATERIAL AND METHODS

The materials used for this study were sterilized by proper technique. All glass materials such as Petri dishes, conical flasks, test tubes, beakers, McCartney bottles, also Laboratory freezers, colony counter, micropipette but material will be washed and sterilized in the hot air oven at 170°C for about 2 h. The media were sterilized at 121°C for about 15-30 min in autoclave The inoculation loop was sterilized by flaming in the Bunsen burner until it turns red hot. Similarly, working surface was sterilized by the application of disinfectant solution (95% ethanol)

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## 2.1 Study site

The study site is within SoAF at kunduchi Region of Dar es salaam that located at Latitude 6o 40’0” S and Longitude 39o13’0” E study site is characterized by Fish ponds stoked with Catfish and Tilapia



2.2Sampling design

Sampling will be conducted at Kunduchi fish ponds. also samples will be collected from Three fish pond designed by give the name as pond A, B, C Analysis of bacteria such E. coli, Salmonella and vibrio cholera should performed in relation to physical chemical parameter of pond water Total bacterial load are compare between those three designed ponds

## 2.3 Sample collection

water samples will be collected from fish ponds A and B and C at the surface about a depth of 20cm using sterile bacteria bottles samples that has been sterilized to prevent bacterial contamination of the sample also PH, Temperature and Dissolved oxygen will be measured Bottles cape should be hold outside of the cap (if you touch the inside of the cap you could contaminate the sample with bacteria). Also 250 ml of water will collected from each pond but 100 ml of water sterilized by put in sterilized bottles then Screw the lid on tightly to prevent leakage. It is important that you do not touch or lay the lid down on the ground to prevent bacterial contamination on the inside of the bottle or cap. Hence samples placed to refrigerator in the laboratory approximately 24 hours -48 hours

## 2.4Sub sampling of untested sample

This step will be performed before microbiological analysis also it involved the isolation of samples collected that stored in refrigerators at Laboratory for the purpose of test microbes present in collected water sample but the process involves recording of the code number as well as identifying the test parameters to be analyze such *Escherichia coli, salmonella sp, Vibrio cholera, Vibrio parahaemolyticus, Staphylococcus,* Total Plate Counting(TPC), determination of coliform,

2.5 Microbiological analysis and Data collection

Microbiological analysis will be performed by test presence of pathogenic microbes Such Coliform, Total coliform E. coli, Salmonella species, Vibrio cholera as well as Total plate count through different methods

The multiple tube fermentation method in which Aliquots of the positive tubes of brilliant green broth were collected and streaked onto MacConkey (MC) agar for *E. coli* confirmation tests (Nikaeen et al 2010) Colonies with various will collected and transferred into tubes containing tryptic soy agar (TSA) and incubated at 37 °C for 24 to 48 h and stored in refrigerator at 4 °C for further analyses. identification. Also The most probable number(MPN) of total coliform counts was calculate using the Hoskins table

Membrane Filtration method

The method will used for Salmonella and *Shigella* identification also 1 liter of water samples will filtered through 0.45 µm cellulose acetate membrane filter (Kadykalo et al 2020) and the filter will dipped in sterile 90 mL buffered peptone water and incubated at 37 °C for 16- 20 hours. For of the coming steps of identification hence identification will be performed by colony appearances and biochemical test

Total plate count (TPC)

Method will be performed by place 1ml of water sample into sterilized petri dish followed by addition of 10-15ml of sterilized nutrient agar medium to petri dish at 34 to 450C (Yin et al 2020) both medium and sample should be shaken thoroughly and finally petri dish incubated at 370C for 24 hours also viable cell formed in plate will used to identify specific bacteria present in water sample

On other side Physicochemical parameter such Temperature, PH and Dissolved oxygen of ponds will be measured to compare its relationship with microbial growth in such way that Dissolved will be measured using Winkler method under specific reagent (Helm et al 2012) such sodium thiosulphate, concentrated sulphuric acid, manganese sulphate, PH measured using PH-meter but Temperature measured using DO - meter

## 2.6 Data analysis

Data will be tested under parametric test for assumption of normality, homogeneity of variance by using one-way ANOVA, two sample t test at a significance level of 0.05 to compare bacterial load from three ponds but if collect data will not conform to parametric-conditions non parametric test Kruskal Wallis, Mann Whitney will be applied. if the P value is < 0.05 The test is not significant different between the variables compared while if p value is >0.05 then there is a significant different between the variables compared All statistical analysis will be performed by using the computer software SPSS (Version 21.0).

3.0 OTHER IMPORTANT INFORMATION

## 3.1 Work plan

Based on research project activities that will be performed planed in table (Figure 1): below to show specific time in which whole research activities will be conducted from starting point up to end of project

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ACTIVITIES | September | December | January | February | March | April |
| Title submission |  |  |  |  |  |  |
| Proposal writing |  |  |  |  |  |  |
| Proposal presentation |  |  |  |  |  |  |
| Proposal submission |  |  |  |  |  |  |
| Data collection |  |  |  |  |  |  |
| Data analysis |  |  |  |  |  |  |
| Result presentation |  |  |  |  |  |  |
| Report writing |  |  |  |  |  |  |
| Report submission |  |  |  |  |  |  |

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## 3.2 Budget

|  |  |
| --- | --- |
| Items | Costs in (TSH) |
| Bottles for sample collection | 13,000/= |
| Transportation | 7,000/= |
| Inoculation reagent and plate | 50,0000/= |
| Balance | 30,000/= |
| Total | 100,000/= |

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Candidate’s name: ………………………………….. Signature……………Date………………..

Supervisor’s comments

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