PHYSIO-CHEMICAL AND MICROBIAL EVALUATION OF MTU FISH POND WASTE WATER

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DECLARATION

I hereby declare that this project report written under the supervision of Dr. O. I. OGUNSUYI and Dr. G. B. Akanni is a product of my research work, information derived from various sources has been duly acknowledged in the text and a list of references provided. This research project report has not been previously presented anywhere for the award of any degree or certificate.

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Date

CERTIFICATION

This is to certify that this research project titled "**PHYSIO-CHEMICAL AND MICROBIAL EVALUATION OF MTU FISH POND (*Vibrio spp., Escherichia coli*)"** was carried out by **ACHIGBUE, ANTHONY**, with matriculation number **18010101010**. This project meets the requirement governing the award of **Bachelor of Science (B.Sc.)** degree in **Microbiology** from the Department of Biological sciences of Mountain Top University, Ogun State, Nigeria and is approved for its contribution to knowledge and literacy presentation.

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Dr. O. I. OGUNSUYI

SUPERVISOR

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Dr. C.I. AYOLABI

Ag. HEAD OF DEPARTMENT

DEDICATION

I dedicate this project to the Almighty God, who has been my strength and provider. Also, to my parents Mr and Mrs A. O Achigbue for their constant prayers, advise and unending support.

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ABBREVATIONS

ABBREVATIONS	MEANING
COD	Chemical Oxygen Demand
BOD	Biological Oxygen Demand
MTU	Mountain Top University
TCBS	Thiosulphate-Citrate-Bile-Sucrose-Agar
V.	*Vibrio*
TAE	Tris-Acetate-EDTA

ABSTRACT

Pollution from pond farm wastewater entering surrounding drainage systems and water bodies is common in the Nigerian community, and might lead to water borne diseases as well as adverse health related problems. This study was designed to assess the presence of *Vibrio spp* and *Escherichia coli* in waste water from three Mountain Top University Fish Ponds waste water samples. Identification and characterization of *Vibrio spp and E. coli* in the waste water samples collected weekly over a period of one month were carried out using morphological, biochemical and molecular tests. In addition, Physico-chemical analysis of the waste water samples was carried out to determine temperature, pH, salinity, conductivity, dissolved oxygen (DO), biological oxygen demand (BOD) levels of the water sample. The morphological test shows that *vibrio* was positive when cultured on TCBS agar and *E.coli* when cultured on SMAC agar, showing their morphological features, for the biochemical test *Vibrio* was positive for catalase, oxidase, Starch hydrolysis, and citrate but was negative for Gram staining. At the molecular level, multiplexed PCR showed the presence of *Vibrio cholera* at 302*bp* (ompW *(Vibrio cholera*)-304*bp)*. The physicochemical parameters were within standard permissible limit. These findings suggest that the fish pond effluent contains possible pathogens, therefore it is critical to monitor it quality before disposing it into the ecosystem. The presence of these organisms may be a sign that the water samples has become contaminated, and when expose to human ecosystem could cause certain water-borne disease

Keyword: Physico-chemical, Wastewater, *Vibrio cholerae*, PCR, *Escherichia coli*

CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

Fish and its product are very important to human population all over the world (Njoku *et al*., 2015). According to the Food and Agricultural Organization (2002), majority (56%) of the population worldwide derives at least 20% from the consumption of fish. This is because it is the preferred source of animal protein as compared to others (i.e. poultry, beef, mutton or pork) (Delgado *et al*., 2017). It is comparatively cheaper and highly acceptable with little or no religious preference which gives it advantage over other proteins, benefiting the body with high biological value in terms of high protein retention in the body, low cholesterol level and presence of essential amino acids (Adewuyi *et al.,* 2010; Emikpe *et al*., 2011). Fishery products are not only important from a nutritional point of view, but also important for international trade, foreign exchange for a number of countries in the world and creation of job opportunities (Wikinson, 2008; Adebayo *et al*., 2012; Béné *et al.,* 2016).

Agriculture is an integral part of the Nigerian economy employing over 70% of the active labour force (Olajide *et al.,* 2012; Adesugba and Mavrotas, 2016). Catfish production plays a major role in Nigeria aquaculture industry given that it is the largest segment of aquaculture in Nigeria (Oluwatayo and Adedeji, 2019; Garlock *et al.,* 2020). Most catfish are cultured in the southern part of Nigeria, and the industry is economically important to several other states (Omofunmi *et al*, 2017). The species of catfish that can be produced include*: Clarias gariepimus, Heteroclarias spp., and Heterobranchus spp*. Besides catfish, Tilapia species like *(Oreochromis niloticus, Sarotherodon galilaues and Tilapia guinessis), Heteriotis niloticus, Gymnarchus niloticus, Mugil cephalus, Chrysichys Nitrodigitatus among others* are also commonly cultured (Adekoya *et al*, 2006).

Fishes are cultivated in different culture media or controlled environment which could be ponds (concrete or earthen), vats (wooden or fiber glass) and plastics (Adebami *et al.,* 2020). The most widely used cultivation medium is the concrete and earthen ponds (Fakorede *et al.,* 2020). Earthen pond culture system has been the traditional method of fish cultivation in Nigeria, until recently the concrete pond culture system is in use as land becomes scarce, expensive and unavailable (Ifeonu *et al.,* 2019). In Nigeria it has been estimated that 73% of fish farmers make use of concrete

ponds compared to the 27% using earthen ponds (Njoku *et al*., 2015). The water quality of a fish farm is determined by its physicochemical and biological properties (Famoofo *et al.,* 2020). The determinants of the water quality may include the questionable sources of water, the feed used for the fish which are produced from animal manure which may serve as a suitable substrate for a wide variety of microbial growth in the pond (Vasile *et al.,* 2017, Njoku *et al*., 2015).

Water sources for earthen ponds are usually untreated surface water runoffs from streams, rivers, lakes, stored waters while underground water source is being utilized for most concrete ponds (Izah *et al.,* 2016; Sule *et al.,* 2016). Water from underground sources such as a well is pumped and stored in storage tank which is used to refill the ponds when the water is to be changed at intervals of about 3-5 days (Kim *et al.,* 2021). The feed used for fish in these ponds contain organic materials from cow dungs and introduces a wide variety of microorganisms into the ponds (Adebami *et al.,* 2020). Bacterial pathogens isolated from the fish feed include: *E. coli, E. coli 0157:H7, Shigella dysenteriae, Staphylococcus aureus, Salmonella typhi, Vibrio cholerae, Klebsiella pneumoniae and Aeromonas hydrophila* (Omojowo and Omojasola, 2013; Ramírez-Castillo *et al.,* 2015). The microbial flora of a cultivated fish is a reflection of its aqueous environment (Njoku *et al*., 2015).

Wastewater comprises liquid waste containing a wide range of potential contaminants (Shah *et al*., 2014). Wastewater contains offensive and possibly dangerous substances which are mostly of anthropogenic origin and causing pollution and contamination in the surrounding environment (Sule *et al.,* 2016, 2011; Unuofin, 2020). Discharge of untreated wastewater pollutes the soil and surface water and this could be worsened during flooding (Edokpayi *et al*., 2017). Heavy metals such as cadmium, zinc, mercury, chromium, copper, cobalt, nickel, manganese, iron, vanadium and molybdenum from industries cause heavy pollution particularly in the ponds, lakes and river systems increasing the Biological Oxygen Demand (BOD) (Obasi *et al.,* 2020).The use of indicator bacteria such as faecal coliforms in water quality determination is widely used (Sibanda *et al.,* 2013). Coliforms and *Escherichia coli* are of great importance among bacterial indicators used in water quality assessment (Dagher *et al*., 2021). Physico-chemical parameters such as dissolved oxygen, pH, temperature, conductivity, total alkalinity, total hardness, total solids, transparency values, carbon dioxide, nitrite-nitrogen, sulphates, carbonates and ammonia are some of the salient factors to consider in relation to fish health (Fafioye, 2011).

1.2 STATEMENT OF THE PROBLEM

Discharge of untreated wastewater containing contaminants (from the water source, the organic fish feed and fish excreta) from fish farms pollutes the soil and surface water encouraging eutrophication and heighten the Biological Oxygen Demand (BOD) (Jana *et al.*, 2018). The spread of a large number of pathogenic bacteria that are non-susceptible to antibiotics by the action of wastewater discharge have been recognized by the World Organization for Animal Health (WOAH), the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) as a serious global human and animal health problem.

Previous studies have evaluated the physico-chemical parameters as well as the microbial community in fish ponds across the world including Nigeria. Therefore, there is a need to evaluate the physico-chemical and microbial community in MTU fish pond.

1.3 JUSTIFICATION OF THE STUDY

Due to knowledge of the harmful effects of pollutants in the environment and the need to maintain public health an extensive study is carried out to elucidate the microbial contaminants in waste water from fish ponds as well as their antibiotics susceptibility patterns. However, it is common to see many fish ponds within our community raising different types of fish especially catfish. The waste water from these fish ponds are often discharged into the surrounding drains. Hence, this study was conducted to determine the quality of this waste water in order to subject such water to proper treatment to protect the environment, plant, animal and man.

1.4 AIM OF THE STUDY

The aim of this research is to identify and characterize the presence of *Vibrio* and *E.coli* and the physico-chemical analysis of wastewater samples from fish pond in Mountain Top University.

1.5 OBJECTIVES OF THE STUDY

The objectives of the study include:

i. Determination of physico-chemical characteristics of the wastewater samples using standard methods

ii. Culturing, colony counting and isolation of bacteria from the wastewater samples

iii. Morphological and biochemical characterizations of the isolated bacteria

iv. Molecular identification of Vibrio using multiplexed PCR method.

CHAPTER TWO
LITERATURE REVIEW

2.1 Overview of Aqua-cultural Practices

Aquaculture is one of the world's fastest increasing industries of agriculture (FAO 2014). Aquaculture is a significant financial activity in many nations and provides possibilities for poverty alleviation, jobs, community development, decrease of natural resource exploitation and food security in tropical and subtropical areas. Aquaculture can also have immediate adverse effects on wild fish, birds and mammals such as seals and sea lions (Siyanbola and Adebayo, 2012). Aquaculture, the fastest increasing significant food sector for centuries, presently generates 53% of the worldwide fish we eat and is mainly liable for increasing the consumption per capital of fish goods since the 1960s (SOFIA, 2018). Global fish production peaked at approximately 171 million tons in 2016, with aquaculture accounting for 47% of the total and 53% excluding non-food uses (including reductions in fishmeal and fish oil). Fishing and aquaculture production's complete first sale value in 2016 was estimated at USD 362 billion, of which USD 232 billion came from aquaculture production. Aquaculture has been liable for the ongoing remarkable development in the supply of fish for human consumption with comparatively static capture fishery manufacturing since the early 1980s (Adekoya *et al*, 2006). Fish and fish products are today some of the world's most widely traded food items. In 2016, for human consumption or non-edible purposes, around 35 percent of worldwide fish manufacturing joined international trade in multiple forms. The 60 million tons of total fish and fish products exported in 2016 (live weight equivalent) constitute a rise of 245 percent over 1976. World trade in fish and fish goods also increased considerably in terms of value during the same era, with exports increasing from USD 8 billion in 1976 to USD 143 billion in 2016. Despite significant modifications in absolute manufacturing, the prevalent uneven pattern of distribution of manufacturing between areas and nations within the same area has stayed pronounced and mainly unchanged over the previous century. For more than two decades, Asia has accounted for around 89 percent of world aquaculture manufacturing. Africa and the Americas have raised their corresponding shares in complete world manufacturing over the same period, while Europe's and Oceania's shares have significantly fallen. Over the previous two decades, Egypt, Nigeria, Chile, India, Indonesia, Viet Nam, Bangladesh and Norway have reinforced to differing degrees their share of regional or world manufacturing. With greater

population growth and growing economically active populations in the agricultural sector, Africa and Asia have shown a usually favorable trend in the amount of individuals involved in fishing and greater rates of development in those involved in aquaculture. Without referring to Nigeria, the increase in African contribution to world fish production and the rapid growth of aquaculture in Africa cannot be discussed. Being the second highest producer of cultured fish in Africa (second to Egypt) and the highest producer of the second most important aquaculture product in Africa (Clarias gariepinus) (FAO, 2012), Nigeria has witnessed a rapid development in aquaculture production in recent time, considering the production level of about 40 000 metric tonnes (only 6% of the total domestic fish production) in 2006 (Adeogun *et al*., 2007) to 200 535 metric tonnes (24.4% of the total domestic fish production) at the end of year 2010 (FAO, 2012).

