# Organic Matter and Benthic Fauna Analysis in a Pond Based Multi Trophic Aquaculture System



**A Thesis**

**By**

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Khulna-9208, Bangladesh.

**November, 2022**

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**November, 2022**

## Declaration

# Organic Matter and Benthic Fauna Analysis in a Pond Based Multi Trophic Aquaculture System

The results presented in the thesis with the above-mentioned title are entirely of the candidate's own investigations and no part of the results has been accepted for any degree nor is it being concurrently submitted for any degree.

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**Dedicated**

**to**

**My Beloved Family**

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## Abbreviations

- IMTA- Integrated Multi Trophic Aquaculture
- EC- Electrical Conductivity
- OC – Organic Carbon
- SQ- Soil Quality
- TN- Total Nitrogen
- SOM – Soil Organic Matter
- OM- Organic Matter
- sp. – Species
- DO- Dissolved Oxygen
- SRDI -Soil Resources Development Institute
- CEC - Cation Exchange Capacity
- *et al.*, – And others
- $T_1$ – Treatment 1; $T_2$ – Treatment 2; $T_3$ – Treatment 3; $T_4$ – Treatment 4

## List of symbols

| Symbol | Meaning |
|---|---|
| µg/g | Microgram per gram |
| % | Percent |
| $NH_3$ | Ammonia |
| $Ca^{++}$ | Calcium ion |
| $Mg^{++}$ | Magnesium ion |
| $Na^{+}$ | Sodium ion |
| $NH_4OH$ | Ammonium hydroxide |
| > | Greater than |
| < | Less than |
| OH | Hydroxyl |
| $H_2O$ | Water |
| $NH_4$ | Ammonium |
| $H_2PO_4$ | Dihydrogen phosphate |
| $HPO_4$ | Hydrogen phosphate |
| $Fe^{3+}$ | Ferric |
| $O_2$ | Oxygen |
| $m^{-2}$ | Per meter square |
| dS/m | deciSimmens per meter |

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## Abstract

The study was carried out to evaluate the variation of soil parameter and benthos diversity in pond based IMTA (Integrated Multi Trophic Aquaculture) system. An experiment was conducted in the research ponds of Fisheries and Marine Resource Technology Discipline, Khulna University from December 2021 to May 2022. Samplings were done at fortnightly intervals. There were four treatments and one control, each of which had three replications. The experiment was designed by incorporating rohu, mola, snail, aquatic vegetable (kolmi) along with main crop prawn and tested in different treatments viz C-only prawn, $T_1$-prawn+rohu, $T_2$-prawn+rohu+mola, $T_3$-prawn+rohu+mola+snail and $T_4$-prawn+rohu+mola+snail+aquatic vegetable (kolmi) with specific stocking densities like prawn at $2/m^2$, rohu at $0.1/m^2$, mola at $2/m^2$, snail at $0.25/m^2$. Same feed was applied in all four treatments and the control. The soil quality parameters of the experimental ponds were analyzed. The highest mean pH value was found to be 8.02 (C), whereas the lowest soil mean pH value 7.88 was found in $T_3$. The highest and the lowest mean electrical conductivity value were found to be 4.58 ($T_3$) and 4.22 ($T_1$), followed by organic matter 3.19 ($T_3$) and 2.93 ($T_4$), total nitrogen 0.18 (C) and 0.16 ($T_3$, $T_4$), phosphorus 10.38 ($T_3$) and 8.53 ($T_1$) respectively during the study. In total 9 species of benthos belonging to 4 genera were identified and among them, *Melanoides* was the most dominant genera. Highest quantity of species was recorded in $T_4$ (*M. maculate*=83.50±15.61, *M. granifera*=56.50±8.71, *M. polymorpha*=69.50±7.41, *P. globosa*=8.33±3.86, *B. angularis*= 10.17±6.34, *Indoplanorbis exustus*=7.83±3.08) followed by $T_1$ (*M. tuberculate*=47.33±13.25) and in $T_2$ (*B.* javanica=16.17±6.52, *P. polita*=10.50±0.50). The highest number of species of macrobenthos species was recorded in $T_4$ and minimum number of species was recorded in control pond.


**Keywords- IMTA, Organic Matter, Macrobenthos.**

# Chapter One

## Introduction

# 1. Introduction

## 1.1. Background of the study

Over the past 15 years, the amount of cultured fish and shellfish produced worldwide has increased. Aquaculture sector is very important because of mitigating nutritional demands for the extended population in Bangladesh (E-Jahan *et al.*, 2010). But conventionally we have only few species i.e. some carps, shrimp, prawn and fin-fishes under aquaculture practice. Even though some new species are currently being produced in aquaculture, the demand from the expanding population cannot be satisfied. Culture of single species known as monoculture has been practiced (Gupta and Acosta, 2004). As a result, only single trophic level of the pond/gher is utilized. Stocking multi-layer fish species could boost fish growth. On the other hand, our aquaculture culture area is decreasing day by day because of overwhelming of growing population (Ahmed and Diana, 2016). Moreover, Bangladesh's fisheries sector is facing considerable losses due to global climate change that endangers the life cycles of many fish species (Islam and Tanaka, 2004; Ahmed and Diana, 2016). River siltation, saline intrusion, rising sea levels and anthropogenic river and pond expansion are among factors contributing to diminishing water area (Ghosh, 2004; Islam and Tanaka, 2004). Under these circumstances, it is become very important to adopt new and alternative methods of fish farming. That is why new aquaculture technique like IMTA is gaining importance that increases fish production by maintaining the environmental integrity. Integrated Multi Trophic Aquaculture (IMTA) is a culture system by utilizing a variety of water bodies with differing trophic levels, where in two or more species are grown together. Lower trophic level species, usually plants or invertebrates, utilize uneaten food from higher trophic level species (typically finfish) as nutrients (Figure 1). The culture system typically known as Integrated Multi Trophic Aquaculture (IMTA) grows fed aquaculture species (such as finfish/shrimp) along with inorganic extractive aquaculture species (such as seaweed) and organic extractive aquaculture species (such as shellfish/herbivorous fish). This is done in order to create a balanced ecosystem management approach to aquaculture for environmental sustainability (bio-mitigation), economic stability and food production for better management practices (Wahab *et al.*, 2012).

IMTA aims to enhance long-term sustainability and profitability per cultivation unit (rather than per species in isolation as is done in monoculture), as the wastes (byproducts) of one crop (fed animals) are converted into fertilizer, food and energy for the other crops (extractive plants and animals) which can then be sold on the market. One of the significant operating costs for firms that are involved in finfish aquaculture is feed. By way of IMTA part of the food, nutrients and energy thought to be wasted in finfish monoculture are recovered and transformed into crops with a marketable value, while bio-mitigation occurs (Figure 1). This is the economic value of all agricultural components, as well as plays a critical role in the system's functions and recycling processes. Harvesting of the various types of crops helps to export nutrients out of the coastal ecosystem (Chopin, 2012). In these conditions, it is important to concentrate on sustainable development goal no-2, which calls for eradicating all kinds of hunger and malnutrition by 2030 and ensuring that everyone especially children has access to enough food throughout the year. This entails encouraging sustainable aquaculture methods which include helping small-scale farmers and granting them fair access to resources including land, equipment and markets.

Bangladesh has the world's largest delta and significant water resources. There are 260 fish and 24 prawn species known to be found in Bangladesh's freshwaters and 12 exotic fish species have been identified here (Rahman, 1989; IUCN, 2000; Shamsuzzaman *et al.*, 2017). Prawn and shrimp have high market demand and economically important species. On the other hand, major carp species such as *Catla catla, Labeo rohita* and *Cirrhinus mrigala* along with exotic carp, such as silver carp (*Hypophthalmichthys molitrix*); grass carp (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*) are the available carp species in the domestic market. Small indigenous fish species include *Puntius ticto, Amblypharyngodon mola, Colisa lalius* and *Anabas testudineus and* some brackish water catfish like *Liza parsia, Mystus gulio* and SIS fish are very nutritious and has high demand on the local market for consumption (Shamsuzzaman *et al.*, 2017).

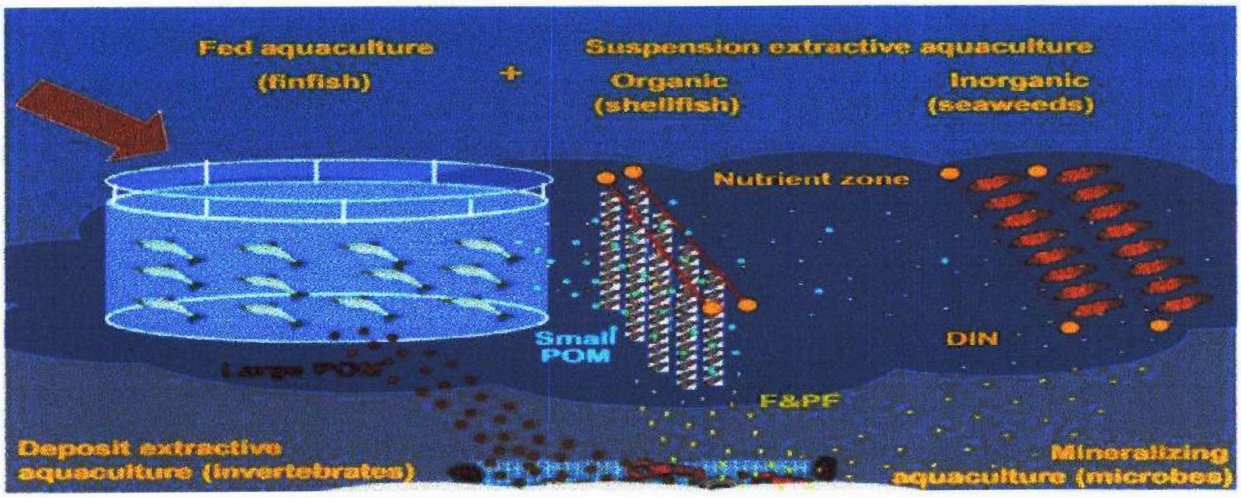


Figure 1: Schematic diagram of Integrated Multi Trophic Aquaculture System. Finfish are fed and suspension feeders like mussels consume their wastes (adopted from Clements and Chopin, 2017).

These fin fish species may have commercially significant values when shrimp and prawn stocked and culture in ponds and gher using IMTA. This practice will utilize all layers of gher that will increase fish production by maintaining eco-friendly environment. As a result, aquaculture is easily accessible to young people and rural women. Farmers will be economically benefited from eco-friendly IMTA and also able to supply local fish demand through culturing multi trophic fish species.

