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De La Salle University - Dasmariñas GRADUATE STUDIES

HEAVY METALS AND PHYSICO-CHEMICAL ASSESSMENT OF WATER,

SEDIMENTS, AND Oreochromis niloticus (TILAPIA) IN MARAGONDON

RIVER, PHILIPPINES

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ABSTRACT

Maragondon River serves as source of water supply for domestic. agricultural, and industrial purposes which also provides livelihood among locals. However, anthropogenic activities might affect the guality of water. The study aimed to determine the physico-chemical characteristics of water and heavy metal concentration of sediments and tissues of Oreochromis niloticus (Tilapia) of Maragondon River in a three-month sampling period. Physical parameters were temperature and transparency while chemical parameters were pH, salinity, dissolved oxygen, phosphate, and nitrate. Physico-chemical assessment revealed that characteristics of water are all within the permissible limit set by DENR standards for Class B water. Both copper (Cu) and lead (Pb) showed no significant difference and within the Klokes Maximum Allowable Limit at 20-100 ppm. The heavy metal concentrations in the tissues of Orechromis niloticus (Tilapia) were able to pass the recommended limits joint by WHO/FAO, Food Standards Programme Codex Committee on contaminants in food. As a whole, Maragondon River can be best used for primary contact recreation such as bathing, swimming, and skin diving. Maragondon River can also be developed for aquaculture purposes and it is suitable for fish growth and propagation. However, regular monitoring must be done to reduce pollution level.





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CHAPTER 1

INTRODUCTION

Background of the Study

The Philippines is blessed with 421 principal river basins and as an archipelago it holds control of over 479 billion cubic meters from ground and surface water (DENR 2012). Rivers are one of the important surface freshwater resources which help in sustaining human life by serving as means of transportation, waterways and as source of water for industrial, domestic use, irrigation, and hydropower generation. However, anthropogenic activities greatly affect and have resulted in significant changes in the quality of water (Bakari 2014). The abuse of rivers through domestic, industrial disposal, and indiscriminate conversions of freshwaters into ecotourism sites deprive the population of more essential purposes of freshwaters (Martinez et al. 2011). The expansions of industrialization, exploding population, agricultural activities and municipal wastes have been considered as major sources of pollutants (Weldemariam 2013). Routine agricultural applications of fertilizers and pesticides are also recognized as significant sources of surface and ground water contamination (Krantz and Kifferstein 2005). Once in the ground water, the contaminants are difficult to remove and this causes persistent water quality problems (Altman and Parizek 1995).



Water plays an essential role in human life. Fresh water is one of the most important resources crucial for the survival of living beings. Human and ecological use of ground water depends upon ambient water quality (Gurunathan 2006). An understanding of water chemistry is the bases of the knowledge which involves the source, composition, reactions and transportation of water since it is directly linked with human welfare (Nagamani 2015). Physical and chemical parameters influencing the aquatic environment are temperature, rainfall, pH, salinity, dissolved oxygen, total suspended and dissolved solids, total alkalinity and acidity and heavy metal contaminants. These parameters are the limiting factors for the survival of aquatic organisms (Amier et al. 2015). The interactions of both the physical and chemical properties of water play a significant role in composition, distribution, abundance, movements and diversity of aquatic organisms (Mustapha and Omotosho 2005). The physicochemical characteristics of water are critical to evaluate the population and distribution of fish (Olalekan et al. 2015). The quality of water may be described according to their physico-chemical and micro-biological characteristics. Effective maintenance of water quality, appropriate control measures and continuous monitoring parameters is essential (Bhandari and Naval 2007).

Heavy metals like Zinc (Zn), Arsenic (As), Copper (Cu), Lead (Pb), Cadmium (Cd), Mercury (Hg), Nickel (Ni), and Chromium (Cr) are being



introduced to rivers either by natural means or anthropogenic activities through discharge from mining, domestic waste agricultural runoff and automobile exhausts (Hariprasad and Dayananda 2013). Toxic heavy metals are taken up by organisms and metals dissolved in water have the greatest potential of causing the most deleterious effects (Garbarino et al. 1995). Fishes remain the important member which often accumulates metals in different body parts and successive levels in biological chain (Akpor and Muchie 2010). Sediments can be sensitive indicators for monitoring contaminants in aquatic environments including heavy metals. These accumulate in sediments via disposal of liquid effluents, terrestrial runoff and leachate carrying chemicals from numerous urban, industrial and agricultural activities (Cohen 2003). Sediments act as scavenger agent, adsorptive sink, and an appropriate indicator of heavy metal pollution. The very fine silt/clay sediment has grain-size less than 63 µm and is considered the most geochemically active fraction of sediment particles that are suitable to gauge potential pollution of sediment by heavy metal (Idris et al. 2007).

Lead is being used as a colouring agent in paint which may accelerate environmental disturbances if the level is higher than the ambient level. Lead concentration might be due to painting of boats and spillage of lead rich gasoline during fishery product transport which accumulates in sediments for time due to its non-biodegradable nature (Kamaruzzaman et al. 2002).



Copper has become the most abundant metals in urban areas and in industrial waste and sewage which pollutes aquatic ecosystem in the form of particulate and dissolved materials. According to Kamaruzzaman et al. (2006) cleaning of boats and ships, ballasting, painting and repairing boats would proportionally increase Cu levels in the coastal environment. Copper and lead pollution is mainly related to mining, smelting, agrochemicals, industrial, and automobile emissions. By the early 1990s, 954,000 and 796,000 tons of Cu and Pb respectively were discharged into the world's aquatic ecosystem (Alloway and Ayres 1993).

Quarrying is a form of land use where non-metallic rocks and aggregates are extracted from land. Unfortunately, this activity can cause significant impact on the surrounding environment. In fact, the extraction process normally depends on heavy machines and explosives, where both processes are normally associated with air pollution, noise pollution, damage to biodiversity, habitat destruction and water pollution (Sayara et al. 2016). Quarry operation results to release of heavy metals in the soils and water which pose health risk (Nzegbule and Ekpo 2007).

Fishes has been utilized as bio-indicators of integrity of aquatic environmental systems in recent years (Tawarifufeyin and Ekaye 2007). Fishes are notorious for their ability to concentrate heavy metals in their muscles and since they play important role in human nutrition, they need to be



carefully screened to ensure that unnecessary high level of some toxic trace metals are not being transferred to man through fish consumption (Adeniyi and Yusuf 2007).

Oreochromis niloticus, locally known as Tilapia, is considered as one of the most critical freshwater species for commercial aquaculture because of its high nutritional qualities and quick development rate (Nakkina 2016). Studies revealed that heavy metals tend to accumulate *in O. niloticus* (Shafei 2015). Heavy metals which may be accumulated in the tissues of fishes are Aluminum (Al), Arsenic (As), Cadmium (Cd), Chromium (Cr), Mercury (Hg), Nickel (Ni) and Lead (Pb) pose health risk when consumed by humans (Hosnia and Mahmoud 2015).