2.2 MERIT AND DEMERIT OF FISH FARMING

Human demand for fish is growing steadily. With fisheries decreasing worldwide, aquaculture is becoming an important socioeconomic alternative and a source of proteins and healthy oils (IUCN, 2007). This is because it is the preferred source of animal protein as compared to others (i.e. poultry, beef, mutton or pork). It is comparatively cheaper and highly acceptable with little or no religious bias, benefiting the body with high biological value in terms of high protein retention in the body, low cholesterol level and presence of essential amino acids (Philips *et al*, 2004; Emikpe *et al*., 2011). The target of every farmer is to produce a wholesome fish of high quality and aesthetically pleasing to the eyes with high yield and economic value towards profit maximization (Ampofo and Clerk, 2010). According to FAO, aquaculture production is already reaching almost 50% of the total fish production for human consumption, including marine and freshwater species. Some even say that the future of fish production lies with aquaculture.

Aquaculture practices are quickly developing. But they raise many concerns too. The impact of aquaculture facilities and infrastructure may affect the local fauna and flora negatively, including threatened species. The effluents from aquaculture farms containing undesired chemicals (e.g. from antifouling products) and therapeutants might distress the local ecosystem (IUCN, 2007). Farm escaped organisms can also have an impact. The use of exotic species in aquaculture is even more important, as they bring some risks such as the introduction of associated forms of life that come together with them (e.g. algae or microorganisms) or new pathogen agents that can spread out to a new environment(IUCN, 2007). Indiscriminate disposal of untreated wastewater from fish

pond on streams results in over- enrichment of water body with nutrients causing eutrophication harmful to the aesthetic value of water body, preventing sunlight penetration and decay of algae weeds which add odorous compound to the aquatic system(Omofunmi *et al*., 2016).

2.3 FISH CULTIVATION SYSTEMS

According to Dauda and Akinwole., 2015 it was noted that the development in aquaculture is not only in output, but also the practices and operations which have cut across the chains of activities in the production, including culture practices, culture systems, and water quality management and feed types and feeding system. Aquaculture systems are classified according to the following criteria these include: types of culture structure (ponds, cages, raceways, pens, enclosure, and tanks), water exchange (open or closed), intensity of culture (extensive, semi-intensive, intensive and high intensive), fish farming methods (monoculture and polyculture).

2.4 TYPES OF CULTURE STRUCTURE

Aquaculture has been practiced successfully in different holding structures ranging from ponds, tanks, raceways, cages and pens.

2.4.1 PONDS:

A pond is a controlled pond, artificial lake, or reservoir that is stocked with fish and is used in aquaculture for fish farming, or is used for recreational fishing or for ornamental purposes. Mostly earthen ponds are used for culture of carps, tilapia, catfishes and sea bass (Ozigbo, 2014). It consists of:

• Rearing ponds: These are large ponds between 0.5 to 1 feddan in size and 1.251.5 m in depth.

2.4.2 AUXILIARY PONDS:

These include ponds for segregation of food stock, Spawning ponds, Fry nursing ponds, Fry holding ponds, Storage ponds for marketable fish, Overwintering ponds. They usually much smaller ponds and may serve different functions in different seasons as, The same pond can be exploited for carp spawning in the spring, fry nursing in the summer, fish retention in the fall,

and fry overwintering in the winter. Often, 85% of the farm's total pond area is made up of growing ponds, while 15% is made up of auxiliary ponds (Magdy, 2016).

2.4.3 RACEWAYS:

One of the pioneering techniques for inland aquaculture is raceway systems. A raceway generally contains either concrete canals or basins that are rectangular in shape and have an inlet and outflow. A continuous water flow through is maintained to provide the required level of water quality, which allows animals to be cultured at higher densities within the raceway (Mirzoyan *et al*., 2010). It usually comprise a parallel sets of a narrow channels constructed in sequential blocks with two to three raceways sets in series. Typically, it is about 30×3×1 m they may be smaller or larger having fast water flow rates (Magdy, 2016). Freshwater species such as trout, catfish and tilapia are commonly cultured in raceways (Gupta and Acosta, 2011).

2.4.4 TANKS:

However, fish farming can be done in concrete or plastic tanks that may be indoor or outdoor. Small tanks (made of glass or plastic) or huge fiberglass tanks can be utilized as tanks. Although production tanks come in a variety of sizes and shapes, round tanks with capacities of 5,000 to 10,000 liters are the most widely used (ALSS, 2013). Tanks must be non-corrosive, hence fiberglass or plastic are recommended materials. Conical-shaped bottoms on smooth circular tanks are thought to be useful because they help with the release of waste materials during draining (Ozigbo, 2014). Water enters the tank through pumps in a way that causes a circular circulation within, and it exits through the centre of the tank through a drain pipe or a bottom drain that is encircled by screen. Draining out is designed to occur through a central outlet made up of a drain pipe that is enclosed by screen. Security is much easier with a tank system because production is concentrated on a small site, which can completely fence in (Magdy, 2016).

2.4.5 FLOATING CAGES:

This refers to the rearing of fish animals in floating cages with a net suspended below, within barriers in natural waterways. They could be circular, square, or rectangular. To keep fish confined and safe until they can be harvested, fish cages are installed in lakes, bayous, ponds, rivers, and oceans (Ozigbo, 2014, Magdy, 2016). When the cages are submerged beneath the water, the method is also referred to as "off-shore cultivation." When they reach market size, they are taken

after being artificially fed and maintained in cages. Several ecosystems, including rivers and streams, saltwater estuaries, and coastal marine zones, are implementing open systems. The size of floating mesh cages, which are anchored to the ocean floor, varies depending on operation's scope and the species cultured (Ozigbo, 2014).

2.4.6 PENS AND NET ENCLOSURES:

In shallow water, usually in ponds, pens and net enclosures (hapas) are used to establish a restricted environment for the culture of fish and some crustaceans. They are usually not really big, measuring only a few tens of square meters or fewer. Tightly packed stakes, such as bamboo stems or mangrove branches, or wire and other mesh can serve as the enclosures' walls. M most of these cultures are practiced in undeveloped countries. The containment of bottom-dwelling scallops with mesh fences or walls is an interesting exception to the shallow water pen (Magdy, 2016).

These pens are high enough to keep the scallops from swimming over the wall. They could use floats to allow the mesh to move with the tide. Hapas are net enclosures made of fine nylon, plastic mosquito netting, or cotton mesh. Harvesting is much easier with haps because the fry cannot escape. Hapas can be any manageable size ranging from 140 m3 to 1-2 m in depth and suspended on poles. Simple squares or rectangles can be used. There are also more complex designs, such as those that use a series of nets to separate brood stock from fry.

Brood stock densities are typically 2-7 fish/m3, with a male:female sex ratio of 1:2 to 1:7. The production rates range from 150 fry per m3 per month to 50 fry/ female/ month to over 880 fry/m3/month of 300-400 fry/female/month. Cleaning nets and harvesting fry on a regular basis are the two most important ways to increase production efficiency. As with any other fry production system, it may be beneficial to rest fish on a regular basis rather than trying to breed from them continuously. Given adequate water circulation by a sufficient depth beneath the nets (0.6 - 1 m) that allows water to pass freely to keep wastes away (Magdy, 2016).

2.5 Escherichia coli

Theodor Escherichia, an Austrian pediatrician, isolated Escherichia coli from a child's faeces for the first time in 1885 under the name "Bacterium coli community" (Escherich, 2015). Gram-negative, mobile, non-spore-shaping bacilli belonging to the Enterobacteriaceae family are E. coli. They measure around 0.5 m

and are between 1.0 and 3.0 m long. The majority of E. coli strains are harmless, but some can have clinical side effects in humans and other highly developed animals. (2011) Mushtaq et al. Enterohaemorrhagic (EHEC), enterotoxigenic (ETEC), entero-intrusive (EIEC), enteropathogenic (EPEC), enteroaggregative (EAEC), and diffuse-follower E. coli (DAEC) are the six pathogenic kinds of E. coli that cause intestinal illnesses, according to Molokwu and Okpokwasili (2002).

2.5.1 Taxonomic Classification of E.coli

Domain	Bacteria
Phylum	proteobacteria
Class	Gammaproteobacteria
Order	Enterobacteriales
Family	Enterobacteriaceae
Genus	*Escherichia*
Species	*Escherichia coli*

2.5.2 Pathogenic strains of *E.coli*

2.5.2.1 Enterohaemorrhagic *E.coli* (ehec)

Humans who are infected with E. coli O157:H7 suffer from a serious intestinal illness. It is the strain that sickens individuals the most frequently. By producing a strong toxin that destroys the lining of the intestinal wall and results in bloody diarrhea, it may be distinguished from other E. coli. Enterohemorrhagic E. coli infection is another name for it. E. coli O157:H7 was first discovered in 1982 as the source of bloody diarrhea from consuming infected raw or undercooked hamburger meat. Since then, E. coli O157:H7 outbreaks have also been linked to well water or surface water, salami, spinach, lettuce, sprouts, unpasteurized milk, apple juice, apple cider, and other types of food and water.

2.5.2.2 Enteroinvasive *E.coli* (eiec)

A small cluster of E. coli is the topic of this lesson. Many secludes lack the H antigen, which prevents them from moving, and they either take longer to mature in lactose or don't (Riley et al., 2020). They are the cause of a condition like bacillary loose stools caused by Shigella and have significant antigens that might react with those of Shigella (Dogan, 2018). Since there are no known creature resources for Shigella, the crucial hotspot for EIEC is undoubtedly contaminated people (Dogan, 2018). Shigella's infective part may only be 10 to a few hundred cells, however

research on volunteers found that around 106 EIEC organisms are needed to infect healthy adults with illness.

