USE OF COLIFORM COUNT AND PLANKTON SPECIES IN LAWAYE RIVER, SAN JUAN BATANGAS AS INDICATORS OF POLLUTION

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The thesis attached hereto, entitled "Use of coliform count and plankton species in Lawaye River, San Juan, Batangas as Indicators of pollution "prepared and submitted by Jose Rafael L. Lopez and Renz Michael F. Pasilan, in partial fulfillment of the requirements for the degree of Bachelor of Science in Biology, is hereby accepted.

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ABSTRACT

The Lawaye River in San Juan Batangas is an important source of water for local residents. However, there have been insufficient researches regarding the water quality of the river. This study was conducted to determine the water quality of the Lawaye River using coliform count and plankton species composition. Four sampling sites were setup along the river; boundary, upstream, midstream and downstream. Fecal coliform count in the river was quantified using the multiple tube fermentation method while detection of the coliform *Escherichia coli* involved the Presumptive, Confirmatory and Completed tests along with the IMViC profiles. Plankton analysis involved the identification of various plankton genus found in the river. Nygaard's and Palmer's indices were then used to determine the river's trophic state and pollution status. The coliform concentration in all sites was found to be well beyond the normal range. Plankton analysis showed that sampling areas in the Lawaye River were found to have different level of organic pollution. The Palmer's and Nygaard's Indices demonstrated that the downstream and the midstream sites registered the highest probability of having organic pollution while the sites found on the boundary and upstream were comparatively normal and oligotrophic. Factors such as improper waste disposal, effluents from drainage and agricultural runoffs might have contributed to the observed results.

INTRODUCTION

Background of the Study

About 75% of the Earth's surface is covered with water. A vast majority of this (97%) is saline and cannot be used for domestic or industrial consumption. The remaining 3% is considered freshwater and is locked within glaciers, found deep as groundwater or occupy the surface as lentic and lotic waters. Rivers only comprise 0.003% of the world's freshwater supply and yet their importance in sustaining life for both organisms and man cannot be ignored. The river as an ecosystem provides habitats for aquatic organisms. It provides water for irrigation, industrial and domestic purposes, and as source of food and navigation routes for man (Miller, 2008).

However, due to irresponsible human activities and other natural sources of contaminants, rivers have become highly polluted. As a consequence of this mismanagement of the river resources, widespread fish kills, heavy siltation, low river productivity, contaminated drinking water and food sources, waterborne diseases have occurred. This unsustainable use of the river has led to serious degradation of water quality.

Here in the Philippines, out of the 421 identified rivers by the DENR, 180 are considered heavily polluted from industrial and agricultural wastes, some could be declared biologically dead in the long run while fifty are now considered dead. Among these are four rivers in Metro Manila including the Pasig River, four in both Cebu and Negros Occidental (Yu, 2008). It has also been identified that 48% of the pollution comes from domestic wastes.

Monitoring of freshwater environments has often been based on pH, dissolved oxygen and carbon dioxide counts, turbidity and other physico-chemical parameters (Suthers, 2009).

These provide a good indication of the chemical quality of the water but do not necessarily reflect the ecological state of the area. Biological parameters such as plankton analysis and microbial counts provide a good alternative since aquatic organisms are sensitive and can have varied responses, incorporating effects of physico-chemical parameters as well as the current condition of the water. These responses made by the organisms can be used to determine the water quality with respect to its life sustaining capabilities.

In San Juan, Batangas for instance, the Lawaye River has provided locals with livelihood, irrigation, and water that are used for domestic purposes. Due to improper waste disposal, the water has been perceived by residents as polluted. However, there has been no previous study regarding the water quality of the river. Given this setting, it is the intention of this research to provide current status of the Lawaye River using microbial and plankton indicators.

Statement of the Problem

Can coliform count and plankton species from the Lawaye River serve as indicators of pollution?

Objectives of the Study

General Objective

To determine whether the coliform count and plankton species from the Lawaye River may serve as indicators of pollution in Lawaye River in San Juan Batangas.

Specific Objectives

To determine the total coliform count using the Most Probable Number

To identify plankton species present in the water samples in the river

To assess the trophic status of various sites along the river using the Nygaard's trophic index and Palmer's algal pollution index

To describe the physico-chemical parameters of the Lawaye river

To describe the topographic and geographic characteristics of the river

To determine the weather conditions present during the time of collection of water samples

Significance of the study

The Lawaye River in San Juan Batangas is an important source of water for local residents. The water is used for agricultural and domestic purposes, as well as routes for locals in trekking the terrain of the area. Also, it is part of an ecotourism development project in the area. However, researches on the water quality conditions of the Lawaye River are deficient. This study may provide baseline data on the algal and microbial species that can be found in the river and serve as indicator organisms to determine pollution in the area.

The sampling sites that are to be studied are located in areas wherein possible sources of pollution along the river bank can be found. This study will also determine whether those sites contribute significantly to the pollution in the river.

The results of this research may also serve as future reference for policy and developmental plans, environmental and health programs of the local government of San Juan, Batangas and other stakeholders.

Scope and Limitation

Water samples were collected from four sites along the river namely, in Muzon located at the boundary of the towns of Rosario and San Juan, at Palahanan, in an upstream area of the river under a bridge, in an area near the marketplace in Poblacion and along the mini dam of Pinagbayanan. Three samplings were done per site; Focus was given to the plankton and microbial tests which were done on the river. The microbial test determined total fecal coliform specifically *Escherichia coli* present in the river using the Multiple Tube Fermentation Method, IMViC and the Presumptive, Confirmatory and Completed tests. Plankton quantification was

done using the hemocytometer counting method while visualization of the plankton species was made under light microscope. Identification of the plankton species used taxonomic keys by Siver (2004), Prescott (1978) and Brown et al.(undated), researches done by Wagner (2006), Celekli (2006), Carr (1966), Suthers (2009) and Perry (2003), as well as illustrated guides for plankton identification by Martin (2008), Microbial Digital Specimens Archive and Microscopy-UK. Identification was only done up to the genus level. These data from the water samples were used to determine the pollution state of the Lawaye River. Physico-chemical tests were also done on the river to provide supporting data. Topographic-geographical as well as climactic information was used only for reference purposes.

REVIEW OF RELATED LITERATURE

Geographical and Economic Features of San Juan, Batangas

The town of San Juan, Batangas is located at the southeastern tip of the province of Batangas. It is approximately 120 kilometers from Metro Manila and 43 kilometers from the provincial capital of Batangas City. It is accessible by land transportation from National Roads coming from the West (through Lipa City and the town of Rosario), and from the East (through the Quezon route). San Juan is bounded on the North by the Quezon towns of Candelaria and Tiaong with the Malaking Ilog river defining the geographical boundary between Batangas and Quezon; on the South by the Verde Island Passage; on the East by the Quezon town of Sariaya and by Tayabas Bay; and on the West by the mountain ranges of the Batangas towns of Rosario and Lobo (Mayo, 2007)

Based form the year 2000 census, San Juan has a population of 78,169. It has 42 barangays including the town center which is the Poblacion. North of the Poblacion along the Malaking Ilog River that divides Batangas and Quezon are the Barangays of Muzon; Palahanan I; Palahanan 2; Sico I; Sico 2; Janao-janao; Calicanto; Maraykit; Lipahan and Tipaz. The other Barangays are all south of the Poblacion. Those that lie alongside Batangas towns (Rosario, Taysan and Lobo) to the West are Libato; Sapangan; Quipot; Pulang Bato; Bulsa; Laiya Aplaya and Hugom. The Barangays with a coast fronting Tayabas Bay are Poctol; Catmon; Pinagbayanan; Ticalan; Puting Buhangin; Abung; Calubcub I; Calubcub II; Subukin; Nagsaulay; Bataan; Imelda; Barualte; Laiya Ibabaw; Laiya Aplaya and Hugom. The interior Barangays southwards of the National Road are Barangays Poblacion and Calitcalit, Mabalanoy, Paling

Uwak; Talahiban I; Talahiban II; Balagbag; Escribano; Sampiro; Buhay na Sapa and Coloconto (Mayo, 2007)

San Juan is predominantly agricultural with about 72% of its total land area devoted to agriculture. In the province of Batangas, San Juan has the largest area planted to rice and coconuts. It also has one of the longest shorelines in the country with areas in Barangays Hugom, Laiya Aplaya, Laiya Ibabao, Imelda and Barualte designated by the Department of Tourism as areas for coastal resort development under the CALABARZON master plan scheme. Except for natural coral reefs near parts of the coast that have been classified as protected sanctuary by DENR, all other coastal areas from a portion of Barangay Barualte down to Barangay Catmon are suitable for aqua-culture industry or farming (Mayo, 2007).

Hydrological Characteristics of Rivers

Rivers are defined as freshwater bodies of water that have a unidirectional current with a relatively high, average flow velocity ranging from 0.1 to 1 ms-1. Thorough and continuous vertical mixing occurs through prevailing currents and turbulence (Meybeck and Helmer, 1996). Rivers drain specific land surfaces known as river basins or watersheds. Thus, the characteristics of a river are related to the size, form and geological characteristics of the basin and the climactic conditions which determine the quantities of water to be drained by the river system (Meybeck et al., 1996).

Rivers can be classified whether regime or non-regime. Regime channels display a variety of patterns and flow characteristics. Non-regime channels on the other hand are controlled by bedrock or older alluvium or they are unstable (Schumm, 2005).

River velocity (flow) and discharge are two important hydrological characteristics of rivers. Velocity is the rate of water movement given as ms-1 or cm s-1. It defines the river's ability to assimilate and transport materials within the river body. In an even river channel, laminar flow occurs. Maximum velocities occur at the center of the channel but are reduced to zero at the bank by frictional forces located at the bank zone. Thus, the velocity gradient tends to force any incoming waters from a tributary to the side of the river. A tributary entering from the right side of the channel remains there while the laminar flow is maintained (Meybeck et al., 1996). In some cases, variations in the river's cross-sectional profile wherein changes in depth occur across the profile, causes bottom friction, resulting in deceleration of the bottom water of the river.

Discharge is determined from the velocity multiplied by the cross sectional area of a river. Most rivers are characterized by a condition called base flow or base discharge. This is the minimum amount of water moving through a river system and is controlled by the groundwater discharge. This base flow can be modified by several factors namely, nature of watershed, climate and vegetation. Small watersheds result in low median discharges with large ratios of peak and low discharge while larger watershed produces more uniform and constant patterns. Climate determines the rainfall distribution over the year thus; variability in discharge over temperate and humid areas is moderate while extreme for subtropical regions. Fluctuations in discharge can be dampened by vegetation cover. In areas with little vegetation, immediate surface runoff occurs (Meybeck et al.,1996).

Physico-Chemical Parameters of Water

Water pH

PH refers to the acidity or alkalinity of a solution (Sawyer, 1967). It expresses the hydrogen activity present in the solution. Water pH is a factor to be considered in chemical coagulation, disinfection, water softening and corrosion control. Water pH determines the solubility and biological activity of nutrients and heavy metals. The pH range goes from 0-14. A pH of 7 is neutral. A pH of below 7 determines the solution to be acidic while above 7 is alkaline. As the value goes down , the solution becomes more acidic and as the value goes up it becomes more alkaline.

High values for pH can become detrimental for water quality. High pH values turns the water bitter and water pipes and other water-using applications become encrusted with deposits. Low pH water will corrode metal and other substances. The standard pH readings for freshwater is 6.5 – 9.0 (DAO, 1995).

Water Temperature

Temperature is a measure of how hot or cold a substance is (Giancoli, 2006). It affects several characteristics of an object. Materials expand or contract as the temperature changes. The electrical resistance of an object changes with temperature as well. Temperature is the result of the motion of particles composing a substance. This motion produces energy which in turn produces heat. As the energy of this motion increases, the temperature also increases (Giancoli, 2006).

Water temperature refers to the amount of heat present in water (Giancoli, 2006). Organisms present in the water can tolerate a certain range of temperatures. If the temperature of

the water either increases or decreases beyond that range, the organisms in the water can become stressed and eventually die. The location of the body of water affects its temperature. If the body of water is in a high altitude, the temperature is lower than that of a body of water in low altitude. The amount of water present also affects the temperature. The standard temperature range for freshwater is 25 – 32 °C (DAO, 1995)

Water Salinity

Water salinity refers to the amount or concentration of salts dissolved in the water (Sawyer, 1967). Salinity changes as the amount of water changes. It can also change if salts are added to the body of water. Organisms can tolerate a certain range of salinity. If the salinity of the water changes beyond this range, the organisms can become stressed and may die.

Water Conductivity

Conductivity is the measure of the ability of water to pass an electric current. It is affected by the presence of inorganic compounds that carry electrical charges (Giancoli, 2006). These charges may either be positive or negative depending on the compound. Organic compounds have low conductivity and cannot conduct electricity as compared to inorganic compounds. Conductivity of streams and rivers is primarily affected by the geology of an area in which it flows. If the water passes through an area that contains compounds that ionise in water, then the conductivity will be affected. Aquatic organisms exist in a certain range of conductivity. Conductivity outside this range may indicate that the water is not suitable for certain aquatic life.

Total Solids

Total solids refer to any solid particle either suspended or dissolved in water. Suspended solids are solids that are suspended in the water and can be obtained by filtering the water. Suspended solids can come from silt, decaying organic matter, industrial wastes and sewage. These have particular relevance for organisms that depend on solar radiation. These solids can reduce the amount of sunlight that enters the water thereby affecting the photosynthetic process. It can contribute to the increase in temperature of the water as it absorbs more heat. It decreases the visibility of fish in the water and may interfere with hunting patterns. The amount of suspended solids is influenced by flow rate, soil erosion, urban run-off, septic and waste water effluent and decaying organic matter.

Dissolved solids are any solids matter dissolved in the water. This includes materials such as bicarbonate, phosphate, nitrate, calcium, organic ions and other ions. These ions and minerals are essential in the survival of life. However, in increased concentrations, these minerals and nutrients can prove to be harmful to aquatic life. They can lead to increased water turbidity, increased water temperature and may interfere with the photosynthetic process. The amount of dissolved solids is influenced by urban and fertilizer run-off, waste water and septic effluent, soil erosion and decaying organic matter. The standard amount of total solids present in freshwater is 80 mg/L (DAO, 1995).

Dissolved Oxygen

Dissolved oxygen (DO) is oxygen gas that is dissolved in water. Oxygen is dissolved in water through diffusion from the atmosphere, wave action or through photosynthesis. Oxygen is removed from water by respiration or by decomposition of organic matter (Sawyer, 1967).

DO is affected by several factors. The volume and velocity of the water affects the amount of DO. The faster the flow of water, the more it is aerated by oxygen in the air. In still waters, oxygen only enters the top layer of water while the deeper layers have low concentration of water due to the decomposition of organic matter. The climate also affects the amount of DO present in the water. Lower temperatures can dissolve more oxygen than higher temperatures. This is why DO is greater in winter than it is summer. The altitude of the water source also affects the amount of DO. Lower altitudes have greater DO because of the higher atmospheric pressure present in the area. The amount of nutrients and living organisms present also affects the concentration of oxygen in the water. Nutrients are required for algae to survive in water. Increased amounts of nutrients in the water encourage the growth of algae. This results in eutrophication. The more organisms are present in the water, the more oxygen they breathe thereby lowering the amount of DO in the water.

Total dissolved gas concentrations in water should not exceed 110 percent (Sawyer, 1967). Concentrations above this level can be harmful to aquatic life. Fish in waters containing excessive dissolved gases may suffer from "gas bubble disease"; however, this is a very rare occurrence. The bubbles block the flow of blood causing death. Aquatic invertebrates are also affected by gas bubble disease but at levels higher than those lethal to fish. The standard amount of dissolved oxygen present in the water is 5 mg/L (DAO, 1995)

Adequate dissolved oxygen is necessary for good water quality. Oxygen is a necessary element to all forms of life. Natural stream purification processes require adequate oxygen levels in order to provide for aerobic life forms. As dissolved oxygen levels in water drop below 5.0 mg/l, aquatic life is put under stress. Oxygen levels that remain below 1-2 mg/l for a few hours can result in large fish kills.

Free Carbon Dioxide

Free carbon dioxide refers to the amount of carbon dioxide dissolved in water. Aquatic plant life and phytoplankton depend on carbon dioxide for growth and survival (Sawyer, 1967). These organisms utilize carbon dioxide in photosynthesis. High free carbon dioxide levels are both beneficial and harmful to aquatic organisms. It is beneficial in a way that it helps promote photosynthesis thereby encouraging the growth of aquatic plants and phytoplankton. It is harmful such that if carbon dioxide levels are high the water may turn acidic. High acidity in water stresses fishes and eventually leads to death.

Total Hardness

Total hardness refers to the amount of calcium and magnesium salts present in the water. Calcium and magnesium salts enter the water through the weathering of rocks. Rainfall is initially soft, as it does not contain any minerals. As it seeps into the grounds, it picks up mineral salts. These salts contribute to the hardness of the water (Sawyer, 1967). Hard water interferes with the cleaning process. The mineral salts interact with the soap, forming scud. Hard water causes pipes to fur up lessening the efficiency of water transport.

Multiple Tube Fermentation Method

The multiple tube fermentation method is based on lactose fermentation with a production of acid and gas within 48 hours indicating a positive result (Eckner, 1998). The multiple tube fermentation method utilizes Lauryl Sufate Tryptose Broth (LSTB) durng the presumptive phase, Brilliant Green Lactose Bile (BGLB) Broth for the confirmatory phase and Eosin Methylen Blue Agar (EMBA) for the completion phase (Jacobs, et al, 1986).

The LSTB tubes are to be inoculated with the sample and incubated for 24 – 48 hours. Gas production and turbidity indicates a positive result. The gas can be seen inside the durham tubes placed during preparation in the tubes. In LSTB, tryptose is used instead of peptone because of its better productivity and selectivity (McCrady, 1943). Sodium lauryl sulphate was used because of its selective effect for the coliform group (Mallmann, 1941). The sodium lauryl sulphate is somewhat similar to oxgall in inhibiting the multiplication of non-coliform organisms. LSTB was used as a substitute for lactose broth in the standard methods and showed an increase in the number of positive indications and a decrease in the number of primary gas positives to be confirmed (McCrady, 1943).

Brilliant Green Lactose Bile (BGLB) broth contains oxgall which is inhibitory to non-coliform organisms (McCrady, 1943). BGLB has long been used as an enrichment and inhibitory medium in the isolation of coliforms (Kelly, 1940). However, it is not productive enough to be used as a primary inoculum. BGLB is considered as the most satisfactory of the selective procedures and yielded results, with the exception of finished waters, which are usually more accurate than those secured by the use of completed test alone (Kelly, 1940).

Eosin methylene blue agar is used as a medium for the differentiation of *Escherichia coli* and other coliforms from *Salmonella* and *Shigella* (Parisi and Marsik, 1969). Typical colonies of *E. coli* are described as blue-black colonies or colonies having a dark center with a colorless periphery and, when viewed under reflective light, a metallic green sheen (Parisi and Marsik, 1969). EMBA is used in the completed test for coliform analysis. Along with EMBA, IMViC profile is determined to ensure that colonies found in EMBA plates are truly *E. coli*.

Coliforms as Indicator Species of Water Quality

Coliforms are found in the digestive tracts of animals, including humans, and their waste as well as in soil and vegetative matter (Morris, Undated). Most coliforms are relatively harmless and some aid in the digestion of animals. Total coliforms comprise all aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas and acid production within 48 hours at 35° C. Within this group, there is a subgroup called the fecal coliforms. Fecal coliforms are defined as those as coliforms which respond to an elevated temperature of 44.5° C. This subgroup indicates that the water has been contaminated by fecal matter which means that the water source has also been contaminated by pathogens and disease causing bacteria or viruses which may also exist in the fecal matter.