The Municipality of Maragondon is a third class municipality in the province of Cavite, Philippines. It is located 54 kilometres south-west from Manila and is subdivided in 27 barangays. According to the 2015 census, it has a population of 37,720 people. One of essential freshwater resources of the municipality is the Maragondon River. It is one of the six major river basins of Cavite and one of the largest, with total area of 357 square kilometers. The river stretches from the upland barangays of the town and ends in Ternate, Cavite where it meets and opens up to Manila Bay. It serves as source of water supply for domestic, agricultural, and industrial purposes. Maragondon River provides livelihood among locals and as important source of food such



as *Brachyura* (crabs), *Oreochromis niloticus* (Tilapia), *Epinephelinae* (grouper), *Teuthida* (squid), *Thunnini* (tuna), and *Rastrelliger brachysoma* (hasa-hasa/mackerel). At present, part of the river is being utilized by local residents for recreation such as bathing, swimming, and floating nipa huts/balsa in Barangay Pinagsanhan-B are available for rent and are used for picnics (Mojica 2013). However, anthropogenic activities specifically piggeries in upstream, domestic wastes from residential area in the midstream and quarrying along the downstream might affect the quality of the water in Maragondon River. Based on DENR standards, Maragondon River has been classified as Class B. Facing the present human activities in the river, this study will determine if Maragondon River may still be used for recreation purposes such as swimming and bathing. It will also provide information for the policy regulation by the local government regarding protection of such river.



Statement of the Problem

Maragondon River is one of the six major rivers in the province of Cavite. It is classified as Class B by the DENR which is intended for recreational purposes such as bathing and swimming. However, at present there are quarrying, piggeries, and other human activities that are being conducted and may affect the quality of the water. This study determined if the intended purpose is still applicable because according to Huang et al. (2014) anthropogenic input related to industrial effluents and domestic wastewater, agricultural activities, and natural weathering process may cause changes in the water quality of Maragondon River by assessing the physico-chemical characteristics of water and heavy metal concentrations of sediments and tissues on *Oreochromis niloticus* (Tilapia).



Figure 1. The research paradigm.



Objectives of the Study

The objectives of the study were to:

- determine physico-chemical characteristics such as water temperature, transparency, salinity, pH, dissolved oxygen, nitrates and phosphates of Maragondon River.
- describe the morphometric characteristics of *Oreochromis niloticus* (Tilapia) collected from Maragondon River
- analyze the heavy metal concentrations of lead and copper in the tissues of Oreochromis niloticus (Tilapia) and sediments of Maragondon River.
- compare for the significant difference among sampling stations of Maragondon River in terms of the physico-chemical characteristics of water and heavy metal concentrations in sediments.
- determine the significant association between the physico-chemical characteristics of the water and heavy metal concentrations in the sediments of Maragondon River.



Scope and Limitations

The study focused on the determination of the physico-chemical characteristics of water, heavy metal analysis of sediments and Oreochromis niloticus (Tilapia). Physical parameters were limited to water temperature and transparency while chemical parameters include salinity, potential Hydrogen (pH), dissolved oxygen (DO) in mg/L, nitrates in mg/L and phosphates in mg/L. Heavy metal analysis was limited to determination of concentrations of copper and lead in the sediments of Maragondon River and fish tissues for 3 consecutive months (January, February, and March). Physico-chemical characteristics observed simultaneously with were the catching of Oreochromis niloticus (Tilapia) fish and collection of sediments in the area. Morphometric variables were limited to the total length, head length, body depth, snout length, eye diameter, and caudal peduncle length.

It is not the intention of the study to determine the relationship of the physico-chemical parameters with the heavy metal concentrations of lead and copper in the tissues of Oreochromis niloticus (Tilapia) of Maragondon River. Physiological effects of heavy metals in Oreochromis niloticus (Tilapia) would not also be covered in the study.



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This study provides relevant information on the physico-chemical characterictics of water and heavy metal analysis of sediments and tissues of *Oreochromis niloticus* (Tilapia) of Maragondon River. The results of the study can be used as an initial reference of data for concerned non-government organizations (NGOs), LGU's, other authorities such as Bureau of Fisheries and Aquatic Resources (BFAR), and the Municipal Environment and Natural Resources Office (MENRO) of Maragondon. Findings will be of great help to future researchers who want to conduct studies on the bodies of water affected by industrial, agricultural, and residential as major contributory factors of pollutants. Lastly, it will change ways, recalibrate minds, redirect steps and develop awareness among people that their micro efforts will soon have macro effect in preserving and conserving Maragondon River.



CHAPTER 2

LITERATURE REVIEW

Effects of Industrial Runoff

Industries are the major sources of pollution in all environments either direct or indirect which include sanitary waste, process wastes from manufacturing, human sewage, pulp and paper industries, slaughter houses, tanneries and chemical industry. High levels of pollutants in river cause an increase in biological oxygen demand, chemical oxygen demand (COD), total dissolved solids, total suspended solids, toxic metals such as Lead, Cadmium, Chromium, Nickel, and fecal coliform make water unsuitable for drinking and for aquatic life (ljeoma 2011). Industrial wastes are released in an ad hoc manner to the environment due to lack of properly equipped plants and sanitary dumping sites operated within the required standards. There is 10% of waste water generated being treated while the rest is discharged into the water bodies (Satter and Islam, 2005). Untreated waste water from industries discharge on surface water indicates that industrialization on the U-tapao River in Thailand basin has contributed to the large scale pollution and this polluted water is not good for human consumption. It is therefore recommended that the disposal untreated wastes should be stopped to save the river water from further deterioration (Gyawali et al. 2012).





Excess use of fertilizers and pesticides in agricultural activities to enhance productivity due to rapid population increase and development of technology threaten the groundwater and surface water on a large scale (Hariprasad and Dayananda 2013). Nutrient concentrations in river systems constitute the keystone of biological productivity and status of aquatic systems. The changes in nutrient concentrations in river systems are often more determined by anthropogenic activities that include discharge of waste inputs, sewage, runoff from the heavily fertilized fields and siltation in the surrounding areas than by natural processes of weathering, and in-situ eutrophication (Carmago et al. 2005).

Heavy metals

Bioaccumulation and magnification is capable of leading to toxic level of metals in fish, even when the exposure is low. The presence of metal pollutant in fresh water disturbs the delicate balance of the aquatic systems. Fishes are notorious for their ability to concentrate heavy metals in their muscles and since they play important role in human nutrition, they need to be carefully screened to ensure that unnecessary high level of some toxic trace metals are not being transferred to man through fish consumption (Adeniyi and Yusuf 2007). For normal metabolism of fish, the essential metals must be taken up from water, food, or sediment. However, non-essential metals like lead are



also absorbed and accumulate in the tissues of fish. Studies have shown that the accumulation of heavy metals in fish tissue is mainly dependent upon concentrations of the metals in surrounding water. Ecological needs and the size of aquatic animals have also been found to affect their inclination towards metal accumulation. Therefore, it is important to better understand the relationships between animal size and the concentrations of heavy metals (Yia and Zhang 2012). A study conducted in Butuanon River in Cebu showed that the highest concentrations of heavy metals recorded were copper (Cu), Lead (Pb), and Zinc (Zn) due to heavy pollution as sink of domestic and industrial wastewater of the city (Paler et al. 2014). Heavy metal (Cd, Cr, Cu, Hg, Pb, Zn) concentrations were identified in the muscle tissue of seven fish species in Yangtze River were measured. The highest concentrations of Cu (1.22) and Zn (7.55) were measured in yellow-head catfish. The catfish also showed strikingly high Cd (0.115) and Hg (0.0304) concentrations. The crucian carp and common carp showed highest levels of Pb (0.811) and Cr (0.239), respectively (Yia and Zhang 2012). Similar findings were observed that heavy metals (Cu, Zn, Pb and Cd) level in sediments were much higher at Port Klang and Port Dickson areas which ships and boats pass through the causeway everyday (Ismail and Yap 2001).



Lead

Lead is a naturally occurring element found in small amounts in the earth's crust. It is being used as pigment for glazing ceramics, pipes and plumbing materials, gasoline, batteries cosmetics roofs, paints, and ammunition (USGS 2015). It is emitted into the environment from industrial sources and contaminated sites such as former lead smelters mining and refining activities which resulted in substantial increase in lead levels in the environment. Exposure to lead can lead to cardiovascular effects, increased blood pressure and hypertension, decreased kidney function and reproductive problems (EPA 2017).