Bangladesh's agro-based economy has been greatly influenced by the sectors of fisheries and livestock. Around 8.0% of the nation's income comes from those sectors, which accounts for 23.11% over all agricultural income. The country gets 60.0% of its animal protein needs from the fisheries industry, which also contributes 3.70% of its GDP and 1.92% of its export revenue. Fisheries generates US$460 million yearly and accounts for 5.60% of total export value, the second-largest export industry is the shrimp and prawn industry as a whole (DOF, 2016). The industry employs 1.2 million people and another 4.8 million household members in the productions of shrimp and prawn sector.

Suitable agroclimatic conditions, enormous amounts of freshwater and brackish-water, sufficient resources, water, soil, available cultivation techniques and the availability of wild PL make Bangladesh to be blessed for its riverine giant prawn (*Macrobrachium rosenbergii* locally known as Golda) culture. After 2000, there was a significant rise in interest in prawn farming among farmers as a result of the White Spot Syndrome Virus (WSSV) attack on shrimp (*Penaeus monodon*) farms and the fact that prawn farms were unaffected by WSSV and thus prawn cultivation received more attention from the farmers. Bangladesh produces 713 kg/ha of shrimp and prawns per year, compared to 2338 kg/ha in Thailand, 1000–1500 kg/ha in Vietnam, 1500 kg/ha in China and 600–1000 kg/ha in India (Ghosh *et al.*, 2016).

In early 1970s, freshwater prawn aquaculture was initially established in the southwest region of Bangladesh (Mazid, 1994). This farming technique started to become commonly used in Bangladesh's south-west in the late 1980s. There are currently over 82,000 prawn farms in the entire southern belt which includes greater Jessore, Khulna, Narail, Bagerhat and Satkhira. These farms cover an area of about 64,000 hectares. A benthic species is the freshwater giant prawn and thus in an aquatic system, they have a relationship with the benthic community (Williams, 2003).

A pond's production is influenced by its soil and water quality. The essential element in aquaculture is the soil. Contact with soil is the source of many chemicals that are dissolved and suspended. The surface layer of pond soil contains chemical and biological processes that have an impact on the water quality and aquaculture. Pond soil also serves as a storage area for a variety of substances that build up in the pond ecosystem. Therefore, pond aquaculture can benefit from knowledge of soil characteristics as well as the reactions and processes that occur there. Poor soil conditions will result in low yield. The existence of suitable environmental factors and the abundance of fish food organisms determine the productivity of fishponds. The primary food creatures in a fish pond's food chain include phytoplankton which obtains its nutrients from the pond's environment and conducts in photosynthetic activity with the help of sunlight. The nature and characteristics of the bottom soil where a series of chemical and biochemical reactions occur continuously and lead to the release of various nutrients in the water above and also their absorption in the soil mass, have a significant effect on the composition of these nutrients in pond water and the preservation of the water's relevant chemical condition (Bhatnagar and Devi, 2013).

Benthic organisms are those that exist either "on" or "in" sediment, since they are invertebrates. Ernest Haeckel, a German artist and naturalist who lived from 1834 to 1919, coined the term "Benthos" along with the phrase "Ecology". Epifauna and infauna are two categories of benthic invertebrates based on where they are found on or in the bottom sand.

- Epifauna - The organisms that inhabit the bottom sediments' uppermost layers; numerous crabs and gastropods are categorized as epifauna.
- Infauna -The organisms that inhabit in sediments; almost all worms and bivalves are categorized as infauna.

As biological indicators, benthic communities are well-known. Benthic organisms are typically found in sediments where water is present; this could be in the sea, river, pond or any other nearby body of water such as a lagoon. They can be easily sampled because they are typically plentiful and available year-round. Benthos is a crucial component of the aquatic ecology. The community of benthic organisms is complex; it includes a wide range of organisms. Benthic organisms are generally classified according to size; viz micro benthos:<.1 mm, macro benthos: >1.0 (not less than 0.5) mm and mega benthos >10.0 mm (Adakole *et al.*, 2003).

The macrobenthos is made up of organisms that are visible to the unaided eye and reside at the base of the water column. These organisms are larger than 1 mm according to some classification schemes, although at least 0.5 mm must be the smallest dimension in another (Link *et al.*, 2006). In this regard, microbenthic communities serve two functions: first, as an interconnected component of the food chain and second as a means of cleaning up contaminated water. The variety of macrobenthos is strongly influenced by the water and soil quality of the water body (Hellawell, 1983; Paul and Nandi, 2003).

Several scholars have argued in favor of using benthos as bio-indicators (Adakole and Ammne, 2003; Edokpayi *et al.*, 2010). Aquatic environments are fundamentally made up of macro-benthic creatures. They uphold varied degrees of contact between the community and environment. The benthic macrobenthic community's structure gives accurate and local information on recent events (Marques *et al.*, 2003). The benthic macrofauna lives on or within the bottom soil deposit and eats detritus. They contribute significantly to the movement and recirculation of nutrients in aquatic environments by speeding the conversion of organic matter in decay into simpler inorganic forms (Idowu and Ugwnmba, 2005).

In transitional ecosystems, benthic invertebrates are important because they filter phytoplankton and then serve as a food supply for larger animals like fish, connecting primary production with higher tropic levels. They significantly contribute to the destruction of organic matter prior to bacterial demineralization, structure the bottom and oxygenate it by reworking sediments. Therefore, an index of the ecosystem can be found in the composition, quantity and distribution of benthic species throughout the year in the natural water body. Benthic organisms are abundant in vitamins, minerals, fatty acids, amino acids and other nutrients. Humans eat some benthic crustaceans particularly clams and crabs (Belal *et al.*, 2014).

Benthic organisms are commonly utilized as biological indicators because they can reveal information about environmental conditions either because they are sensitive to individual species (indicator species) or because of some general characteristics that allow them to integrate environmental signals over a long period of time. These characteristics include being exposed to chemical contaminants that are regularly deposited in soil and being exposed to low dissolved oxygen levels (hypoxia/anoxia) which typically occur close to the bottom surface as a result of organic matter decay (Tagliapietra and Sigovini, 2010).

## 1.2. Objectives of the study

The specific objectives of the study as follows:

- ❖ To analyze the soil quality among control and treatments of Integrated Multi Trophic Aquaculture system.
- ❖ To identify and quantify the macrobenthos among control and treatments of Integrated Multi Trophic Aquaculture system.

# Chapter two

## Literature review

# 2. Literature Review

## 2.1. Integrated Multi Trophic Aquaculture

Integrated Multi Trophic Aquaculture (IMTA), a farming method that eliminates waste from culture systems, lowers the risk of illness and generates additional income, is both environmentally and economically sound. The IMTA system of shrimp, spotted scat and water spinach was given starch, which increased output, profitability and water quality. In addition to boosting shrimp life, production and nitrite-N and TAN reduction, the adoption of the IMTA model in combination with biofloc technology has a significant favorable direct effect on FCR and overall economic benefit (Liu *et al.*, 2014).

## 2.2. Soil quality

Soil quality (SQ) is defined as the ability of a particular type of soil to function, within unmanaged or controlled ecosystem boundaries, in a way that supports plant and animal productivity, maintains or improves water and air quality and supports human health and habitation (Doran and Parkin, 1994). Functions of the soil could include its physical, chemical and biological characteristics and processes. Since a soil's processes and physical characteristics cannot be evaluated, evaluating SQ as a whole is challenging. These markers could be physical (soil structure, aggregate stability, hydraulic conductivity and pore space), biological (microbial biomass, enzyme activity and microbial communities) or chemical (cation exchange power, pH, carbon content etc. (Bronick and Lal, 2005). The choice of indicators to calculate SQ depends on the soil functions for which measurement is carried out (Karlen *et al.*, 1997). Main nutrients in soil are consisting of:

- Major nutrients (macronutrients): Nitrogen, phosphorus and potassium.
- Minor nutrients: Calcium, magnesium and sodium.
- Micronutrients: Cobalt, copper, manganese and zinc.

The primary and secondary nutrients are the most important since a lack of them in fish pond soil reduces the pond's productivity. Transferrable cations are the term for secondary nutrients ($Ca^{2+}$, $Mg^{2+}$, $Na^{+}$) (Hodges, 2010). Ammonium hydroxide is used to remove these cations from the soil during agitating (Shanwal and Kapoor, 2004). The characteristics and functions of a soil are interconnected. For example, a soil's enzymatic activity is essential to nutrient cycling, organic matter decomposition, soil health and the environment (Das and Varma, 2011). The quality of the soil and water affect the production of a pond (Bhatnagar and Devi, 2013). Fish and shrimp production are often higher in fertile soils than in ponds located in less productive agricultural soils (Boyd and Tucker, 2012). A healthy pond soil is one in which nutrients are continuously taken in, held in place and released over an extended period of time while organic matter is quickly mineralized (Anderson, 1980). In addition, numerous chemical and biological processes occur in the lower soil, which either cause nutrients from the soil to be released into the water or cause the soil and the microbial population to absorb nutrients from the water (McCauley *et al.*, 2009). To understand the ponds' ecosystem, understanding the quality of the soil and water is crucial in order to enhance the productivity of the ponds and number of fish and shrimp as a whole (Boyd and Tucker, 2012).



## 2.3. Soil pH

Soil pH is a measure of the hydrogen concentration in the soil solution (Thomas, 1996). The pH is the hydrogen ion concentration ($H^+$) negative logarithm which can be defined by the following equation: pH = -log ($H^+$) (Wexler and Clegg, 2002). Low alkalinity waters are acidic, moderate alkalinity waters are nearly neutral and basic waters with high alkalinity are alkaline (Yan *et al.*, 2008). The actions of plants, animals and bacteria cause a regular cycle of pH. During the afternoon, when conditions are ideal for quick photosynthesis and carbon dioxide removal, high pH (>9) is frequently observed in aquaculture ponds (Boyd and Tucker, 1998). Because of the low pH of the water and general alkalinity, there is a loss of inorganic phosphorus and carbon dioxide for plant growth (Boyd and Tucker, 1998). However, rapid photosynthesis generally causes a severely high pH in water with little alkalinity (Boyd and Tucker, 2016). Ponds are additionally given a liming application to increase alkalinity (Boyd and Tucker, 1982). Boyd (1988) found that the optimal pH values of freshwater animals for good health and high growth rates is between 6.5 and 9.0 health and high growth rates.