Coliforms may contaminate the water from many different sources. The most common ways of contamination are excretions from humans and other animals, surface run-off and multiplication of non-fecal coliform on vegetative matter.

Among all the species in the coliform group, there are four genera that are commonly used. These are *Escherichia*, *Klebsiella*, *Citrobacter* and *Enterobacter*. Some of these are

commonly found in the intestinal tracts of animals such as *Escherichia* while others are found in soil and plants such as *Klebsiella*. These organisms, along with other coliforms, may be accompanied with disease causing organisms. Coliforms are used to assess water quality rather than pathogens because the detection of coliforms is easier and more reliable. Pathogens appear in small concentration and less likely to be isolated and detected.

In addition to other bacteria, the human body produces about 100 to 400 billion coliform organisms per day (Metacalf and Eddy, 1991). The absence of coliform organisms is a sign that the water is free from contamination of disease producing organisms.

Coliforms have shown to have a correlation with pathogens. Goldreich (1978) have found that for a contamination of less than 200/dl (100ml = 1dl), *Salmonella* occurrences ranges from 6.5 -31%. However when contamination reaches 1000/dl, the occurrence of *Salmonella* doubles.

There have also been cases in which non-fecal coliforms found in soil and vegetative matter that have a positive result in total and fecal coliform tests. Fecal coliform tests have also shown to be positive in industries and agricultural areas with little mammalian or avian waste. Elevated concentrations of organic materials can support bacterial populations that are capable of responding positively to total and fecal coliform tests

Plankton Ecology and Morphology

Plankton refers to any small biota that is living in a body of water and drifts with the currents. They form a wide range of species and are defined by their ecological niche. Even jellyfish and krill, both active swimmers are incorporated in this definition though they are more aptly termed as nekton.

Plankton can be divided in terms of function and size. In terms of function, plankton can be classified as phytoplankton; unicellular organisms that live near the water's surface and undergo photosynthesis, zooplankton; animal-like protists and metazoans that feed on phytoplankton, and bacterioplankton; bacteria and related taxa which recycle nutrients in the trophic cycle (Weis, 2008). Individual members of plankton also vary in body size ranging from minute bacteria to large gelatinous zooplankton like jellyfishes. Megaplankton are organisms that exceed 20cm in length. Examples of these are the large jellyfishes and related species. Species from 2-20cm in length are considered as macroplankton and are comprised of krill and comb jellies. Mesoplankton which are from 0.2-20mm, include copepods, cladocerans and larval fish. Microplankton (20-200μm) and nanoplankton (2-20μm) comprise the large and small phytoplankton, radiolarians, diatoms, dinoflagellates and foraminiferans. Bacteria and marine viruses make up the picoplankton whih range from 0.2-2 μm (Suthers, 2009).

Plankton abundance and distribution are affected by several factors, one of which is nutrient concentration of the water. The most important nutrients for phytoplankton growth are nitrogen and phosphorus while diatoms need silica in their diet. According to Suthers (2009), the Redfield ratio demonstrates that phytoplankton species take up dissolved forms of the nutrients C, N and P in a given ratio of 106C:16N:1P. Though this ratio may slightly differ across species, this is important for accounting abnormal algal growth in an area. If located in areas of low phytoplankton productivity, growth is primarily sustained through regeneration of nutrients. This occurs through remineralisation of organic matter to dissolved inorganic nutrients via microbes in the plankton. Production will occur in response to external nutrient inputs. Zooplankton grazers present a trophic pathway to channel organic carbon from phytoplankton to fish and

nutrient cycling that occurs in the water though it is now accepted that a significant portion of phytoplankton is not consumed directly by zooplankton grazers but is cycled by microbes before being available to consumers.

Zooplankton usually conducts diel (24 hour) vertical migrations in freshwater and marine environments. This involves rising to surface waters at dusk and grazing heavily on phytoplankton cells throughout the night, before descending to deeper waters well before dawn. For phytoplankton, while light for photosynthesis declines exponentially from the surface of the ocean, the availability of nutrients typically increases down the water column. Thus, they migrate down the water column at night to obtain nutrients, but return to the surface during daylight. This migratory behavior is believed to be triggered by changes in light intensity, and is largely an adaptation to avoid visually feeding predators, particularly fish. (MacKenzie, 2002).

Plankton as Indicator Organisms for Water Quality

Biological indicators have been used together with physical and chemical methods to determine water quality. One of these biological parameters is plankton. The role of plankton has recently gained widespread use due to their simplicity, availability, sensitivity and rapidity of analysis (Kumar, 2004). Palmer (1969) has reported various forms of algal taxa that are pollution tolerant, five of which are classified as extremely tolerant; *Chlorella*, *Chlamydomonas*, *Euglena*, *Oscillatoria* and *Scenedesmus*. He lists down an algal pollution index wherein 20 algal genera are noted with their corresponding pollution values (Lobban et al., 1988). A score of 20 or more for a sample is evidence of high organic pollution though a low rating on the scale does not necessarily mean no pollution is occurring in the area.

Zooplanktons have been widely used as indicators to monitor and assess various forms of pollution including acidification, eutrophication and pesticide pollution. Acidification due to acid rain, resulting in airborne pollutants has had adverse effects on a variety of organisms in freshwater ecosystems. Zooplanktons richness is reduced with increasing acidity. The water flea or *Daphnia* is eliminated while smaller crustaceans and rotifers become dominant (Suthers, 2009). Eutrophication also changes the size structure, species composition and biomass of the zooplankton. Typically, total zooplankton biomass increases with increasing eutrophication and increased importance of rotifers and ciliated protozoans. The rotifer *Asplanchna brightwelli* is listed as an indicator for eutrophy in an Australian river by Shiel (1982).

Cyanotoxins concentration released by Microcystis and Anabaena can also be assessed using zooplanktons. Daphnia, copepods and rotifers are used ecotoxicollogically as indicator organisms to determine direct and indirect effects of this cyanotoxins. High concentrations of cyanotoxin kill zooplankton like *Daphnia* while low concentrations of reduce growth and reproduction among zooplanktons according to De Mott (1991).

Phytoplankton such as diatoms and dinoflagellates has also been utilized to monitor the quality of surface water. Battarbee et al. (1997) have discussed the relationship between diatoms and water quality in Norway specifically the factors controlling the abundance and occurrence of aquatic organisms in unpolluted acid-sensitive systems. Relationships between the two were explored using the multivariate technique of canonical correspondence analysis (CCA). They found out that the principal variables influencing species composition of periphytic diatoms were found to be pH and water colour. Furthermore, the relationship between species abundance and pH was sufficiently strong to enable reconstruction of water acidity from diatom data.

Gromisz and colleagues studied the impact of the Oder River on the phytoplankton composition and biomass in the Pomeranian Bay. They found out that because of the high N/P ratio in the Oder waters, phosphorus was very probably the factor limiting phytoplankton primary production in the Pomeranian Bay during periods of intensified inflow of riverine waters. The species dominating the phytoplankton of the Pomeranian Bay during the present study were found to be the same as those recorded in this region 40 years earlier (Gromisz et al., 1999).

Wu (1984) also studied algal samples to determine the water quality present in different sampling sites in Taiwan. An association of five algal species as well as genera was used as pollution indicators. A pollution syndrome was observed with fairly rapid and marked reduction of certain species and possible proliferation of another species. In some cases, the diversity of the algal community is correlated with the degree of pollution.

In a study done by Perez and colleagues, biological indicators for water quality were identified from the algal species that could tolerate toxic levels of Nickel, chromium and Iron. They identified species such as *Bulbochaete* sp., *Oedogonium* sp., *Chlorococcum* sp., *Fragilaria* sp. and *Stigeoclonium* sp. for zinc; *Navicula* sp. and *Scytonema* sp. for chromium (VI); *Pachycladon* sp. and *Zygnema* sp. for nickel; *Oedogonium* sp., *Lyngbya* sp., *Scytonema* sp., *Stigeoclonium* sp., and *Phormidium* sp. for cobalt; *Bulbochaete* sp. for cadmium; and *Chlorococcum* sp. and *Spirogyra* sp. for mercury (Perez et al., 1999). However based form their regression analysis, they were unable to identify an algal species that could be used as heavy metal indicator, though these algal species does indicate a marked presence of the heavy metals that they tolerate.

Palmer's Index of Algal Pollution and Nygaard's Trophic State Index

Biotic indices are based mainly on the tolerance of certain organism to pollution and assigning them a score based on the level of pollution. The total score of all the species observed would then represent the index for the level of pollution. Quantitative studies using indices utilize the relative density and abundance of species while qualitative studies use the presence or absence of the taxa (Goel, 2006).

In 1957, Mervin Palmer approached 56 authors and asked them to assign two points for very high and one point for high pollution tolerant organisms. Palmer further improved the system and described a procedure using twenty algal genera and species, generally considered pollution tolerant, for a method to estimate the algal pollution index of a water sample (Agarwal, 2005). A pollution index of 1-5 was assigned to each of 20 types of algae that are most tolerant to organic pollution. The most tolerant algae were assigned a factor of 5. Less tolerant types were assigned a lower number. A score of 20 or more is taken as evidence of high organic pollution, while a score from 15-19 represents probability of organic pollution. Lower scores indicate that pollution is not high.

Another index is used to determine the organic pollution in water bodies using five algal groups. Nygaard's trophic state index uses a quotient of the relation of numbers of species of Cyanophyta, Chlorococcales, Centric Diatoms and Euglenophyta on the one side and Desmidiales on the other. The quotients can then be used to classify whether a freshwater body is oligotrophic or eutrophic. These set of quotients have been developed on the fact that various algal groups have different tolerance to organic pollution and nutrient enrichment. For example

cyanophycean and euglenophycean members can tolerate pollution and categorise the water body as eutrophic. Pennate diatoms and desmids usually reside in oligotrophic water (Mahesh, 2008). A compound quotient can also be used to indicate the total degree of pollution (Water Pollution Indices, 2002)

Several researches have utilized the Palmer's Nygaard's Biotic Indices as basis to determine the level of organic pollution present in freshwater bodies, including rivers. Bhat (1998) conducted a research to assess the biotic composition, phytoplankton density and diversity in the rivers and to assess levels of water pollution, considering Palmer's algal species index and species diversity, in rivers Kali and Aghanashini, in Karnataka state, India. He found that Palmer's index of species was 8 in river Aghanashini and was 11 in river Kali indicating closeness of river Kali to organic pollution and eutrophication.

Biological assessment of the Mutha River in Pune, India using Palmer's and Nygaard's indices was done by Jafari and Gunale, (2006) showed that several sampling sites along the river showed ratings of 20 and above for the Palmer's index, indicating high probability of organic pollution while the Nygaard's index confirmed eutrophication along similar sites across the river.

Ramakrishnan (2003) utilized phytoplantktons and periphytes to assess the water quality in two freshwater bodies at Tiruvannamalai. He used Palmer's algal index and other pollution scales like Nygaard's index and Sapropobic index to quantify the water quality of the bodies. Results showed that the chemical analysis and the pollution indexes were highly correlated and both suggest that upon comparison of the two bodies of water, water body I was more polluted than water body II.

Nandan and Aher (2005) assessed quality of water of Haranbaree dam and Mosam river of Maharashtra in India using algal communities. By using Palmer's index of pollution for rating of water samples, the total score of each station of study area was greater than 20 indicating the confirmed high organic pollution. Out of the 34 pollution tolerant genera, 27 genera and 33 genera were observed at the dam and river sites respectively.

METHODOLOGY

A. Habitat Analysis

Topographic-geographical aspect of the river and the area around the river was obtained using a Garmine Brand Global Positioning System (GPS). Temporal and spatial data were recorded. These included the date and time of day. Spatial information was recorded using a Global Positioning System (GPS) to determine the exact coordinates of each site.

B. Determination of Weather Conditions

Weather conditions were observed at each sampling site. Measurements were taken two times during the day. The weather conditions observed were: aerial temperature, light intensity and relative humidity.

Aerial Temperature

A thermometer was suspended for 3 minutes at 3 random sites around each sampling site. The readings were observed and recorded. All measurements were recorded in degrees Celsius.

Light Intensity

A light meter was used to measure light intensity. The light meter was held with the sensory plate facing the sun. Light intensity was observed at 3 random sites around the sampling site. All measurements were recorded in lux.

Relative Humidity

A slingshot psychrometer was used to determine the relative humidity. The cotton around the bulb was moistened with water. The initial reading was recorded. The psychrometer was rotated above the head for 2 minutes. This was done at 3 random sites around the sampling site. Relative humidity was determined using the table for relative humidity based on the wet and dry bulb readings (Hallare, 2006)

C. Microbial Analysis

Five hundred ml water samples were obtained at each sampling site using a sterile 500 ml bottle. Water samples were obtained underwater to avoid any contamination. Water samples were placed in a cooler with ice and transported to UP Manila Biology Department Laboratory for inoculation. Samples were kept in the cooler with ice for a maximum of 24 hours.

Three different dilutions were made from the water samples. The dilutions were 1ml, 0.1ml and 0.01ml. Five tubes were used for each dilution. Single strength medium was used because samples are classified under surface water. Culture media was formulated beforehand and autoclaved to ensure sterility. Amount of dehydrated media is subject to change due to varying sources of culture media.

Ten ml of the sample was transferred using a pipette into a 100ml flask containing 90ml of phosphate-buffered dilution water. The resulting dilution was the 0.1ml dilution. 10 ml was obtained from the 0.1ml dilution using a pipette and transferred to a 100ml flask containing 90ml of dilution water. The resulting dilution was the 0.01ml dilution. 100ml of the original water sample was used as the 1ml dilution.

One ml of each dilution was transferred to each of 5 test tubes containing 9ml of lauryl sulfate tryptone broth using a pipette. Each test tube was labelled with a reference number and dilution. The test tubes were incubated for 48 hours at 37 degrees Celsius. Test tubes were observed after 24 hours for growth. Gas production, colour change and turbidity indicated growth. The tubes were returned to the incubator and re-examined after 48 hours. The number of positive tubes per dilution series per site was compared with a MPN table to determine the MPN/100ml. The value obtained from the table was then multiplied by then 10 to obtain a rough estimate. This is because the dilution series is not in the MPN table.

Positive tubes were inoculated into brilliant green lactose bile broth using a sterile wire loop. The wire loop was sterilised between transfers by heating in a flame. The tubes were labelled with same code used in the presumptive test. The tubes were incubated for 48 hours at 37 degrees Celsius. After 48 hours, the tubes were observed for gas production and turbidity. Gas production and turbidity indicates a positive result. The results were compared to a most probable number table.

Eosin methylene blue agar was inoculated with the positive tubes to check for the presence of *Escherichia coli*. Presence of metallic green on the colonies indicates the presence of E. coli.

Each EMBA plate was inoculated unto Simmon's Citrate agar and MRVP broth and stained with gram stain for complete coliform analysis.

Each EMBA plate was smeared unto a glass slide and stained with gram stain set. Blue or violet colonies indicated gram positive bacteria. Red colonies indicated gram negative colonies.

Inoculum was obtained from each EMBA plate and inoculated unto a slant of Simmon's Citrate agar. Blue coloration on the media represents utilization of citric acid as a carbon source.

D. Plankton Analysis

Plankton collection

Twenty bucketful of surface water were passed through the plankton net with a mesh size of 25µm. The filtered water sample was then transferred into a sterile bottle. This was done at each of the sampling sites twice a day, morning (8-10am) and afternoon (1-4pm), following diurnal patterns. The bottles were preserved with 4 drops of 10% buffered formalin solution. The samples were viewed under a microscope in the lab for the identification and quantification of plankton species (Asis et al., 2006).

Counting of Plankton Using Hemocytometer Method

Using a clean pipette, a drop of well mixed algal samples was placed in the two inner grooves of the hemocytometer. Evenness of cell distribution was checked under low power magnification (10x) in an Olympus light microscope. The cells were allowed to settle for 3-5 minutes before counting. Counting was made at the four corner squares of the counting block starting at the top left square and only those cells that lie within or touching the boundary line was included in the count. Calculation of the cell density was given in the formula:

$$\text{Plankton Density} = \text{Average of total count} \times 10^4$$

Microscopic Examination of Planktonic Organisms

With a medicine dropper, a drop of the plankton sample was placed on a glass slide. A cover slip was then placed on the drop gently with a toothpick. The slide was examined under the low and high power objectives (10 and 40x) on an Olympus light microscope to locate and identify the organisms. Various references such as taxonomic keys by Siver (2004), Prescott (1978) and Brown et al.(undated), researches done by Wagner (2006), Celekli (2006), Carr (1966), Suthers (2009) and Perry (2003) as well as illustrated guides for plankton identification by Martin (2008), Microbial Digital Specimens Archive and Microscopy-UK were used to identify organisms.

E. Physico-Chemical Analysis of Water

Collection of Water Samples

Five hundred ml of water was collected using previously labelled 500 ml clean glass bottles. Three samples were taken from each sampling site, namely the border, upstream, midstream and downstream. Sampling bottles were completely submerged and filled underwater. After filling the bottles with water to the brim, the bottles were tightly sealed underwater to avoid any air from entering the bottles. After collecting the samples, the samples were stored in a cooler with ice and transported to the UP Manila Biology laboratory for analysis for a maximum of 1 day. Samples were collected once in the morning, at 8:00AM – 10:00AM, and in the afternoon, at 1:00PM – 4:00PM.

Turbidity

Water samples were placed in small vials. The transmittance reading of each water sample was obtained using a spectrophotometer. The wavelength was set 540nm.

Temperature, Salinity, Conductivity and pH

Conductivity (ms), pH, salinity (ppm) and temperature (°C) were measured using HORIBA q-10 portable water checker. Values were taken in each of the four sampling sites. Readings were taken 3 times.

Total Solids

To determine the amount of filterable solids, 50 – 100 ml of well-stirred sample was passed though an oven dried pre-weighed filter paper (0.45 micrometer pore Whattman) fitted into a Millipore filtration unit. The filter paper was removed and placed in a clean Petri dish. The filter paper was oven dried at 105°C overnight. It was placed in a dessicator and weighed. The amount of filterable solids was determined by subtracting the weight of the filter paper before filtration from the weight of the filter paper after filtration.

To determine the amount of dissolved solids, 25-50 ml of the filtrate was poured in a clean, pre-weighed evaporating dish. The filtrate was evaporated until the evaporating dish is dry. The evaporating dish was placed in a dessicator and weighed. The amount of dissolved solids was determined by subtracting the weight of the evaporating dish before heating from the weight of the evaporating dish after heating.

Dissolved Oxygen

Dissolved oxygen was determined using the Winkler with Azide modification test

Winkler with Azide Modification

0. 2ml of $MnSO_4$ was added to the water sample immediately followed by two (2) ml of alkali-iodide-azide reagent. The bottle was then inverted for at least 15 times allowing a precipitate to settle each time. Before removing the stopper and adding H_2SO_4, the precipitate was allowed to settle first. Once the precipitate settled, two ml of concentrated H_2SO_4 was immediately added by allowing the acid to run down the neck of the bottle and immediately restoppered. Then the solution was mixed until the floc dissolves and a clear yellow-orange solution remained. This represents the iodine that has replaced the dissolved oxygen in the sample. The sample was titrated afterwards.

Titration proceeded as follows; 203ml of the sample (which corresponds with 200ml of the original) was placed in a 250ml Erlenmeyer flask. Burette was rinsed with 0.025 $Na_2S_2O_3$ and then the sample was titrated to a very pale yellow color. Once the color was obtained, 1-2ml of starch solution was added. This made the solution turn blue. Titration was again carried out until the solution became colorless. It was made certain that the color persisted under agitation. The amount of thiosulfate was then solved for.

Free Carbon Dioxide

Ten ml of cold-water sample was placed in a 50ml beaker. Then 10 drops of phenolphthalein was added to sample and titrated with N/44 NaOH solution until a pink colour appeared. The volume of NaOH used was multiplied by 10 to obtain the amount of free carbon dioxide in ppm.