Copper

Copper is also a naturally occurring element in the environment. It is ideal for electrical wiring because it can be drawn into fine wire and has high electrical conductivity. Copper is released both naturally and through human activities such as mining, metal production, and phosphate fertilizer production. It is often found near mines, industrial settings, landfills, and waste disposals. Most Copper compounds will settle and be bound to either water sediment or soil particles which cause threat to human health. Industrial exposure to copper fumes, dusts, or mists may result in metal fume fever with atrophic changes in nasal mucous membranes. Chronic Copper poisoning results in





Physical Parameters

Water Temperature

Water is an essential requirement of human life and activities associated with industry, agriculture, and others. Water temperature is a physical property expressing how hot or cold water is. One important aspect of water temperature is its effect on the solubility of gases, such as oxygen. More gas can be dissolved in cold water than in warm water. Increased water temperature can also increase in the photosynthetic rate of aquatic plants and algae. This can lead to increased plant growth and algal blooms, which can be harmful to the local ecosystem. Change in river water temperature has important consequences for the environment and people (Garner 2013). A change in water temperature of only 2°C has been shown to stimulate the metabolism, appetite, and growth of some stream fish by 30-60% (Morgan et al. 2001). Fish are vulnerable because the body temperature of fish varies with the ambient temperature due to their ectothermic nature, affecting physiological processes as a result (Pang et al. 2011). When water temperature becomes too hot or too cold, organisms become stressed, lowering their resistance to pollutants, diseases, and parasites. Fish are believed to be among the animals that could be affected to the greatest degree



by climate change (Morgan et al. 2001). Fish regulate body temperature because of the optimal temperature that they need to survive. Every species has an optimal temperature range at which they can be active, grow, reproduce, and metabolize (Dodson 2005).

Transparency

Transparency is how easily light can pass through a substance. In lakes, this means how deep sunlight penetrates through the water. Water transparency depends on the amount of particles in the water. Particles can be inorganic (e.g. sediment from erosion) or organic such as algae, phytoplankton (Citclops). One of the primary requirements of submerged plant community restoration is to improve the water transparency. Light field below the water surface is a significant factor of the aquatic ecosystem, which has direct effect on the growth of submerged plants (Lian et al. 1996). The low water transparency hinders the photosynthetic activity and results in the death of submerged plants (Gaevsky et al. 2002). Water transparency is an important parameter describing the optical properties of the water body and a key index for assessing the eutrophication status of the water body, and it can visually reflect the degree of clarity and turbidity of the water body (Zhang et al. 2003). The water transparency reflects the projection deepness of the sunbeams, it is closely related to solar radiation, physical and chemical properties of water body, composition and content of suspended sediment, weather condition and



so on, and it is influenced by various environmental factors (Figueroa et al. 1997).

Chemical Parameters

Potential Hydrogen (pH)

The pH of river water is the measure of how acidic or basic the water is on a scale of 0-14. It is a measure of hydrogen ion concentration. The optimum pH for river water is around 7.4. Water's acidity can be increased by acid rain but is kept in check by the buffer limestone. Extremes in pH can make a river inhospitable to life. Low pH is especially harmful to immature fish and insects. The livable pH range is from 5.5 to 10 (Moyle 1993). A low pH can result in death as well as a variety of more subtle effects. Values less than 6 can result in a marked decrease in some fish oogenesis, egg fertility or growth of fry, or egg hatchability and growth (Matthews 1998). Matthews (1998) also discovered that in spite of the possible stress of water with a low pH, most fish failed to discriminate between the ranges of 5.5 to 10. The most productive waters, however, are those that are slightly alkaline (pH 8) (Moyle 1993). Dissolved oxygen and pH affects directly or indirectly other limnological parameters such as transparency, viscosity, total dissolved solids and conductivity, all of which constitute the very important physical and chemical parameters that form the basis for an enlightened fisheries and water resources management (Araoye et al. 2007).



Salinity

Salinity is the measure of all the salts dissolved in water. Salinity is usually measured in parts per thousand. There are many different dissolved salts that contribute to the salinity of water. Most lakes and rivers have alkali and alkaline earth metal salts, with calcium, magnesium, sodium, carbonates and chlorides making up a high percentage of the ionic composition. Freshwater usually has a higher bicarbonate ratio while seawater has greater sodium and chloride concentrations (Fondriest nd). The average ocean salinity is 35ppt and the average river water salinity is 0.5ppt or less. Salt enters aquatic systems from groundwater, terrestrial material via the weathering of rocks or from the atmosphere, transported by wind and rain (Baldwin 1996). Evaporation, combined with intrusions of groundwater often caused natural salinity levels to be high for periods of time (Kay et al. 2001). Salinity affects the types of organisms and kinds of plants living in bodies of water. Increase in salinity may affect fishes as salt in the bodies of water is greater than their body. Fishes may continuously lose water through osmosis to have equal concentration of salt inside and outside their body (Shere 2012).

Dissolved Oxygen

Dissolved oxygen is a measure of how much oxygen is dissolved in the water. According to U.S. Geologic Survey (USGS), the oxygen dissolved in lakes, rivers, and oceans is crucial for the organisms. When dissolved oxygen



drops below 5.0 mg/l, the water quality is harmed and creatures begin to die off. Water body can "die", a process called eutrophication (USGS 2016). Adequate dissolved oxygen is necessary for good water quality. Oxygen is a necessary element to all forms of life but may fall if the water is polluted. Oxygen levels that remain below 1-2 mg/l for a few hours can result in large fish kills. Scientific studies suggest that 4-5 parts per million (ppm) of DO is the minimum amount that will support a large, diverse fish population. The DO level in good fishing waters generally averages about 9.0 parts per million (ppm). Dissolved oxygen depletion could suppress respiration, depress feeding or affect embryonic development and hatching, reproductive failure, stock-recruitment failure at the population level or changes in the composition, abundance and diversity of species at the community level. Natural stream purification processes require adequate oxygen levels in order to provide for aerobic life forms (Clark 1996).

Nitrates

Nitrate is a common contaminant of surface water and groundwater and it can cause health problems in infants and animals as well as eutrophication of water bodies. High concentrations of nitrates and phosphates lead to eutrophication of water bodies (Taylor et al. 1997). Such environmental problems are increasingly occurring on a worldwide basis and now affect marine as well as freshwater ecosystems (Jens et al. 2000). As nitrates and



phosphates are added to water bodies, they can lead to overgrowth of plant life, which in turn, leads to depletion of dissolved oxygen; which may through their effects on the aquatic life and vegetation, be transmitted to humans (Wolfe and Patz 2002). The World Health Organization and the U.S. Environmental Protection Agency have established a maximum contaminant level for nitrate of 10 mg/L as NO3---N in drinking water. Many studies have shown that agricultural activities are a significant source of surface and ground water pollution due to long-term and excessive fertilizer use (Zhang 2015). Nitrogen fertilizers are applied extensively in agriculture to increase crop production, but excess nitrogen supplies can cause air, soil, and water pollution. Arguably one of the most widespread and damaging impacts of agricultural over application of nitrogen fertilizers is the degradation of groundwater quality and contamination of drinking water supplies, which can pose immediate risks to human health (Schroeder et al. 2004). Researchers have identified several factors affecting nitrate groundwater contamination, including fertilizer levels and build-up of soil organic matter, which can result in a large mineral nitrogen pool and thus in a higher risk of nitrate leaching (Sieling and Kage 2006).