## 2.4. Electrical Conductivity (EC)

Electrical conductivity (EC) is a material's potential to transmit (conduct) an electrical current and is usually expressed in milliSiemens per meter (mS/m) units. Measurements of soil EC can also be recorded in units of Siemens decimals per meter (dS/m), which is equivalent to the mS/m reading divided by 100 (Grisso *et al.*, 2005). Soil electrical conductivity (EC) is a calculation that corresponds with soil properties that influence crop productivity, including soil texture, ability of cation exchange (CEC), drainage conditions, amount of organic matter, salinity and characteristics of the subsoil (Doerge, 2001). It is the most common indicator of soil salinity which means that an aqueous solution is capable of bearing an electrical current.

## 2.5. Organic Matter

Soil organic matter (SOM) refers to a heterogeneous composition (Jimenez-Montealegre *et al.*, 2002) and materials from the microbiological and chemical alteration of organic wastes make up this substance (Nebbioso and Piccolo, 2013). The rates of in situ development of organic material that is deposited and sinks to the bottom before being mineralized and the rate of mineralization of organic matter in the sediment all influence how so much organic matter is added to or eliminated from sediments. (Arndt *et al.*, 2013). The rate of particulate matter sedimentation was calculated using the size, shape and composition of the particles as well as the density and viscosity of the water (Jorgensen, 1989). Sand and silt settle quickly, whereas microscopic particles like clay and finely split organic compounds settle more slowly. (Boyd *et al.*, 2002; Whitehouse *et al.*, 2013) reported that since soil organic matter contains between 45-50% carbon, multiplying soil organic carbon by 2% gives an approximation of the amount of organic matter in the soil (Tapader *et al.*, 2017). In terms of their link to aquaculture, Boyd *et al.*, (2002) provided the following classification of pond soil organic matter concentration (Table 1).

Table 1: Relation between organic carbon and organic matter

| Organic carbon (%) | Comment |
|---|---|
| >15 | Organic soil |
| 3.1-15 | Mineral soil, high organic matter content |
| 1.0-3.0 | Mineral soil, moderate organic matter content, best range for aquaculture |
| <1 | Mineral soil, low organic matter content |

Organic matter and soil bottom composition are connected (Ellerbrock *et al.*, 2005). Since it provides the metabolic energy source for the biological processes in soil that affect the availability of nutrients. Long-term soil fertility maintenance depends greatly on soil organic matter (Rahman *et al.*, 2013). Organic matter has an impact on the soil's environmental health as well as its chemical and physical characteristics. Organic matter affects the soil's structure, capacity to hold moisture, diversity and activity of soil organisms which can be both beneficial and detrimental to crop productivity as well as the availability of nutrients (Dungait *et al.*, 2012). It affects the effects of pesticides, fertilizers and herbicides as well as those of chemical changes (Prashar and Shah, 2016).

## 2.6. Nitrogen

Two significant sources of nitrogen for ponds are fertilizer and prepared feed (Hargreaves, 2006). A surplus of nitrogenous inputs will result in a decline in soil quality (Fenn *et al.*, 1998). The possible harm of nitrogenous compound such as nitrite and ammonia to marine organisms is less growth (Schroeder *et al.*, 2011). The primary sources of ammonia in wetlands are often fish excretion, sediment flow from the mineralization of organic materials and reduced sediment diffusion (Jiménez-Montealegre *et al.*, 2005). Nitrogen is often used to fertilize wetlands in the form of urea, which easily breaks down the manure to produce ammonia, ammonium and nitrate ions or organic nitrogen (Boyd and Tucker, 1998). In a temperature and pH-dependent equilibrium, ammonia ($NH_3$) and ammonium ($NH_4^+$) usually occur in water: $NH_4^+ + OH = NH_3 + H_2O$ (Sheehan and Lewis, 1986).

## 2.7. Phosphorus

Phosphorus is a crucial resource that lowers aquatic productivity in most of the natural waters (Boyd and Tucker, 1998). The phosphorus cycle is affected by the combination of pond sediment and the water column of natural water (Palmer-Felgate *et al.*, 2011). In pond aquaculture, this relationship is also a big factor (Boyd and Musig, 1981; Boyd, 1995). Boyd and Tucker (1998) suggested that plants absorb phosphorus as orthophosphate ions, also known as ionization products of orthophosphoric acid. Boyd and Tucker (1998) have disclosed that much of the orthophosphate occurs as a mixture of $H_2PO_4$ and $HPO_4$ that can be found similarly accessible to plankton at a typical environmental pH level in aquaculture ponds of 7 to 9. Most of the phosphorus in aquaculture pond waters comes from phytoplankton cells and other debris of an algal origin. Pond soil can be used as a phosphorus source or sink and aerobic soils are essential as a phosphorus sink (Boyd and Musig, 1981). Typically, calcium and aluminum interact with phosphorus in sediments to form complexes with very low solubilities (Li *et al.*, 2017). With declining pH, the solubility of aluminum and iron phosphates increases, while with increasing pH, the solubility of calcium phosphate compounds decreases. The amount of oxygen in the mud affects the composition of iron phosphate (Søndergaard *et al.*, 2003). The majority of phosphorus in aerobic environments is largely coprecipitated as phosphorus in the layer of ferric oxide covering silt or clay particles or mixed with amorphous ferric ($Fe^{3+}$) oxyhydroxide gels. Ferric iron is typically converted to soluble ferrous soluble ($Fe^{2+}$) iron under anaerobic conditions and the corresponding phosphorus is soluble (Majzlan, 2011).

## 2.8. Benthic fauna

Understanding the species distribution, variety and abundance of benthic macro-invertebrate fauna as well as their links to environmental factors are crucial components of understanding the structure and operation of aquatic ecosystems. Distribution of macrobenthic populations is directly related to sediment qualities which are linked to a wide range of environmental conditions (Buchanan, 1984 and Gray, 1974).

Numerous juvenile fish and crustacean species depend greatly on benthic fauna for food which is essential for the survival of many commercially farmed species. One of the largest rivers in Bangladesh's south-west region is the Ruspha river and has enormous potential for fisheries resources. However, such resources are in danger due to various activities, particularly effluents from discharges from various industries located adjacent to the river Rupsha. No such studies have been conducted in any freshwater system in Bangladesh. Few articles are published in this aspect and outcomes of such studies are summarized below:

We also looked through the literature on the benthic fauna of other bodies of water such as rivers, lakes, estuaries etc. as followes:

Ahmed (1990) and Jahan *et al.*, (1990, 1993) reported that there are 212 species of snails classified into 80 genera, 40 families, and 5 orders occurred in the coastal waters of Bangladesh.

Ali and Begum (1979) reported that the aquatic food chain is significantly influenced by the chironomid larvae. Many fish, especially those that live on the bottom, eat these bug larvae. Three subfamilies and six species of chironomid larvae were discovered in Dhaka, Bangladesh.

Ali and Chakraborty (1992) reported that *Pila globosa*, a freshwater snail is found in abundance in the districts of Faridpur, Gopalgong, Madaripur, Jashore, Narail, Khulna, Bagerhat, Satkhira, Kushtia and Jhenaidah.

Ali *et al.*, (1987) found significant correlation between water temperature and pH and the growth of mollusks (*Viviparus bengalensis*).

Alimov *et al.*, (1994) conducted a study on the lake ecosystem's reaction to different plankton stocking concentrations (*Coregonus* fishes). The outcomes of the experiment demonstrate that there are numerous fishes in the lake that are both plantivorous and carnivorous. Despite causing noticeable structural and some functional changes in the benthos and plankton communities, this did not fundamentally alter the ecosystem's ability to function.

Asadujjaman *et al.*, (2012) observed the abundance of macrobenthos of Hatiya and Nijhum Dweep, Bangladesh. During pre-monsoon a total of 10 major groups/taxa were observed; the highest density (4,511 individuals/$m^{-2}$) was discovered at Nijhum Dweep namar bazar, while the lowest density (433 individuals/$m^{-2}$) was discovered at Nalchira ghat. Shrimp larvae (13.93%), oligochaetes (16.65%) and polychaetes (45.03%) made up the macro benthos. Gastropods (3.56%), crab (9.63%), isopods (1.15%), bivalves (1.15%), copepods (0.73%), amphipods (0.63%), annelids (0.42%), other organisms (7.12%), crab, shrimp larvae, polychactca, oligochaetea made up 85.24% of the overall population. With a contribution of 45.03% to the whole macro-benthos polychaetes dominated.

Bais *et al.*, (1992) found that the most abundant benthos group, with a maximum 91.80%, was oligochaeta. While summer was less suited for benthos growth, winter was the best time for it. The lake's rich tropical state is indicated by the abundance of macroinvertebrates.

Basu. A. *et al.*, (2013) reported that macrobenthic fauna is in responsible of effectively utilizing the sediments, and the diversity of this species is a good indicator of a wetland's health in relation to sediment quality. *Bellamya* and *Thiara* were the two most prevalent species, indicating that the pond's water was pure, out of the 12 mollusk taxa and 1 annelid taxonomic that were discovered.

Bose and Lakra (1994) studied the macrobenthos of two freshwater ponds in Rachi in connection to several ecological parameters and discovered a strong association between the quantity of chironomus larvae in the population and the overall alkalinity content. In the middle bottom zone of both ponds, chironmid predominated.

Chakrabarti (1984) found highest amount of macrobenthic fauna with DO levels between 6.2 and 6.8 mg/l in pond treated with manure and inorganic fertilizer.

Hamilton (1971) stated that zoobenthos of 15 lakes in the experimental lake in northwest Ontario and discovered that species from the chironomid and chaoborid groups dominated each lake with average depth below than 10 inches.

Hershey (1992) conducted an experiment to determine how fertilization influenced an arctic lake's benthic macroinvertebrate fauna and discovered that choniomids were not food-limited and appeared to be limited by predators, in contrast to snails which appeared to be food-limited.

Hirabayashi and Hayashi (1994) worked on horizontal distribution of benthic macroinvertebrates in lake Kizaki, Japan and observed that the Oligochates inhibited the whole lake bottom, with higher concentration in shallower region (less than 10 m). In contrast, densities of chaoborid larvae were higher in the deeper regions (>20 m) than in the shallow water region. Chironomid larvae, on the other hand, were distributed in higher population in the transitional regions (10-20 m) and a marked declined in the number of species was observed as the deep increased.