Total Hardness

To obtain total hardness, a titrant was formulated with 50% concentrated detergent and 50% distilled water. 20 ml from each stock solution was obtained and titrated until the soap bubbles are firm and stopped collapsing. This is the true endpoint of the total hardness. To standardize this, a lather factor was computed. This was done by taking 20ml of distilled water and titrated as well. To solve for total hardness or $CaCO_3$ in mg/L, this formula was employed:

$$\text{Total Hardness } (CaCO_3 \text{ mg/L}) = \frac{\text{Volume of titrated detergent - lather factor (0.04ml)}}{\text{Volume of sample (20 ml)}}$$

Productivity

Two extra water samples were collected. One bottled was covered with aluminum foil to prevent sunlight penetration. Another was left open for light to enter. Both bottles were left out in the sun light for 24 hours. After which, dissolved oxygen was determined using Winkler with Azide modification test. Respiration, gross productivity, and net productivity were solved afterwards (Appendix A).

RESULTS

Habitat Analysis

Sampling was done on twice for both morning and afternoon of December 18, 2009 for the boundary, upstream and market samples while the downstream site sampling was done on December 19, 2009. Location for the sites as well as the time of collection was also recorded (Table 1).

Collection of samples was done along four sites of the Lawaye River. The boundary site at Muzon was located between the towns of Rosario and San Juan. In the site, the river formed a small stream in the middle of a forest clearing. Residents living nearby use the river for various domestic needs such as washing clothes (Plate 2). Piggeries near the area also derive their water sources from the river. Motorized pumps are used to draw water to the piggeries nearby (Plate 3).

Locals attest to occasional floods that occur during the rainy season as well as the unpleasant odour that come with it. Most of them attribute it to local piggeries found at the Rosario tributary of the Lawaye. Algal blooms were found to be present in the river (Plate 6).

The upstream site was situated in a part of the river that passes underneath a bridge. Locals live near the area and use this part of the river for washing clothes (Plate 9) and recreative purposes such as swimming (Plate 10). Improper waste disposal was observed as various wastes and garbage were noted at the river body and river bank (Plate 11).

The river body running through the San Juan Public Market served as the midstream site. It is characterized by walls that run along the left side of the river. The walls are lined with

drainage pipes coming from the nearby market of San Juan (Plate 12 and 14). Effluents coming from the pipes are deposited directly to the river. Piggeries and slaughterhouse were found in the marketplace (Plate 15). Locals also live nearby as villages were observed on the right side of the river (Plate 17).

The downstream sampling site was done in the mini-dam in Pinagbayanan, San Juan Batangas (Plate 18). The site is located in the vicinity of rice fields and irrigation canals (Plate 19). Agricultural livestock such as cows grazed near the grasslands around the site (Plate 22).

Locals utilize the river for recreational purposes (Plate 21) although algal blooms (Plate 20) were observed in the river.

Determination of Weather Conditions

The weather condition during the time of sampling ranged from partly cloudy to clear skies. Air temperature was from the four sites fell within the range of 26°C to 29.5°C (Table 2). It was consistent with the climatic temperature in San Juan, Batangas that ranged from 23°C to 30°C (EIA Hopewell Power Corporation, 1992). The relative humidity measured ranging from 78% to 92% in the four sites (Table 2) was also consistent with the mean annual relative humidity at 87% (EIA Hopewell Power Corporation, 1992). Light readings in the areas were from 247 lux to 292.33 lux (Table 2).

Coliform Analysis

All sites were tested using the multiple tube fermentation method. In the presumptive phase, lauryl sulfate tryptose broth. Five tubes were inoculated with samples from each dilution. The tubes were allowed to incubate for 24-48 hours. After the incubation period, turbidity and

gas production was observed in all five tubes from each sampling site (Plates 25 - 36). Based on the number of positive tube and the MPN table (Appendix B), the amount of coliforms present in the water is greater than 1600 per 100ml (Table 4).

The presences of coliforms from the previous test were confirmed in the confirmatory phase using brilliant green lactose bile (BGLB) broth. Each positive tube from the presumptive test was inoculated into the BGLB broth. The tubes were incubated for 24-48 hours. After the incubation, turbidity and gas production was observed in all the tubes (Plates 37 - 48).

The positive tubes were then inoculated into eosin methylene blue agar plates for a completed test. All plates were incubated for 24-48 hours. After incubation, metallic green colonies were observed in some of the plates. While the other plates did not contain any metallic green colonies (Tables 5 – 8. Plates 49 and 50).

The metallic green colonies were inoculated into Simmon's citrate agar and MRVP broth to determine the IMViC profile (Tables 5 – 8. Plates 51 - 54). The tubes were inoculated for 24-48 hours. The colonies were also smeared unto clean, glass slides and stained with gram stain. The colonies were determined to be gram-negative bacteria (Table 5 – 8. Plates 55 - 56).

Plankton Analysis

A total of 34 planktonic species were identified during the study (Table 14 , Plates 57 - 88). The results showed that *Ankistrodesmus*, *Closterium*, *Cosmarim*, *Pandorina*, *Navicula*, *Tabellaria* were the most prevalent of the all the genus that were identified during the study (Table 14).

The morning and afternoon samplings of the midstream along with the afternoon sampling of the downstream obtained high scores on the index, based on Palmer's algal pollution index of the Lawaye (Table 11). This indicates a high probability of organic pollution. The upstream and the border sampling sites had relatively normal indices.

The boundary and upstream samples, both morning and afternoon, were found to be oligotrophic based on all the indices of Nygaard's trophic state index (Table 9). Based from the compound index, the upstream site is still considered as oligotrophic though its euglenophycean index is eutrophic. The midstream however is weak eutrophic, in its cyanophycean, euglenophycean and compound indices. This shows that the midstream site might subject to excessive productivity and even blooms. The downstream site is considered fairly oligotrophic in nature.

Plankton count in the sampling areas showed the downstream and border sites had the highest counts in all the areas. Afternoon counts were more numerous compared to morning samples except for the upstream site (Table 13).

Physico-Chemical Analysis

The results show that all morning and afternoon samples have a slightly basic pH reading with the midstream portion having the highest pH reading, 9.52 and 9.64 respectively (Table 15 and 16). The salinity readings of all sites were constant with a reading of 0.01 ppm (Table 15 and 16). The conductivity readings of the samples have a mean of 0.379 (Table 15 and 16). The temperature of all sites ranges from 25.3 - 25.6° C (Table 15 and 16). Dissolved oxygen concentrations are within the normal values in the upstream and boundary sites while Midstream

site has the lowest reading followed by the downstream site (Table 15 and 16). Free carbon dioxide concentration is highest in the downstream and midstream sites with a mean of 11.25ppm while it is lowest in the upstream and boundary sites with a mean of 4ppm (Table 15 and 16). Total hardness of the water is at a mean of 0.002 (Table 15 and 16). Productivity is variable among the sites. Afternoon samples contain the highest readings with a mean of 0.05ppm while morning samples have a mean of 0.04ppm (Table 15 and 16).

DISCUSSION

Physico-Chemical Properties

The pH readings in border site, upstream site and downstream site are all within the normal range of 6.5 – 9.0 (DAO 1995). The midstream site, however, is slightly greater than the normal range with a reading of 9.52. These readings indicated that the water in the sites were slightly basic. High pH readings can be very detrimental to fishes and other aquatic organisms. The slightly basic reading of the water is due to the amount of decaying matter and organic waste present. The decaying matter can come from natural sources, such as dead leaves and animals, and human intrusion such as the disposal of organic waste into the river. The wastes dumped into the river can be nitrogenous or basic in nature. With the presence of piggeries in all sites and the market in the midstream site, it can be assumed that the organic matter present in the water is nitrogenous in nature since fecal matter contains ammonia. High pH readings can also be attributed to the use of detergents in the water as locals use the river water for washing clothes.

The salinity, temperature and conductivity of the samples are within the normal range for freshwater. The salinity readings in the downstream site were low because the data was collected during low tide. As such, there was no mixing of freshwater and seawater. Freshwater salinity levels should not go beyond 1000ppm. The temperature is also within the standard range of 25 – 31 °C (DAO, 1995)

The DO readings of the border and upstream sites are similar and within the normal reading for freshwater of 5 ppm while the DO readings of midstream and downstream sites are

relatively lower than the standard (DAO 1995). All sites have decaying matter as verified with the presence of fallen leaves and dead plants. However, there is an increase in the amount of organic matter in the water in the midstream site due to its proximity to the marketplace and residential areas. The flow of the wastes from these sources goes through pipes which lead directly into the river. This contributes to the bloom of bacterial species which utilize oxygen. The increased levels of such bacterial species add to the oxygen consumption of the river. With the increased levels of organic and decaying matter, the amount of decomposition increased as well.

The downstream and midstream sites had very high concentration of free carbon dioxide relative to the upstream and border sites. This may be due to high concentration of bacterial species and other aerobic organisms that utilize oxygen and give off carbon dioxide.

Coliform Analysis

The total coliform count was found to be very high in all sites when compared to the standard of 200/100 ml (DAO, 1994). This can prove to be very detrimental to the health of humans and livestock that utilize the water. High coliform concentration indicates that disease-causing pathogens are very likely to be present in the water.

There were always five positive tubes for each dilution series in each site. From this, we could assume that there is a high concentration of coliforms present in the water. However, this is not very conclusive. Based on the MPN table (Appendix B), each site has more than 16,000/100 ml. With a MPN this high, the amount of pathogens present in the water can also be

expected to be high. With this in mind, the water from Lawaye River may pose a serious health problem for the local people of San Juan, Batangas.

The tubes that formed the presumptive phase were inoculated into BGLB tubes to confirm the presence of coliform bacteria. BGLB is selective for coliform bacteria due to the presence of Oxgall in the media (McCrady 1943). Oxgall inhibits the growth of non-coliform bacteria. The results showed that all tubes per dilution per site showed positive results. This confirmed the presence of coliform bacteria.

Afterwards the BGLB tubes were inoculated into EMBA plates for the completion phase. EMBA determines the presence of *Escherichia coli* when the media contains dark colonies with a metallic green sheen. The colonies are then stained with gram stain to determine whether they are gram positive or gram negative. The IMViC profile should also be determined. *E. coli* is a gram negative bacteria with an IMViC profile of ++--.

The EMBA plates showed that some plates contained colonies with a metallic green sheen. This indicates that *E. coli* is present. The other plates did not have any colonies with a metallic green sheen. This indicated that *E. coli* is not present in the plate though other coliforms may be present.

The positive plates were stained with gram stain and resulted in red coloration. This indicated that the colonies were gram negative bacteria. The same plates showed positive results in indole test and methyl red test and negative results in Voges -Proskauer test and citrate test. With this information, the IMViC profile of the colonies with metallic green sheen was determined to be the same as the IMViC profile of *E. coli*. The negative plates showed various results but none of the results matched the criteria required to identify them as *E. coli*.

High coliform concentration can be attributed to the presence of piggeries in the upstream and border site of the river. According to locals, the wastes of these piggeries pass into the water and on rainy days produces a very bad stench coming from the river (Anonymous, Pers. Comm., 2009). The dumping of waste from piggeries can be the primary cause for high coliform concentrations. The improper disposal of waste may also attribute to the increased concentration of coliforms in the water (Plate 11).

There is a marketplace within the vicinity of the midstream site. The wastes of the pigpens and market stalls found in the midstream (Plate 15) emptied into a drainage system that was seen flowing into the river (Plate 12 and 14). This can cause an increased amount of organic matter for bacterial species to utilize. In the downstream site, numerous agricultural and residential areas are found within the vicinity of the river (Plate 19). The runoff from the agricultural areas increases the amount of organic matter in the river. Numerous livestocks, such as cows, have also been spotted grazing near the river (Plate 22). These run-offs and livestock wastes can be the primary cause for high coliform concentration in the area. The downstream site serves as the sink before the river reaches the sea. As such, all the wastes from the other sites flow into the downstream site and accumulate. This might also explain the high coliform concentration found.

Plankton Analysis

Based on Palmer's algal genera index, *Ankistrodesmus*, *Chlorella*, *Navicula*, *Euglena*, *Closterium*, *Pandorina*, *Phacus*, *Synedra*, *Oscillatoria* were found to be present in the samples (Table 14). These algae are members of an algal list determined by Palmer to be the most pollution tolerant or the most sensitive genera to organic pollution.

Ankistrodesmus, *Chlorella*, *Closterium* and *Pandorina* are members of the green algae Chlorophyta which lives in freshwaters such as lakes and rivers. Large scale growth of members of this group can cause waterbloom in nutrient enriched waters and eventually cause eutrophication upon death; although none of the members of the group are toxic (Chatterjii, 2007). *Oscillatoria* belongs to the blue green algae, Cyanophyta, a genera that possesses some similarity with bacteria. Under low concentrations of nitrogen in the water, most of the algae can fix atmospheric nitrogen into organic nitrogen. They also possess lipopolyssacharides in their cell wall posting a potential health hazard as contact irritants (Suthers, 2009). The cyanophytes can also cause blooms called cyanophycean blooms (Chatterjii, 2007).

The genus *Oscillatoria* has been noted by Tripathi and Pandey (2009) and Agarwal (2005) to be a good indicator or organic pollution. It has also been placed as the second most pollution tolerant taxa out of 60 in Palmer's algal index (Tseng and Wang, 1982). Along with *Euglena* it is commonly found in waters with high nitrogen concentration (Person, 1989). The diatom taxa, Bacilliarophyta, have also been widely used as biomonitoring species for water pollution (Cicek et.al., 2009). *Navicula* and *Synedra* have been determined by Jafari and Gunale (2005) as being a dominant algal flora in polluted waters. *Synedra* also ranks as one of the 10

most pollution tolerant genus in the algal index (Agarwal, 2005). Most of the euglenoid species like *Euglena* and *Phacus* inhabit freshwater environments and can exist in areas of varied pH and light levels with an abundance of decaying organic matter thus thriving under high nutrient levels (Siver, 2004); as such they also grow in areas abundant in animal wastes (Baddour et.al., 2003). According to authorities, *Euglena* is considered as the most pollution tolerant genus and is commonly found in areas of high nitrogen concentration.

Eutrophication is the process of increased productivity of a body of water. This is due to excessive nutrient loading and is accelerated by human influence and is termed cultural eutrophication. Eutrophic waters have high productivity and may be brought about by natural or anthropogenic factors. Oligotrophic waters on the other hand, have low productivity due to low nutrient content and are usually characterized by clarity of waters (www.des.nh.gov, 2010).

Both the Palmer's and Nygaard's indices point out to the midstream and downstream sites having a high probability of organic pollution and eutrophication. This may be attributed to the setting the two sites have. The midstream is located near the San Juan marketplace and is an outlet for surface runoff and waste discharge from the market and the nearby Poblacion. During rains, it can be observed that sludge and other wastewater from the marketplace flows out of the drainage pipes in near the river. The downstream site is located near a dam near the river opening to the sea. It is the final dumping area of the all the waters that flow along the Lawaye River. Actual observation along the site revealed that there were algal blooms that occur around the site (Plate 20), indicative of eutrophication. The site is also close to agricultural lands which utilize fertilizers which can result in agricultural runoffs carrying excess nutrients. Coupled with

wastewater effluents carried down the river path, it might have resulted into the blooms found and the indices measured.

Plankton densities were also measured; the downstream had the highest number followed by border, upstream and midstream. The nutrient loading was evident in the area as was previously mentioned. These nutrients that were abundant combined with favourable physico-chemical parameters like high free carbon dioxide created and environment suitable for phytoplankton growth. The border site was near piggeries that may have influenced the plankton count. There were observed blooms in the area, a sign of nutrient enrichment; this in turn can lead to favourable conditions for plankton to thrive in (Suthers, 2009).

Physico-chemical parameters play an important role in the growth and distribution of plankton in freshwaters. The pH recorded at the river areas except midstream and downstream were slightly basic but within the DAO specifications of freshwater rivers. The two latter sites showed a pH range of 8.8-9.5 in the morning samples and it increased to a range of 9.3-9.6. It has been noted that pH 5-8.5 represents the optimal pH for phytoplanktons and greater than 8.8 is detrimental to algae (Tripathi and Pandey, 2009). Although the pH shown in the results seem to be adverse to algal growth, some factors might have led to some taxa surviving in it; for example, all of the cyanophyta samples were found in the midstream and downstream areas along with *Euglena*. This might be attributed to two factors, first, the cyanophyta and euglenophyta represents the more pollution tolerant taxa of the algae based from algal pollution ratings (Agarwal, 2005) and second, the blue-green algae are found to thrive in higher pH, turbidity, alkalinity, total solids, dissolved oxygen and temperature (Tripathi and Pandey, 2009).

Temperature plays an important role in aquatic systems especially plankton. Certain taxa such as Euglenophyta are inhibited by very high temperature while some like Chlorococcales favoured high values (Tripathi and Pandey, 2009). Turbidity in the river also has a direct effect on plankton growth. High turbidity restricts light passage in the water, thus preventing limiting phytoplankton capacity for photosynthesis. High turbidity can be brought about by algal blooms, effluents and soil erosion. The results from the physico-chemical measurements showed that the values from both temperature and turbidity were both relatively constant and normal.

The relationship between phytoplankton and dissolved oxygen is one of the best indicators of water quality in freshwaters (http://courses.washington.edu, 2009). In the presence of sunlight, phytoplankton use carbon dioxide to undergo photosynthesis and release oxygen in the water. Thus it is usually typical to find high levels of dissolved oxygen in areas of large phytoplankton growth and vice versa. However there are some instances wherein there are areas of low dissolved oxygen concentration but with significant numbers of phytoplankton. This occurs in the dissolved oxygen counts for downstream wherein they ranged from 3.2-3.67mg/li in morning and afternoon sampling and still had a high plankton density count relative to the upstream and border sites that had normal dissolved oxygen counts.

There are some possible explanations for this; first, there might be an elevated water turbidity restricting light or the nutrients in the water are found in limiting quantities. The dissolved oxygen would therefore be the net production of oxygen by some phytoplankton and bacteria minus the oxygen used by other organisms during respiration. Second, some plankton species are pollution tolerant that they thrive in areas of low dissolved oxygen levels, an example would be the blue green algae and third, there is a large amount of bacteria and other

decomposers found in the waters that they consume large amounts of oxygen thus contributing significantly to the reduced dissolved oxygen levels. Since the water turbidity measured in the midstream and downstream were normal, the latter explanations are plausible in explaining the larger phytoplankton species numbers in the area. Also, it can be seen in table productivity that the downstream site had a relatively high amount of respiration compared to the upstream and border, pointing out to high amounts of decomposers such as bacteria, who utilize oxygen. Zooplankton on the other hand is less sensitive to lower oxygen concentrations and is therefore less affected, although very low concentrations can kill off zooplanktons and bacteria (http://courses.washington.edu, 2009).

Carbon dioxide is present usually as a result of animal respiration, the decay of organic matter, and the decomposition of certain minerals. Surface waters typically contain less than 10 ppm (mg/l) dissolved CO_2, while ground waters, particularly if deep, may contain several hundred ppm (mg/l) (APHA Standard Methods, 1998). Generally, higher amounts of free carbon dioxide favour algal growth (Tripathi and Pandey, 2009) since plankton that undergo photosynthesis will need carbon dioxide. This trend is shown in the result of the downstream, wherein it has a high free carbon dioxide count and also has a relatively greater plankton density counts compared to the other sites.

Measured productivity was observed to be highest in both the border and downstream sites both morning and afternoon. This may be attributed to the recorded plankton densities in the downstream and border sites; both had the highest density counts in all of the sites. The high numbers of plankton located in those areas may have contributed to the increased productivity in the area.