Phosphates

Phosphorus is a common constituent of agricultural fertilizers, manure, and organic wastes in sewage and industrial effluent. It is an essential element



for plant life, but too much of it in water can result in eutrophication of rivers and lakes. Soil erosion is a major contributor of phosphorus to streams. Phosphorus tends to attach to soil particles and moves into surface-water bodies from runoff (USGS 2016). Unlike nitrogen, phosphate is retained in the soil by a complex system of biological uptake, absorption, and mineralization. Eutrophication problems are caused by high nutrient (nitrogen, N, and phosphorus, P) fluxes from population pressure and intensive agriculture. For watershed management, P is regarded as the primary limiting nutrient for nuisance algal growth in freshwaters (Smith and Schindle 2009).

Oreochromis niloticus (Tilapia)

Oreochromis niloticus locally known as Tilapia is considered as one of the most critical freshwater species for commercial aquaculture because of its high nutritional qualities and quick development rate (Nakkina, 2016). Tilapia is freshwater fish that belongs to the family Cichlidae. According to FAO (2004), tilapias (*Oreochromis* sp.) are among the most cultured fish worldwide after the carps (Ezzat et al. 2016). Its reputation for fast growth and ability to grow under wide range of environmental conditions has assisted its adoption in many countries. The global production of tilapia was 4.85 million tonnes in 2014 and is expected to exceed 7 million tonnes by 2030 with Nile tilapia (*Oreochromis niloticus*) representing more than 70% of the total production (Mekkawy et al. 2017). Tilapia meat has good organoleptic characteristics, with



a fillet yield of up to 33% (Borghesi 2006). But, *Oreochromis niloticus* is one of the aquatic organisms affected by heavy metals, so it was frequently used as a metal biological marker in toxicological studies (Rashed 2001). Studies have shown that accumulation of heavy metals in fish tissues is mainly dependent on concentration of metals in water and exposure period (Jovanovic et al. 2011).

Related Studies

There are several studies conducted on physico-chemical characteristics on heavy metals both local and international. In Philippines, Mercurio (2016) studied the physico-chemical characteristics of aquaculture and non-aquaculture in Taal Lake. Transparency, temperature, pH, phosphates, nitrates, salinity, total dissolved solids, and dissolved oxygen were observed in a 10-month period. Results revealed that nitrates, phosphates, and total dissolved solids were observed in an area with fish cages compared to aquaculture sites.

Revilla (2016) conducted heavy metals analysis in sediments and tissues of *Oreochromis niloticus* in Boac River, Marinduque. Cu concentration in sediments showed significant differences while Pb showed no significant difference and within the Klokes Maximum Allowable Limit at 100ppm. Cu and Pb passed the recommended limits by WHO/FAO, Food Standards Programme Codex Committee on contaminants in food.



Yacoub and Gad (2012) conducted heavy metal analysis in tissues (gills, intestine and muscles) of *Oreochromis niloticus* collected from different sites of the River Nile at Upper Egypt. Using the SHIMADZU atomic absorption spectrophotometer model AA- 6800, results revealed that the abundance of heavy metals in fish organs followed the order Mn>Zn>Pb>Cu. The highest level of heavy metal was recorded in the intestine and the lowest in the muscles. The concentrations of Cu in the fish muscle were below the maximum permissible limit while Mn, Pb and Zn exceeded the permissible limits.

Mohsien and Mahmoud (2015) evaluated heavy metals Cd, Pb, Cr, and Al in muscle of fresh water fish *Oreochromis niloticus* from ten provinces in Egypt. The analyzed metals were detected in order as Pb>Cr>Cd>Al. The concentrations of Pb, Cd and Cr in fish samples were several times higher than their concentration in water and the bioaccumulation Factor (BAF) ranged from 8.22 - 122.6. The estimated weekly intake of Cd, Pb and Cr for a 70 kg person consuming fish in Egypt (7.94, 15.84 and 9.8 µg) is well below the Provisional Permissible Tolerable Weekly Intake (PTWI) recommended by FAO/WHO. Although heavy metal levels in Egypt exceed the maximum permissible limits recommended by Egypt and WHO in some fish samples, the consumption of Nile *O. niloticus* from Egypt is safe on human health.



Another study was conducted by Sangoremi (2013) on Tilapia (*Oreochrornis niloticus*) and Cat fish (*Clarias gariepimus*) Ala River, Nigeria using their different organs such as gills, scale and flesh. The value of the heavy metals ranges (mg/100g), Fe (33.4-81.5 mg/l00g), Zn (8.3-20.5 mg/l00g), Cu (0.3-0.8 mg/100g), Mn (19.0-65.0 mg/l00g), Pb (0.3-1.5 mg/100g). The highest value of Pb was found in Tilapia Fish Flesh (1.5 mg/100g) while the lowest value was found in Cat Fish Flesh (0.3 mg/l00g). This may be attributed to the level of metals in soil sediment and the water in which they lived. Fish species that appeared to be capable of bioaccumulating heavy metals with low concentration are still good for human consumption.

Moreover, Abowei (2009) studied the condition of in terms of salinity, dissolved oxygen, pH and surface water temperature in Nkoro River, Nigeria for one year. Dissolved oxygen and temperature were measure using OxyGuard Handy MK II, pH by pH meter, and salinity by salinometer (model New S-100). Dissolved Oxygen values ranged from 3.2±0.1 (mg/l) to 7.3±0.16 mg/l, pH values ranged from 7.3±0.17 to 7.7±0.14 and temperature values ranged from27.3±0.24 to33.7±0.21. There was no significant difference in salinity and pH between stations, but dissolved oxygen, and temperature showed significant differences between stations (P#0.05). The results showed significant correlation between variables which means that Nkoro River was similar from the same source from Atlantic Ocean.



CHAPTER 3

METHODOLOGY

Research Design

The study utilized both the quantitative and qualitative methods for data collection and analysis. Physico-chemical analysis of water, heavy metal analysis of sediments and tissues of *O. niloticus* were determined using the established standard procedures. Quantitative data on water quality parameters and metal concentrations among sites were compared using the one way-ANOVA and Tukey's HSD test as post hoc test. In addition, the correlation of the physico-chemical parameters of water and heavy metal concentrations in sediments was determined using the Pearson's r.

Study Site

Maragondon River is located in the municipality of Maragondon, Cavite. It is one of the largest with total area of 357 square kilometers with and irrigable area of around five percent (5%). It receives a total of 850,000,000 cubic meters of water annually. This river, together with Salipit stream (Bucal 1 – Kapantayan) and Mambog River in Barangay Pinagsanhan and Malibiclibic River in Tulay serves as sources of water supply for domestic, agricultural and industrial purposes. Maragondon River is still a healthy habitat for aquatic life such as shrimp, seaweeds, and fish species include tilapia, mackerel, nememterids, grouper, squid, tuna and hasa-hasa (Mojica 2013). The river is



being used for bathing, fishing, and floating balsa with nipa huts for picnics. The waste may be carried to the river due to the following activities. In this study, three sampling sites were included: the Upstream (located at Brgy. Mabato), Midstream (located at Brgy. Bucal IV-A and Downsteam (located at Brgy. Pinagsanhan). Each of the sampling sites had three substations of approximately 100 meters distance from each other. Coordinates of the stations were recorded using GPS (Garmin 010-00631-00 model e Trex H. portable GPS Receiver) unit. Field tape was used to determine the three substations which are 100 meters away from each other. Each station was divided into substations. All of the 9 substations were gathered with physico-chemical measurements. Measurements were recorded in triplicates.