Hossain *et al.*, (2009) reported that the post-monsoon and monsoon seasons in Chandpur and Hatiya respectively, had the highest and lowest frequency. Additionally, it was noted that 20 faunal taxa including oligochaetes (53.75%), polychaetes (33.31%) and meso-gastropods (4.94%) were present in the studied areas of the Meghna river estuary.

Jahan (1993) reported that thirteen terrestrial and ten freshwater gastropod species were identified from various locations in Bangladesh.

Jonasson (1972) reported that the growth of chironomids was limited to two very short periods in lake Esrom. One occurred in the spring during the phytoplankton maximum when the water was abundant and the other done so after the autumn overturn when there was plenty of oxygen but food production was reduced. He observed that chironomid growth was slowed down during the summer by the drop-in oxygen levels in the hypolimnion.

Jonasson (1978) suggested that food cannot be properly digested at low oxygen levels, sufficient oxygen is crucial for benthos formation.

Kullberg (1992) worked on pond humic content and the community structure of benthic macro invertebrates in streams have been compared.

Mandal and Moitra (1976) studied the change in total benthos volume over time in a freshwater fishpond in Burdwan. The volume of the entire benthos was found to be largest in winter and lowest in summer.

Martien and Benke (1977) reported that water must have more than 1 mg/l of dissolved oxygen in order to maintain positive benthos production.

Nelson (1990) worked on four wetlands in the Missouri state of the USA. The researchers also studied the seasonal abundance and life cycle of chironomids. They discovered that two species- *Chironomusten sand* and *Glyptotendipens paripes* were dominated the flora of semi-permanent wetlands. These species were found in seasonal wetland but made up a smaller part of the total population.

Rahman *et al*., (2003) Bangladesh's Bakkhali, Gotibanga, Chakaria and Teknaf estuaries are habitats for two kind of clams: *Anadara granosa* and *Anadara rhomba*.

Reid and Wood (1976) reported that a number of variables including sludging, microscopic organisms, organic material, latitude, season and the angle and strength of incident light are all factors have an impact on the transparency of water.

Shaha *et al*., (2003) reported that tribal people from Bangladesh were the Bawm, Chakma, Garo, Hajong, Marma, Monipuri and Murang. In their diets, Rajbangshi, Santa, Tanchanga, Tripura and other peoples include some types of mollusks.

Sing *et al*., (1994) analyzed the qualitative and quantitative benthic fauna of Kararia Lake of Monthihari, Bihar, India. They reported that in case of qualitatively the benthic fauna was found to be varied with months and the highest value was observed in the month of August and the lowest was in January. In quantitative analysis, they found that the area was mainly dominated with chironomid larvae, oligochaetes, gastropods, pelecypods and ostracods. However, few shrimps, leeches and other insect larvae were also noticed.

Sinha and Das (1993) worked on effect of sewage pollution on the distribution and abundance of benthos in the Ganga river, India. They investigated the ecological imbalances using benthic organisms as biological indices. The study showed that in rivers with low levels of pollution, a variety of organisms were present, with a diversity index of up to 9.1, while in rivers with high levels of organic pollution, monocultures of *tubficid* were found, along with a few insect larvae like as *Ehironofnus*, *Psychodasp* etc. and a diversity index of less than 1.

Van-Den-Brink *et al*., (1994) discovered a connection between seasonal factors and the abundance of all macrobenthos, such as the duration of flood pulses, water level, water temperature and vegetation cover in lower Rhine flood plain lakes. They came to the conclusion that in the frequently flooded lake, hydrological elements were most significant whereas in the isolated, non-flooded lake, vegetation cover and water temperature were more significant.

Wasilewska *et al*., (1979) concluded that carp feeding conditions are significantly affected by the changes to the bottom fauna's qualitative composition and growth in biomass.

# Chapter three

## Materials and methods

# 3. Materials and methods

## 3.1. Study area and period

The research work was carried out in the experimental pond complex II (Figure: 2 ) of the Fisheries and Marine Resource Technology Discipline at Khulna University in Khulna, Bangladesh, from December 2021 to May 2022. All of the ponds were rectangular in shape, with a depth of 1.5 m and a surface area of 240 $m^2$.

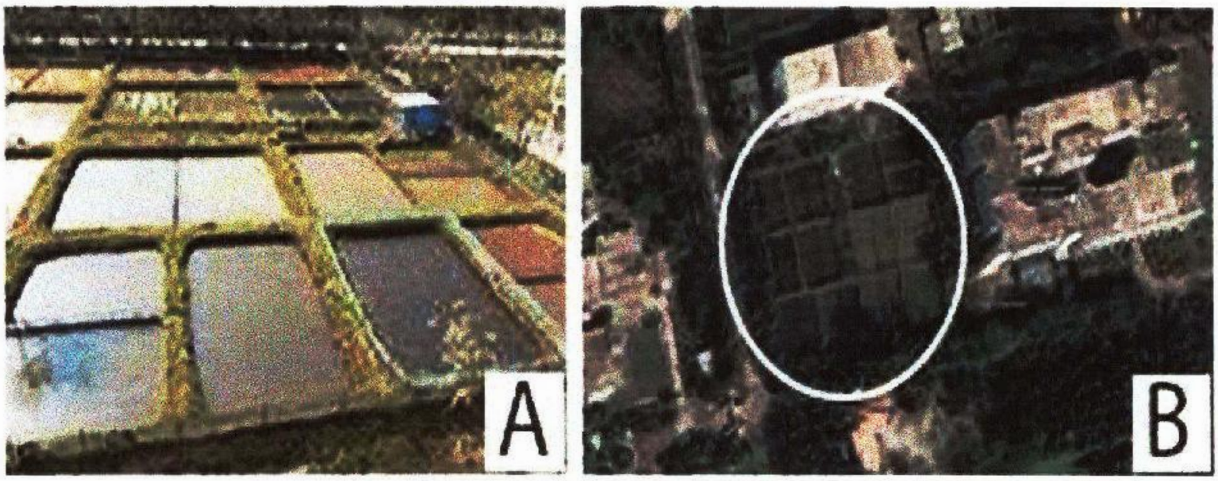


Figure 2 : A. Camera view and B. Google Map, 2022 view of and experimental ponds and study area.

## 3.2. Pond preparation

Ponds were not dewatered before the test. Rotenone was first used to kill all of the non-target fish. Then, repeated fishing was done using a seine net to make sure there were no real arachnids because they are not affected by rotenone. After that, quick lime was applied in the pond water at the certain dose depending on pH. Every pond was fully exposed to the dominating sunlight.

## 3.3. Feeding

Same feed was applied in all treatments and control pond during the study period. For nursery period (0-3 g body live weight) quality feed-1 having protein level of 32% was given thrice a day with 10% body weight of prawn. At the weight of 3-10 g, quality feed-2 having 32% protein level was also given to the fishes thrice a day. Prawn attaining 10g of body weight were given grower feed containing 30% protein at the rate of 8% body weight. Fertilizer were used named urea, TSP, molasses, rice polish and yeast with certain dose during the study period in all four treatments.

## 3.4. Experimental design

The design of the experiment is shown in (table 2) as follows:

Table 2: Design of the experiment

| Species | Stocking Density | Control | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
|---|---|---|---|---|---|---|
| Prawn(*Macrobrachium rosenbergii*) | $02/m^2$ | + | + | + | + | + |
| Fishes (*Labeo rohita*) | $0.1/m^2$ | - | + | + | + | + |
| SIS (*Amblypharyngodon mola*) | $02/m^2$ | - | - | + | + | + |
| Mollusk (*Pila globosa*) | $0.25/m^2$ | - | - | - | + | + |
| Vegetable (*Ipomoea aquatica)* | ……… | - | - | - | - | + |
| | Replication | 3 | 3 | 3 | 3 | 3 |

**(+) denotes as present and (-) denotes as absent.**

## 3.5. Process of sampling

**3.5.1. Pond soil:** Samples were taken on a bimonthly basis. Every pond in our experimental pond complex had five random locations from where the upper layer soil at the bottom of the pond was taken. The sample allowed to air dry for a number of days in plastic bowls and then reduced to a fine powder kept in airtight polythene bags and the samples were then transported to the Soil Resource Development Institute (SRDI) Laboratory, Daulotpur, Khulna for the analysis of organic matter, EC, total nitrogen, phosphorus and pH (Table 3).

Table 3: Determination of pond soil parameter

| Parameters | Measurement Process | Apparatus | Unit |
|---|---|---|---|
| Organic matter | Standard method | Burette, Pipette, Conical flask, Dispenser, Measuring, Cylinder, Indicator. | % |
| Nitrogen | Micro kjeldhal method | Kjeldhal digestion tube, Steam distillation unit, Burette | % |
| Phosphorus | Olsens method | Spectrophotometer | µg/g |
| pH | Soil and water solution | pH meter | - |
| EC | Soil and water solution | EC meter | dS/m |

**3.5.2. Benthos:** A total of 6 samples were collected from a particular culture period. Benthic samples were collected from five treatments. Sample collection usually started around 11 am. The nature of the bottom was also noted. To make things easier, three sites from each pond were identified to consider as the sampling sites. Bottom dredging machine was used sampling of bottom sediments. The sediments were transferred to a polybag along with any objects caught. Each sample was washed through water in a set of three standard brass sieves with mesh sizes of 4.0 mm, 2.0 mm and 1.0 mm respectively. Benthic organisms that remained on the sieves from the residue were collected using fine forceps and preserved in separate vials containing 5% formaldehyde. The vials were correctly marked using a marker pen before being brought into the laboratory of FMRT Discipline at Khulna University for separation and identification.

**Separation of macrobenthos >1.0 (not less than 0.5) mm:** The sample was kept for 48 hours in the laboratory to allow the animals to be harden (Maitland and Heebspith, 1974). The preserved animals were then transferred to a petridish. The benthic species were then divided into many major taxonomic groups by using a magnifying glass.

**Identification of benthos:** The organisms were identified using a check list of morphological characteristics and sources from various books, articles and the internet. For the purpose of identifying the organisms at least to the species level, many groupings of animals were studied. Macrobenthos was recognized with the unaided eye or with a hand-held operated magnifying lens. The benthic organisms were counted and analyzed after identification.

### 3.6. Statistical analysis

All sampling data was recorded in Microsoft Excel-2016. Both the SPSS-2022 and Microsoft Excel-2016 software's were used to analyze the physiochemical characteristics of the soil samples. To compare the various treatments in this study as well as to determine significance difference among them. A one-way Analysis of Variance (ANOVA) was used. A Sharpio-wilk test was used to determine whether the data are normally distributed. All Statistical analyses were considered significant at 5% ($p < 0.05$).