The experiment was conducted twice to observe diel vertical migrations of plankton in the river. Plankton sample counts were in higher numbers in the afternoon sampling compared to the morning. Both zooplankton and phytoplankton show vertical migrations in the water column. According to Ashjian et al. (1997), downward migration of zooplankton begins to occur at the time nearest to sunrise and was measured at 5-7am in the morning and upward migration during 4-6pm in the evening. Bai et al. (2007) demonstrated that there are distinct population of phytoplankton that dwell near the bottom at near midnight then as the sun rises they slowly migrate towards the surface, eventually reaching peak of distribution after midday through the afternoon. Phytoplanktons exhibit vertical movement down the water column as a response to various environmental stimuli such as water turbulence, buoyancy regulation and nutrient requirements (Cullen and McIntyre, 1998).

CONCLUSION

The physico-chemical properties of each site are within normal range except for pH, DO and free carbon dioxide. The increased levels of pH and free carbon dioxide and reduced levels of DO can be attributed to high concentration of organic matter present and coming into the water. Human intrusion and agricultural runoffs are the main causes of heightened readings.

The coliform concentration in all sites was found to be well beyond the normal range. All sites are registered to having organic pollution coming from surface runoffs and wastes. Due to high concentration of coliforms, there is a very high risk of pathogens and other disease causing organisms to be present in the water.

Plankton analysis showed that sampling areas in the Lawaye River were found to have different level of organic pollution. The Palmer's and Nygaard's Indices demonstrated that the downstream and the midstream sites registered the highest probability of having organic pollution while the sites found on the upper river sites border and upstream were comparatively normal and oligotrophic. Factors such as improper waste disposal, effluents from drainage and agricultural runoffs might have contributed to the observed results.

Different genera of plankton species provided probable indicators of the level of organic pollution that was present in the river. Plankton counts demonstrated the diel vertical migration undergone by plankton throughout the day as well as suggestive of the nature of the waters they were found.

Thus, this study showed that plankton and microbial species were good biological indicators of pollution in the Lawaye River.

RECOMMENDATION

Further research is suggested on the Lawaye River. Regular monitoring or longer sampling period should be done to obtain a more accurate and consistent data regarding the physico-chemical parameters, microbial and plankton species.

Plankton research should be done more times at least four times a day to better observe the vertical movements of plankton. Species diversity count is also recommended to determine the population makeup of individual species present as well as identify key plankton species that may play an important role in the environmental niche.

Dissemination of information among the locals is recommended in order to make them aware of the current condition of Lawaye River. This will also make them aware of the potential risks involved when utilizing the river.

Determining the amount of fecal coliforms is recommended to provide more conclusive analysis. Identification of other important coliform species is recommended to determine other health hazards that the river water may pose. Determining the presence of other microbial groups is also recommended to better understand the current situation of the river.

Restoration of the river should also be undertaken. Immediate action by the local government is recommended as well as regular monitoring of the river to ensure that the water quality does not worsen.

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TABLES

Table 1 – GPS Coordinates of Sampling Sites

	GPS Coordinates	Date and Time of collection	
		Morning	Afternoon
Boundary	13°50'39.81"N 121°21'34.22"E	8:30am December 18 2009	4:34pm December 18 2009
Upstream	13°50'32.71"N 121°23'54.49"E	10:25am December 18 2009	3:46pm December 18 2009
Midstream	13°49'30.18"N 121°23'32.77"E	9:20am December 18 2009	3:40pm December 18 2009
Downstream	13°47'56.98"N 121°25'07.57"E	6:20am December 19 2009	5:29pm December 19 2009

Table 2 – Weather Conditions during Morning Sampling

Morning Samples	Psychrometer Reading	Relative Humidity	Light	Air Temperature	Water temperature
Boundary	Dry bulb- 29.8°C	78%	260.67 lux	29.5°C	28.5°C
	Wet bulb- 26.8°C				
Upstream	Dry bulb- 26.33°C	85%	246 lux	26°C	24.5°C
	Wet bulb- 24.33°C				
Midstream	Dry bulb- 26°C	85%	225.33 lux	27°C	24.5°C
	Wet bulb- 24°C				
Downstream	Dry bulb- 25°C	84%	227 lux	26.83°C	24°C
	Wet bulb- 23°C				

Table 3 – Weather Conditions during Afternoon Sampling

Afternoon Samples	Psychrometer Reading	Relative Humidity	Light	Air temperature	Water temperature
Boundary	Dry bulb- 27.83°C	85%	247 lux	27.5°C	24.5°C
	Wet bulb- 26.83°C				
Upstream	Dry bulb- 27.5°C	92%	292.33 lux	27.5°C	26.5°C
	Wet bulb- 26.83°C				
Midstream	Dry bulb- 28°C	78%	285.67 lux	27.33°C	23.5°C
	Wet bulb- 25°C				
Downstream	Dry bulb- 26.5°C	92%	253.67 lux	27.33 °C	24.33°C
	Wet bulb- 25.1°C				

Table 4 – Most Probable Number (MPN) per 100ml

	Number of Positive Tubes			MPN Count per 100 ml
	1	0.1	0.01	
Downstream	5	5	5	>1600
Midstream	5	5	5	>1600
Upstream	5	5	5	>1600
Boundary	5	5	5	>1600

Table 5 – Completed Test for Detection of E. Coli in Downstream Site

Dilution, Plate number	EMBA	Indole	Methyl Red	Voges-Proskauer	Citrate	IMViC Profile	Gram Stain
1, 1	+	+	+	-	-	++--	-
1, 2	+	+	+	-	-	++--	-
1, 3	+	+	+	-	-	++--	-
1, 4	-	-	+	+	+	-+++	+
1, 5	-	-	-	+	+	--++	+
0.1, 1	+	+	+	-	-	++--	-
0.1, 2	+	+	+	-	-	++--	-
0.1, 3	+	+	+	-	-	++--	-
0.1, 4	-	-	-	+	+	--++	-
0.1, 5	-	-	+	-	+	-+-+	+
0.01, 1	+	+	+	-	-	++--	-
0.01, 2	-	+	-	+	+	+-++	+
0.01, 3	-	-	+	+	+	-+++	-
0.01, 4	-	+	+	-	+	++-+	-
0.01, 5	-	+	+	+	+	++++	+

Table 6 – Completed Test for Detection of E. Coli in Midstream Site

Dilution, Plate number	EMBA	Indole	Methyl Red	Voges-Proskauer	Citrate	IMViC Profile	Gram Stain
1, 1	+	+	+	-	-	++--	-
1, 2	-	-	+	+	+	-+++	+
1, 3	-	-	-	-	+	---+	+
1, 4	-	-	-	+	+	--++	-
1, 5	-	-	-	-	+	---+	+
0.1, 1	+	+	+	-	-	++--	-
0.1, 2	+	+	+	-	-	++--	-
0.1, 3	+	+	+	-	-	++--	-
0.1, 4	-	+	+	+	+	++++	+
0.1, 5	-	+	+	-	+	++-+	+
0.01, 1	+	+	+	-	-	++--	-
0.01, 2	+	+	+	-	-	++--	-
0.01, 3	+	+	+	-	-	++--	-
0.01, 4	+	+	+	-	-	++--	-
0.01, 5	+	+	+	-	-	++--	-

Table 7 – Completed Test for Detection of E. Coli in Upstream Site

Dilution, Plate number	EMBA	Indole	Methyl Red	Voges-Proskauer	Citrate	IMViC Profile	Gram Stain
1, 1	+	+	+	-	-	++--	-
1, 2	-	-	+	+	+	-+++	-
1, 3	-	-	+	+	+	-+++	+
1, 4	-	-	+	-	+	-+-+	+
1, 5	-	-	-	-	+	---+	-
0.1, 1	+	+	+	-	-	++--	-
0.1, 2	+	+	+	-	-	++--	-
0.1, 3	-	-	+	+	+	-+++	-
0.1, 4	-	-	-	-	+	---+	-
0.1, 5	-	+	+	-	+	++-+	+
0.01, 1	+	+	+	-	-	++--	-
0.01, 2	+	+	+	-	-	++--	-
0.01, 3	-	-	+	+	-	-++-	-
0.01, 4	-	+	-	-	+	+--+	-
0.01, 5	-	-	-	+	+	--++	+

Table 8 – Completed Test for Detection of E. Coli in Boundary Site

Dilution, Plate number	EMBA	Indole	Methyl Red	Vogues-Proskauer	Citrate	IMViC Profile	Gram Stain
1, 1	+	+	+	-	-	++--	-
1, 2	-	+	-	-	+	+--+	+
1, 3	-	-	+	+	+	-+++	+
1, 4	-	-	+	+	+	-+++	+
1, 5	-	+	+	-	+	++-+	+
0.1, 1	+	+	+	-	-	++--	-
0.1, 2	+	+	+	-	-	++--	-
0.1, 3	-	-	-	+	+	--++	-
0.1, 4	-	+	-	+	+	+-++	+
0.1, 5	-	+	-	-	+	+--+	+
0.01, 1	+	+	+	-	-	++--	-
0.01, 2	-	-	-	+	+	--++	-
0.01, 3	-	+	+	-	+	++-+	+
0.01, 4	-	-	+	+	+	-+++	+
0.01, 5		-	+	+	+	-+++	+

Table 9 – Nygaard's Trophic State Index

INDEX	OLIGOTROPHIC	EUTROPHIC
cyanophycean	0-0.4	0.1-3
chlorophycean	0-0.7	0.2-9
Diatom	0-0.3	0-1.75
euglenophycean	0-0.7	0-1
Compound	<2	2-6 (weak) <6 (eutrophic)

Table 10 - Nygaard's Trophic State Index of the Lawaye River

Nygaard's Algal Index								
	Border		Upstream		Midstream		Downstream	
INDEX	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
Cyanophycean	0	0	0	0.5	0.5	1	0	0.5
Chlorophyceean	0	0	0	0	0	0	0	0.5
Diatom	0	0	0.5	0.33	0	0	0	0
Euglenophycean	∞	0	1	∞	1	1	0	∞
Compound	0.5	0	1	1.5	1	2	0	1

Table 11 –Palmer's Algal Pollution Index

Points	Level of Pollution
20 or above	High organic pollution
15-19	Probable evidence of high organic pollution
Below 15	No or very low organic pollution

Table 12 – Palmer's Algal Index of the Lawaye River

Palmer's Algal Pollution Index								
	Border		Upstream		Midstream		Downstream	
PLANKTON	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
Ankistrodesmus	2	-	2	2	2	2	-	2
Chlorella	-	-	-	-	-	-	3	-
Closterium	1	1	1	1	1	1	1	1
Euglena	-	-	-	-	-	5	-	5
Navicula	3	3	3	3	3	3	-	3
Oscillatoria	-	-	-	-	4	-	-	4
Pandorina	1	1	1	-	1	1	1	1
Phacus	2	-	2	2	2	2	-	-
Synedra	-	2	-	-	2	2	2	-
Total	**9**	**7**	**9**	**8**	**15**	**16**	**7**	**16**

Table 13 – Plankton Count of the Lawaye River

PLANKTON COUNT								
Plankton density count	Border		Upstream		Midstream		Downstream	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
Cells/ml	28×10^4	32×10^4	20×10^4	18×10^4	16×10^4	18×10^4	25×10^4	33×10^4

Tables 14 – Plankton Composition

	PLANKTON COMPOSITION							
	Border Morning	Border Afternoon	Upstream Morning	Upstream Afternoon	Midstream Morning	Midstream Afternoon	Downstream Morning	Downstream Afternoon
ROPHYTA								
rodesmus	x		x	x	x	x		x
occus								x
lla							x	
bora						x		
ium	x	x	x	x	x	x	x	x
rium	x	x	x	x	x	x	x	x
ia					x	x		
spora					x			
xia			x					
na	x	x	x		x	x	x	x
rum								x
ra		x	x	x				
ix								x
OPHYTA								
occus				x		x		
toria					x			x
na						x		
OPHYTA								
m					x			
ROPHYTA								
um				x		x		
LLARIOPHYTA								
odiscus			x	x				
lla	x							
aria	x			x	x			
la		x	x	x	x	x		x
aria	x	x	x	x			x	x
a		x			x	x	x	
ENOPHYTA								
a						x		x
s	x		x	x	x	x		
OZOA								
ia						x		
POD		x	x	x				
SOPHYTA								
riella				x				
ROPODA								
ius	x							
FERA								
chia			x					
ayla			x	x		x	x	
EBOZOA								
	x			x	x	x		
gyxis				x		x		

Table 15 – Physico-Chemical Parameters of Morning Samplings

Parameter	Border	Upstream	Midstream	Downstream
pH	8.46	8.41	9.52	8.84
Salinity	0.01	0.01	0.01	0.01
Conductivity	0.427	0.397	0.363	0.339
Temperature (°C)	25.5	25.4	25.4	25.6
Turbidity	0.004	0.001	0.003	0.001
Dissolved Oxygen (mg/li)	5.1	5.3	3.8	3.2
Free Carbon Dioxide (ppm)	4	5	11	11
Water Hardness (mg/li)	0.002	0.002	0.002	0.003
Total Solids (g)	1.06	1.07	1.08	1.06
Productivity	0.1	0.04	0.07	0.43

Table16 – Physico-Chemical Parameters of Afternoon Samplings

Parameter	Border	Upstream	Midstream	Downstream
pH	8.46	8.95	9.64	9.32
Salinity	0.01	0.01	0.01	0.01
Conductivity	0.354	0.364	0.372	0.413
Temperature	25.3	25.6	25.5	25.3
Turbidity	0.001	0.004	0.002	0.009
Dissolved Oxygen	5.3	5.73	1.96	3.67
Free Carbon Dioxide	3	4	12	11
Water Hardness (mg/li)	0.001	0.002	0.002	0.003
Total Solids (g)	1.07	1.06	1.06	1.04
Productivity	0.5	0.06	0.06	0.3

PLATES

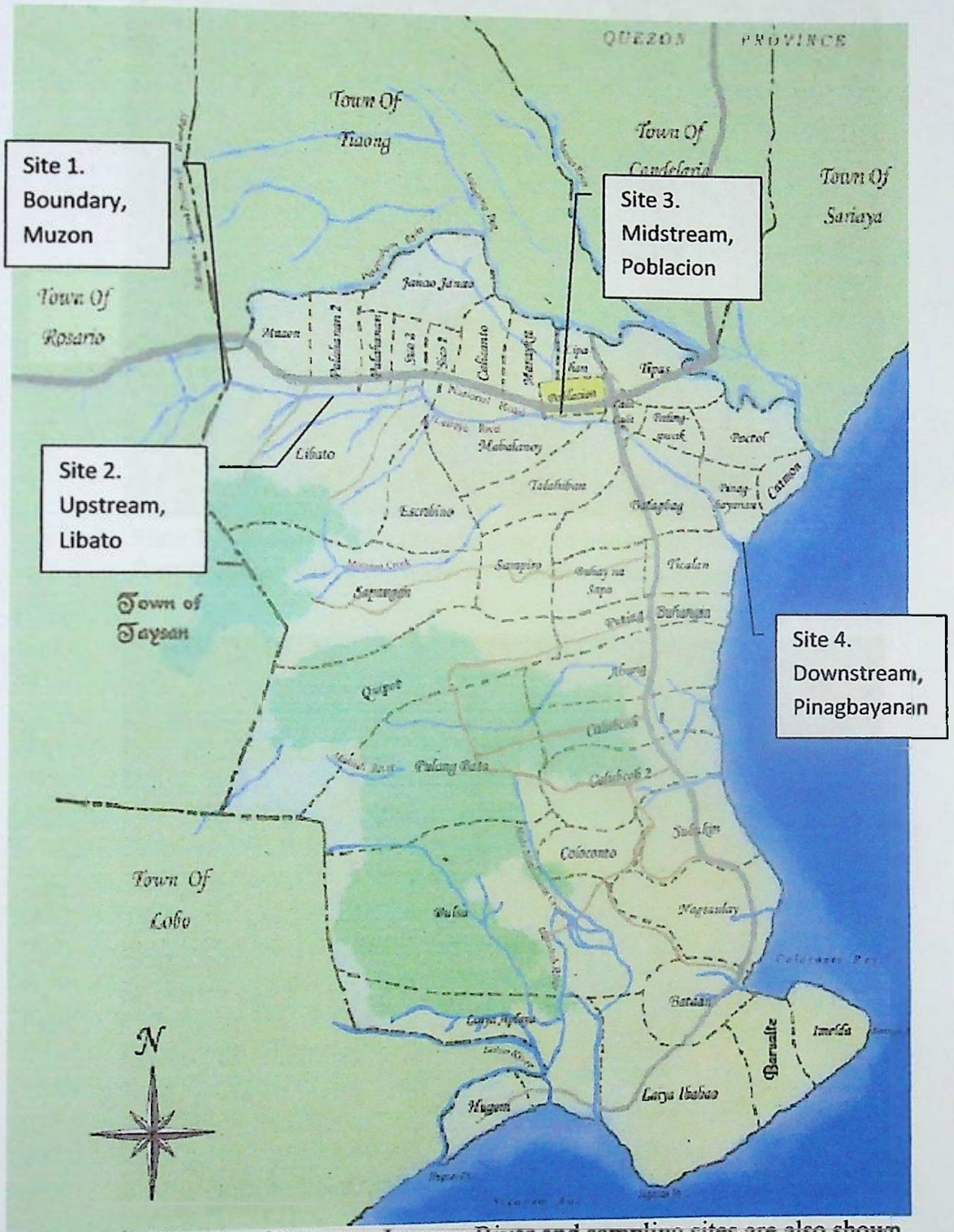

Plate 1. Map of San Juan Batangas. Lawaye River and sampling sites are also shown

Plate 2. Boundary collection site near the borders of San Juan and Rosario, Batangas. Two women are seen washing their clothes.

Plate 3. Boundary collection site. Motorized pumps used to pump water to piggeries near the river

Plate 4. Boundary collection site. Local houses that are found along the river.

Plate 5. Boundary collection site. Piggeries that are found near the river.

Plate 6. Boundary collection site. Algal bloom found in the river.

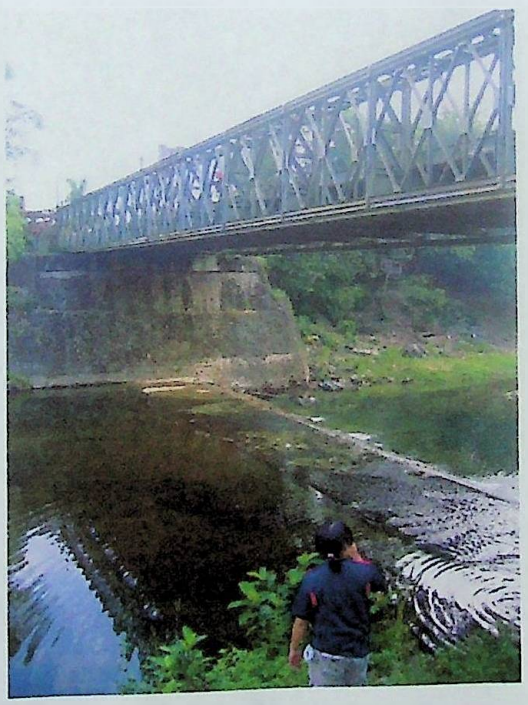

Plate 7. Upstream collection site found underneath a bridge.

Plate 8. Upstream collection site. Local children swimming in the river.