Collection Sites

Brgy. Mabato (Upstream)

The following were the coordinates of the 3 substations: N 14°14' 41.3"N E 120°46' 54.7"E (Substation1), 14°14' 44.9"N 120°46' 55.6" E (Substation2), 14°44' 45.6"N 120°46' 56.7"E (Substation3). The station was located in the upper most part of Maragondon River (Appendix A, Figure 2). The river is surrounded by different plants like bamboo, mango trees, and other shrubs which gives a relaxing and soothing feeling. The river has falls which makes it more attractive to the residents. The water is clear and cold which is suitable for swimming, bathing, and source of food since fishes and


other freshwater organisms are present. It is also a source of income for the residents since bamboo and nipa huts along the sides and in the middle part of the river are available for rent. Sediments and fish samples were taken at the middle part of the river same with the exact location of the station.

Brgy. Bucal IV (Midstream)

Brgy. Bucal IV was the estimated middle part of the river. The coordinates of the 3 substations are: 14°16' 20.4"N 120°45' 35.9"E (substation1) 14°16' 22.4" N 120°45' 36.6"E (substation 2), 14°16' 23.7" N 120°45' 37.1" E(substation 3). The water is also clear, refreshing, and deep in some parts of the river. The residents are also using the river for swimming, laundry, fishing, and for income since floating Nipa huts are available for rent and are perfect for picnic lunches especially during summer season. Unlike Brgy. Mabato, Brgy. Bucal is not surrounded by bamboos and trees which makes it more exposed to sunlight. Sediments and fishes were collected in the area.

Brgy. Pinagsanhan (Downstream)

The coordinates of the 3 substations in station 3 were: 14°15' 54.5" N 120°42' 45.6" E(substation 1), 14°15' 55.3"N 120°42' 44"E (substation 2), 14°15' 55.9" N 120° 42' 42.5 " E (substation 3). Brgy. Pinagsanhan was almost part of the Ternate, Cavite. It is located in the down part of Maragondon River.



Trees and other shrubs surround the river which give a relaxing feeling. The water in the upper part of the station is clear but becomes murky as one goes along the down part of the river where quarrying is ongoing. Mud on the sides of the river is slippery compared to the normal mud. Sediments and fish were collected in the area.

Collected samples were brought to DLSU-Dasmariñas Laboratory. Drying of sediment was done in an oven and ashing of *O. niloticus* tissue was done using controlled furnace (Nabertherm). Heavy metal analysis of the sediments and tissue of *O. niloticus* were done in Chemistry laboratory using Atomic Absorption Spectrophotometer (Shimadzu AA-7000).

Field Collection

Measurement of the Physico-chemical Characteristics of Water, Collection of Tilapia and Sediments in Maragondon River (Umaly and Cuvin 1988)

Determination of physico-chemical characteristics as well as collection of sediment and *Oreochromis niloticus* (Tilapia) was conducted once every last week of the month from January 2018 to March 2018. Physico-chemical characteristics were observed in-situ, simultaneous with the fishing of *Oreochromis niloticus* (Tilapia) fish and collection of sediments in the area.

The physical parameters were limited to water temperature and transparency while chemical parameters include salinity, pH, dissolved oxygen



(mg/L), nitrates (mg/L0, and phosphates. The water temperature was measured using a thermometer. Transparencies of the water were determined using a Secchi disk. The depths at which the disk will disappear and reappear were measured and averaged.

Dissolved oxygen of the water was determined by dipping the probe of the DO meter (YSI 550) into the surface not more than deeper 1 meter below the water for one minute until the reading is stabilized. Similarly, the probe of the SCT meter (ESI 300) was dipped in the water for one minute to determine the pH and salinity. The nitrates and phosphates were measured using the colorimeter (HACH DR/890). The nitrate and phosphate contents of the river water were measured using nitrates and phosphates reagents tested in a 10ml vials collected from each sampling station.

Simultaneous with the measurement of physico-chemical characteristics were the collection of sediments from each substation of upstream, midstream, and downstream. Grab sampler was used to collect sample of sediments in each station. The samples were placed in zip lock for preservation and were labelled accordingly.

Collection of Tilapia

A total of 9 samples of *Oreochromis niloticus* (Tilapia) (3 in each station) for every sampling for three consecutive months were collected from the



upstream, midstream, and downstream of the river. Local fishermen were hired to collect samples of the fish in the stations. Samples were packed in zip locks and labelled accordingly. The samples were transported in an ice chest to the DLSU-D Laboratory. Morphometric measurements started immediately before shrinkage starts. Each specimen was given serial identification number after the water was drained off using filter paper. The morphometric features analyzed the total length (TL), head length (HD), body depth (BD), snout length (SnI), eye diameter (ED), and caudal peduncle length (CPL). All measurements were taken using a ruler and measurement was determined to the nearest centimetre. Body parts were measured with the head pointing left. The size, length, and weight of the fishes were also determined.

Preparation of Fish samples (Environmental Bureau Laboratory, 1995)

The weight of the fish was determined before it was digested. Fish was placed in a dissecting pan. Using a scalpel or knife, pectoral of the fish was removed and skin was cut near the dorsal fin starting from the head to tail. Gills were cut across the body along the ventral edge from the gills to the tail. The skin was pulled from flesh with the aid of tweezers. Precaution is important to have a minimal contamination of outer skin with the flesh of the fish. Fillet was cut from the vertebral column starting from the cut near the gills with a clean knife. Pair of tweezers was used to lift the fillet in a way that it





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would not touch the other parts of the flesh. Samples were placed in a clean plastic sampling vial to determine the total weight.

Preparation of Sediment Samples (EPA) Method 3010A)

Before heavy metal analysis, sediment samples were dried in an oven at 105°C for 4 hours or until it was fully dried. After drying, sediment samples were sieved using the Market Grade Sieve (size 60), weighed (5 grams) using the analytical balance (Shimadzu AUW220D), and placed in a 150mL beaker in 3 trials.

Preparation of the Reagents and Solutions for Acid Digestion of Sediments and Fish Tissues (EPA Method 3010A)

The working solution was prepared in reference to USEPA 3010A. A ratio of 3:1 of analytical-reagent grade (70%) nitric acid (HNO₃) and analytical-reagent grade (70%) hydrochloric acid. HCl was prepared in a 500 mL (PYREX) Erlenmeyer flask. For the acid digestion of one sample, a ratio of 15 mL to 5 mL of HNO₃ and HCl was mixed into the flask under the fume hood, and with safety gear. Preparations were done in batch of replicates.



Heavy Metal Analysis of Fish Samples (EPA Method 3010A)

About 5 grams of fish muscle was placed in a crucible and marked corresponding to the samples in it. It was then transferred in a controlled furnace (NABERTHERM) slowly preheat 110°C to 125°C up to 500°C until it became white ash. Under the fume hood, the samples were mixed with HNO₃ and HCl solution. A 20 mL of the solution was poured into individual crucibles. This was done on each of the replicate batch. The crucibles were covered with lid covers. The digested solutions were heated on Corning hot plate for 1 hour at 110°C. Simultaneously, 50 mL volumetric flasks were prepared with filter paper-lined glass funnels to sieve the digested solution. The volumetric flasks were labelled based on the specimens heated up. After 1 hour of heating, digested solutions were poured out into the prepared volumetric flask with filter paper. After sieving the solution, deionized distilled water was slowly added to reach the final volume of 50 mL. The solutions were filtered using ordinary filter paper to avoid instrument clogging.