# Chapter four

# Results

# 4. Results

## 4.1. Soil quality parameter

Table no 4: Mean ±SD values of different soil quality parameter in control and treatment ponds.

| Parameter | Optimum values | Control (Mean±SD) | Treatment 1 (Mean±SD) | Treatment 2 (Mean±SD) | Treatment 3 (Mean±SD) | Treatment 4 (Mean±SD) |
|---|---|---|---|---|---|---|
| **pH** | **7.0 to 8.0** Ref: Ravichandran *et al.*, (2006) | $8.02\pm0.12^{a}$ | $7.95\pm0.13^{a}$ | $7.90\pm0.14^{a}$ | $7.88\pm0.19^{a}$ | $7.92\pm0.25^{a}$ |
| **EC** | **<4 ds/m** Ref: Boyd, 1995 | $4.55\pm1.78^{a}$ | $4.22\pm1.60^{a}$ | $4.58\pm1.59^{a}$ | $4.43\pm2.03^{a}$ | $4.40\pm1.75^{a}$ |
| **Soil organic matter** | **2.0 %- 4.0 %** Ref: Jahiruddin and Satter 1995 | $3.00\pm0.48^{a}$ | $3.11\pm0.36^{a}$ | $3.11\pm0.38^{a}$ | $3.19\pm0.93^{a}$ | $2.93\pm0.42^{a}$ |
| **Soil total nitrogen** | **(0.1% to 0.3%)** Ref: Haque ,2006 | $0.18\pm0.01^{a}$ | $0.17\pm0.01^{a}$ | $0.17\pm0.01^{a}$ | $0.16\pm0.02^{a}$ | $0.16\pm0.02^{a}$ |
| **Soil phosphorus** | **5.5 µg/g -15 µg/g** Ref: Rana *et al.*, (2017) | $10.38\pm3.35^{a}$ | $9.94\pm3.81^{a}$ | $9.21\pm4.20^{a}$ | $8.98\pm3.68^{a}$ | $8.53\pm4.39^{a}$ |

In a row same letter in superscript are not significally different (p>.05)

## 4.2. Soil pH

Among the treatments, the pH value was observed to vary. The mean pH values of the were varied from 7.88±0.19 to 7.95+0.13. Soil pH was not significantly varied (p>.05) among the treatment ponds and control pond. In $T_1$ pond, the pH value was the highest among the treatments and the lowest value was in $T_3$ whereas in comparison with control, the value was the highest.

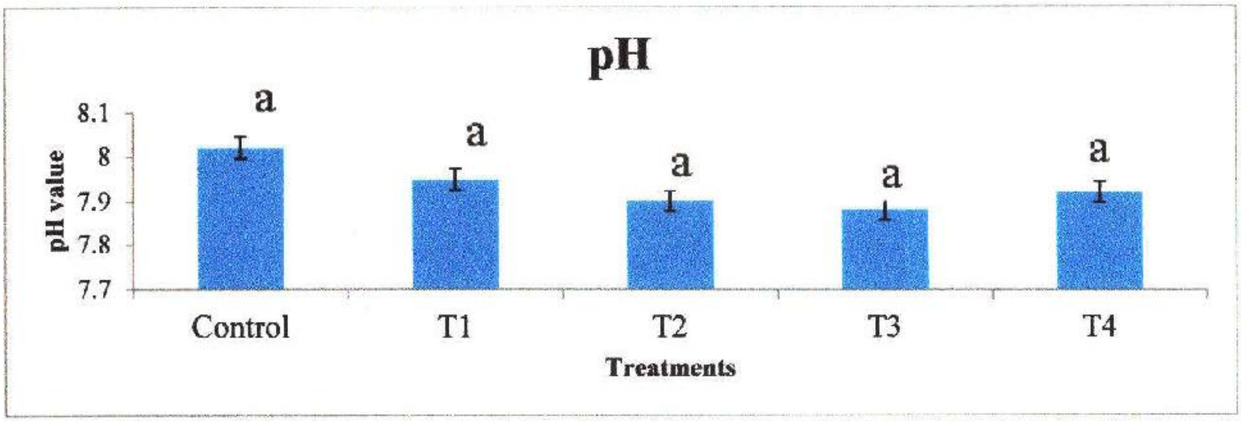


Figure 03: Variation of soil pH value in the control and treatment ponds.

## 4.3. Soil electrical conductivity

It was observed that the EC differed between the different treatments. The mean EC value between treatments varied from 4.22±1.60 to 4.40±1.75 dS/m.

Soil electrical conductivity was not significantly varied ($p>.05$) between treatment pond and control pond. In $T_2$ pond was the highest among the treatments and $T_1$ the lowest among the treatments but less than control.

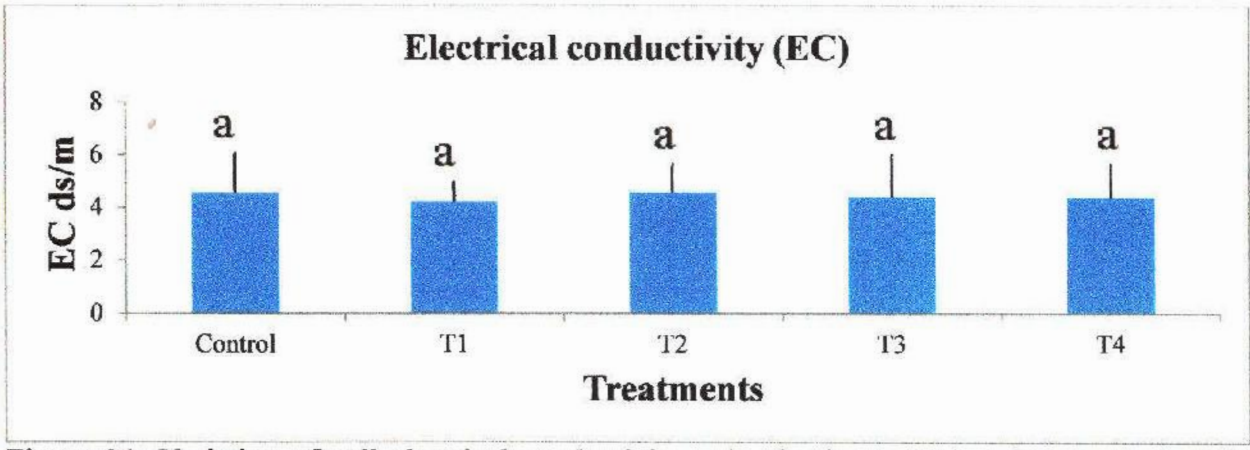


Figure 04: Variation of soil electrical conductivity value in the control and treatment ponds.

## 4.4. Soil organic matter

Soil organic matter has been shown to vary between various treatments. The mean SOM values ranged from 2.93±0.42 to 3.19±0.93 percent among the treatments.

Soil organic matter was not significantly varied ($p>.05$) between treatment pond and control pond. In $T_4$ pond was the highest among the treatments and $T_3$ the lowest among the treatments but less than control.

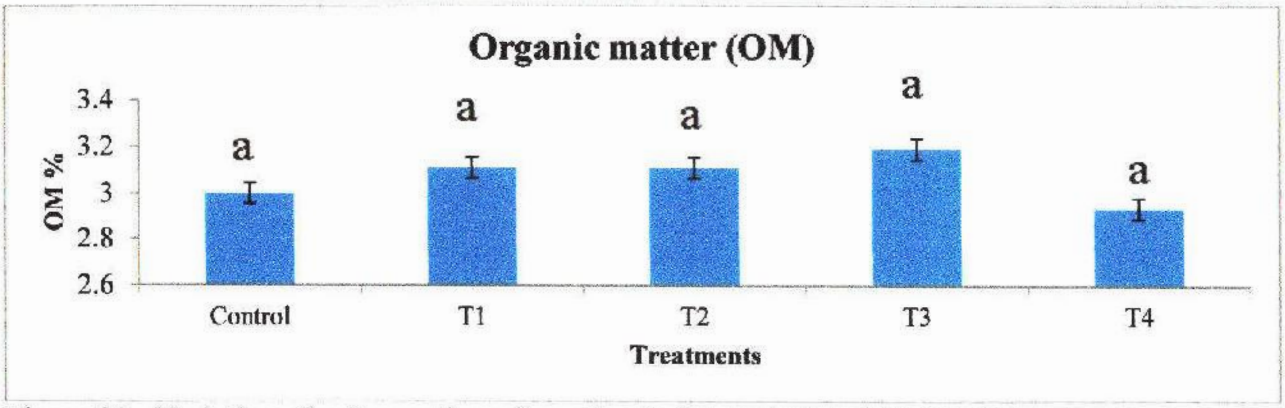


Figure 05 : Variation of soil organic matter value in the control and treatment ponds.

## 4.5. Soil total nitrogen

Total nitrogen has been shown to vary between various treatments. The mean TN values between treatments ranged from 0.17±0.01 to 0.16±0.02%. Soil total nitrogen was not significantly varied ($p>.05$) between treatment pond and control pond. In $T_1$, $T_2$ pond was the highest among the treatments and $T_3$, $T_4$ the lowest among the treatments but less than control.

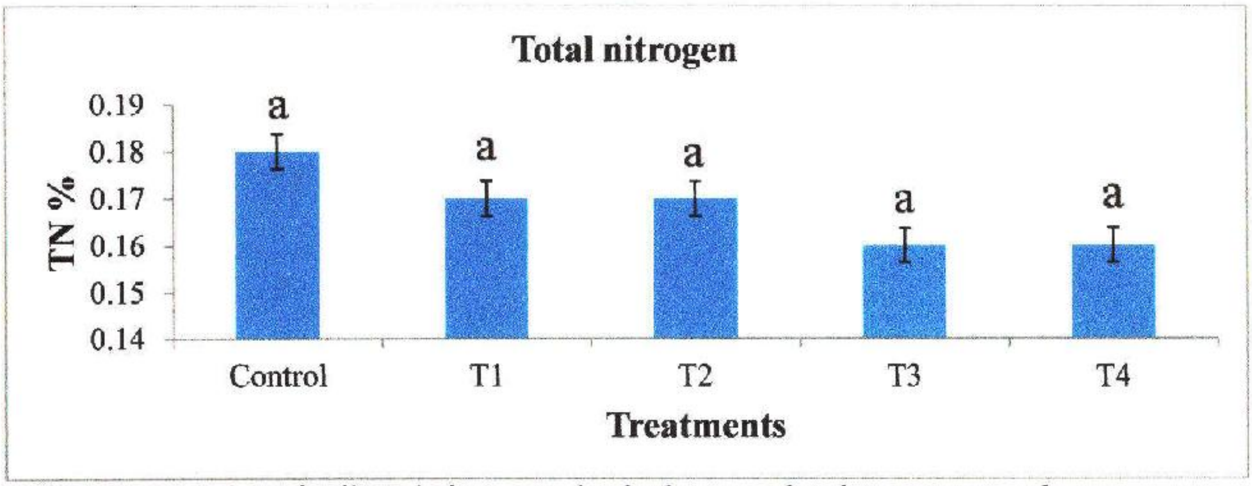


Figure 06: Variation of soil total nitrogen value in the control and treatment ponds.