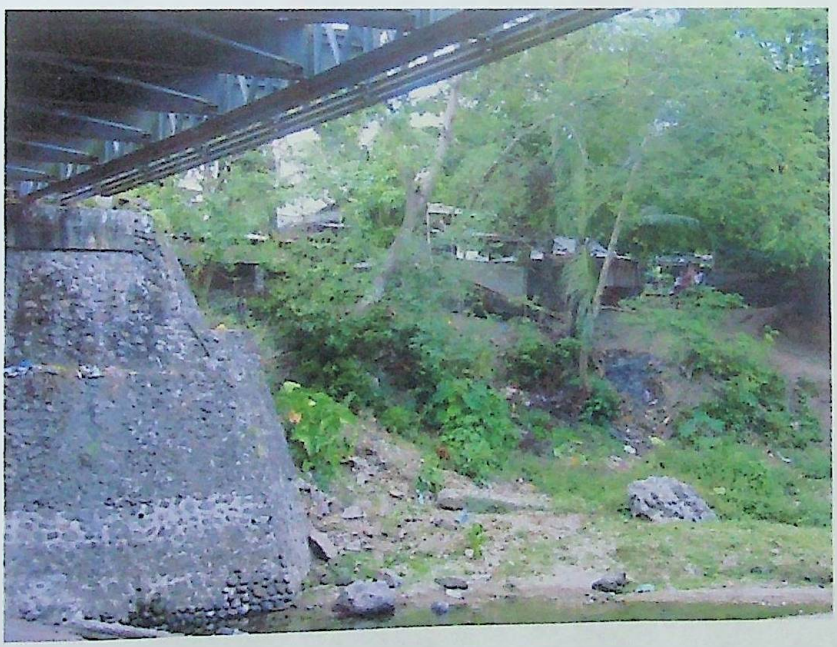

Plate 9. Upstream collection site. Local houses found along the river.

Plate 10. Upstream collection site. Locals washing their clothes in the river

Plate 11. Upstream collection site. Wastes and garbage found in the river.

Plate 12. Midstream collection site near the San Juan Public Market in Poblacion, San Juan, Batangas. Drainage pipes are found on the market's wall facing the river.

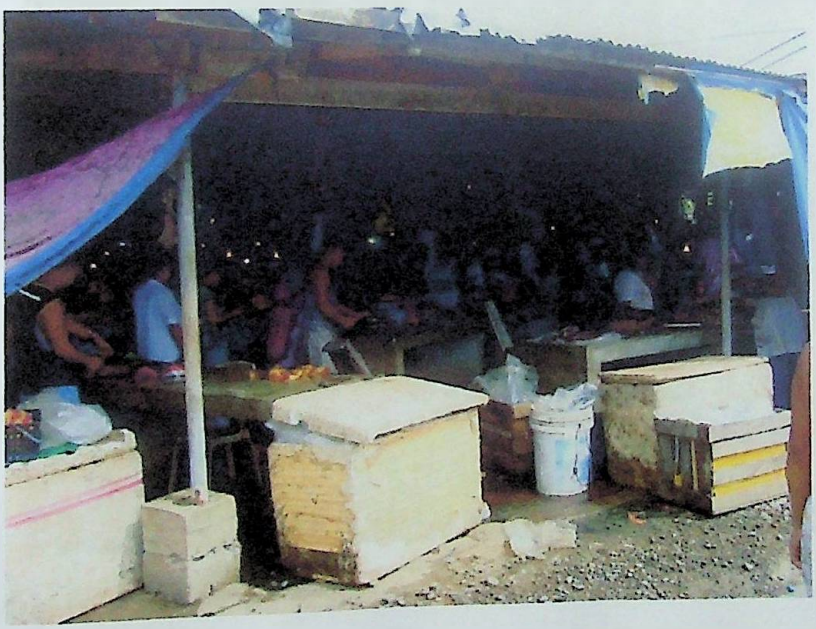

Plate 13. Midstream collection site. San Juan Public Market.

Plate 14. Midstream collection site. Drainage pipes lining the market's wall.

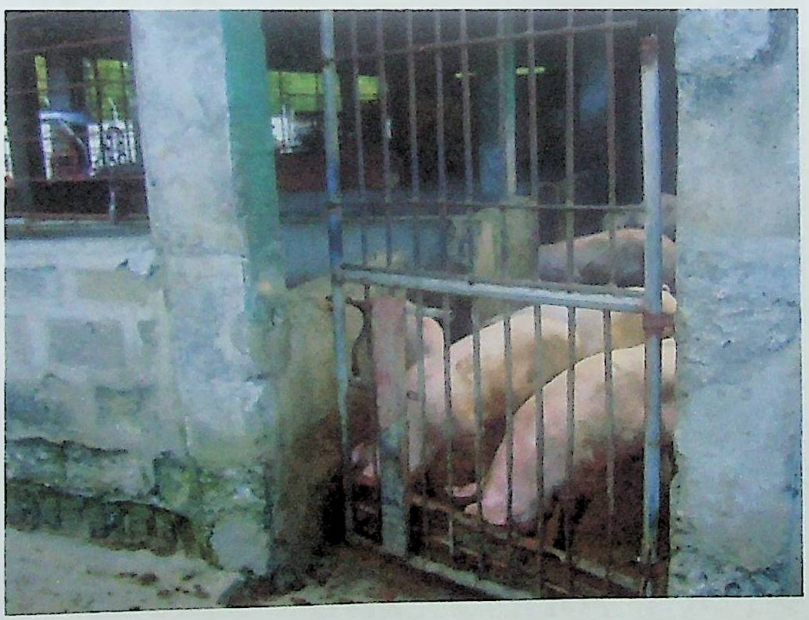

Plate 15. Midstream collection site. Piggery and slaughterhouse found in the marketplace.

Plate 16. Midstream collection site. Wastes and garbage found in the river and riverbank.

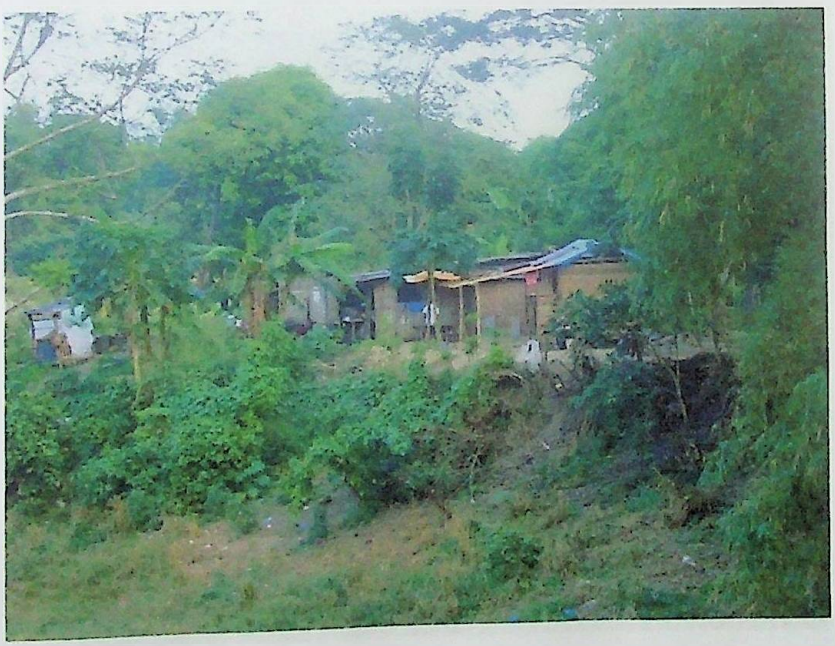

Plate 17. Midstream collection site. Local houses found near the river.

Plate 18. Downstream collection site at the Pinagbayanan mini dam.

Plate 19. Downstream collection site. Rice fields and irrigation canals near the dam.

Plate 20. Dowstream collection site. Algal bloom found in the river.

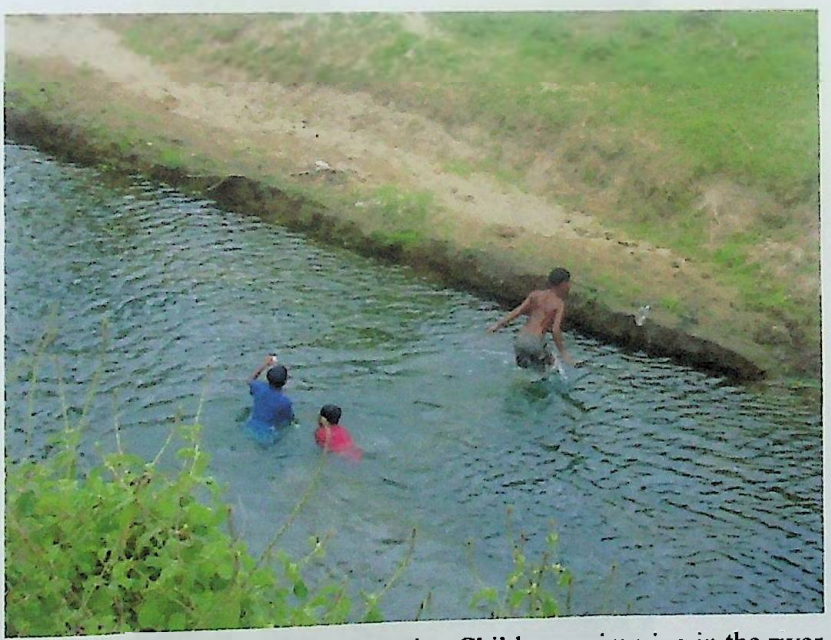

Plate 21. Downstream collection site. Children swimming in the river.

Plate 22. Downstream collection site. Cows grazing on the grass field near the river.

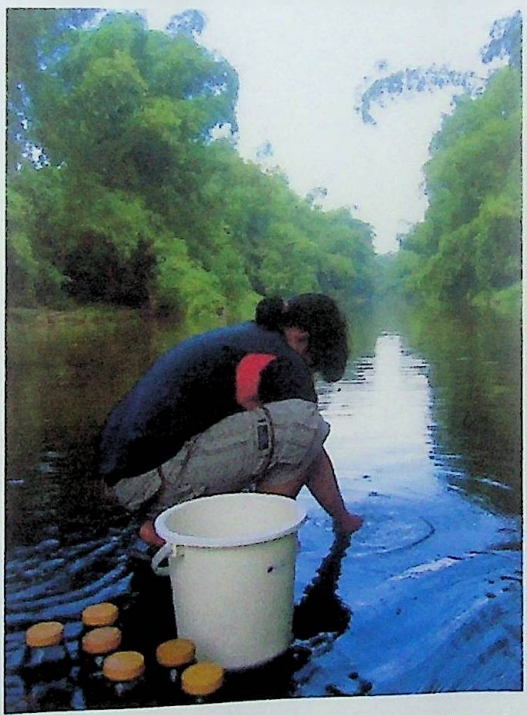

Plate 23. Water collection in the sampling sites.