2.6 Vibrio species

2.6.1 Taxonomic classification of *Vibiro spp*

Domain Bacteria
kingdom Bacteria
Phylum Proteobacteria
Class Gammaproteobacteria
Order Vibrionaceae
Family Vibrionaceae
Genus Vibrio
Species Vibrio paraheamolyticus
Vibrio choleare
Vibrio vulnificus
Vibrio mimicus

2.6.2 *Vibrio Choleare*

The gram-negative bacteria Vibrio cholerae is a member of the Vibrionaceae family and is often regarded as the cause of cholera (Willey et al.,, 2008). Only the O1 and O139 serogroups of the V. cholerae, which has more than 200 identified serogroups, have been linked to the diarrheal illness commonly known as cholera (Gaffga et al.,, 2007). Any surviving non-O1/non-O139 serogroups are considered to be the primary culprits b33nd sporadic, localized outbreaks of an illness that is similar to cholera (Elhadi, 2012). Water has a key role in the transmission of the disease and the research of cholera disease transmission in marine climates, where V. cholerae is typically found (Tamrakar, 2009). Therefore, it is important to see this microorganism in water sources (Choopun, 2002).

Vibrio cholerae, Vibrio mimicus, Vibrio fluvialis, Vibrio parahaemolyticus, Vibrio alginolyticus, Vibrio cincinnatiensis, Vibrio vulnificus, Vibrio furnissii, Vibrio metschnikovii, and Vibrio

harveyi (carchariae) are among the 10 species of the Vibrio family that are harmful to (Tarr et al.,. 2007)

2.6.3 *Vibrio mimicus*

A bacterium called Vibrio mimicus resembles Vibrio cholerae in many ways, including its ability to thrive at low salt concentrations and its antigenic makeup. It has been implicated in isolated instances and gastroenteritis flare-ups, and certain strains can also transmit the cholera toxin (Lee et al., 2008).

2.6.4 *Vibrio fluvialis*

Vibrio fluvialis is known to cause both isolated cases and outbreaks of gastroenteritis. Likewise, consumption of raw or undercooked seafood, especially raw clams, is frequently linked to sickness in many different vibrio species (Lee et al., 2008). Halophilic bacteria Vibrio fluvialis is frequently found in aquatic environments or marine-related objects. Due to the fact that V. fluvialis' clinical symptoms of gastroenteritis are almost identical to those caused by V. cholerae, it is now known to be a major disease-causer. After the recent characterization of an enterotoxigenic El Tor-like haemolysin in V. fluvialis, which tackles one of the harmfulness parts of this, it turned out to be much more significant.

2.6.5 *Vibrio alginolyticus*

The halophilic bacteria Vibrio alginolyticus, which has been found in marine and estuary environments, is responsible for sepsis and other extraintestinal illnesses as well as severe contamination of sensitive tissue. People consume contaminated fish or come into touch with the microbes through open water (Kiratisin, 2012). One of the about twelve Vibrio species that can seriously depress health and result in fatality is V. alginolyticus (Scallan, and Black, 2012). V. alginolyticus was the third most common Vibrio species found in human illness for a long time, but starting in 2007, when the overall vibriosis rate increased, it became the second most common normally normal Vibrio species.

2.6.6 *Vibrio parahaemolyticus*

V. parahaemolyticus is a Gram-negative halophilic bacterium that is widely distributed (Nelapati et al., 2012; Ceccarelli et al., 2013; Zhang and Orth, 2013). A single polar flagellum attached to inanimate and living surfaces, such as zooplankton, fish, shellfish, or any suspended object underwater, gives V. parahaemolyticus the ability to move freely (Gode-Potratz et al., 2011). V. parahaemolyticus occasionally causes septicaemia, ear infections, or wound infections that can be fatal to those with existing medical issues (Zhang and Orth, 2013).

2.7 CONCEPT OF WASTEWATER

Wastewater is a term used to describe a complex mixture of wastes comprising all water discharged directly from domestic homes, industrial and agricultural business, road run-off, and any materials that may leak through damaged sewerage systems (Mayowa and Ademola 2017). These waste waters contain a wide range of potential contaminants. Waste water contains offensive and potentially dangerous substances which are mostly of anthropogenic origin and causing pollution and contamination of receiving water bodies (Ikpi and Offem, 2011). The types of wastewater are domestic, industrial and storm run-off. Domestic wastewater is a complex and dynamic composition of mixture of natural and man-made organic and inorganic materials, and changes in response to factors such as the weather and the diet of the local community For this reason, the monitoring of wastewater can be a complicated and in exhaustive process (Mayowa and Ademola, 2017). Industrial wastewaters are wastewater generated from industries and associated processes that utilize water (Alebel, 2010). These kinds of wastewater are mixtures of various suspended solids, nutrients, biodegradable organics, pathogens, heavy metals, refractory organics, and dissolved inorganic solids. The relative compositions vary widely depending on the type of activity producing the wastewater. The amount and toxicity of waste released by an industry is also directly related to its own specific activity (Nkonyeasua, 2010).

2.8 CHARACTERISTICS OF WASTEWATER

2.8.1 PHYSICOCHEMICAL CHARACTERISTICS

The physicochemical characteristics of wastewater that are of special concern are pH, dissolved oxygen (DO), oxygen demand (chemical and biological), solids (suspended and dissolved), nitrogen (nitrite, nitrate and ammonia), phosphate, and metals (Akpor and Muchie, 2011). The hydrogen-ion concentration is an important quality parameter of both natural and waste waters. It is used to describe the acid or base properties of wastewater. A pH less than 7 in wastewater influent is an indication of septic conditions while values less than 5 and greater than 10 indicate the presence of industrial wastes and non-compatibility with biological operations. The pH concentration range for the existence of biological life is quite narrow (typically 6-9). An indication of extreme pH is known to damage biological processes in biological treatment units (Akpor and Muchie, 2011; Gray, 2002).

Another parameter that has significant effect on the characteristics of water is dissolved oxygen.

All of these should be inspected at various times: To determine the effect of photosynthesis on concentrations, oxygen and temperature are measured at least twice daily. Nitrate, ammonia, and nitrite can be measured 2-3 times per week, and salinity and pH are measured daily. Pesticides (known to be used in the watershed) should be tested on a regular basis and at various rainfall levels to determine the impact of runoff on concentrations. Check for rising water flow during periods of high rainfall, which affects water quality and may boost pesticides, soil particles, and organic loading. Other parameters should be monitored several times over a month to discern ranges. Know what pesticides are used in the watershed and when, fully comprehend their toxicity, and test water for those compounds on a regular basis (Ozigbo, 2014). The BOD measures only the biodegradable organics and requires a relatively long time to obtain test results (Gray, 2002; Metcalf and Eddy, 2003).

Similarly, the COD test measures the oxygen equivalent of the organic material in wastewater that can be oxidized chemically. The COD will always be higher than the BOD. This is because the COD measures substances that are both chemically and biologically oxidized. The ratio of COD: BOD provides a useful guide to the proportion of organic material present in wastewaters, although some polysaccharides, such as cellulose, can only be degraded anaerobically and so will not be

included in the BOD estimation. There are different ways of classifying solids in wastewater, the most common types are total dissolved solids (TDS), total suspended solids
(TSS), settle able, floatable and colloidal solids, and organic and inorganic solids (Akpor and Muchie, 2011). Heavy and trace metals are also of importance in water.

They are the most important persistent pollutants in wastewater, the metals of importance in wastewater treatment are As, Cd, Ca, Cr, Co, Cu, Fe, Pb, Mg, Mn, Hg, Mo, Ni, K, Se, Na, V and Zn. Living organisms require varying amounts of some of these metals (Ca, Co, Cr, Cu, Fe, K,

Mg, Mn, Na, Ni and Zn) as nutrients (macro or micro) for proper growth. Other metals (Ag, Al,

Cd, Au, Pb and Hg) have no biological role and hence are non-essential (Metcalf and Eddy, 2003; Hussein *et al*., 2005). Unlike organic pollutants, they cannot be degraded, but accumulate throughout the food chain, producing potential human health risks and ecological disturbances. Their presence in wastewater is due to discharges from residential dwellings, groundwater infiltration, and industrial discharges. The accumulation of these metals in wastewater depends on many local factors, such as the type of industries in the region, way of life and awareness of the impact on the environment through the careless disposal of wastes (Hussein *et al*., 2005; Silvia *et al*., 2006).

2.8.2 MICROBIOLOGICAL CHARACTERISTICS

Municipal wastewater contains the most common types of microorganisms, including viruses, bacteria, fungus, protozoa, and helminthes. Although different aquatic microbes are thought to have a significant part in a number of waterborne epidemics, including pneumonia, diarrhea, meningitis, degenerative heart disease, and stomach ulcers, they also have a number of positive effects on wastewater influents (Kris, 2007). In secondary wastewater treatment, dissolved organic matter is often removed using microorganisms. The microorganisms are utilized in fixed film systems, suspended film systems, or lagoon systems depending on the treatment plant's preference. Their presence can promote solids breakdown, resulting in decreased sludge generation, during the various treatment phases (Ward-Paige et al., 2005).

Aside from solid reduction, wastewater microbes also recycle nutrients such as phosphate, nitrogen, and heavy metals. If nutrients trapped in dead materials are not broken down by microbes,

they will never be available to help other organisms survive. Microorganisms are also in charge of detoxifying acid mine drainage and other toxins in wastewater (Ward-Paige et al., 2005b). Microbial pollutants can also be used as water quality indicators. Detecting, isolating, and identifying various types of microbial pollutants in wastewater is always difficult, expensive, and time consuming. In order to avoid this, indicator organisms are always used to assess the relative risk of the presence of a specific pathogen in wastewater (Paillard et al., 2005).

For instance, *Enteric bacteria*, such as *coliforms*, *Escherichia coli*, and faecal *Streptococci* are used as indicators of faecal contamination in water sources (Akpor and Muchie, 2011; Momba and Mfenyana, 2005). To indicate viral contamination, bacteriophages (somatic and F-RNA coliphages) are used. Also, Clostridium *perfringens*, a faecal spore-forming bacterium, which is known to live longer in the environment and reported to be resistant to chlorine, is used as an indicator for the presence of viruses, protozoa or even helminthes eggs (Akpor and Muchie, 2011). Furthermore, diatoms are used to indicate the general quality of water with respect to nutrient enrichment, and they provide valuable interpretations with respect to changes in water quality, such as turbidity, conductivity, COD, BOD and chloride (Dela *et al*., 2002).

2.9 WASTEWATER FROM FISH CULTIVATION SYSTEMS

Aquaculture is like any other production enterprise where there are inputs to generate products. There are always wastes in such systems, which are either unused inputs or by-products. These wastes have little or no economic value and are often a nuisance to the environment. The waste generation from aquaculture has made its sustainability a public concern (Dauda *et al*., 2018)

2.10 SOURCES OF WASTE FROM AQUACULTURE

2. 10.1 FEED

Depending on the culture technique utilized, feed plays a different role in aquaculture productivity (Dauda et al., 2018). The principal source of waste in aquaculture systems has been recognized as feed (Martins et al.,2010; Akinwole et al, 2016;). The amount of extra feed has an impact on the amount of fish feed waste produced. Nutrient content, manufacturing method (extruded vs. pelleted), feed size to fish size ratio, quantity of feed per unit time, feeding method,

and storage period are just a few of the variables that affect waste formation from feed (Miller&Semmens,2002).