## 4.6. Soil phosphorus

Phosphorus has been shown to vary between different treatments. The mean phosphorus values between treatments ranged from 9.94±3.81 to 8.53±4.39 µg/g.

Soil phosphorus was not significantly varied ($p>.05$) between treatment pond and control pond. In $T_1$ pond was the highest among the treatments and $T_4$ the lowest among the treatments but less than control.

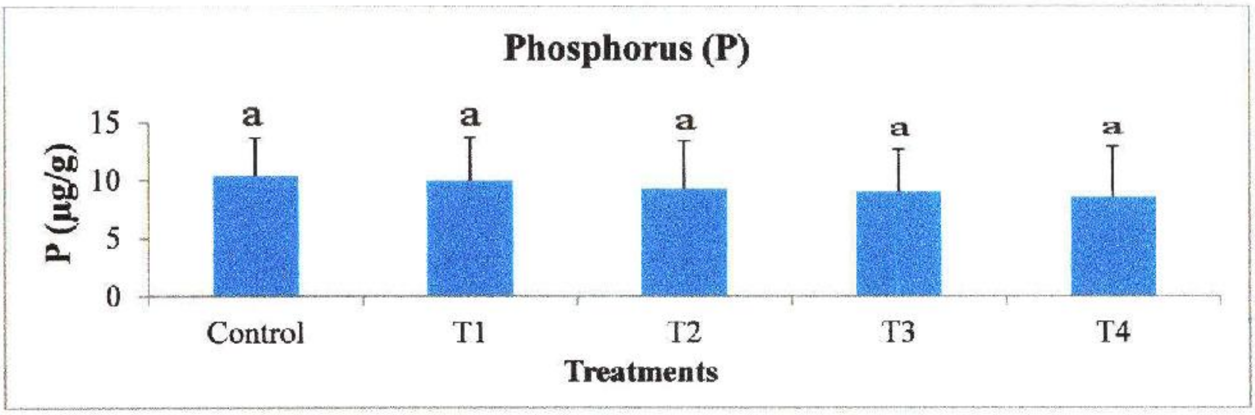


Figure 07: Variation of soil phosphorus value in the control and treatment ponds.

## 4.7. Benthos

In this study, the identification and quantification of macrobenthos under the five experimental treatments were done in pond-based Integrated Multi Trophic Aquaculture system.

### 4.7.1. Identification of macro benthos

Nine macrobenthos species under 4 genera in all were identified at the end of the experiment and these are i. Genus *Melanoides* (four species-*Melanoides maculate, Melanoides granifera, Melanoides tuberculate* and *Melanoides polymorpha)* ii. Genus *Bellamya* (two Species-*Bellamya javanica* and *Bellamya angularis*) iii. Genus *Pila* (two species *Pila polita* and *Pila globosa*) and iv. Genus *Indoplanorbis* (one species- *Indoplanorbis exustus)* was identified (table 5, figure 8).

Table no 5: Classification of different species of macrobenthos in experimental pond.

| No | Genus | Species | Kingdom | Phylum | Class | Family |
|---|---|---|---|---|---|---|
| 1 | *Melanoides* | *Melanoides maculate* | Animalia | Mollusca | Gastropoda | Thiaridae |
| 2 | *Melanoides* | *Melanoides granifera* | Animalia | Mollusca | Gastropoda | Thiaridae |
| 3 | *Bellamya* | *Bellamya javanica* | Animalia | Mollusca | Gastropoda | Viviparidae |
| 4 | *Melanoides* | *Melanoides tuberculate* | Animalia | Mollusca | Gastropoda | Thiaridae |
| 5 | *Bellamya* | *Bellamya angularis* | Animalia | Mollusca | Gastropoda | Viviparidae |
| 6 | *Pila* | *Pila polita* | Animalia | Mollusca | Gastropoda | Ampullariidae |
| 7 | *Melaoides* | *Melaoides polymorpha* | Animalia | Mollusca | Gastropoda | Thiaridae |
| 8 | *Pila* | *Pila globosa* | Animalia | Mollusca | Gastropoda | Ampullariidae |
| 9 | *Indoplanorbis* | *Indoplanorbis exutus* | Animalia | Mollusca | Gastropoda | Bulinidae |


Figure 8: Different species of macrobenthos in the experimental pond.

## 4.7.2. Quantification of macrobenthos

The number of different macrobenthos species, viz *Melanoides maculate, Melanoides granifera, Melanoides tuberculate, Melanoides polymorpha, Pila polita* and *Pila globosa* and *Indoplanorbis exustus* was significantly varied ($p<.05$) among the treatment ponds and control pond but *Bellamya javanica* and *Bellamya angularis* was not significantly varied ($p>.05$) between treatment ponds and control pond.

Table no 6: Mean ±SD values of different species of macrobenthos in control and treatment ponds

| Name of the species | Control (Mean±SD) | Treatment 1 (Mean±SD) | Treatment 2 (Mean±SD) | Treatment 3 (Mean±SD) | Treatment 4 (Mean±SD) |
|---|---|---|---|---|---|
| ***Melanoides maculate*** | $27.17\pm11.96^{a}$ | $64.50\pm24.20^{b}$ | $67.83\pm25.92^{c}$ | $69.17\pm16.26^{c}$ | $83.50\pm15.61^{d}$ |
| ***Melanoides granifera*** | $29.83\pm7.24^{a}$ | $51.17\pm8.63^{b}$ | $56.33\pm8.69^{cd}$ | $51.83\pm15.49^{cd}$ | $56.50\pm8.71^{dd}$ |
| ***Bellamya javanica*** | $16.00\pm3.56^{a}$ | $15.50\pm5.85^{a}$ | $16.17\pm6.52^{a}$ | $15.33\pm4.96^{a}$ | $12.80\pm2.79^{a}$ |
| ***Melanoides tuberculate*** | $26.00\pm9.07^{a}$ | $47.33\pm13.25^{b}$ | $27.00\pm7.44^{bd}$ | $36.17\pm11.62^{bd}$ | $28.00\pm6.06^{ad}$ |
| ***Bellamya angularis*** | $4.50\pm2.14^{a}$ | $7.00\pm1.73^{a}$ | $6.83\pm2.97^{a}$ | $4.67\pm3.86^{a}$ | $10.17\pm6.34^{a}$ |
| ***Pila polita*** | $3.17\pm2.11^{a}$ | $5.83\pm3.44^{b}$ | $10.17\pm5.70^{c}$ | $10.50\pm0.50^{c}$ | $8.50\pm2.50^{d}$ |
| ***Melaoides polymorpha*** | $24.67\pm12.51^{a}$ | $60.50\pm13.24^{b}$ | $71.33\pm18.55^{c}$ | $59.00\pm11.12^{d}$ | $69.50\pm7.41^{e}$ |
| ***Pila globosa*** | $0.00^{a}$ | $1.83\pm2.03^{b}$ | $6.83\pm3.58^{c}$ | $8.33\pm3.25^{d}$ | $8.33\pm3.86^{d}$ |
| ***Indoplanorbis exustus*** | 0.00 | 0.00 | 0.00 | $0.00^{a}$ | $7.83\pm3.08^{b}$ |

In a row same letter in superscript are not significally different ($p>.05$)

### 4.7.3. Quantification of macrobenthos at control pond

In control pond, *Melanoides granifera* was the highest in abundance, *Pila polita* was lowest in (Figure 09). However, *Pila globosa* and *Indoplanorbis exustus* were not found. Overall, the lowest number of macrobenthos species were recorded in the control.

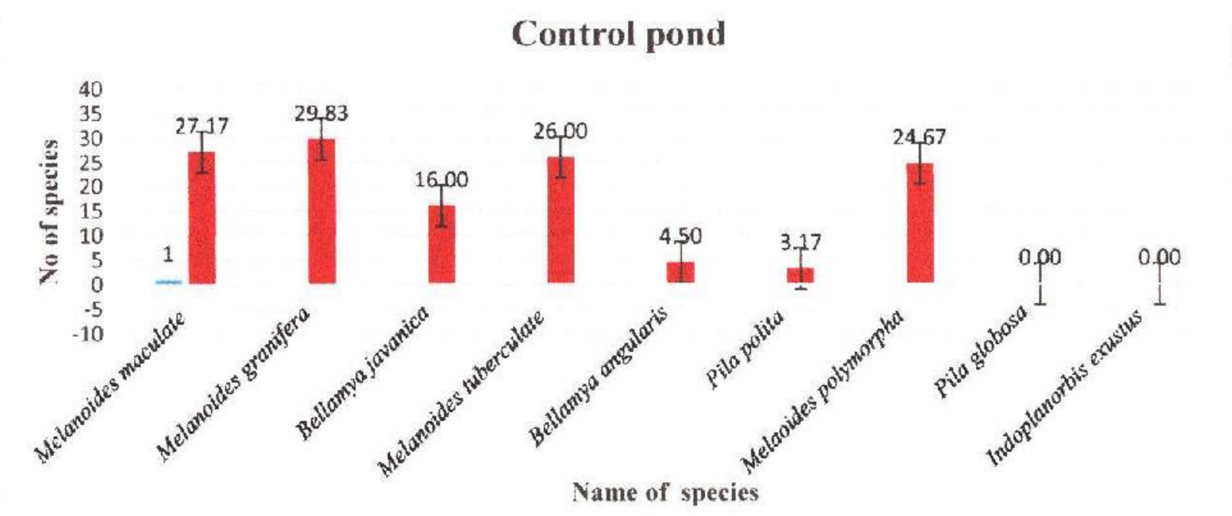


Figure 09: Variation of different benthos species in control pond

### 4.7.4. Quantification of macrobenthos in Treatment 1 pond

In Treatment 1 pond, *Melanoides maculate* was the highest in abundance and *Pila globosa* was the lowest in abundance, but *Indoplanorbis exustus* was not found (Figure 10).