Plate 24. Plankton collection at the sampling sites

Microbial Analysis Plates

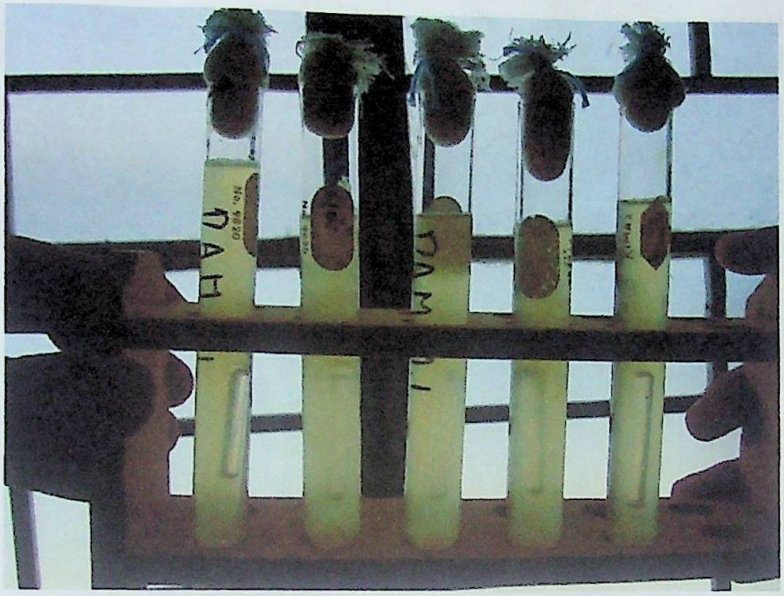

Plate 25. LSTB Tubes for Downstream site, Dilution 1

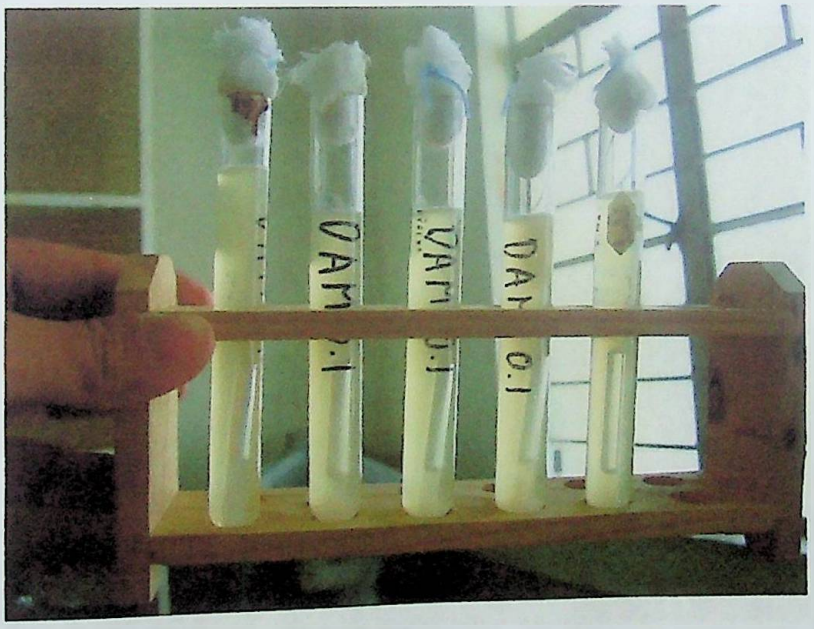

Plate 26. LSTB Tubes for Downstream site, Dilution 0.1

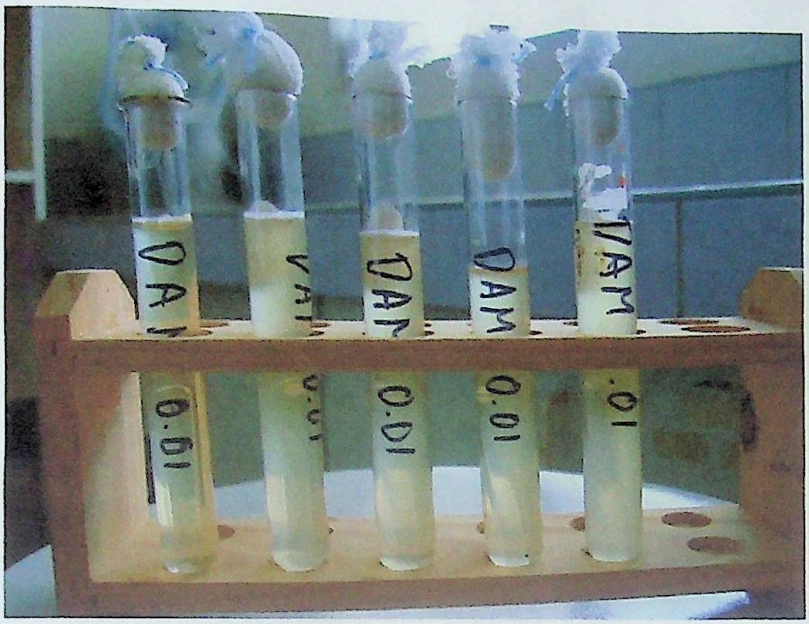

Plate 27. LSTB Tubes for Downstream site, Dilution 0.01

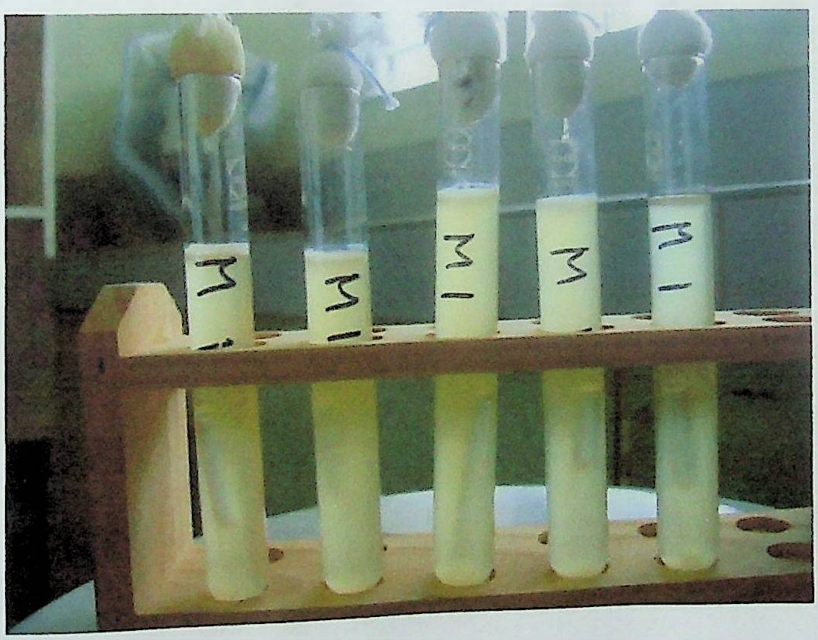

Plate 28. LSTB Tubes for Midstream site, Dilution 1

Plate 29. LSTB Tubes for Midstream site, Dilution 0.1

Plate 30. LSTB Tubes for Midstream site, Dilution 0.01

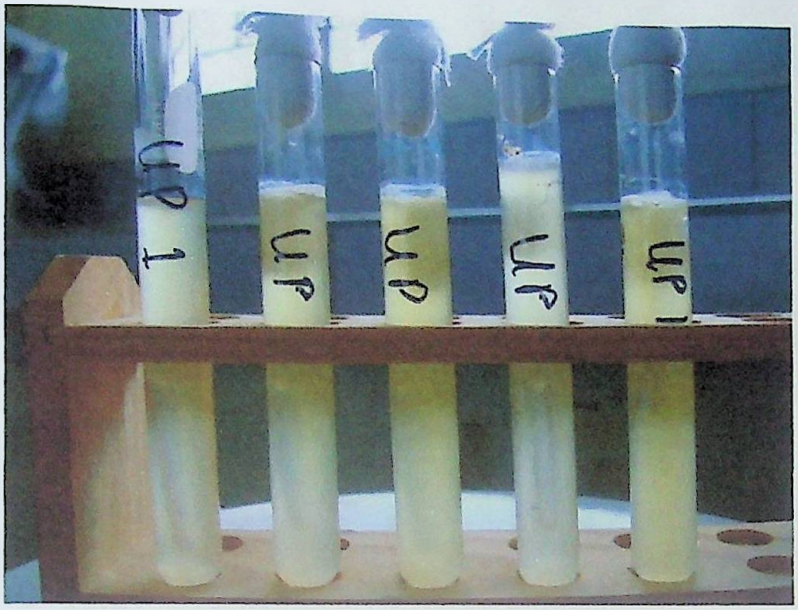

Plate 31. LSTB Tubes for Upstream site, Dilution 1

Plate 32. LSTB Tubes for Upstream site, Dilution 0.1

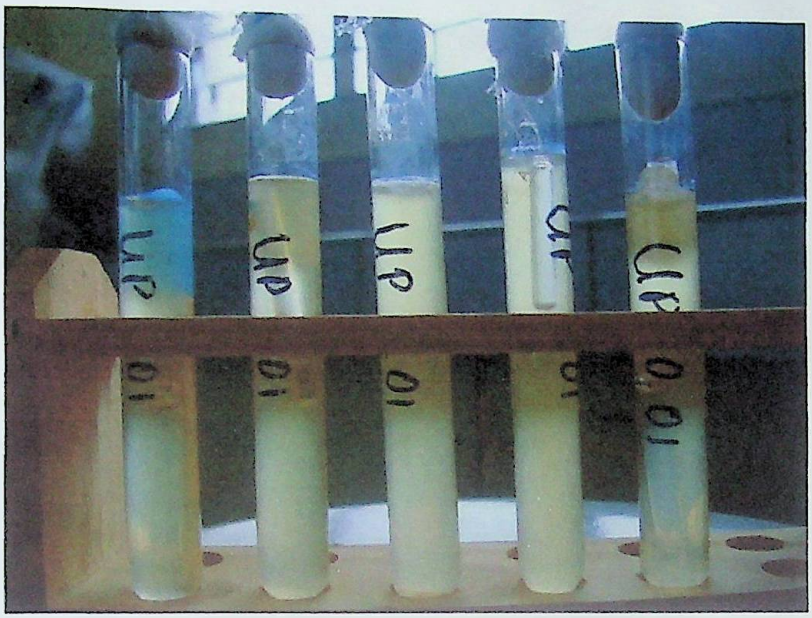

Plate 33. LSTB Tubes for Upstream site, Dilution 0.01

Plate 34. LSTB Tubes for Boundary site, Dilution 1

Plate 35. LSTB Tubes for Boundary site, Dilution 0.1

Plate 36. LSTB Tubes for Boundary site, Dilution 0.01

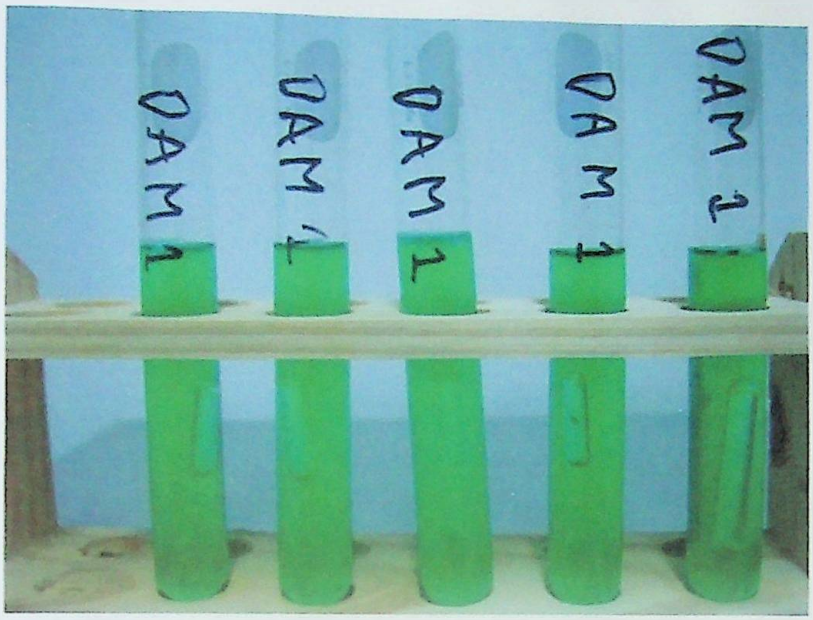

Plate 37. BGLB Tubes for Downstream site, Dilution 1

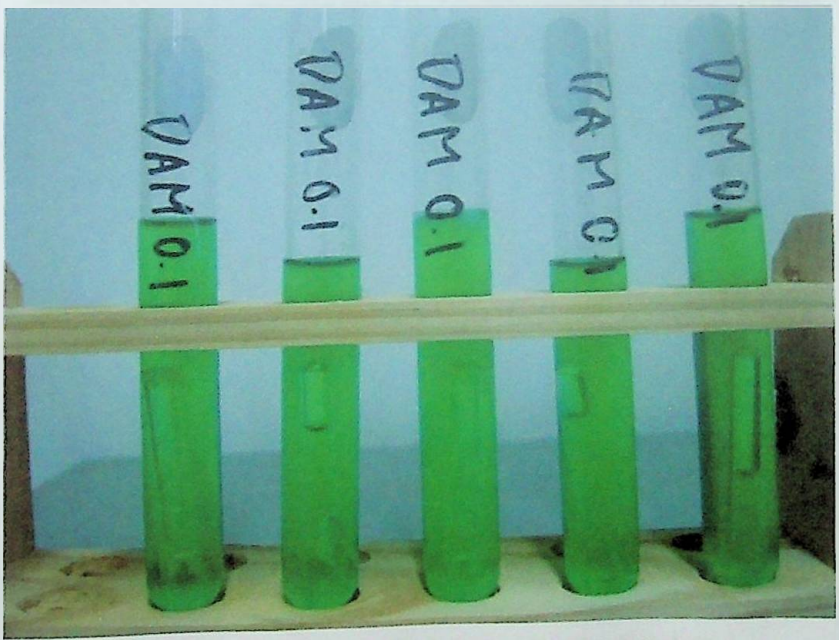

Plate 38. BGLB Tubes for Downstream site, Dilution 0.1

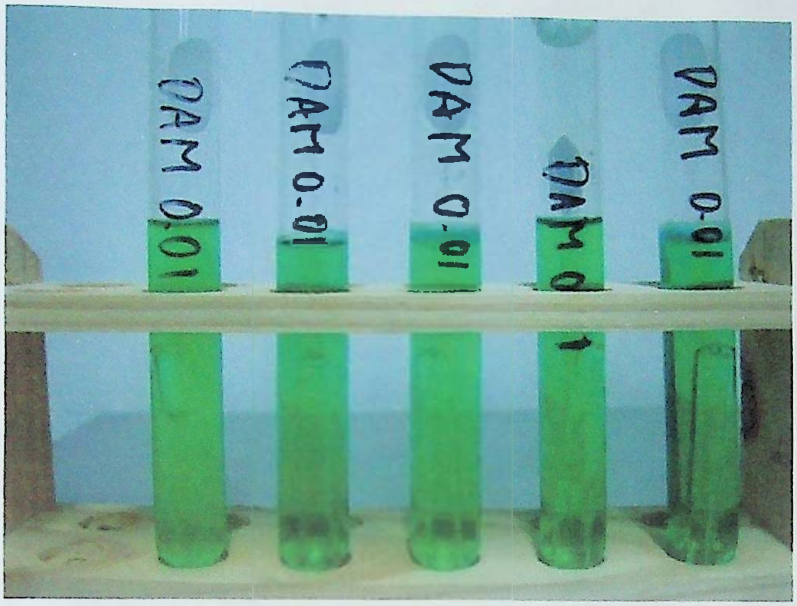

Plate 39. BGLB Tubes for Downstream site, Dilution 0.01