Heavy Metal Analysis of Sediment Samples (EPA Method 3010A)

Sediments were digested in 20 mL aquaria (1:3 of HNO_3 and HCI respectively) in a hot plate for 1 hour under 90°C. Simultaneously, 50 mL volumetric flasks were prepared with filter paper-lined glass funnels to sieve the digested solution. The volumetric flasks were labelled based on the



specimens heated up. After one hour of heating, digested solutions were cooled and poured out into the prepared volumetric flask with filter paper. After sieving the solution, deionized distilled water was slowly added to reach the final volume of 50 mL. The solutions were filtered using ordinary filter paper to avoid instrument clogging.

Data Gathering and Statistical Analysis

The physical characteristics of the river water such as temperature and water transparency were carried out using the SCT meter and Secchi Disk. Chemical characteristics of water like pH, dissolved oxygen, nitrates, and phosphates were carried out using the DO meter and calorimeter (HACH DR/890). The data was compared with the DENR standards for Class B waters and with previous studies on condition of Maragondon River.

The readings of Cu and Pb concentration in sediments and fishes were carried out after all the samples were prepared into digested solutions. The Shimadzu AA-7000 Atomic Absorption Spectrophotometry was used to analyse the total Cu and Pb concentration in sediments and fish tissues. The analyses of the solution standards and sample were carried out in triplicates.

Single factor ANOVA was used to determine the significant differences between physico-chemical characteristics of water and lead and copper concentrations in sediments among stations. Tukey's Test as post-stat test



was used among samples with significant differences. Pearson r correlation coefficient was used to determine the correlation of physico-chemical characteristics of water toward heavy metals on sediments. Gathered data of the physico-chemical characteristics of water of Maragondon River was compared to the DENR standards for Class B water, heavy metals in sediments to Kloke Guideline Values, and heavy metals of tissues in WHO/FAO Maximum Tolerable Intakes 2011.



CHAPTER 4

RESULTS AND DISCUSSION

Assessment of Water Quality

In terms of temperature, the highest mean value is the upstream and lowest in the downstream which is 28.62 °C and 27.48°C respectively (Table 1). However, statistically there is no significant difference among the stations. The recorded water temperatures from the three sampling stations were within the permissible limit set by DENR since there was no recorded increase of more than 3 °C. Whereas for transparency, the highest mean value 0.76 m is in the midstream and lowest 0.26 m in the downstream. Statistically, there is a significant difference among the sampling stations.During the sampling, no visible algae growth and runoffs were observed. Khan and Chowdhury (1994) reported that higher transparence occurred during summer due to absence of rain, runoff and flood water as well as gradual settling of suspended particles.

The results of temperature of Maragondon River is comparable to the study conducted by Maglangit et al. (2014) in Bulacao River, Cebu wherein temperature readings from 28.05°C – 29.53°C were not significant among the sampling stations. It is also similar to the study of Maglangit (2014) in Buhisan River, Cebu where water temperature from upstream to downstream was not significant. It means that the present



temperature readings will not cause stress to aquatic ecosystems since at this temperature range the water still maintains its ability to hold essential dissolved gases like oxygen (Lawson 2011). Touchart et al. (2012) reported that water holds more oxygen which supports more aquatic organisms if it is cold. Contrary, as temperature rises, the rate of photosynthesis by algae and larger aquatic plants also increases. The faster the plants grow, the faster the plants die and so the decomposition of bacteria that will consume the oxygen. As a result, increasing photosynthesis will decrease the level of dissolved oxygen in the water (Maxim et al., 2011). Other factors that contributed to the temperature variations include season, water depth, air temperature, amount of shade, and thermal pollution from human activities (Chapman 1996).

Table 1. Physical Characteristics of Water

PHYSICAL PARAMETERS	Units	UPSTREAM	MIDSTREAM	DOWNSTREAM	MEAN AVERAGE	DENR STANDARDS
Temperature	°C	28.62 ^A	27.97 ^A	27.48 ^A	28.03	3°C
Transparency	m	0.73 ^A	0.76 ^A	0.26 ^B	0.59	_

The superscripts (A and B) indicate significant difference (p<0.5) on the mean values of water temperature and transparency among the three stations of Maragondon River.

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There is no significant difference in the salinity, dissolved oxygen, total dissolved solids, and nitrates in the chemical characteristics of water among the three sampling stations (Table 2). However, there is a significant difference in the pH and phosphates of Maragondon River. The chemical characteristics of water in Maragondon River were still in the acceptable limit set by the DENR standards except for phosphates with a very slight difference of 0.1mg/L in the mean average. The highest mean pH value 7.62 is the upstream and lowest 7.06 in the downstream. The mean pH value of Maragondon River is comparable to the pH of Buhisan River in Cebu (7.17-7.60) of Maglangit et al. (2014). In general, the pH values were almost at the neutral level to slightly alkaline. pH increased significantly towards upstream direction. The high pH in the upstream site could be attributed to the release of bicarbonates. pH also depends on many factors such as air, temperature, and wastewater discharges (US EPA, 1997). The pH of water is a vital chemical parameter since most of the aquatic organisms are adapted to an average pH and cannot tolerate sudden pH changes (Rankhamb and Raut 2012).

Salinity remains constant throughout the sampling period with a mean value of 0.1ppt from upstream to downstream. Statistically, there is no significant difference among the sampling stations. The mean average of salinity still meet the standards of DENR. Low salinity implies the sites



have more dissolved oxygen which is required for the aquatic life Chakraborty et al. (2017).

In the present study, the dissolved oxygen mean value was found highest in the midstream 7.77 mg/L and lowest mean value 6.96 mg/L in the downstream. Statistically, there is no significant difference among the stations and were still in the acceptable 5.0 mg/L minimum standards of DENR. The dissolved oxygen is comparable to the results conducted by Martinez et al. (2011) in Mamba River, Mt. Palay Palay with a 6.77mg/L mean average during the entire year of study. Dissolved oxygen is an important aquatic parameter, whose presence is vital to aquatic fauna. It plays crucial role in life processes of animals. Rani et al. (2004) reported that lower values of dissolved oxygen in summer season could be attributed to higher rate of decomposition of organic matter and limited flow of water due to high temperature.

In terms of total dissolved solids, the midstream has the highest mean value and upstream has the lowest mean value. In the present study, the TDS values ranged from 90.17-127.47 mg/L and were found to be below the 1000mg/L limit for Class B surface waters. This study is also similar to Martinez et al. (2011) findings on TDS with mean average of 210mg/L for a year of study. The total concentration of dissolved solids or ions in water body is an important parameter to maintain the water balance in cells of aquatic organisms. The values of TDS could be the



result of increased precipitation, salt-water intrusion, and impacts of anthropogenic activities in the area such as small-scale quarrying activities (Weber-Scannell and Duffy 2007).

Nitrates in the upstream has the highest mean value of 2.2 mg/L while downstream has the lowest mean value of 1.5 mg/L. Statistically, there is no significant difference among the stations. At the time of sampling, domesticated animals such as cows and horses were found at the upstream near the vicinity of river. But despite this, the mean rate values are within the DENR standard and did not cause significant aquatic plant growth in the area. When it rains, water moves as runoff across the surface of the soil and carries the untreated sewage which is a significant source of nitrates (Asriningtyas and Rahayuningsih 2012). The presence of little higher value of nitrates in water is an indication of river pollution and will cause eutrophication, thus reducing water quality. Algal bloom and aquatic plant growth will decrease pH, alkalinity, oxygen, light penetration so less rate of photosynthesis (Chauhan and Sagar, 2013).