2.10.2 CHEMICALS

Chemical use in fish farms is strictly limited in current aquaculture practices; however, some chemicals are still used in the form of medications, disinfectants, and antifoulants (Read & Fernandes, 2003). Antibiotics are used for prophylaxis and curative purposes (Ajadi et al., 2016), as are anesthetics, ectoparasiticides, endoparasiticides, and vaccines, which are used for the treatment and control of parasites (internal and external), as well as microbial infections (Dauda et al., 2018). Salts are primarily used to reduce stress in fish, while lime is used to treat pond bottoms for acidity during pond preparation, and other chemicals that are not harmful to fish are also used. Although these chemicals are beneficial to fish culture, they may also be harmful to humans. the environment (Boyd & McNevin, 2015). As the water is released from the ponds, it flows into natural water bodies. The effect of these chemical wastes upon these natural water systems depends on the concentration of chemicals used, the farm size, and the size of the receiving water bodies.

2.10.3 PATHOGENS

This group of waste is rarely considered in aquaculture systems, especially when it is below the level that affects the cultured fish. However, discharging pathogens with the wastewater may negatively affect the aquatic organisms in the natural water bodies (Dauda *et al*., 2018). Natural water bodies have their own pathogenic load and receiving additional loads from fish culture systems may cause stress or the outright death of aquatic organisms. The discharge of pond effluent is rampant in semi-intensive pond aquaculture, which is more common in Africa, where organic fertilizers used in aquaculture resulted in a high level of pathogens (Ansah,2010).Four organic fertilizers (blood cow waste, cow manure, pig manure, and poultry manure) contribute to a high level of fecal streptococci (Ampofo &Clerk,2003).

2.11 COMPONENTS OF WASTEWATER FROM AQUACULTURE SYSTEMS

Our focus will be narrowed to those major aquaculture wastes from feed. Generally, wastes from aquaculture can be classified into solid wastes and dissolved wastes.

2.11.1 SOLID WASTES

Solid wastes are primarily derived from the uneaten feed and fecal droppings of cultured fish (Akinwole *et al*., 2016). They occasionally include those fish that do not survive the culture process. Solid wastes can be further classified as suspended solids and settled solids. The suspended solids are fine particles and remained suspended in the water, except when a method of coagulation or sedimentation is employed, and are the most difficult type of solids to remove from culture systems (Dauda *et al*., 2018). The settled solids are larger particles that settle within a short period of time and can be easily removed from the culture column (Dauda *et al*., 2018). Solid wastes have been classified as the most dangerous waste in fish culture systems and should be effectively removed as quickly as possible (Akeem *et al*., 2018). Solid wastes are regarded to be very dangerous because they can clog the fish gills and lead to death, especially in the case of large settled particles (Akinwole *et al*., 2016).If left for a long time and allowed to decompose, these wastes lead to increases in both the total suspended and total dissolved solids. They may also increase the nitrogenous compounds and stress the cultured fish (Akinwole *et al*., 2016).

2.11.2 DISSOLVED WASTES

Dissolved wastes are products of food metabolism in fish or decomposed, uneaten feed. In dissolved wastes, the two major components of concern are nitrogen (N) and phosphorus (P) products (Dauda *et al*., 2018). These two elements constitute important components of protein, which is the main component of fish feed. Fish, irrespective of species, require a high dietary crude protein ranging from 25 to 50%. The high protein fish feeds contain high amounts of nitrogen and phosphorus, yet less than 50% of these potential water pollutants (nitrogen and phosphorus) are retained in the body of the fish (Piedrahita, 2003). Hence, a large percentage is transferred into the culture water, where it becomes a nuisance, and, when finally released, have a lot of environmental impacts. Piedrahita (2003) went further to reveal that fish fecal droppings contained 3.6%–35%N and 15%–70%P, while the amount of N and P as the excretory products were 37%–72% and 1%–62%, respectively.

The nitrogen is mainly excreted in dissolved form as ammonia, while phosphorus is excreted as particulate matter (Dauda *et al*., 2018). Fish are unable to utilize a substantial percentage of N and P, which are primarily the main nutrients (components) of the feed, giving aquaculture a high potential for environmental pollution (Lazzari & Baldisserotto, 2008), hence its categorization as industrial waste. It is possible that these dissolved nutrients have little or no significant effect on the cultured fish, depending on the concentrations (Ansah, 2010). However, releasing of culture water of poor quality may have a significant impact on the aquatic organisms in the receiving water bodies (Dauda *et al*., 2018). Nitrogen is released into culture water in the form of ammonia, which may be further decomposed to nitrite and nitrate (Dauda *et al.*, 2014 ; Piedrahita, 2003), depending on the biological activity in the culture column. Ammonia (NH3) is highly toxic to both the fish cultured in the system and those in receiving water bodies, if not treated before released into the environment (Romano & Zeng, 2013).

Cultured fish have varying tolerances of ammonia-nitrogen which depends on fish species, age, and physiological status. Warm water fish are more tolerant than cold water fish, while adult fish are more tolerant than the fingerlings and juveniles. Ammonia is generally recommended to be below 1mg/L in culture tanks (Ajani *et al*., 2011). According to Boyd (2003), Global aquaculture alliance (GAA) recommended total ammonia nitrogen (un-ionized ammonia + ionized ammonia) of 5mg/L in the aquaculture effluents as part of the guidelines for aquaculture effluents management. Nitrite is the intermediate product of ammonia oxidation to nitrate, is also toxic, and the level below 0.5mg/L is generally desirable in fish culture systems (Ajani *et al*., 2011). However, nitrite is not stable and further oxidizes to nitrate. Nitrate is the end product of ammonia oxidation and it is generally regarded as safe because it is not toxic to most fish species even at a concentration as high as 200mg/L (Dauda & Akinwole, 2015). However, it constitutes a nuisance to the environment because it is capable of enriching the receiving water and, with phosphorus, causing eutrophication (Dauda *et al*.,2014).

Phosphorus is another important metabolite or decomposed product of aquaculture feed that is also poorly utilized. Unlike ammonia, phosphorus is not toxic to cultured fish, but when released to the environment, it enriches natural water bodies and leads to eutrophication, depending on its concentration, frequency of release, and the size of the receiving water body (Dauda *et al*., 2018). Unlike nitrogen that is released into the water mainly in dissolved form, a larger percentage of P

is released as particulates in feces. This varies with species, with Tilapia hybrid releasing major phosphorus (60–62%) in dissolved form through excretion (Piedrahita, 2003). Phosphorus in culture water is primarily released as phosphate, which is an important nutrient for receiving water along with its nitrate counterpart from nitrogen (Lazzari & Baldisserotto, 2008). Unfortunately, when the concentration is high, the two cause eutrophication in the receiving water bodies.

2.11.3 MICROBIAL WASTE

The fish, their biotic and abiotic environments are inextricably linked, and changes in one may reflect and affect the other (Wurt, 2000). Many microorganisms in pond water or water used in intensive fish rearing could be pathogenic or opportunistic pathogens to fish, humans, and planktons (Zmyslowska et al., 2003). These contaminating microorganisms have been linked to poor water quality, which can be traced back to contaminated water sources and high stocking densities (Sule et al., 2016). Water sources for earthen ponds are typically untreated surface water runoffs from streams, rivers, lakes, and stored waters, whereas most concrete ponds use an underground water source.

Water is pumped from underground sources, such as a well, and stored in a storage tank, which is used to refill the ponds when the water needs to be changed every 3-5 days. The fish feed used in these ponds contains organic materials and introduces a wide range of microorganisms. Omojowo and Omojasola (2013) isolated six bacterial pathogens from cow dung used as feed in the fish pond: E. coli, E. coli 0157:H7, Shigella dysenteriae, Staphylococcus aureus, Salmonella typhi, and Aeromonas hydrophila. A cultivated fish's microbial flora reflects its aqueous environment ((Sule et al., 2016; Oni et al., 2013).

Freshwater fish in ponds commonly suffer from bacterial diseases such as various kinds of skin ulcerations, albinoderma, erythroderma, furunculosis, and verticle-scale disease, primarily caused by *Aeromonas sp*. and *Pseudomonas sp*. (Das, 2004). Some of these diseases were reported to be most severe during the dry season, when declining water quality is a problem. In Arizona, significant mortalities of cultured tilapia resulted from infection with a salt-tolerant strain of the

bacterium *Aeromonas hydrophilia* (Sule *et al*., 2016). Some prevalent pathogenic bacteria of milkfish and tilapia isolated in Taiwan include species of the genera: *Aeromonas, Edwardsiella, Flavobacterium, Pseudomonas,* and *Streptococcus* (Sule *et al*., 2016). A ricketssia-like bacterium has also been identified as a causative agent for disease outbreaks in tilapia cultured in Taiwan.

2.12 IMPACTS OF FISH FARM EFFLUENTS

Pollution Omofunmi et al. investigated the impact of pond effluents on soils and found that it generates offensive odors in the immediate environment, has a negative impact on aesthetic value, and affects the physical parameters and mineral composition of the soil in the intermediate vicinity. The effluent from the fish pond contains oxygen-demanding waste that competes for available oxygen in the soil and water for the decomposition of organic matter. There are two methods for disposing of catfish effluents that have been observed: land disposal and dilution technique. The former method allows effluent to flow over cultivable land (integrated farming) or bared land. In the latter method, effluent is discharged into a body of water or a stream. There were mixed feelings about reusing catfish effluent irrigation (integrated system).

2.12.1 PUBLIC HEALTH RISK

According to Ravikant et al. (2006), hospital effluents have a high load (0.58 to 40%) of multiple drug resistant (MDR) bacteria, in addition to deviations in physico-chemical parameters such as pH, total suspended solids (TSS), biological oxygen demand (BOD), and chemical oxygen demand (COD). As a result, effluent discharged into the environment can pose a serious public health risk. MDR bacteria could infect community members and pose a threat and serious therapeutic problem and can be more dangerous if their drug resistance is transferred to other sensitive pathogens in the environment. The development and spread of resistance to antibiotics by pond bacteria is a major public health threat, it could have serious medical and economic implications. These resistant pathogens may be transmitted to humans and farm animals causing infections that cannot be treated by conventional antibiotics The World Health Organization (WHO) estimates that 3.4 million people, mostly children die every year from water-related diseases (Abu and Wondikom, 2018).

2.12.2 IMPACT IN WATER BODY

Water in catfish ponds usually has higher concentrations of nitrogen, phosphorus, organic matter and biochemical oxygen demand than natural surface waters in the vicinity (Boyd et-al, 2000). Researchers likes Boyd, (2005) and Boyd et-al, (2008) reported that concentrated aquatic animal production (CAAP) facilities such as hatchery and fish ponds to mention few are major sources of wastewater effluent that contain high level of oxygen demanding waste, producing objectionable odor in the receiving adjacent streams which most fish farmer consider an ease waste disposal methods. However, indiscriminate disposal of untreated wastewater from fish pond on streams results in over- enrichment of water body with nutrients causing eutrophication harmful to the aesthetic value of water body, preventing sunlight penetration and decay of algae weeds which add odorous compound to the aquatic system.

2.13 EFFECT ON AQUATIC ECOSYSTEM

The release of toxic substances from wastewater into receiving water bodies has direct toxic impacts on terrestrial plants and animals. The toxic impacts may be acute or cumulative. Acute impacts from wastewater effluents are generally due to high levels of ammonia and chlorine, high loads of oxygen-demanding materials, or toxic concentrations of heavy metals and organic contaminants. Cumulative impacts are due to the gradual buildup of pollutants in receiving water, which only become apparent when a certain threshold is exceeded (Akpor and Muchie, 2011). In addition, eutrophication of water sources can lead to nutrient enrichment effects.