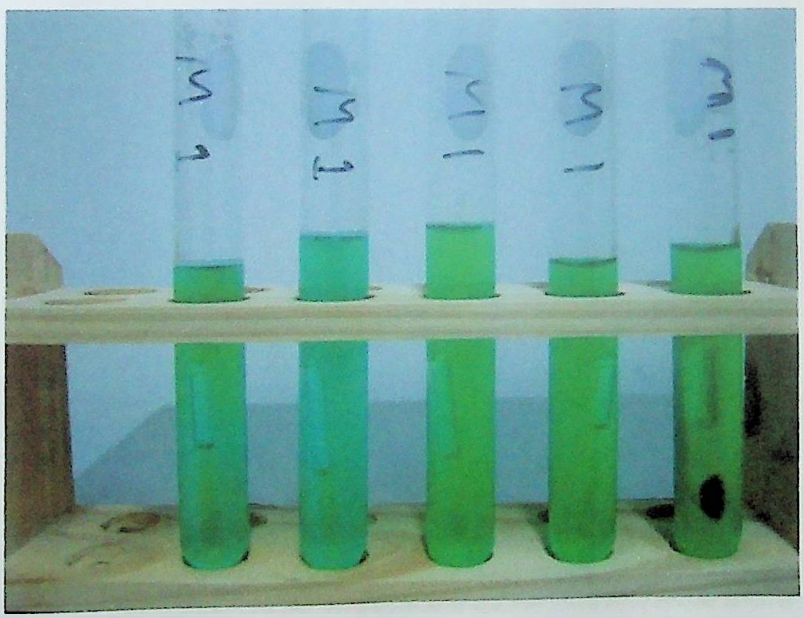

Plate 40. BGLB Tubes for Midstream site, Dilution 1

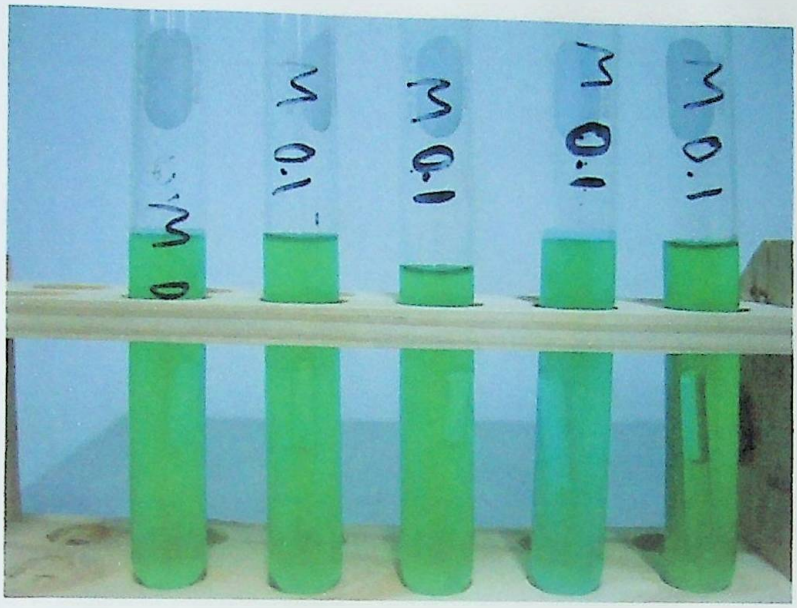

Plate 41. BGLB Tubes for Midstream site, Dilution 0.1

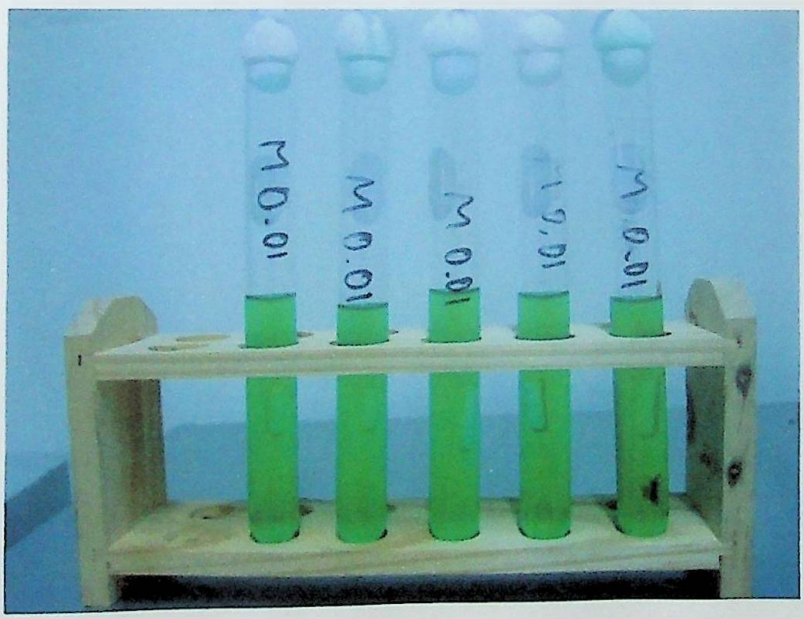

Plate 42. BGLB Tubes for Midstream site, Dilution 0.01

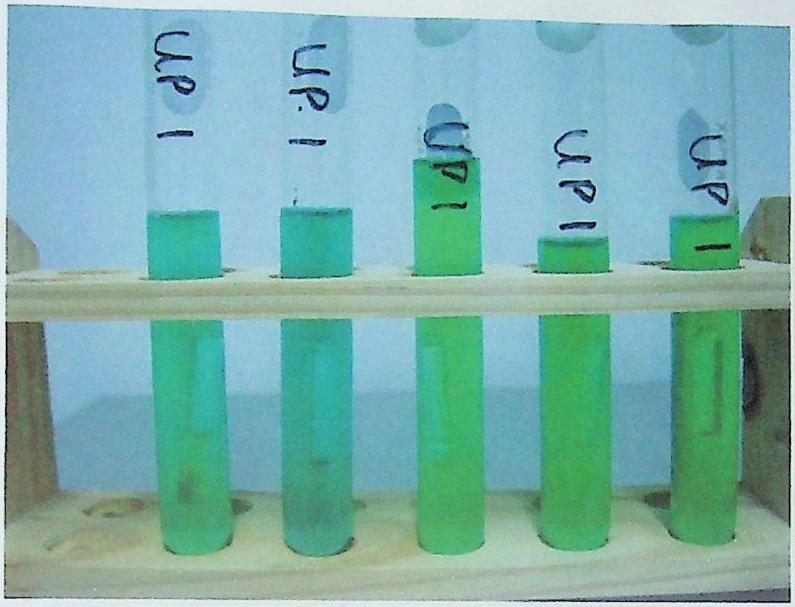

Plate 43. BGLB Tubes for Upstream site, Dilution 1

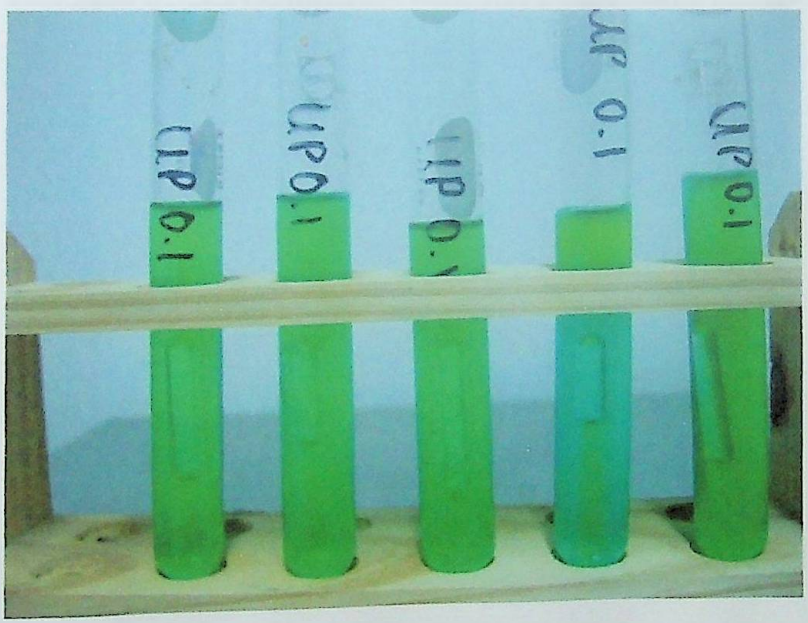

Plate 44. BGLB Tubes for Upstream site, Dilution 0.1

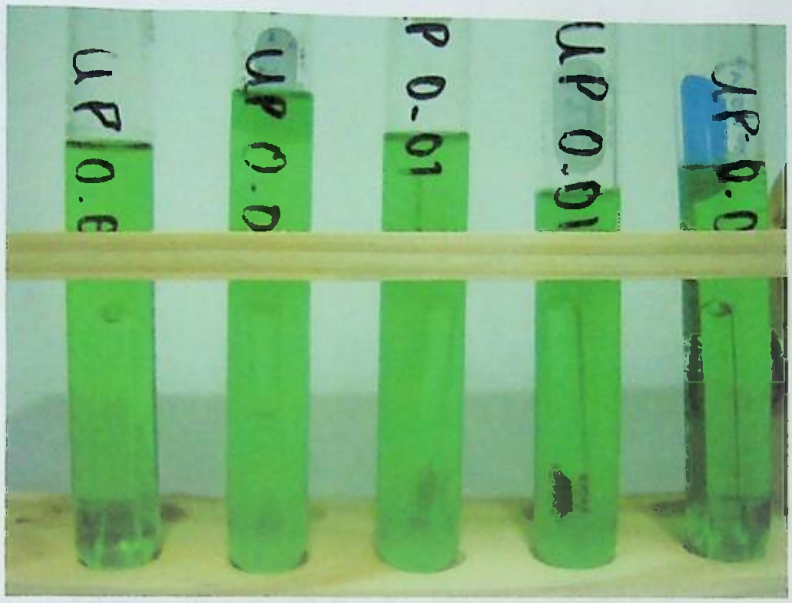

Plate 45. BGLB Tubes for Upstream site, Dilution 0.01

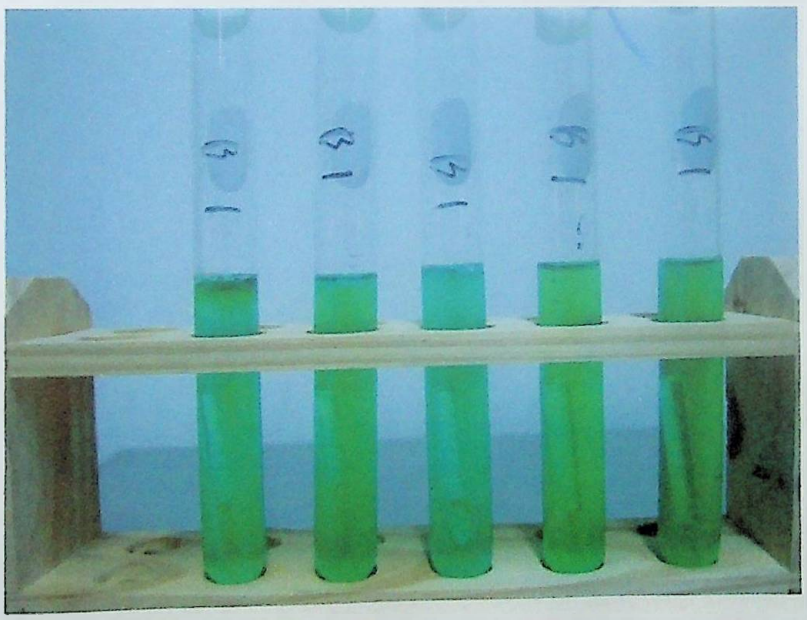

Plate 46. BGLB Tubes for Boundary site, Dilution 1

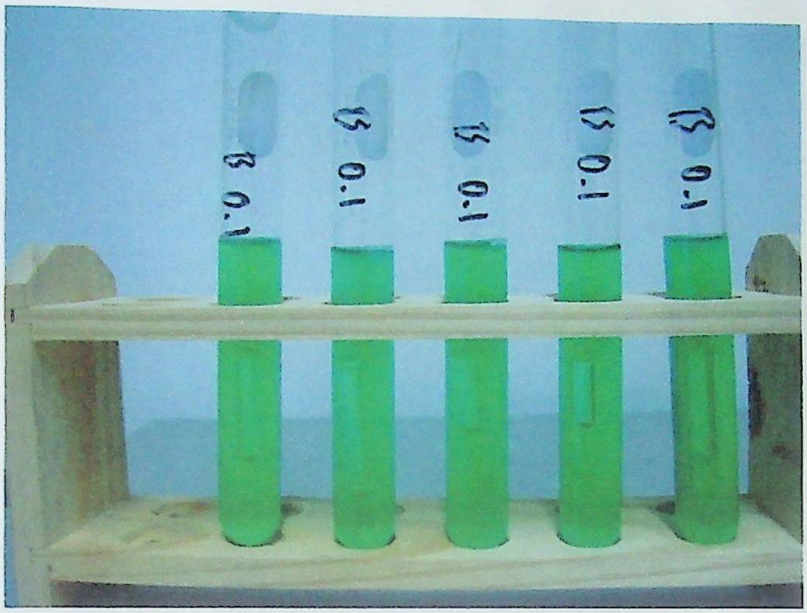

Plate 47. BGLB Tubes for Boundary site, Dilution 0.1

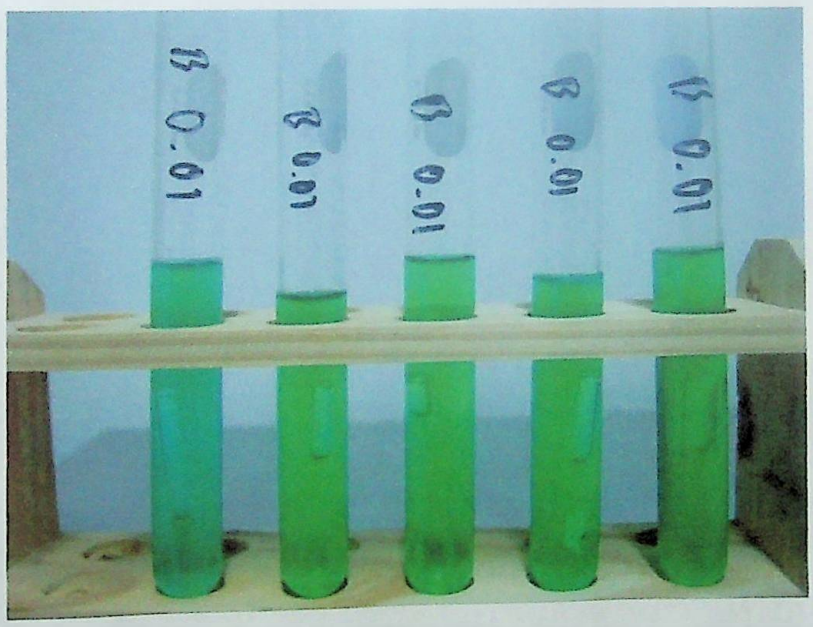

Plate 48. BGLB Tubes for Boundary site, Dilution 0.01

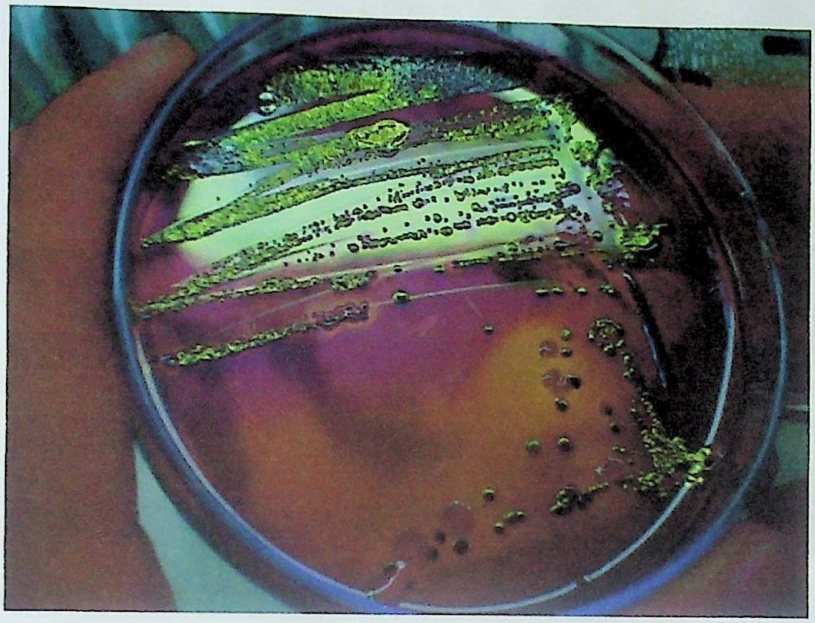

Plate 49 . Positive EMBA Plate Showing Metallic Green Colonies

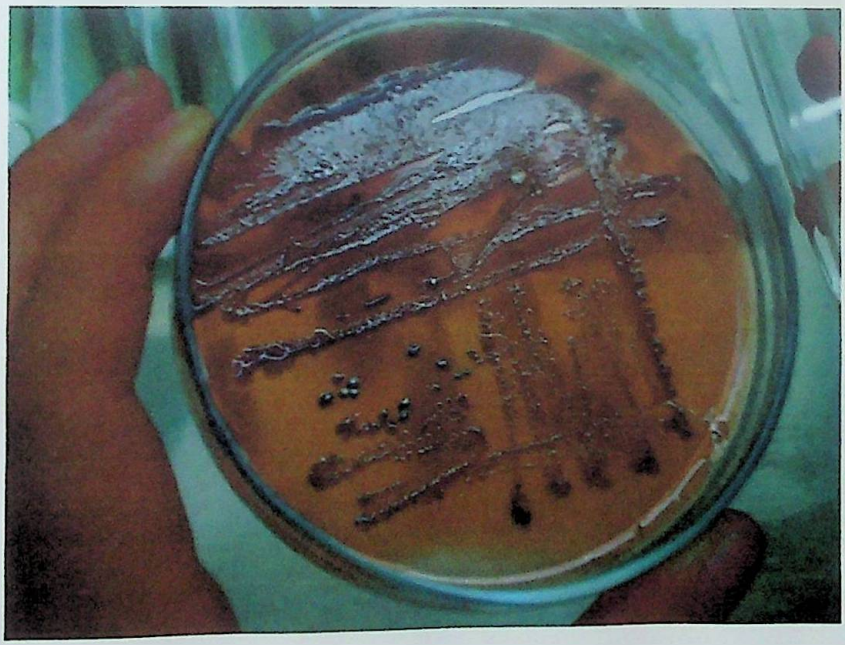

Plate 50. Negative EMBA Plate, Absence of Metallic Green Colonies

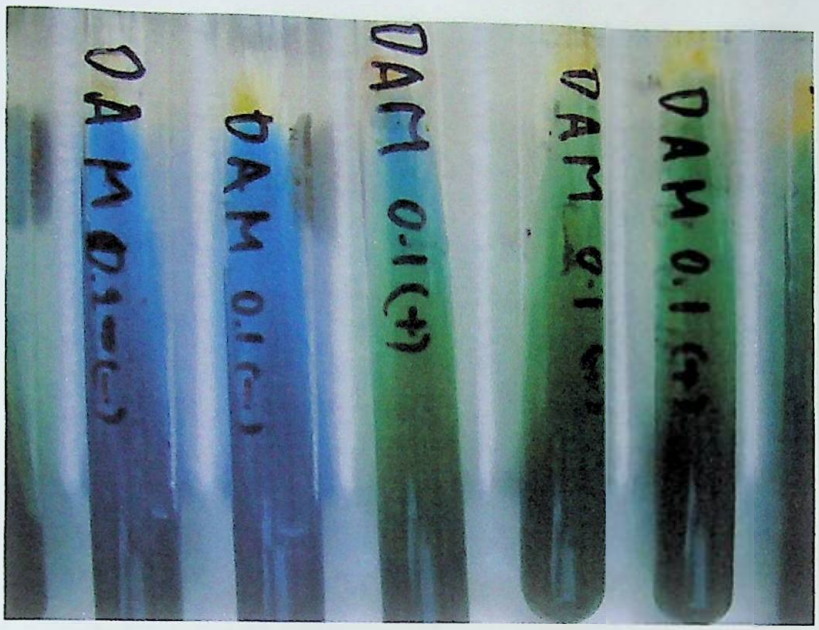

Plate 51. Simmon's Citrate Agar Slants. Leftmost is Positive, Rightmost is Negative

Plate 52. Indole test. Leftmost is negative. 2nd from the right to Rightmost is Postive