The phosphate mean values of Maragondon River is higher in the upstream 1.58 mg/L and lowest in downstream with a mean value of 0.34 mg/L. Statistically, there is a significant difference among the stations. This study is similar to the findings of Manlangit et al. 2014 (0.14-1.94) in Buhisan River, Cebu. An increased level of phosphate in the upstream and midstream is due to the presence of people washing clothes and



taking a bath during the sampling. Detergent contributes to the increase of phosphate in domestic wastes (Ling et al. 2012). It was also observed that the upstream and midstream are surrounded by ricefield and vegetation. With these, varying amounts of phosphates are washed from fertilizers used in ricefields and contribute to the high phosphate level (Cojocariu et al. 2011).

Most of the physicochemical properties of Maragondon River were within the tolerable range and are not harmful to the aquatic resources. Only phosphates exceeded the minimum but not the maximum limit of DENR Class B Standards for the physico-chemical parameters of water.

CHEMICAL PARAMETERS	Units	UPSTREAM	MIDSTREAM	DOWNSTREAM	MEAN AVERAGE	DENR STANDARDS
рН		7.62 ^A	7.41 ^A	7.06 ^B	7.37	6.5-8.5
Salinity	ppt	0.10 ^A	0.1 ^A	0.10 ^A	0.10	1 (max.)
Dissolved Oxygen	mg/L	7.75 ⁴	7.77 ^A	6.96 ^A	7.49	5 (min.)
Total Dissolved Solids	mg/L	90.17 ^A	127.47 ^A	102.26 ^A	106.63	1000 (max.)
Nitrates	mg/L	2.20 ^A	1.89 ^A	1.50 ^A	1.86	10 (max.)
Phosphates	mg/L	1.58 ^A	1.39 ^A	0.34 ^B	1.10	0.05-1.00

 Table 2. Chemical Characteristics of Water

The superscripts (A and B) indicate significant difference (p<0.5) on the mean values of chemical parameters among the three stations of Maragondon River.



The mean values of *Oreochromis niloticus* (Tilapia) in Maragondon River showed a higher difference in the total length, head length, body depth, snout length, eye diameter and caudal peduncle from the study conducted by Kosai et al. (2014) on the morphometric characteristics of Tilapia from Chacheongsao , Thailand. The snout length, eye diameter, and caudal peduncle of Tilapia from Maragondon River are comparable to the morphometric characteristics of Tilapia in Boac River conducted by Revilla (2016). The reason for the wide variation in morphometry of the same species may be due to the immediate response to the environmental changes in the study locations where fish samples were obtained and may be due to the genetic makeup which has a fundamental role in the expression of the genes (Azua et al. 2017).

CHARACTERISTICS (cm)	UPSTREAM	MIDSTREAM	DOWNSTREAM	MEAN AVERAGE	Kosai et al. (2014)
Total Length	17.74	17.56	16.94	17.41	12.00
Head Length	5.37	5.43	5.10	5.30	3.37
Body Depth	5.12	5.57	5.29	5.33	3.59
Snout Length	2.04	2.08	1.33	1.82	0.78
Eye Diameter	1.03	1.08	1.00	1.04	0.76
Caudal Peduncle Length	1.93	1.94	1.76	1.88	1.41

Table 3 Morphometric characteristics of Orechromis niloticus (Tilapia)

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Figure 1. Calibration curve for Lead (Pb) analysis

Figure 1 shows an acceptable curve which means that any test for Lead in the instrument (AAS) is acceptable. The sediments and fish sample solutions were subjected to the analysis and it yield results (See Appendix F) that is below detection limit, the detection limit is 0.003ppm.

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Figure 2. Calibration curve for Copper (Cu)

Figure 2 shows an acceptable curve which means that any test for Copper in the instrument (AAS) is acceptable. The sediments and fish sample solutions were subjected to the analysis and it yield results (See Appendix F) that is below detection limit, the detection limit is 0.006ppm.



The mean average of copper and lead -0.3ppm and -0.43ppm respectively were lower than the study conducted by Kaoud and El-Dashan (2010) (2.54ppm and 1.52ppm) (Table 4). This study is also comparable to the study of Revilla (2016) in Boac River where lead and copper concentrations the recommended limits by WHO/FAO. Lead passed and copper concentrations in the tissues of Oreochromis niloticus (Tilapia) collected from three samplings were detected at a very low concentration. The lead concentrations in the tissues of Tilapia which is -0.43ppm were below the standard limit (0.5ppm) set by Fisheries Administrative Order No. 210 (2001). Statistically, both copper and lead have no significant difference among the three sampling stations. Comparing the mean metal concentrations obtained in this study with WHO/FAO, Food Standards Programme Codex Committee on contaminants in food (5th Session, 21-25 March 2011, Netherlands) standard for fish muscles indicated that the mean of copper and lead are safe for human consumption. Otherwise, possible adverse health effect in consuming lead contaminated Tilapia includes chronic damage to the Central Nervous System and gastrointestinal tract as reported by Duruibe et al. (2007).





(Tilapia)

HEAVY METALS	UNIT	UPSTREAM	MIDSTREAM	DOWNSTREAM	MEAN AVERAGE	WHO/FAO 2011
Copper	ppm	-0.31 ^A	-0.32 ^A	-0.30 ^A	-0.31	0.30
Lead	ppm	-0.46 ^A	-0.46 ^A	-0.37 ^A	-0.43	0.50

The superscripts (A and B) indicate significant difference (p<0.5) on the mean values of heavy metals in the tissue of Oreochromis niloticus (tilapia) among the three stations of Maragondon River.

The mean average of copper and lead concentrations in sediments -0.15ppm and -0.32ppm respectively did not exceed Kloke's values (20-100ppm) (Table 5). Statistically, copper has no significant difference among stations. Lead concentration on sediment has also no significant difference among stations which is similar to study conducted by Revilla (2016) on the heavy metals of sediments in Boac River, Marinduque. Lead and copper concentrations in sediments of Maragondon River were below detectable limit in three sampling stations. This indicates that river is not contaminated with heavy metals. If sediments with heavy metal concentration are disturbed, heavy metals will spread in the water column and will pose hazards to animal, plant life, and human health (Sarinas et al. 2014).



HEAVY METALS	Units	UPSTREAM	MIDSTREAM	DOWNSTREAM	MEAN AVERAGE	Kloke's Guideline Values
Copper	ppm	-0.11 ^A	-0.14 ^A	-0.20 ^A	-0.15	20-100
Lead	ppm	-0.30 ^A	-0.33 ^A	-0.33 ^A	-0.32	20-100

Table 5. Heavy metal concentrations in the sediments of Maragondon River

The superscripts (A and B) indicate significant difference (p<0.5) on the mean values of heavy metals in sediments among the three stations of Maragondon River.

The study revealed that transparency, pH, dissolved oxygen, total dissolved solids, nitrates, phosphates, flow rate, depth, and air temperature have weak positive correlation toward lead concentration on sediments. Salinity has no correlation with the lead concentration on sediments (Table 6). While, the water temperature and air temperature have strong positive correlation toward the lead concentration of sediments which means that as the mean average value of water temperature and air temperature increases, the mean average of lead concentration in sediments also increases. The present study is similar to the results of Hasan et al. (2010) which showed positive correlation between water and air temperature, transparency, TDS, and phosphates with lead (Pb) concentration in sediments in Euphrates River, Iraq. The behavior and distribution of heavy metals in sediments may be affected by water hydrology, domestic discharge and many biological, chemical, and geological operations in aquatic system Hasan et al. (2010).