Nutrient-induced production of aquatic plants in receiving water bodies has the following detrimental consequences: Algal clumps, odours and decolouration of the water, thus interfering with recreational and aesthetic water use; extensive growth of rooted aquatic life interferes with navigation, aeration and channel capacity; dead macrophytes and phytoplankton settle to the bottom of a water body, stimulating microbial breakdown processes that require oxygen, thus causing oxygen depletion; extreme oxygen depletion can lead to the death of desirable aquatic life; siliceous diatoms and filamentous algae may clog water treatment plant filters and result in reduced backwashing, and algal blooms may shade and submerge aquatic vegetation, thus reducing or eliminating photosynthesis and productivity (Kurosu, 2001; Alm, 2003; Mbewele, 2006; McCasland *et al*., 2008).

Although nitrogen and phosphorus are beneficial to aquatic life in small amounts, when in excess they contribute to eutrophication. Eutrophication leads to algal blooms and plant growth in streams, ponds, lakes, reservoirs and estuaries and along shorelines (Akpor and Muchie, 2011). In lakes, rivers, streams and coastal waters where large algal blooms are present, the death of the vast numbers of phytoplankton that make up the blooms may smother the lake bottom with organic material. The decay of this material can consume most or all of the dissolved oxygen in the surrounding water, thus threatening the survival of many species of fish and other aquatic life (Akpor and Muchie, 2011). In most surface waters, total ammonia concentrations greater than 2 mg/L are toxic to aquatic life, although this varies between species and life stages, they also inhibit photosynthesis. Nitrate is believed to cause a reduction in amphibian populations.

Adverse effects are reported to be poor larval growth, reduced body size, and impaired swimming ability (Environmental Canada, 1999).

2.14 FISH POND WASTEWATER TREATMENT METHODS

Wastewater treatment is a process to renovate wastewater before its reuse or discharge. The goal is to reduce or remove organic matter, solids, nutrients, disease causing organisms and other pollutants from wastewater. A number of physical, chemical, and biological methods used in wastewater treatment have been applied in aquaculture systems (Cao and Wang, 2010).

2.14.1 PHYSICAL PROCESS

SEDIMENTATION

In intensive pond culture, there is an abundance of suspended solids derived from feces and uneaten food. Sedimentation refers to the processes that allow suspended solids with a higher density or specific gravity than water to settle out of suspension and be separated from the main flow. Gravity, along with other complicating factors, causes particulate waste matter to sink. The settling velocity is determined by the viscosity of the fluid (water) and the particle diameter (if the particle is assumed to be spherical) (Cao and Wang, 2010).

2.14.2 MECHANICAL FILTRATION

Mechanical filtration canemove suspended solids as well. There are several types of filtration, including drum filtration, screen filtration, and sand filtration. These filters have mesh sizes as small as 40 um, but due to the large amount of wash water required, filters with mesh sizes of 70 um or larger are usually preferred. Small suspended solids tend to accumulate in recirculating systems despite these highly improved filtration methods (Cao and Wang, 2010). Small suspended solids can be removed using either chemical or biological oxidation. Foam fractionation is also categorized as a type of mechanical filtration (Chen et al., 1992). Through air-stripping, it can remove and separate soluble organic substances and suspended solids inside the rising air bubbles. Foam fractionation can prevent accumulation of the toxic substances in the aquaculture wastewater.

AERATION

Aeration is widely used in most rural areas of China to provide oxygen to the effluent being treated as well as to remove odorous gases from bottom sediment. Some methods for bringing water into contact with air include allowing it to fall down a set of steps, splashing and breaking up into films and drops; spraying it into the air; and blowing or drawing air bubbles through it (Cao and Wang, 2010). Pumping by air lift has a minor aerating effect. After exchanging the oxygen-rich surface water with the pond's bottom water, some organic-rich sediment could be effectively decomposed (Cao and Wang, 2010)

2.14.3 CHEMICAL PROCESS

Chemical removal is a wastewater treatment method that involves the addition of chemicals to form particles that settle and remove contaminants. Chemical treatment is still an important part of many wastewater treatment plans. The general goals of chemical treatment are as follows: removal of suspended solids (turbidity) from water; pH adjustment; removal of dissolved material in water; and improvement of water quality. Coagulation/flocculation, chlorination, chloramination, ozonation, and ultraviolet light (UV) are the most commonly used chemical treatment methods (Gray, 2002). Flocculation reduces pathogen levels while also removing

particles that could protect pathogens from chemical or thermal destruction, as well as organic matter that could bind up chlorine added for purification (Akpor, 2011).

Adding coagulation chemicals such as alum will then increase the rate at which the suspended particles settle out by combining many smaller particles into larger floc which will settle out faster. In bulk water treatment, the alum dose is varied until the required dose is found (Gomez *et al*., 2006). Chlorination is the most prevalent practice of disinfection. Although it requires a relatively long contact time, because of its high oxidation potential, it is still a disinfectant of choice. However, chlorine does not only disinfect, but also rapidly reacts with contaminants such as NH^{4+}, NO^{2-}, H_2S, Fe^{2+} and other organic compounds, thus leading to the formation of compound called trihalomethanes, which are considered health hazards Chloramination (use of chloramines as disinfectant) has the benefit of reduction of formation of trihomethanes but because of their relatively longtime after discharge to receiving water environment, always lead to toxicity problems. It is mostly used in treatment of wastewater with high organic compounds (Tchobanoglous *et al*., 2003).

Ozonation is primarily employed in secondary wastewater treatment. It has good bactericidal and virucidal properties, is colorless, and does not produce toxic byproducts. Its disadvantages include its high cost and lack of maintenance. Because there is always the possibility of microbial re-growth in the water after treatment. UV and chlorine have been shown to be effective in wastewater disinfection. Ultraviolet light does not produce toxic byproducts, and its faecal indicators are extremely sensitive. Its drawbacks are that it is costly, increases the volume of sludge produced, and usually results in sludge with poor dewatering and settling characteristics. The main advantages of chemical treatment over biological processes are: mineralization of non-biodegradable compounds and smaller reactor volume (Tchobanoglous *et al*., 2003).

2.15 REUSE OF WASTEWATER

The use of urban wastewater in agriculture is a centuries-old practice that is receiving renewed attention with the increasing scarcity of fresh water resources in many arid and semi-arid regions of the world (Nkonyeasua, 2010). According to the International Water Management Institute (IWMI) research in Pakistan, Ghana, Vietnam and Mexico which examined both positive and negative impacts of wastewater reuse for agriculture. It stated that the positive effects of waste water irrigation include the conservation of water, low-cost method for sanitary disposal of

municipal wastewater provides a reliable water supply to farmer, increases crop yield, conserves nutrients thereby reducing the need for artificial fertilizer; reduces pollution of rivers, canals and other surface water resources. They also pointed out some potential negative effects such as health risks for irrigators and communities with prolonged contact with treated wastewater and consumers of vegetables irrigated with wastewater contamination of ground water, creation of habitat for disease vectors and buildup of chemical pollutants in the soil (heavy metals).

Aquaponics combines aquaculture and hydroponics. Hydroponics is the process of planting crops in nutrient-rich water rather than in soil. It was developed during the 1980s became more popular in subsequent years. As a result, others scientist are now researching about, developing, and diversifying the method. The combination of the two methods is known as a close-loop food production system, which results in little or no waste. The close-loop means that the waste obtained from one method becomes the nutrients, or input, for the other method. This is possible because aquaponics takes advantage of the natural nitrification process that is carried out by bacteria (Nicolae *et al*., 2015). Basically, in aquaponics, the waste obtained from fish water in aquaculture serves as fertilizer for plants

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS AND EQUIPMENT

Petri-dishes, beakers, conical flasks, hockey stick, measuring cylinder, Eppendorf tubes, micro pipette (with their tips), test tubes (with their racks), spatula, filter paper, inoculating loop, wash bottles, Autoclave, incubator, weighing balance, thermal cycler, centrifuge, stomacher blender, distiller, Lamina air flow cabinet, Magnetic stirrer water bath (set at 50°C and 100°C), Bunsen burner

3.2 REAGENTS

The reagents used during the experiment include: methyl red indicator, iodine, safranin, crystal violet, 70% alcohol, Kovac's reagent, oxidase reagent, TCBS Agar, Nutrient Agar, Sorbitol MacConkey Agar.

3.3 PREPARATION OF CULTURE MEDIA

For the enumeration, isolation and identification of microorganisms, different selective and differential media were used for enhancement of their viability and isolation. Selective media are made up of ingredients that allow the growth of target microorganisms and inhibit the growth of unwanted microorganisms, they contain sugars, salts, antibiotics and dyes that only the selected microorganism can utilize because of the way it changes the metabolic systems of microorganisms, these ingredients could be the only carbon or nitrogen sources and this results in the inhibition of other unwanted or screened out microorganisms due to their inability to assimilate these sources of nutrients. Also, differential media are the ones that have the ability to differentiate or group microorganisms based on their varying appearance and patterns of growth and morphology.

Buffer Peptone Water (BPW) was used for primary enrichment of samples for the detection of fastidious organism and its presence in foods may go undetected of not enriched). Buffered Peptone Water (0.1% BPW) was used for serial dilution of samples for isolation. To obtain 0.1 %

BPW, 1g of peptone powderwas dissolved in 1 litre of water, then autoclaved at 121°C for 15 minutes.

Nutrient Agar was used for identification of Total Viable Count. It was prepared according to the manufacturer's instruction (Ritcher). 14g of powdered media was dissolved in 500ml of distilled water, then autoclaved at 121°C for 15minutes. MacConkey Agar was used for the identification and enumeration of Coliforms in the isolate and it was prepared based on the manufacturer's instruction Sorbitol MacConkey Agar was used for the presumptive identification, enumeration and isolation of *Escherichia coli*. It was prepared according to the manufacturer's instruction (Lilfilchem). 51.5 g of powdered media was dissolved in 1 litre of distilled water which was autoclaved at 121°C for 15minutes

3.4 TCBS Agar

TCBS Agar is a differential medium used to culture *Vibriospp.*

Preparation

Dehydrated TCBS agar of 26.7g was weighed and Poured in to a conical flask containing 100mL of distilled water. The flask opening was Sealed with an aluminum foil and Placed the TCBS solution on heating stirrer until it started boiling and completely dissolved. (autoclaving was not needed) the solution was allowed to cool down to 45°C. The TCBS solution was Poured into petri dish aseptically and allowed to solidify.

3.5 Sorbitol MacConkey Agar

Sorbitol MacConkey Agar is a selective medium used to isolate coliforms. It provides a color indicator distinguishing between organisms that ferment lactose (e.g., E. *coli*) and those that do not (e.g., *Salmonella*).

Preparation

The medium 36g was suspended in 1000mL distilled water and mixed thoroughly. The mixture was heated with frequent agitation to completely dissolve the powder and autoclaved at 121^{O}C for 15minutes. The agar was allowed to cool to 45^{O}C and poured aseptically into sterile petri dishes and left to solidify.