Plate 53. Methyl Red Test. Leftmost is Positive, Rightmost is Negative

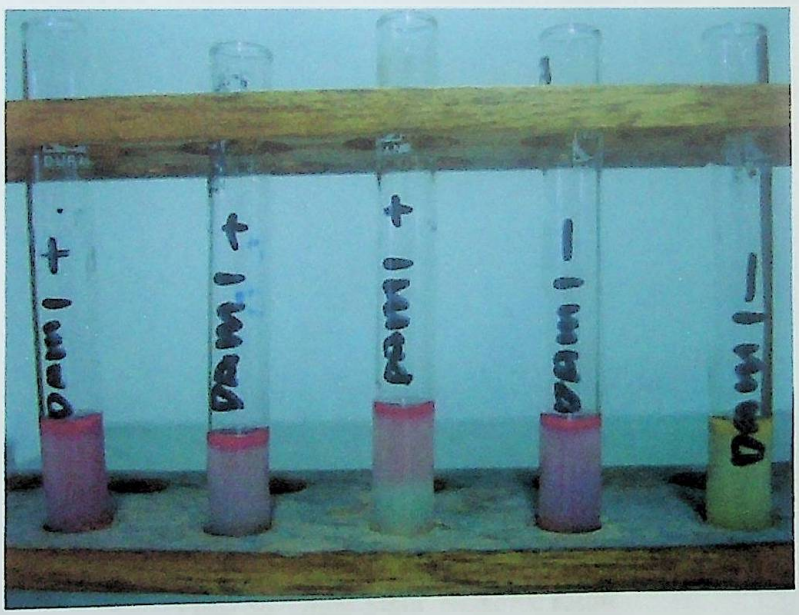

Plate 54. Voges-Proskauer Test. Leftmost is Positive, Rightmost is Negative

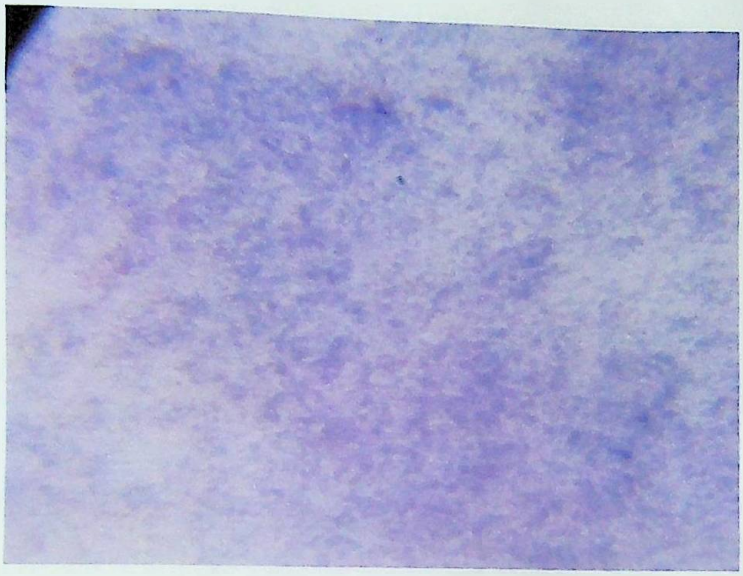

Plate 55. Gram Stain. Positive result

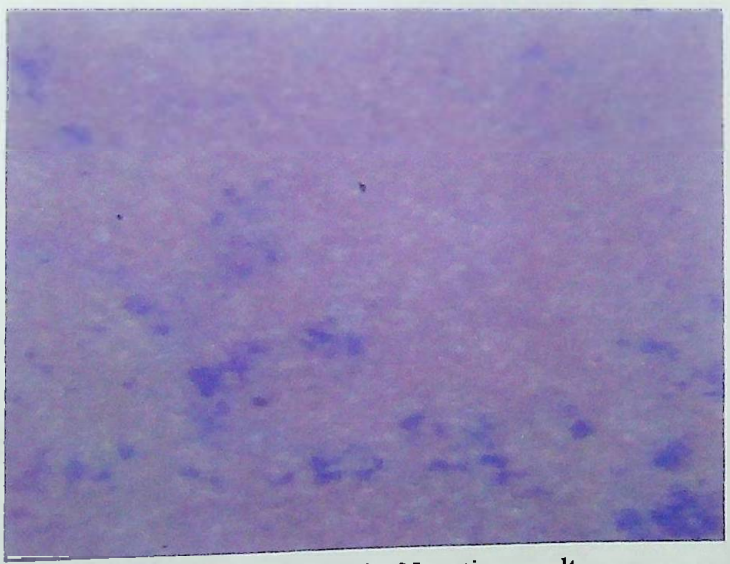

Plate 56. Gram Stain. Negative result

Plankton plates

Plate 57. *Ankistrodesmus*

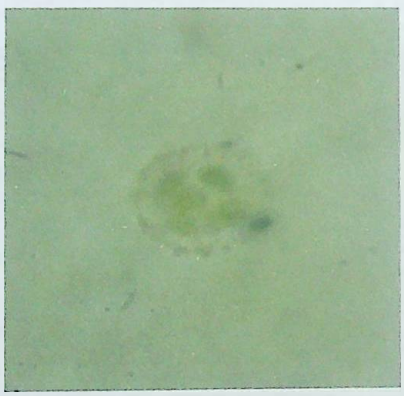

Plate 58. *Asterococcus*

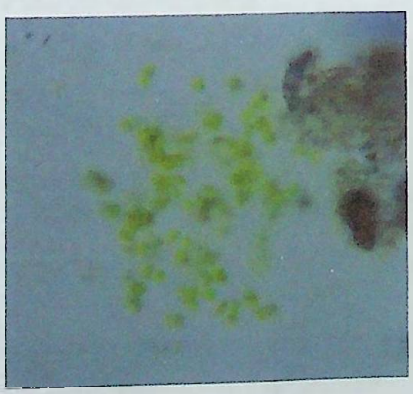

Plate 59. *Chlorella*

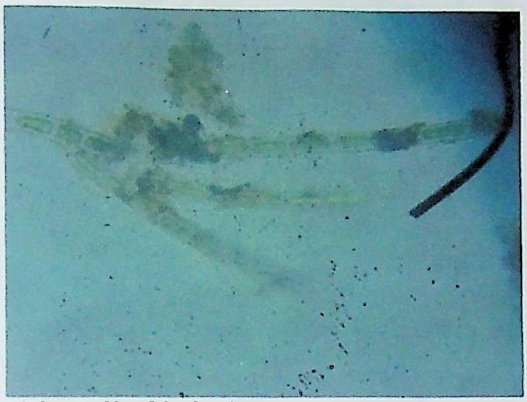

Plate 60. *Cladophora*

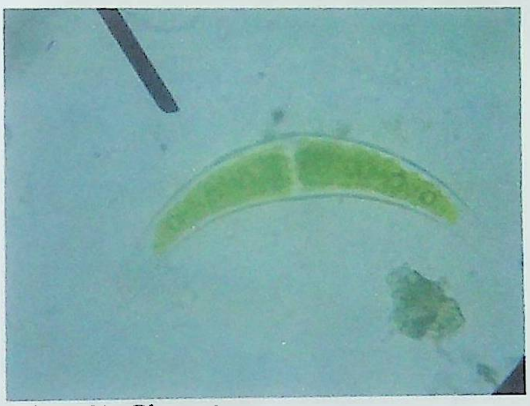

Plate 61. *Closterium*

Plate 62. *Cosmarium*

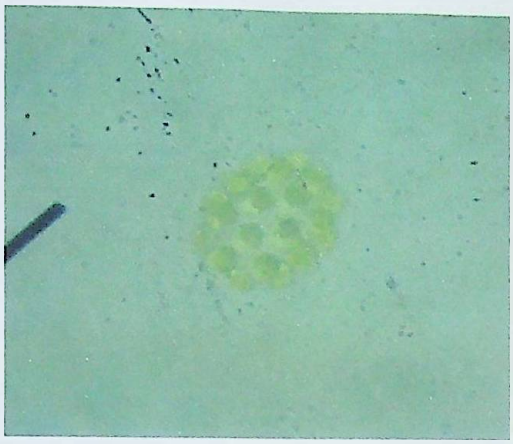

Plate 63. *Eudorina*

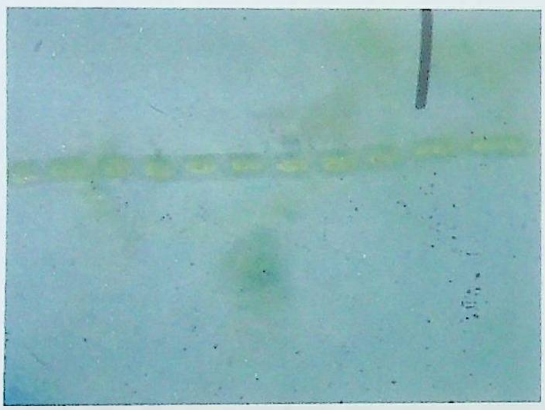

Plate 64. *Microspora*

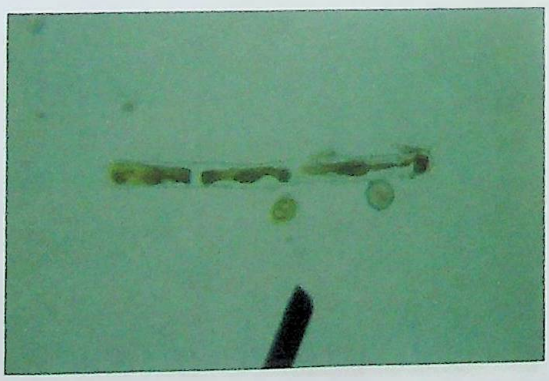

Plate 65. *Mougeotia*

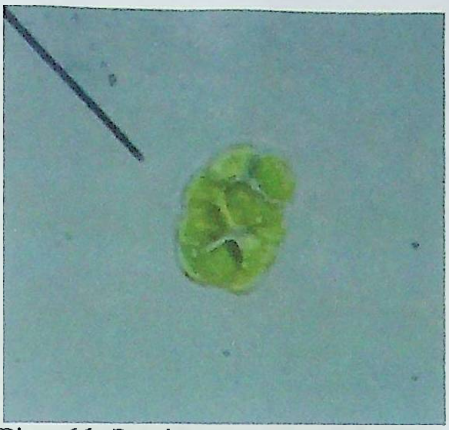

Plate 66. *Pandorina*

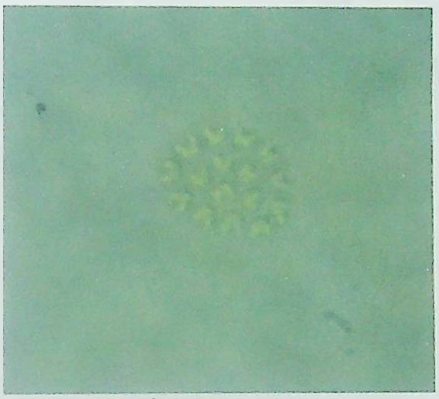

Plate 67. *Pediastrum*

Plate 68. *Spirogyra*

Plate 69. *Ulothrix*

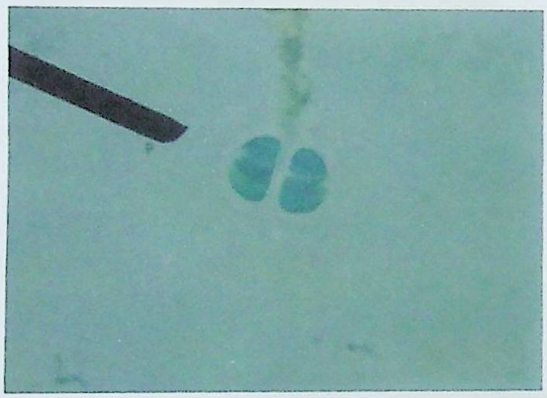

Plate 70. *Chroococcus*

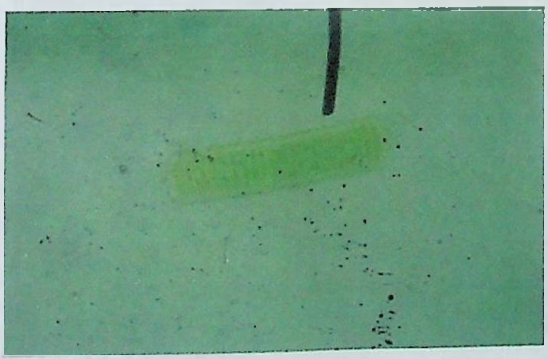

Plate 71. *Oscillatoria*

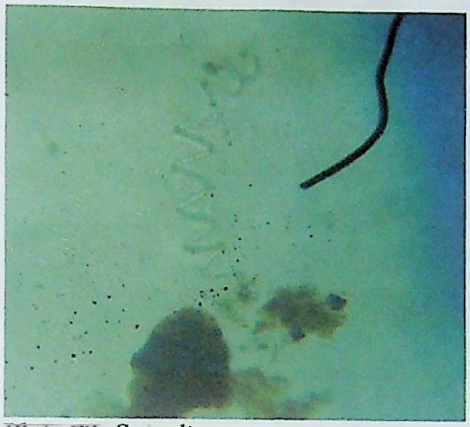

Plate 72. *Spirulina*

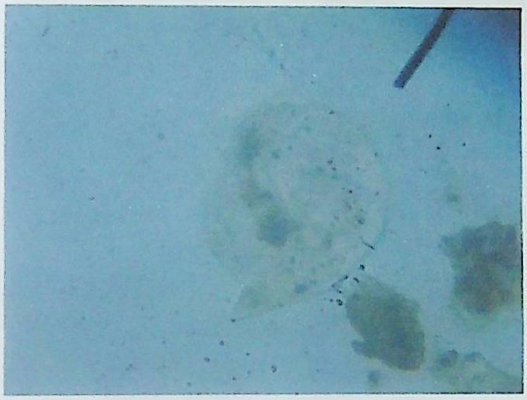

Plate 73. *Ceratium*

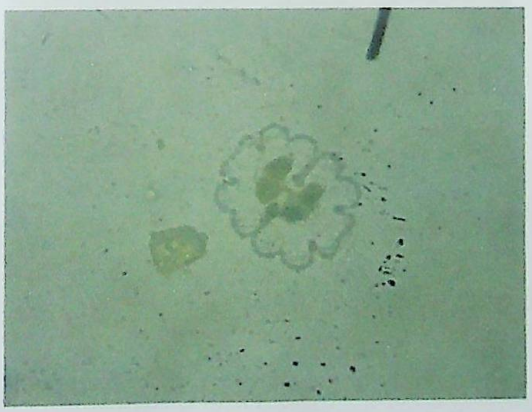

Plate 74. *Euastrum*

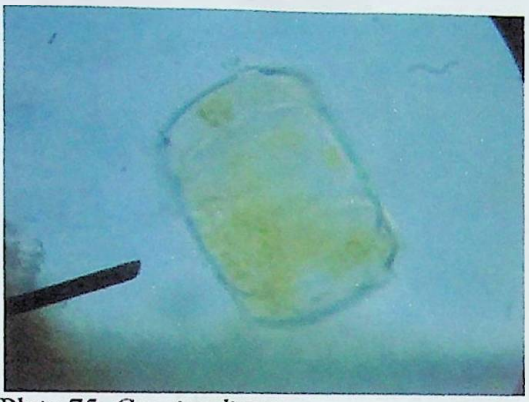

Plate 75. *Coscinodiscus*

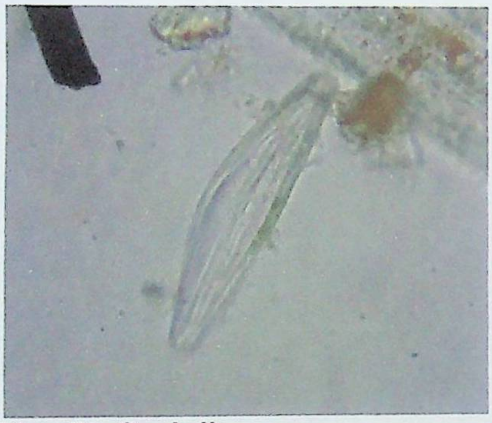

Plate 76. *Cymbella*

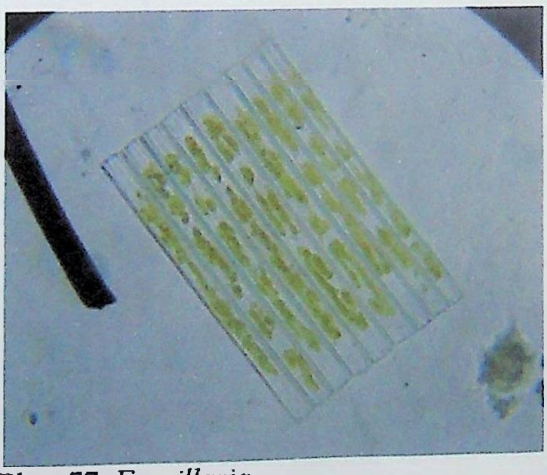

Plate 77. *Fragillaria*

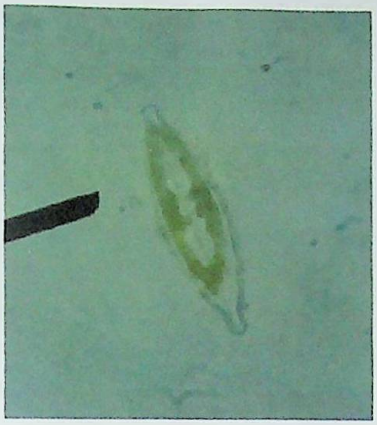

Plate 78. *Navicula*

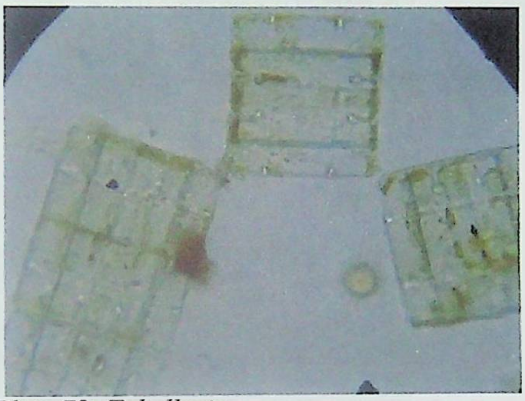

Plate 79. *Tabellaria*

Plate 80. *Synedra*

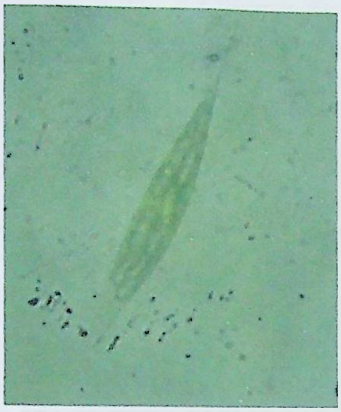

Plate 81. *Euglena*

Plate 84. *Chaoborus*

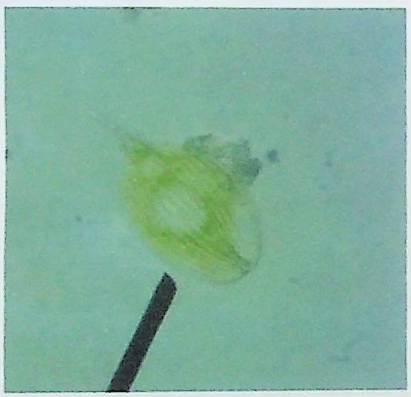

Plate 82. *Phacus*

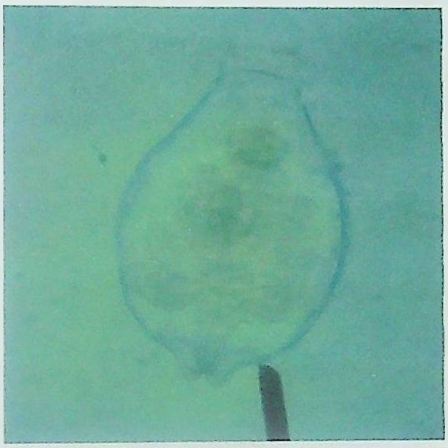

Plate 85. *Asplanchna*

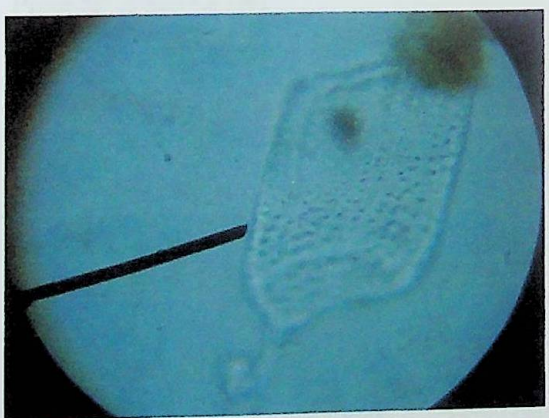

Plate 83. *Tetraediella*

Plate 86. *Monostyla*

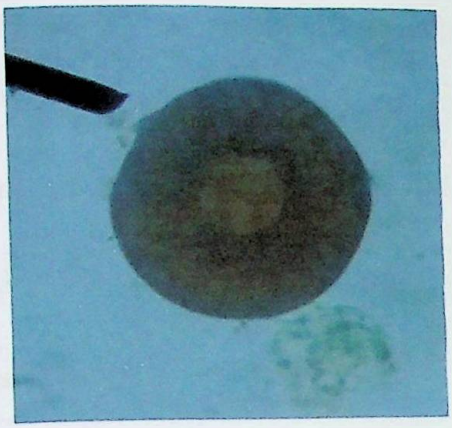

Plate 87. *Arcella*

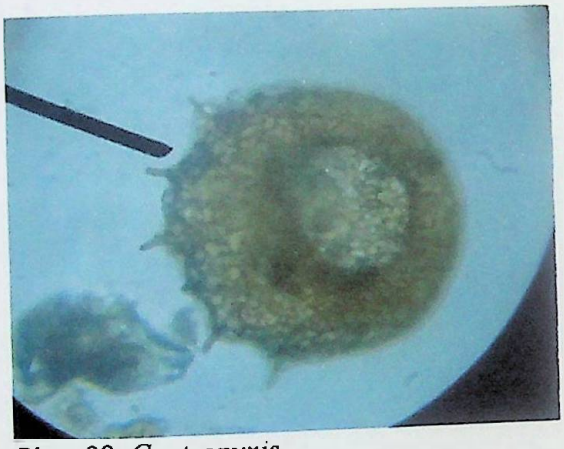

Plate 88. *Centropyxis*

APPENDIX A. Productivity Calculation (Hallare, 2006)

Initial bottle = Dissolved oxygen in Initial bottle

Dark bottle = Dissolved oxygen in Dark bottle

Light bottle = Dissolved oxygen in Light bottle

Respiration = Initial bottle – Dark bottle

Gross Primary Production (GPP) = Light bottle – Dark bottle

Net Primary Production (NPP) = GPP – Respiration

APPENDIX B – Most Probable Number Table

Positive Tubes			Mpn	Positive Tubes			Mpn	Positive Tubes			Mpn
10ml	1ml	0.1ml		10ml	1ml	0.1ml		10ml	1ml	0.1ml	
0	0	0	<1.8	1	0	0	2	2	0	0	4.5
0	0	1	1.8	1	0	1	4	2	0	1	6.8
0	0	2	3.6	1	0	2	6	2	0	2	9.1
0	0	3	5.4	1	0	3	8	2	0	3	12
0	0	4	7.2	1	0	4	10	2	0	4	14
0	0	5	9	1	0	5	12	2	0	5	16
0	1	0	1.8	1	1	0	4	2	1	0	6.8
0	1	1	3.6	1	1	1	6.1	2	1	1	9.2
0	1	2	5.5	1	1	2	8.1	2	1	2	12
0	1	3	7.3	1	1	3	10	2	1	3	14
0	1	4	9.1	1	1	4	12	2	1	4	17
0	1	5	11	1	1	5	14	2	1	5	19
0	2	0	3.7	1	2	0	6.1	2	2	0	9.3
0	2	1	5.5	1	2	1	8.2	2	2	1	12
0	2	2	7.4	1	2	2	10	2	2	2	14
0	2	3	9.2	1	2	3	12	2	2	3	17
0	2	4	11	1	2	4	15	2	2	4	19
0	2	5	13	1	2	5	17	2	2	5	22
0	3	0	5.6	1	3	0	8.3	2	3	0	12
0	3	1	7.4	1	3	1	10	2	3	1	14
0	3	2	9.3	1	3	2	13	2	3	2	17
0	3	3	11	1	3	3	15	2	3	3	20
0	3	4	13	1	3	4	17	2	3	4	22
0	3	5	15	1	3	5	19	2	3	5	25
0	4	0	7.5	1	4	0	11	2	4	0	15
0	4	1	9.4	1	4	1	13	2	4	1	17
0	4	2	11	1	4	2	15	2	4	2	20
0	4	3	13	1	4	3	17	2	4	3	23
0	4	4	15	1	4	4	19	2	4	4	25
0	4	5	17	1	4	5	22	2	4	5	28
0	5	0	9.4	1	5	0	13	2	5	0	17
0	5	1	11	1	5	1	15	2	5	1	20
0	5	2	13	1	5	2	17	2	5	2	17
0	5	3	15	1	5	3	19	2	5	3	26
0	5	4	17	1	5	4	22	2	5	4	29
0	5	5	19	1	5	5	24	2	5	5	32

Positive Tubes			Mpn	Positive Tubes			Mpn	Positive Tubes			Mpn
10ml	1ml	0.1ml		10ml	1ml	0.1ml		10ml	1ml	0.1ml	
3	0	0	7.8	4	0	0	13	5	0	0	23
3	0	1	11	4	0	1	17	5	0	1	31
3	0	2	13	4	0	2	21	5	0	2	43
3	0	3	16	4	0	3	25	5	0	3	58
3	0	4	20	4	0	4	30	5	0	4	76
3	0	5	23	4	0	5	36	5	0	5	95
3	1	0	11	4	1	0	17	5	1	0	33
3	1	1	14	4	1	1	21	5	1	1	46
3	1	2	17	4	1	2	26	5	1	2	64
3	1	3	20	4	1	3	31	5	1	3	84
3	1	4	23	4	1	4	36	5	1	4	110
3	1	5	27	4	1	5	42	5	1	5	130
3	2	0	14	4	2	0	22	5	2	0	49
3	2	1	17	4	2	1	26	5	2	1	70
3	2	2	20	4	2	2	32	5	2	2	95
3	2	3	24	4	2	3	38	5	2	3	120
3	2	4	27	4	2	4	44	5	2	4	150
3	2	5	31	4	2	5	50	5	2	5	180
3	3	0	17	4	3	0	27	5	3	0	79
3	3	1	21	4	3	1	33	5	3	1	110
3	3	2	24	4	3	2	39	5	3	2	140

3	3	3	28	4	3	3	45	5	3	3	180
3	3	4	31	4	3	4	52	5	3	4	210
3	3	5	35	4	3	5	59	5	3	5	250
3	4	0	21	4	4	0	34	5	4	0	130
3	4	1	24	4	4	1	40	5	4	1	170
3	4	2	28	4	4	2	47	5	4	2	220
3	4	3	32	4	4	3	54	5	4	3	280
3	4	4	36	4	4	4	62	5	4	4	350
3	4	5	40	4	4	5	69	5	4	5	440
3	5	0	25	4	5	0	41	5	5	0	240
3	5	1	29	4	5	1	48	5	5	1	350
3	5	2	32	4	5	2	56	5	5	2	540
3	5	3	37	4	5	3	64	5	5	3	920
3	5	4	41	4	5	4	72	5	5	4	1600
3	5	5	45	4	5	5	81	5	5	5	>1600

Positive Tubes			Mpn	Positive Tubes			Mpn	Positive Tubes			Mpn
10ml	1ml	0.1ml		10ml	1ml	0.1ml		10ml	1ml	0.1ml	
0	0	0	<1.8	1	0	0	2	2	0	0	4.5
0	0	1	1.8	1	0	1	4	2	0	1	6.8
0	0	2	3.6	1	0	2	6	2	0	2	9.1
0	0	3	5.4	1	0	3	8	2	0	3	12
0	0	4	7.2	1	0	4	10	2	0	4	14
0	0	5	9	1	0	5	12	2	0	5	16
0	1	0	1.8	1	1	0	4	2	1	0	6.8
0	1	1	3.6	1	1	1	6.1	2	1	1	9.2
0	1	2	5.5	1	1	2	8.1	2	1	2	12
0	1	3	7.3	1	1	3	10	2	1	3	14
0	1	4	9.1	1	1	4	12	2	1	4	17
0	1	5	11	1	1	5	14	2	1	5	19

0	2	0	3.7	1	2	0	6.1	2	2	0	9.3
0	2	1	5.5	1	2	1	8.2	2	2	1	12
0	2	2	7.4	1	2	2	10	2	2	2	14
0	2	3	9.2	1	2	3	12	2	2	3	17
0	2	4	11	1	2	4	15	2	2	4	19
0	2	5	13	1	2	5	17	2	2	5	22
0	3	0	5.6	1	3	0	8.3	2	3	0	12
0	3	1	7.4	1	3	1	10	2	3	1	14
0	3	2	9.3	1	3	2	13	2	3	2	17
0	3	3	11	1	3	3	15	2	3	3	20
0	3	4	13	1	3	4	17	2	3	4	22
0	3	5	15	1	3	5	19	2	3	5	25
0	4	0	7.5	1	4	0	11	2	4	0	15
0	4	1	9.4	1	4	1	13	2	4	1	17
0	4	2	11	1	4	2	15	2	4	2	20
0	4	3	13	1	4	3	17	2	4	3	23
0	4	4	15	1	4	4	19	2	4	4	25
0	4	5	17	1	4	5	22	2	4	5	28
0	5	0	9.4	1	5	0	13	2	5	0	17
0	5	1	11	1	5	1	15	2	5	1	20
0	5	2	13	1	5	2	17	2	5	2	17
0	5	3	15	1	5	3	19	2	5	3	26
0	5	4	17	1	5	4	22	2	5	4	29
0	5	5	19	1	5	5	24	2	5	5	32

Positive Tubes			Mpn	Positive Tubes			Mpn	Positive Tubes			Mpn
10ml	1ml	0.1ml		10ml	1ml	0.1ml		10ml	1ml	0.1ml	
3	0	0	7.8	4	0	0	13	5	0	0	23
3	0	1	11	4	0	1	17	5	0	1	31
3	0	2	13	4	0	2	21	5	0	2	43
3	0	3	16	4	0	3	25	5	0	3	58
3	0	4	20	4	0	4	30	5	0	4	76
3	0	5	23	4	0	5	36	5	0	5	95
3	1	0	11	4	1	0	17	5	1	0	33

3	1	1	14	4	1	1	21	5	1	1	46
3	1	2	17	4	1	2	26	5	1	2	64
3	1	3	20	4	1	3	31	5	1	3	84
3	1	4	23	4	1	4	36	5	1	4	110
3	1	5	27	4	1	5	42	5	1	5	130
3	2	0	14	4	2	0	22	5	2	0	49
3	2	1	17	4	2	1	26	5	2	1	70
3	2	2	20	4	2	2	32	5	2	2	95
3	2	3	24	4	2	3	38	5	2	3	120
3	2	4	27	4	2	4	44	5	2	4	150
3	2	5	31	4	2	5	50	5	2	5	180
3	3	0	17	4	3	0	27	5	3	0	79
3	3	1	21	4	3	1	33	5	3	1	110
3	3	2	24	4	3	2	39	5	3	2	140
3	3	3	28	4	3	3	45	5	3	3	180
3	3	4	31	4	3	4	52	5	3	4	210
3	3	5	35	4	3	5	59	5	3	5	250
3	4	0	21	4	4	0	34	5	4	0	130
3	4	1	24	4	4	1	40	5	4	1	170
3	4	2	28	4	4	2	47	5	4	2	220
3	4	3	32	4	4	3	54	5	4	3	280
3	4	4	36	4	4	4	62	5	4	4	350
3	4	5	40	4	4	5	69	5	4	5	440
3	5	0	25	4	5	0	41	5	5	0	240

3	5	1	29	4	5	1	48	5	5	1	350
3	5	2	32	4	5	2	56	5	5	2	540
3	5	3	37	4	5	3	64	5	5	3	920
3	5	4	41	4	5	4	72	5	5	4	1600
3	5	5	45	4	5	5	81	5	5	5	>1600