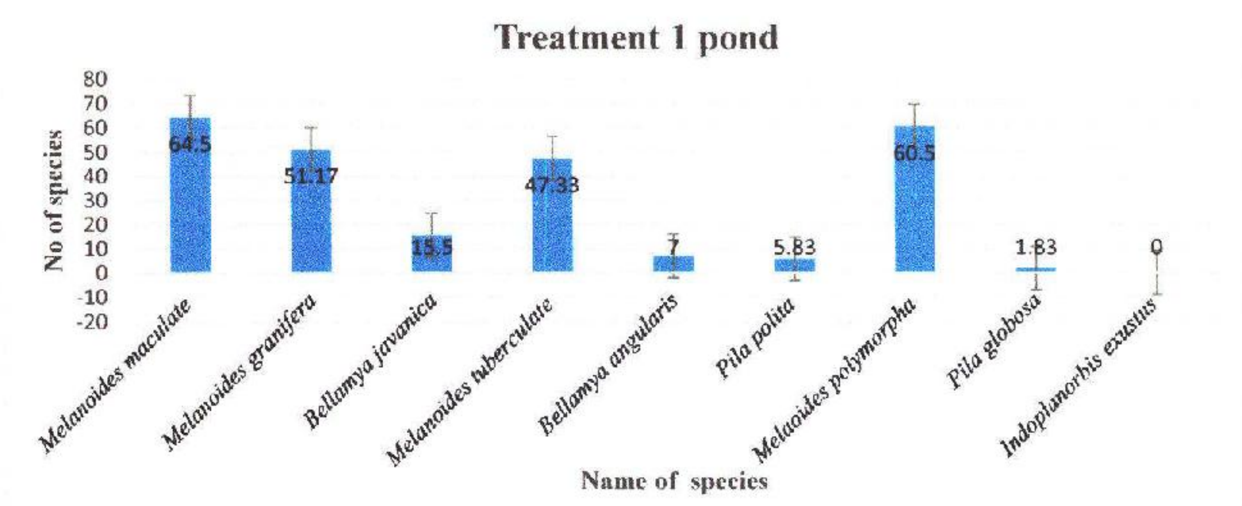


Figure 10: Variation of different benthos species in $T_1$ pond

## 4.7.5. Quantification of macrobenthos in Treatment 2 pond

In Treatment 2 pond, *Melaoides polymorpha* was the highest in abundance and *Pila globosa* was the lowest, but *Indoplanorbis exustus* was not found (Figure 11).

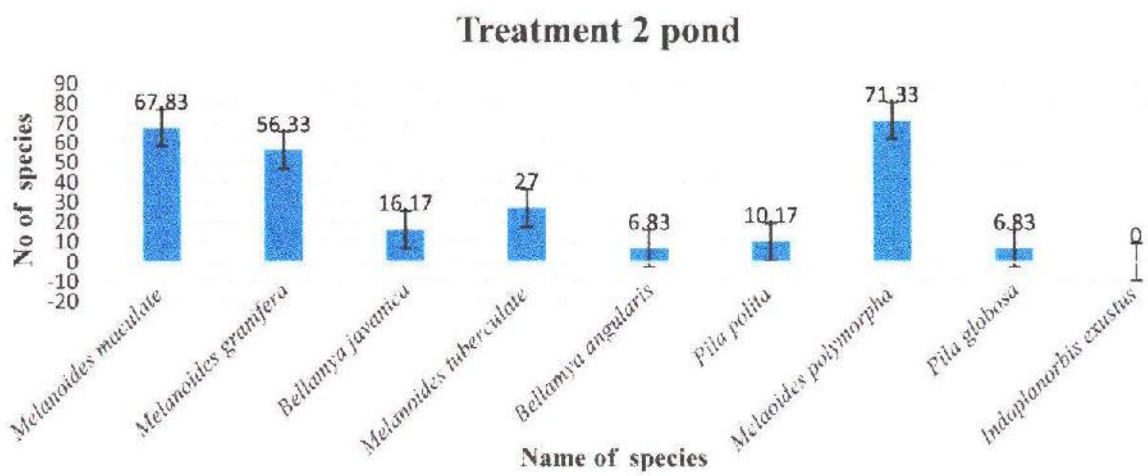


Figure 11: Variation of different benthos species in $T_2$ pond

### 4.7.6. Quantification of macrobenthos in Treatment 3 pond

In Treatment 3 pond, *Melanoides maculate* was highest in abundance and *Bellamya angularis* was lowest in abundance. But *Indoplanorbis exustus* was not found (Figure 12).

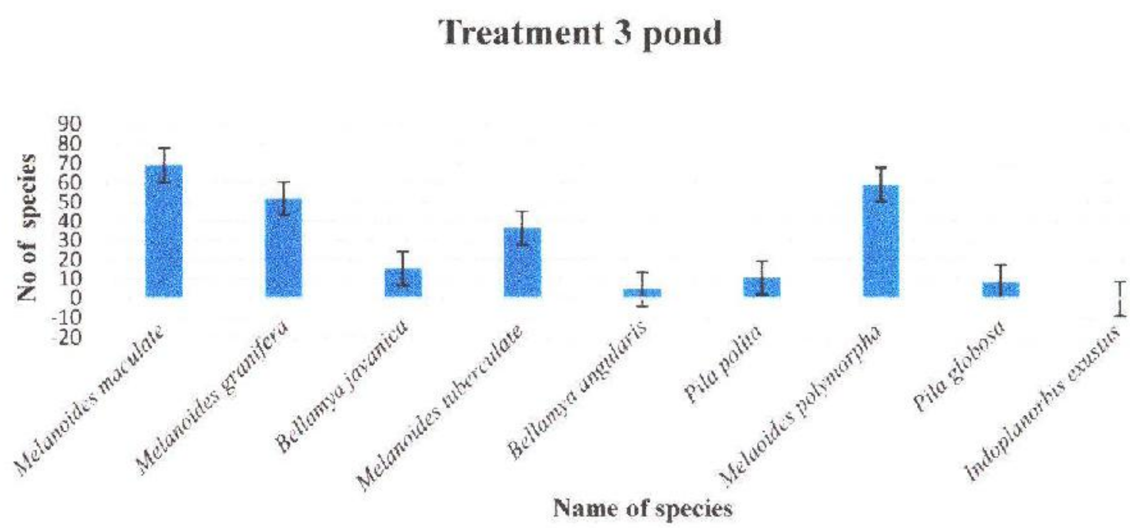


Figure 12: Variation of different benthos species in $T_3$ pond

### 4.7.7. Quanatification of macrobenthos in Treatment 4 pond

In Treatment 4 pond, *Melanoides maculate* was the highest in abundance and *Indoplanorbis exustus* was the lowest, but maximum number of species was recorded (Figure 13).

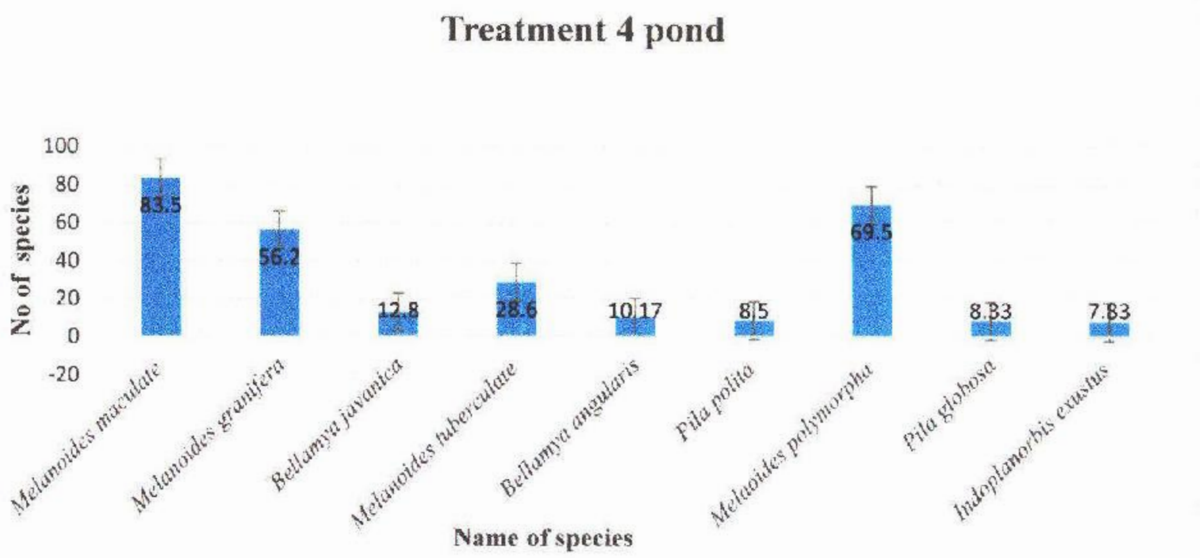


Figure 13: Variation of different benthos species in $T_4$ pond

# Chapter five

# Discussion

# 5. Discussion

## 5.1. Soil pH

pH is a crucial factor in determining the acidity and alkalinity of soil and aids in understanding the organic, biological, and indirect physical characteristics of soil, including both nutrients and toxins (Šlmek *et al.*, 2002). Based on the pH values which range from 0 to 14, soils are classified as acidic, neutral, or alkaline (basic). pH values more than 7.0 is alkaline, less than 7.0 is acidic, and 7.0 is neutral (McCauley *et al.*, 2009). Banerjea (1967) indicated that pond soils should have a pH between 6.5 and 7.5, while land soils should have a pH between 5.5 and 8.5. Zhou *et al.* (2009) supposed that the ideal pH range for fish growth and health is 6-9. Fish growth would be slowed down if the pH values were to exceed this level. When the pH value is greater than 10 or less than 4.5, death occurs (McLuckie *et al.*, 2020). A supply of hydrogen ions would be provided by the nitrification of ammonium ions created by the mineralization of organic matter, which might result in a drop of the pH in the soil (Matheyarasu *et al.*, 2016). The mean value of Soil pH was found to vary among the treatment ponds 7.88±0.19 to 7.95±0.13. Our results are more or less similar to the findings of Nupur *et al.* (2013).