3.6 Nutrient agar

Nutrient agar is a general-purpose nutrient medium used for cultivation of microbes supporting growth of a ride range of non-fastidious organisms.

Preparation

The medium 28g was suspended in 1000mL distilled water and mixed thoroughly. The mixture was heated with frequent agitation to completely dissolve the powder and autoclaved at 121^{O}c for 15minutes. The agar was allowed to cool to 45^{O}C and poured aseptically into sterile petri dishes and left to solidify.

3.7 COLLECTION SITE

The wastewater samples were collected from Mountain Top University, Ogun state.

3.8 SAMPLE COLLECTION

The wastewaters were collected into plastic containers at a depth 10 – 15 cm from the surface of the water. During collection the containers were rinsed 2 to 3 times with the sample water which was then collected and closed in the water. The containers were properly labeled and transported to the laboratory on ice for analysis within 8 hours (Abu and Wondikom, 2018).

3.9 PHYSIOCHEMICAL ANALYSIS OF WATER SAMPLES

Physical and chemical properties of the wastewater samples were determined. The properties included: Temperature, pH, total suspended solids, conductivity, salinity were determined according to methods described by (A.P.H.A.,2005).

3.9.1 DETERMINATION OF WASTEWATER TEMPERATURE

Temperatures of pond water samples were determined at the sampling location using ordinary thermometer. This was done by fully inserting the tip of the thermometer into the water sample. The reading was immediately recorded.

3.9.2 Determination of pH

pH values of water samples was determined by means of electrometric pH meter by dipping the electrode into the water (pHep® HI 98107- Italy).

3.9.3 Determination of dissolved oxygen

The dissolved oxygen (DO) was determined by Winkler's iodometric titration method. Water sample for DO was collected at each location in 100 ml DO sample bottle without agitating. The stopper was carefully removed. 1ml each of sodium iodide (Nal) solution and magnesium Sulphate

(MgSO4) solution were added with aid of 1ml pipette, the stopper was replaced and the content was thoroughly mixed, 2.0 ml of concentrated Sulphuric acid (H_2SO_4) was added mixture, 50 ml of the solution was titrated with 0.025N of Sodium thiosulphate ($Na_2S_2O_3$) with starch solution as indicator of the colorless end point. The dissolve oxygen (mg/l) is expressed as follows: DO (mg/l) = ml of 0.025(N) $Na_2S_2O_3$ used x 4 (APHA, 2005).

3.9.4 Determination of biochemical oxygen demand (BOD)

The BOD was determined by Winkler's method. Water sample for BOD was collected at each location in 100 ml BOD bottles without agitating. The initial DO content is determined as stated; stopper will be carefully removed. 1ml each of sodium iodide (NaI) solution and magnesium Sulphate ($MgSO_4$) solution was added with aid of 1ml pipette, the stopper was replaced and the content was thoroughly mixed, 2.0 ml of concentrated Sulphuric acid (H_2SO_4) was added to the mixture, 50ml of the solution was titrated with 0.025N of Sodium thiosulphate ($Na_2S_2O_3$) with starch solution as indicator of the colorless end point. After 5 days, incubated bottles, DO were determined using the above procedure. The BOD_5 (ppm): Initial DO of sample – DO of sample after 5 day X 100 /ml of percentage of sample added (APHA, 2005).

3.10 MICROBIAL ANALYSIS OF THE WATER SAMPLE

3.10.1 ISOLATION OF TOTAL HETEROTROPHIC BACTERIA

Five-fold serial dilutions of the water samples were prepared aseptically in sterile physiological saline up to 10^{-5} and 0.1 mL of the diluent was inoculated using the pour plate method in a disposable petri-dish with *TCBS* agar for isolating bacteria in duplicates. The *TCBS* agar plates were incubated at 37ºC for 24 - 48 hours under aerobic conditions. Sterility of the media was confirmed by incubating an un-inoculated agar plate labeled as the control. At the end of incubation period, the observable colonies were counted and the numbers of colony forming units per ml (cfu/ml) were calculated by multiplying the number of colonies per dilution factor (Njoku *et al*., 2015).

3.10.2 Enumeration of Total Coliform

Similar to total heterotrophic bacteria, five-fold serial dilutions of the water samples were prepared aseptically in sterile physiological saline up to 10^{-5} and 0.1 mL of the diluent was inoculated using the pour plate method in a disposable petri-dish with MacConkey agar for isolating coliform bacteria in duplicates. The MacConkey agar plates were incubated at 37ºC for 24 – 48 hours under aerobic conditions. Sterility of the media was confirmed by incubating an un-inoculated agar plate labeled as the control. At the end of incubation period, the observable colonies were counted and the numbers of colony forming units per ml (cfu/ml) were calculated by multiplying the number of colonies per dilution factor (Njoku *et al*., 2015).

3.10.3 ISOLATION OF *Vibrio spp.*

The thiosulphate citrate bile salt agar (TCBS) was prepared and poured onto sterilized petri-dishes. On solidification, 0.1ml of each pond water was transferred to the solidified agar in duplicate and spread evenly with a sterile hockey stick. It was incubated at 35oC for 24-48 hours. After incubation, Vibrio colonies were enumerated for Vibrio count and identified using biochemical reactions. Isolation of .coli, coliforms and total viable count.

Isolation of *E.coli*, coliforms and total viable count

A sterile test tube with 9mL of 1% buffered sterile peptone water was filled with 1mL. The combination was serially diluted up to 10-4. The spread plate technique was used to plate 0.1 mL in duplicate onto Sorbitol MacConkey Agar, Nutrient Agar, and nutrient agar for the isolation of

enteropathogenic E. coli, coliforms, and for the Total Viable Count. The plates were then incubated for 18–24 hours at 35–37°C.

3.10.4 PURE CULTURE TECHNIQUE

Morphologically different colonies were randomly selected from both Nutrient and MacConkey agar plates. The colonies were picked with sterile inoculating loop and streaked on sterile nutrient agar plates. The cultures were later transferred to nutrient agar slants for storage in refrigerator at 4^{o}C until needed for further analysis.

3.10.5 IDENTIFICATION OF THE ISOLATES

The isolated bacteria were identified using morphological characteristics and biochemical tests.

3.10.6 MORPHOLOGICAL IDENTIFICATION OF THE ISOLATES

Morphological characterizations were done using their colonial, cellular and pigment appearances on culture plates.

3.10.7 BIOCHEMICAL CHARACTERIZATION OF THE ISOLATES

Standard biochemical tests, such as Gram's stain, catalase, Methyl red-Voges proskauer (MRVP), coagulase, citrate utilization, indole, starch hydrolysis, oxidase tests, and sugar fermentation tests, were used to identify pure colonies of each bacterial species (Olutiola et al., 2000; Forbes et al., 2007). Each isolate from the stock culture was added to nutrient agar to create the test cultures for the biochemical assays. This was incubated at 37 oC for 18 to 24 hours.

GRAM STAINING PROCEDURE

The most common and useful staining procedure is the gram stain which separates bacteria into two groups according to the composition of their cell walls and were done as described by William *et al*.,(2001). A film was made on a clean slide by emulsifying part of a colony in loop full of distilled water. The film was then air dried and fixed by slight flaming and stained as with crystal violet solution for 1-2 minutes. The smear was rinsed rapidly with water and gram’s iodine solution was added and left for 1-2 minutes. Iodine was poured off and the slide was washed with 70% ethanol for 5-15 sec. The smear was then washed with tap water and stained with safranin solution for 20 sec. The slide was washed with water and allowed to dry. On microscopic examination the gram positive organisms appeared purple and gram negative organisms appeared pink (Ogeneogaga and Solomon, 2017).

Catalase test

Catalase enzyme protects bacteria from hydrogen peroxide (H_2O_2) accumulation, which can occur during aerobic metabolism. Catalase test is aimed at identifying organisms that produce the enzyme catalase, which converts hydrogen peroxide to water and oxygen bubbles. In this test small amount of the test organism was smeared from the petri-dish onto the head of a sterile slide using a sterile wire loop. Then a drop of hydrogen peroxide was added to the smear and mixed. If bubbles become visible, this concludes that the organism produces catalase. Lack of bubbles indicates negative result (Abu and Wondikom, 2018).

Oxidase test

Some amount of the pure culture was swabbed into one of the ends of an oxidase dry slide using sterile wire loop. Colour changes to purple or blue after 30s to 1 min is evidence that the result is positive. The lab test is based on detecting the production of enzyme cytochrome oxidase by

Citrate utilization test

The citrate utilization test was carried out to determine the ability of the isolates to use citrate as sole source of carbon and energy. Tubes of Simon's citrate agar were each inoculated with a test organism and incubated at 35 ^{o}C for 48 hrs. A change in the medium from green to royal blue was recorded as a positive test (Abu and Wondikom, 2018).

Starch hydrolysis test

20ml of molten starch agar was aseptically poured into each sterile petridish, allowed to set and was inverted in an incubator at 37^0C. The organism was streaked across the surface of the plate and incubated at 37^0C for 24-48 hours. Afterwards, the plates were flooded with some quantity of Gram's Iodine. Unhydrolysed starch formed a blue black colour, hydrolysed starch appeared as a clear zone and reddish brown zones around the colony indicated partial hydrolysis of starch (Olutiola *et al*., 2000).

3.11 MOLECULAR IDENTIFICATION

3.11.1 ACTIVATION OF ISOLATES

1ml of pure BHI was prepared into 2ml Eppendorf tubes and sterilized using the autoclave at 121°C for 15 minutes. 100µl of each thawed stock culture was added into the various Eppendorf tubes containing sterile BHI after it was allowed to cool, it was then incubated at 37°C for 48hours.

3.11.2 PREWASHNG

Each isolate was centrifuged in Eppendorf tubes at 5000rpm for 3 minutes. The BHI supernatant was discarded into a waste container, leaving the pellet in the tubes. 1.5ml of sterllized distilled water was added into the tubes, vortexed and then centrifuged at 5000rpm for 3 minutes. The supernatant was discarded and 200 µl of sterilized distilled water was added to the tubes and vortexed.

3.11.3 DNA EXTRACTION BY BOILING USING HEATING BLOCK

The heating block was switched on and allowed to reach 100°C. The Eppendorf tubes containing the prewashes isolates were placed into the heating block and the lid was gently placed over it to prevent the tubes from popping open. It was allowed to boil for 15 minutes, the boiled DNA were then placed into ice to cool for 5 minutes. The already cooled DNA was centrifuged at 7000rpm for 6 minutes after which 150 µl of the DNA supernatant was carefully transferred into an already properly coded fresh Eppendorf tube.

3.11.4 POLYMERASE CHAIN REACTION (PCR)

The component of the PCR used for the characterization of *E. coli* pathotypes is shown in table 3.2 below. After the PCR cocktail has been prepared it was placed in a thermocycler. The PCR was carried with initial denaturation at for ; cycles of for ; for and for ; and a final enlongation step at for . the PCR product were confirmed by gel electrophoresis and visualized under UV light with a gel documentation system.

3.11.5 AGAROSE GEL ELECTROPHORESIS

The agarose gel was prepared using dry agarose powder, 1g of agarose powder was dissolved in 50ml od TAE buffer the mixture was then boiled until a clear solution was gotten 3µl of ethidium bromide was added to the mixture using a micropipette. The mixture was swirled and allowed to

cool slightly but not left to solidify. The mixture was then poured into a gel cast with the combs in place and left to solidify. The gel is gently removed and transferred in an electrophoresis tank and TAE beffer was poured over it. 4µl of the PCR products are pipetted into each well of the already well formed gel after removing the comb. The tank is connected to a pwer source and allowed to run. The gel is viewed under the UV transilluminator.

Table 3. 1*:* PCR Cycling conditions for v. cholerae

No of cycles	Step	Temperature	Time
1	Initial denaturation	94^0c	10 min
35	Denaturation	94^0c	1 min
35	Annealing	59^0c	1 min
35	Elongation	72^0c	2 min
1	Final elongation	72^0c	10 min

Table 3. 2 : PCR Cycling conditions for *V. parahaemolyticus*

No of cycles	Step	Temperature	Time
1	Initial denaturation	95^0c	10 min
35	Denaturation	92^0c	40 sec
35	Annealing	57^0c	1 min
35	Elongation	72^0c	1.5 min
1	Final elongation	72^0c	10 min

Table 3. 3: Multiplex PCR reaction components

TREATMENT 1

No.	Reagents	Initial concentration	Final concentration	Volume per rxn(v/r)	n=20
1	Master mix	5x	1x	2	20
2	Ompwf	20um	0.25	0.125	1.25
3	Ompwr	20um	0.25	0.125	1.25
4	Aaef	20um	0.25	0.125	1.25
5	Aaer	20um	0.25	0.125	1.25
6	700f	20um	0.05	0.25	0.25
7	1325r	20um	0.05	0.25	0.25
8	Mgcl2	25mm	0.5	0.2	2
9	dH_20			5.25	52.5
10	DNA			2	

Table 3**.** 4: Oligonucleotide primers used for PCR amplification of *Vibrio* species-specific gene fragments

Target organism	Primer sequence (5′-3′)	Targeted gene	Amplicon size (bp)	PCR cycling conditions
v.cholerae	F: CACCAAGAAGGTGACTTTATTGTG R: GGTTTGTCGAATTAGCTTCACC	ompW	304	
V. parahaemolyticus	F: GCAGCTGATCAAAACGTTGAGT R: ATTATCGATCGTGCCACTCAC	flaE	897	
700 F	CGGTGAAATGCGTAGAGAT			
1325 R	TTACTAGCGATTCCGAGTTC			

Table 3. 5 :**primer sequences16S rRNA amplification**

	NAME: (TARGET GENE)	SEQUENCE	TARGET ORGANISM
1	700 F	CGGTGAAATGCGTAGAGAT	
2	1325 R	TTACTAGCGATTCCGAGTTC	
3	OMPW F	CACCAAGAAGGTGACTTTATTGTG	v.cholerae
4	OMPW R	GGTTTGTCGAATTAGCTTCACC	v.cholerae
5	FLAE F	GCAGCTGATCAAAACGTTGAGT	V. parahaemolyticus
6	FLAE R	ATTATCGATCGTGCCACTCAC	V. parahaemolyticus

CHAPTER FOUR

RESULT AND DISCUSSIONS

4.1 THE PHYSICO-CHEMICAL RESULT

The physicochemical parameters of wastewater form the three fish pond samples are presented in table 4.1 and these parameters includes pH, Conductivity, Temperature, Salinity.

4.2 Colony count on TCBS Agar.

Figure 4.1 shows the total colony counts of the different ponds' wastewater. Wastewater sample $W3T3^{-1}$ had (3 colonies), $W2B2^{-4}$ had highest total colony count (23 colonies), $W2T2^{-3}$ (3 colonies), $W2G2^{-2}$ (5 colonies), $W2G2^{-1}$ (4 colonies), $W2B2^{-3}$ (2 colonies), $W2T2^{-1}$ (6 colonies), $W2T2^{-4}$ (4 colonies), $W2G2^{-3}$ had the least (1 colonies).

4.3 Morphological characteristics

Table 4.3 and Figure 4.2 Shows the Morphological characteristics of bacterial isolates on TCBS Agar, all samples had yellow, round, small, raised, shiny, smooth, colony 2 to 4mm of slightly flattened colonies with opaque centers. Based on the selective media used for identification, isolates were suspected to be *Vibrio spp.*

Morphological characteristics of suspected *E.coli*. All samples had pink and white, convex, circular, smooth and shiny colonies (Table 4.4).

4.4 Biochemical characteristics

Biochemical test was performed on all the purified isolates, all the isolates were positive (+) to catalase test, which was aimed at identifying organisms that produce the enzyme catalase, which converts hydrogen peroxide to water and oxygen bubbles. Bubbles become visible and the result was recorded as positive. The isolated organisms were Gram negative (-) as they appeared pink, retaining the colour of the counter stain- safranin. Oxidase test Colour changed to purple or blue after 30s to 1 min was evidence that the result was positive. Starch hydrolysis test came out positive (+). Citrate test had a change in the medium from green to royal blue and was recorded as a positive (+) result. The results are summarized in table 4.

4.5 Agarose Gel Electrophoresis

Figure:4.3 shows the result of Agarose gel electrophoresis. Amplicons of fragments of *flaE* (*V. parahaemolyticus*) and *ompW* (*V. cholerae*) 304bp genes were amplified during the study. Lane *L* = 300dp DNA marker, lane 2 = fragment of the *ompW* gene *V. cholerae* isolates was positive in the test.

Table 4. 1: Physico chemical result from the water samples

Parameters/Samples Sites	ICT	CBAS	GIRLS HOSTEL
Temperature (° c)	28.7	28.6	28.8
pH	6.8	6.4	7.0
Salinity(ppt)	094	155	120
Conductivity(Ms/cm)	0.12	0.17	0.16

Table 4. 2: Sample identification table

Samples	Isolate ID	
Sample 1 (water)	W1G1	Water sample, week one, girls' hostel, sample one
	W1T1	Water sample, week one, ICT, sample one
	W1B1	Water sample, week one, CBAS, sample one
Sample 2 (water)	W2G2	Water sample, week two, girls' hostel, sample two
	W2T2	Water sample, week two, ICT, sample two
	W2B2	Water sample, week two, CBAS, sample two
Sample 3 (water)	W3G3	Water sample, week three, girls' hostel, sample three
	W3T3	Water sample, week three, ICT, sample three
	W3B3	Water sample, week three, CBAS, sample three
Sample 4 (water)	W4G4	Water sample, week four, girls' hostel, sample four
	W4T4	Water sample, week four, ICT, sample four
	W4B4	Water sample, week four, CBAS, sample four

Table 4. 3 Morphological characteristics of bacterial isolates on TCBS Agar

Samples	Isolate ID	Colour	shape	size	Elevation	appearance	texture	opacity	margin
Sample 1	W1G1	Yellow	Round	Small	Raised	Shiny	smooth	Opaque	Entire
	W1T1	Yellow	Round	Small	Raised	Shiny	smooth	Opaque	Entire
	W1B1	Green	Round	Small	Raised	Shiny	smooth	Translucent	Entire
Sample 2	W2G2	Black	Round	Small	Raised	Shiny	smooth	Translucent	Entire
	W2T2	Yellow	Round	Small	Raised	Shiny	smooth	Opaque	Entire
	W2B2	Yellow	Round	Small	Raised	Shiny	smooth	Translucent	Entire
Sample 3	W3G3	Yellow	Round	Small	Raised	Shiny	smooth	Opaque	Entire
	W3T3	Yellow	Round	Small	Raised	Shiny	smooth	Opaque	Entire
	W3B3	Green	Round	Small	Raised	Shiny	smooth	Translucent	Entire
Sample 4	W4G4	Yellow	Round	Small	Raised	Shiny	smooth	Opaque	Entire
	W4T4	Yellow	Round	Small	Raised	Shiny	smooth	Opaque	Entire
	W4B4	Yellow	Round	Small	Raised	Shiny	smooth	Opaque	Entire

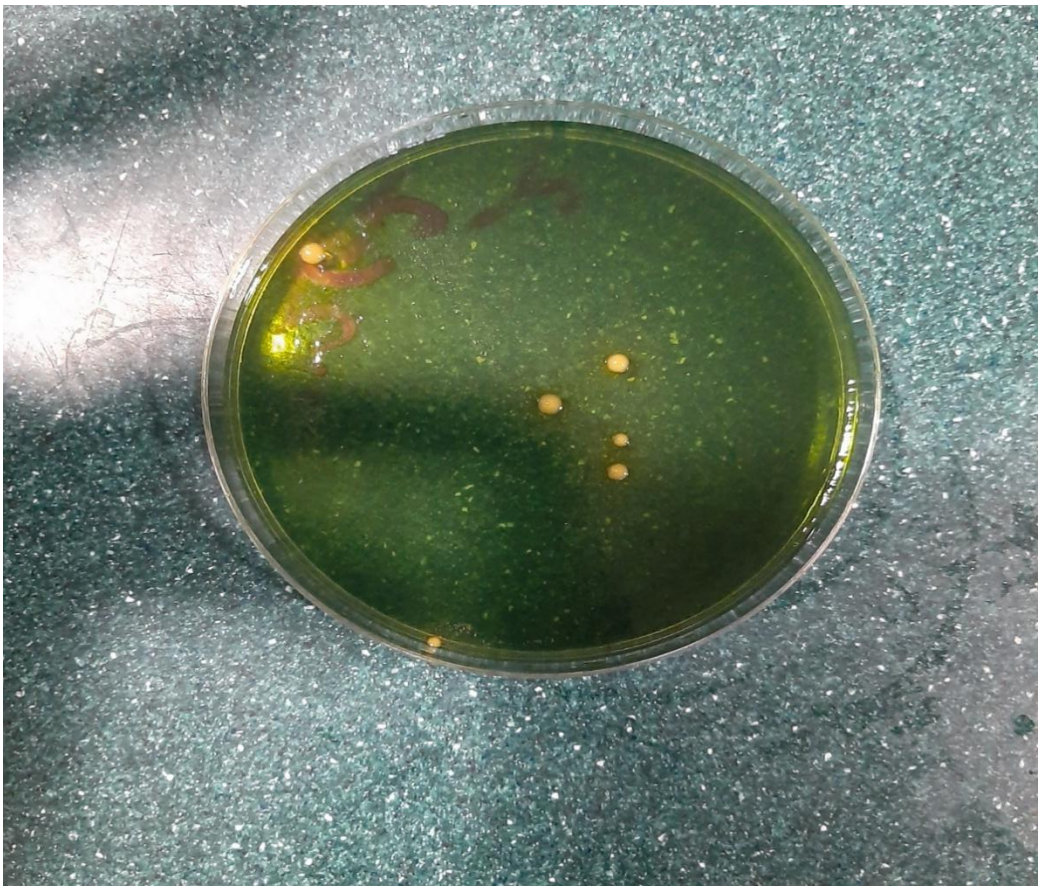

Figure 4. 1 *Vibrio* growth on TCBS plate

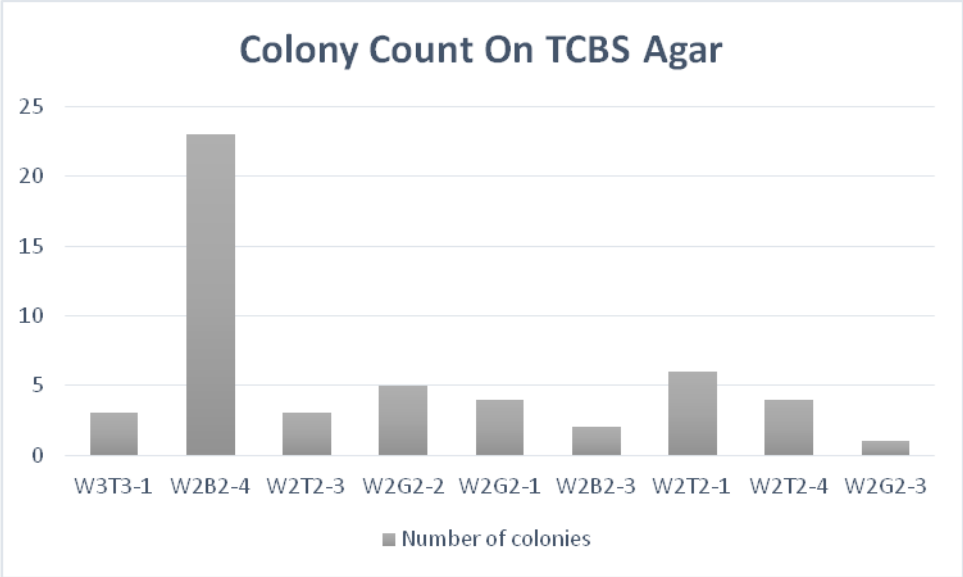

Figure 4. 2: Colony count on TCBS Agar

Table 4. 4 Morphological characteristics of bacterial {*E.coli*} isolates on Sorbitol MacConkey Agar