## 5.2. Electrical conductivity (EC)

Electrical conductivity (EC) is a material's potential to transmit/conduct an electrical current and is usually expressed in milliSiemens per meter (mS/m) units. Measurements of soil EC can also be recorded in units of Siemens decimals per meter (dS/m), which is equivalent to the mS/m reading divided by 100 (Grisso *et al.*, 2005). The mean value of electrical conductivity was found to vary among the treatment ponds 4.22±1.60 to 4.40±1.75 dS/m. Similar result was reported in the findings of Ghosh *et al.* (2016).

## 5.3. Soil organic matter (SOM)

The most significant indicator of soil fertility is organic matter. Organic loads build up in the bottom of the pond as a result of unused feed, carbonaceous waste, fecal matter, dead plankton (Kumar *et al.*, 2016). In Bangladesh, the majority of soils have less organic matter than 1.5% and some have less than 1%, however a healthy soil should have at least 2.5% organic matter (BARC, 2005). The mean values of SOM in the present study was ranged from 2.93% to 3.19% among the treatment ponds, which is suitable fish culture. Aquaculture pond soil organic matter concentrations range from less than 1% in severely leached mineral soils in large aquaculture ponds to over 20% in ponds based on organic soils (Boyd, 1995). Aquaculture ponds are purposefully fertilized with organic manure, synthetic fertilizers, or agricultural wastes, which causes an accumulation of organic matter (Rengpipat *et al.*, 1998). More feed input, metabolites produced by aquatic animals, and further feed spills all contribute to an increase in the amount of organic matter in the marine environment (Boyd, 2017). Organic matter is broken down by microorganisms, either in aerobic or anaerobic processes, depending on the environment (Philben *et al.*, 2020). Similar result was reported by Nupur *et al.* (2013) and Islam *et al.* (2008)

## 5.4. Soil nutrients

The nitrogen content in the present study showed to vary among the treatment ponds from 0.160 to 0.170 which was more or less similar to the findings of Islam *et al.* (2008). Haque (2006) estimated the contents (0.1%-0.3%) nitrogen in the coastal region of Bangladesh which is higher than those obtained in the present study. The result showed high nitrogen content in control treatment 3 pond. This may happen because most of the nitrogen in bottom soil is contained in organic matter. Organic material is mineralized by heterotopic bacteria into ammonia that can be utilised by phytoplankton. Most of the nitrogen in soil is found in organic forms, which are then converted by microorganisms into simpler inorganic molecules (Geisseler *et al.*, 2010). Boyd *et al.* (1998) reported that reduction in nitrogen concentrations is caused by probiotics. The high content of nitrogen in the ponds bottom soil in control pond was possibly caused by the old age of ponds and the accumulation of inputs of nitrogen fertilizers and aqua feed containing nitrogen (Xia *et al.*, 2004). The current soil samples contained high levels of nitrogen that were adequate for fish productivity. Phosphorous promotes the growth of plankton and aquatic plants that fish eat, the element phosphorus is essential for the growth of plants and animals (Boyd and Musig 1981; Boyd, 1995). The mean value of phosphorus was found to vary from among the treatment ponds 8.53±0.4.39 to 9.94±03.81 μg/g. The current study showed higher phosphorus content than the findings of Ghosh *et al.* (2016). More or less similar results were also reported by findings of Nupur *et al.* (2013). The phosphorus content of pond soil should be 3-6 μg/g for optimal fish growth. More than 6 μg/g is considered to be productive for fish culture (Jahiruddin and Satter, 2010) which is very close to our findings. The decrease in phosphorus level suggests that phosphorus consumed during prawn growth was more readily ingested (Beristian, 2005). Unused food and organic metabolites are broken down by microorganisms, and minerals are produced via phosphorus and nitrogen (Fouz, 2018).

## 5.5. Macrobenthos

Species living on or in the sediment at the bottom of a body of water are referred to as benthic macrofauna (Odum, 1971; Barnes and Hughes, 1988; Idowu *et al.*, 2005). The term "macro-invertebrates" or "macro-zoobenthos" refers to large benthic organisms that are easily seen without the need of a microscope. They are a crucial and essential part of any aquatic environment (Hossain *et al.*, 2011). The macrobenthic fauna is important for the food chain, biological water cleaning and enhancing aquatic resources (Quasin *et al.*, 2009). Several macrobenthos groups including plankton, macrophytes, protozoa, fish and other creatures have been used as indicators of water quality or environmental changes in freshwater bodies (Chukuwu and Nwankwo, 2003; Atobatele *et al.*, 2005). The biotic and abiotic components of the freshwater environment are probably influencing the composition and abundance of macrobenthos. The biotic aspects included competition for resources like food and space, predation, reproduction and substrate preference, whereas the abiotic influences included temperature, air and light exposure, dissolved oxygen levels and natural disasters (Ramamurthy, 1953; Weyl and Peter, 1970).

In this study, 9 macrobenthos species were identified. These are belonging to four different genera. These were: i. Genus *Melanoides* (four species- *Melanoides maculate*, *Melanoides granifera*, *Melanoides tuberculate* and *Melanoides polymorpha)*; ii. Genus *Bellamya* (two Species- *Bellamya javanica* and *Bellamya angularis*) iii. Genus *Pila* (two species- *Pila polita* and *Pila globosa*) and iv. Genus *Indoplanorbis* (one species *Indoplanorbis exustus)* was identified. The variations of the quantities of macrobenthic organisms among the experimental ponds were likely to be influenced by the soil quality. Among the four different genera, *Melanoides* was found to be dominant. This dominance may be linked to favourable ecological conditions, such as a higher intake of nutrients from the sediments. Benthic organisms may be found in greater quantity as a result of the relatively soft surface and rich detritus or organic matter (Ahmed *et al.*, 1991). Pawar *et al.,* (2017) also found highest species diversity in rocky substratum where supply of the required food was higher. Fujioka *et al.* (2007) found higher diversity of macrobenthos species in the mangrove swamps than the aquacultural ponds because of the higher productivity in the mangrove area. Similar results were also reported by Syuja *et al.* (2018). While dissimilar results were found by Califano *et al.* (2020) in their respectively studies as well.

# Chapter Six

# Conclusion

# 6. Conclusion

The ranges of pH, organic matter (%), phosphorus (μg/g) and total nitrogen (%) of the bottom-soil quality parameters of the experimental ponds were within the productive ranges for the growth of macrobenthos during the experiment. However, such variation of soil quality could attributed be different stocking densities and cultured species of fish.

Benthic fauna is frequently used as biological indicators because they either have a general characteristic that allows them to integrate environmental signals over a long period of time. The present study revealed that microbenthic fauna and their abundance were variable among the experimental ponds. During the study nine different species of macrobenthos were identified. The experiment also revealed that stocking density and soil quality had influence on the abundance of macrobenthos. However, the data collected can be utilized to further analysis of the seasonal variations in macrobenthos and preserve their biodiversity as well as used as water quality indicators in freshwater pond.

# Chapter Seven

# References

# 7. References


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# Chapter Eight

# Appendix

# 8. Appendix

## Soil Quality Analysis

**ANOVA**

| | | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| pH | Between Groups | .075 | 4 | .019 | .519 | .723 |
| | Within Groups | .900 | 25 | .036 | | |
| | Total | .975 | 29 | | | |
| EC | Between Groups | 5.105 | 4 | 1.276 | .344 | .845 |
| | Within Groups | 92.625 | 25 | 3.705 | | |
| | Total | 97.730 | 29 | | | |
| OM | Between Groups | .250 | 4 | .063 | .169 | .952 |
| | Within Groups | 9.249 | 25 | .370 | | |
| | Total | 9.499 | 29 | | | |
| Nitrogen | Between Groups | .000 | 4 | .000 | .386 | .817 |
| | Within Groups | .007 | 25 | .000 | | |
| | Total | .008 | 29 | | | |
| Phosphorus | Between Groups | 13.256 | 4 | 3.314 | .181 | .946 |
| | Within Groups | 456.952 | 25 | 18.278 | | |
| | Total | 470.208 | 29 | | | |

# Benthos Analysis

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| *Melanoides_maculate* | Between Groups | 10597.867 | 4 | 2649.467 | 5.784 | .002 |
| | Within Groups | 11451.500 | 25 | 458.060 | | |
| | Total | 22049.367 | 29 | | | |
| *Melanoides_granifera* | Between Groups | 2940.133 | 4 | 735.033 | 5.910 | .002 |
| | Within Groups | 3109.333 | 25 | 124.373 | | |
| | Total | 6049.467 | 29 | | | |
| *Bellamya_javanica* | Between Groups | 293.867 | 4 | 73.467 | 1.261 | .312 |
| | Within Groups | 1456.833 | 25 | 58.273 | | |
| | Total | 1750.700 | 29 | | | |
| *Melanoides_tuberculate* | Between Groups | 1952.533 | 4 | 488.133 | 4.193 | .010 |
| | Within Groups | 2910.167 | 25 | 116.407 | | |
| | Total | 4862.700 | 29 | | | |
| *Bellamya_angularis* | Between Groups | 126.467 | 4 | 31.617 | 1.845 | .152 |
| | Within Groups | 428.500 | 25 | 17.140 | | |
| | Total | 554.967 | 29 | | | |
| *Pila_polita* | Between Groups | 225.867 | 4 | 56.467 | 2.956 | .040 |
| | Within Groups | 477.500 | 25 | 19.100 | | |
| | Total | 703.367 | 29 | | | |
| *Melaoides_polymorpha* | Between Groups | 7828.667 | 4 | 1957.167 | 9.038 | .000 |
| | Within Groups | 5413.500 | 25 | 216.540 | | |
| | Total | 13242.167 | 29 | | | |
| *Pila_globosa* | Between Groups | 363.533 | 4 | 90.883 | 8.933 | .000 |
| | Within Groups | 254.333 | 25 | 10.173 | | |
| | Total | 617.867 | 29 | | | |
| *Indoplanorbis_exustus* | Between Groups | 294.533 | 4 | 73.633 | 32.390 | .000 |
| | Within Groups | 56.833 | 25 | 2.273 | | |
| | Total | 351.367 | 29 | | | |

# Chapter Nine

## Photo Gallery

# 9. Photo Gallery

Experimental Pond

Figure: Gold plus nursery 1 feed (Left) Gold plus nursery 2 feed (Right)

Feed Bag

Bottom Sampling Dredge

Figure:Sieve Pan

Figure:Benthos Collection


Figure:Soil Resource Development Institute, Doulatpur ,Khulna