Samples	**Isolate ID**	**Colour**	**shape**	**size**	**Elevation**	**appearance**	**texture**	**opacity**	**margin**
Sample 1	W1G1	White	Circular	Small	Low convex	Butyrous	smooth	Opaque	Entire
	W1T1	Pink	Circular	Small	Raised	Butyrous	smooth	Opaque	Entire
	W1B1	White	Circular	Small	Low convex	Butyrous	smooth	Opaque	Entire
Sample 2	W2G2	Pink	Circular	Small	Low convex	Butyrous	smooth	Opaque	Entire
	W2T2	White	Circular	Small	Low convex	Butyrous	smooth	Opaque	Entire
	W2B2	Pink	Circular	Small	Convex	Butyrous	smooth	Opaque	Entire
Sample 3	W3G3	White	Circular	Small	Convex	Butyrous	smooth	Opaque	Entire
	W3T3	Pink	Circular	Small	Low convex	Butyrous	smooth	Opaque	Entire
	W3B3	White	Circular	Small	Convex	Butyrous	smooth	Opaque	Entire
Sample 4	W4G4	Pink	Circular	Small	Low convex	Butyrous	smooth	Opaque	Entire
	W4T4	White	Circular	Small	Raised	Butyrous	smooth	Opaque	Entire
	W4B4	Pink	Circular	Small	Low convex	Butyrous	smooth	Opaque	Entire

Table 4. 5 :BIOCHEMICAL TEST RESULT

ISOLATES	ISOLATE ID	CATALASE	GRAM-STAINING	OXIDASE	STARCH	CITRATE
1ST samples	W1G1	+	-	+	+	+
	W1T1	+	-	+	+	+
	W1B1	+	-	+	+	+
2nd Samples	W2G2	+	-	+	+	+
	W2T2	+	-	+	+	+
	W2B2	+	-	+	+	+
3RD Samples	W3G3	+	-	+	+	+
	W3T3	+	-	+	+	+
	W3B3	+	-	+	+	+
4TH Samples	W4G4	+	-	+	+	+
	W4T4	+	-	+	+	+
	W4B4	+	-	+	+	+

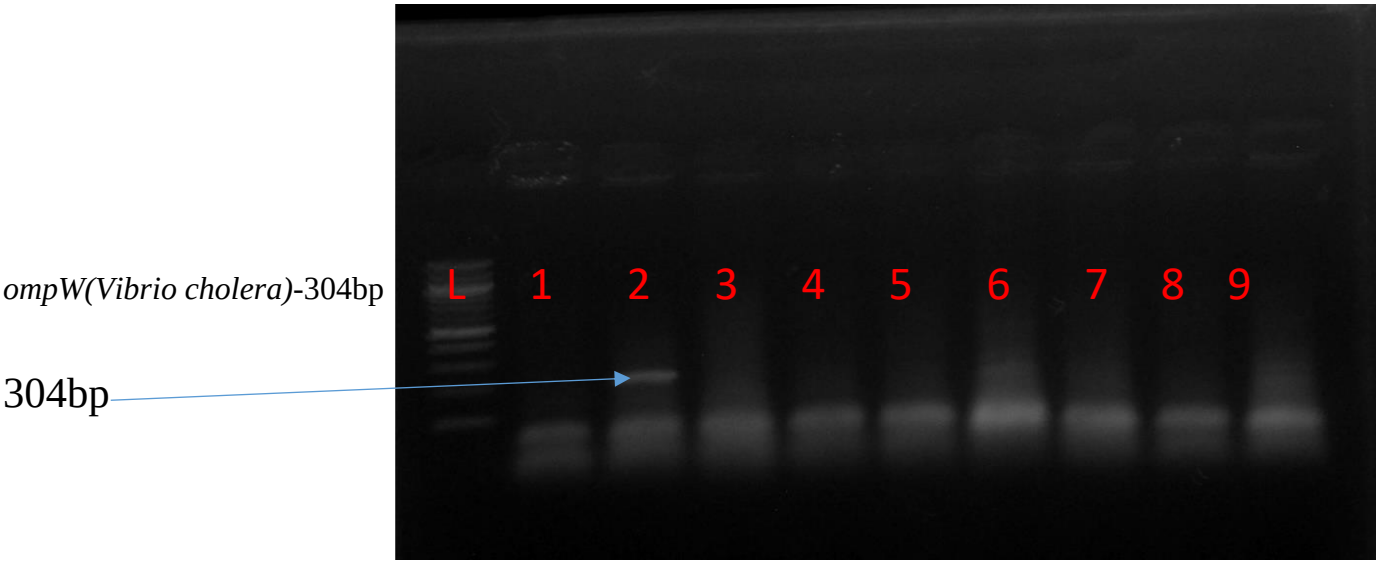

Plate 4. 3 Agarose Gel Electrophoresis

Agarose gel electrophoresis image of multiplex-PCR product, showing amplicons of fragments of *flaE* (*V. parahaemolyticus*) and *ompW* (*V. cholerae*) 304bp genes amplified during the study. Lane *L* = 300dp DNA marker, lane 2 = fragment of the *ompW* gene of positive isolates of *V. cholerae.*

4.5 DISCUSSION

Temperature of an organism is defined as the level of hotness or coldness in the body of a living organism either in water or land. The finding on the temperature range of pond water in this study normal and it is consistent with the report of Adebami (2020) where he recorded temperature range 23.83 – 24.67 °C which were within the recommended limits of WHO respectively.Thus, *Escherichia coli* cells will grow at a normal level because the temperature values in the result support it growth. According to (Anne and Frederick, 1998) *Escherichia coli* cells will grow over a temperature range of about 40°C, and remarkably, the cell growth rate increases in response to increasing temperature like a simple chemical reaction in a central normal range of its growth temperatures (20 to 37°C).

The desirable range for pond pH is 6.5 - 9.5 and acceptable range is 5.5 - 10.0 (Ehiagbonare and Ogunrinde, 2010). The range of the pH obtained from this study was 6.4 - 7.0. This agrees with Ehiagbonare and Ogunrinde (2010). Thus, *E. coli* will grow well at this ph level because, *e.coli* will grwo at pH of 6-8 according to (Andrew T. schilling, 2008).

Salinity represents the total concentration of dissolved inorganic ions, or salt, in water. It plays a significant role for the growth of culture organisms through osmoregulation of body minerals from the water. For better survival and growth an optimum range of (50-300ppm) according to (WHO) salinity should be maintained in the pond water. If salinity is too high, fish and shrimp will start to lose water to the environment. The range of salinity in these studies ranges from (94 - 155ppm). which illustrate that the water level is according to the WHO standard of fish pond water, The optimum salinity range for maintenance of fish health has been reported to be in the range of 15 – 32 ppm (Andrew, 2007). which will enhance the growth of fish production.

Electrical conductivity is a measure of the capacity of water to allow the passage of electricity through it. The results of the electrical conductivity obtained in this study ranged from 0.12mol/cm to 0.17mol/cm. According to (Adebami *at el.,* 2020) he reported that the electrical conductivity values recommended for fish farming range from 20 to 150 µS/cm. The World Health Organization also has a standard limit for conductivity of water is 100 µS/cm (WHO, 2006). Moreover, the conductivity of the pond in this study is below the standard range, this could be due to the dilution of the ions in wastewaters, which is often the case during wet season. Previous studies have shown

that dilution of water during the rainy seasons lowers the levels of electrical conductivity. Conductivity helps to increase growth and production of fish; to improve their feed utilization and reduce feeding costs through better management practices such as a well-adjusted daily feeding ration or an increased availability of natural food through fertilization/liming.

The result above shows the presence of *Vibrio* and *E.coli* in the fish pond wastewaters, This concludes that the fish pond waste water is contaminated. This outcome maybe as a result of contamination through the fish feeds, source of water etc. Other research also acknowledged the presences of familiar microbial evaluation result. It was found that gram negative bacterial genera were predominant and agrees with my findings of {Adebami *et al.,* 2020}. The discovery of of *Escherichia coli* in the samples is as a result of fecal oral route contamination and also through fertilization of the pond with manure that was introduced directly into the fish pond or excretion by the fish into the pond.

CHAPTER 5
CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The physio chemical evaluation carried out in this research with the microbial analysis shows the presence of pathogenic microorganism in the of the fish pond waste water which might have negative effect i.e water-borne illness, diseases and death. Moreover, the findings of this study suggest the need for regular monitoring of fish pond waste water released into the environment. The waste water contains both *Vibrio cholerae* and *E. coli* if introduced into nearby water bodies can be of negative effect on plants, animals, and human beings close to that area, by consumption and domestic use, which is common in under developed communities. It also affects plants when they absorb these waste water through their roots due to the toxic concentration of the waste water. As a result, there is a need for the treatment of fish pond waste water and proper drainage system. Also, organic manure should be avoided for pond fertilization because it alters the physical and chemical properties of the water and also acts as a source of contamination of fish ponds. Environmental education should be included in all levels of school curricula to enlighten individuals on the effect of pathogenic *E. coli* to them and how they can abstain from being infected with the organism.

5.2 RECOMMENDATIONS

Physiochemical and microbial test of waste water should be highly employed in fish pond activities to lessen the possible outcome of epidemics in the country. More research on personnel, fish surface, body organs, and fish feeds is needed to determine the microbial load and heavy metal contamination. Water treatment campaigns should be organized in the studied area to educate residents and fish farmers about the safety of portable water for fish farming. The water in the fish pond should be analyzed on a regular basis. This is a quality assurance procedure that ensures there are no toxic substances in the ponds that could lead to bio-accumulation and toxicity of waste water. This ensures the health of the aquatic ecosystem, humans, and the environment.

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