Table 6. Correlation between Physico-chemical Parameters and Lead(Pb) concentration on Sediments

PHYSICO-CHEMICAL PARAMETERS	MEAN AVERAGE	Pb concentration (mean Average)	r values (correlation at 0.05 level)	INTERPRETATION
Temperature	28.03	-0.3210	0.7912 (p<0.05)	strong positive correlation
Transparency	0.59	-0.3210	0.0087 (p>0.05)	weak positive correlation
рН	7.37	-0.3210	0.4508 (p<0.05)	weak positive correlation
Dissolved Oxygen	7.49	-0.3210	0.0668(p<0.05)	weak positive correlation
Salinity	0.1	-0.3210	0	no relationship
Total Dissolved Solids	106.63	-0.3210	0.0071 (p>0.05)	weak positive correlation
Nitrates	1.86	-0.3210	0.331(p<0.05)	weak positive correlation
Phosphates	1.10	-0.3210	0.2294 (p<0.05)	weak positive correlation
Flow rate	214.73	-0.3210	0.0878 (p<0.05)	weak positive correlation
Depth	0.94	-0.3210	0.1087 (p<0.05)	weak positive correlation
Air Temperature	33.06	-0.3210	0.7094 (p<0.05)	strong positive correlation

The present study showed that the transparency, pH, total dissolved solids, nitrates, and depth have weak negative correlation while dissolved oxygen, phosphates, and flow rate have weak positive correlation toward the copper concentration in sediments of Maragondon River. Salinity has no correlation toward the copper concentration in sediments. However, the water temperature and air temperature have moderate negative correlation which means as the mean average of water temperature and air temperature increases, the mean average of copper concentration in sediments decreases.



The present study is comparable to the results of Revilla (2016) of Boac River in terms of temperature and pH which have weak negative correlation and phosphates which has a positive weak correlation. The transparency, TDS, and nitrates in Maragondon River have weak negative correlation while in the study by Revilla (2016) the transparency, TDS, and nitrate have weak positive weak correlation. The results conducted by Hassan et al. (2010) in Euphrates River, Iraq also showed positive correlation between copper and air temperature, water temperature, dissolved oxygen, and nitrates. The increase of heavy metal concentration may be due to multiple source of pollution such as sewage, pesticides, and industrial waste Hassan et al. (2010).

The results of the physico-chemical characteristics of water and heavy metals on sediments and tissue of *Orechromis niloticus* (Tilapia) will provide initial reference for the recommendation of policy on the conservation and protection of Maragondon River.



Table 4.7 Correlation between Physico-chemical Parameters and Copper (Cu)

concentration on Sediments

PHYSICO-CHEMICAL PARAMETERS	MEAN AVERAGE	Cu concentration (mean Average)	r values (correlation at 0.05 level)	INTERPRETATION
Temperature	28.03	-0.1508	-0.7059 (p>0.05)	moderate negative
Transparency	0.59	-0.1508	0.2309 (p<0.05)	weak negative
рН	7.37	-0.1508	-0.0567 (p>0.05)	weak negative
Dissolved Oxygen	7.49	-0.1508	0.0131 (p>0.05)	weak positive
Salinity	0.1	-0.1508	0	no relationship
Total Dissolved Solids	106.63	-0.1508	-0.1932 (p>0.05)	weak negative
Nitrates	1.86	-0.1508	-0.1176 (p>0.05)	weak negative
Phosphates	1.10	-0.1508	0.1813 (p<0.05)	weak positive
Flow rate	214.73	-0.1508	0.3958 (p<0.05)	weak positive
Depth	0.94	-0.1508	-0.0675 (p>0.05)	weak negative
Air Temperature	33.06	-0.1508	-0.5752 (p>0.05)	moderate negative



CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The study provided information about the current condition of Maragondon River. Physico-chemical assessment of water, determination of the morphological characteristics of *Oreochromis niloticus* (Tilapia), and heavy metal analysis specifically lead and copper in sediments and tissues of *Oreochromis niloticus* (Tilapia) were done.

The physico-chemical assessment of Maragondon River for a period of three months indicated that water temperature, pH, salinity, dissolved oxygen,total dissolved solids, and nitrates were within the DENR standards set for Class B surface waters, while phosphate level exceeded a very slight difference on the 0.5-1.0 mg/L limit which could be attributed to domestic sewage, agricultural effluents with fertilizer, and detergents from bathing and washing of clothes by the residents nearby.

Tissues of *Oreochromis niloticus* (Tilapia) from upstream to downstream were able to pass the WHO/FAO, Food Standards Programme Codex Committee on contaminants in foods (5th Session, 21-25 March 11, The Netherlands). The heavy metals (lead and copper) also passed the Klokes Guideline Values for sediments. Though the results were positive, there is still a need for regular monitoring to reduce the polution level.





Based on the results of this study, it was recommended to

- conduct heavy metal analysis of water, sediments, and tissues of Oreochromis niloticus (Tilapia) other than lead and copper;
- determine heavy metal analysis in other parts of fish such as liver, gills, intestines;
- conduct lead and copper analysis in tissues of other fishes or organisms such as freshwater crustaceans like shrimps and crabs;
- conduct study of physico-chemical assessment and heavy metal analysis of sediments and tissues of *Oreochromis niloticus* (Tilapia) during wet and dry seasons.
- use bioindicators, the natural indicator of environmental pollution in Maragondon River
- regular monitoring and assessment of water condition for the protection of Maragondon River.



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Figure 1. Map of Maragondon River showing sampling stations marked with google map pushpin icon. Adopted and modified from Google Earth 2017.



APPENDIX B

LOCATION AND SAMPLES COLLECTED AT MARAGONDON RIVER

STATION	SUBSTATION	LATITUDE	LONGITUDE	ELEVATION	SAMPLE COLLECTED
	1	N 14°14' 41.3"	E 120°46' 54.7"	19	Sediment/ <i>O. niloticus</i>
BGY. MABATO (Upstream)	2	N 14°14' 44.9"	E 120°46' 55.6''	22	Sediment/ <i>O. niloticus</i>
	3	N 14°14' 45.6"	E 120°46' 56.7''	23	Sediment/ <i>O. niloticus</i>
	1	N 14°16' 20.4"	E 120°45' 35.9"	7	Sediment/ <i>O. niloticus</i>
BGY. BUCAL IV (Midstream)	2	N 14°16' 22.4"	E 120°45' 36.6"	13	Sediment/ <i>O. niloticus</i>
	R	N 14°16' 23.7"	E 120°45' 37.1"	8	Sediment/ <i>O. niloticus</i>
	1	N 14°15' 54.5"	E 120°42' 45.6"	8	Sediment/O. niloticus
BGY. PINAGSANHAN (Downstream)	2	N 14°15' 55.3"	E 120°42' 44''	14	Sediment/ <i>O. niloticus</i>
(Soundareality	3	N 14°15' 55.9"	E 120° 42' 42.5 ''	10	Sediment/ <i>O. niloticus</i>


STATION 1 UPSTREAM (Brgy. Mabato)



Substation 1



Substation 2



Substation 3

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STATION 2 MIDSTREAM (Brgy. Bucal IV)



Substation 1



Substation 2



Substation 3

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STATION 3 DOWNSTREAM (Brgy. Pinagsanhan)



Substation 1



Substation 2



Substation 3

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APPENDIX D

WATER USAGE AND CLASSIFICATIONS

(DENR Administrative Order No. 34 Series 1990)

For Fresh Water (river, lakes, reservoir, etc.)

Classification	Beneficial Use
Class AA	Public Water Supply Class 1. This class is
	intended primarily for water having watersheds
	which are uninhabited and otherwise protected
	and which require only approved disinfection in
G	order to meet the National Standards for
	Drinking Water (NSDW) of the Philippines
Class A	Public Water Supply Class 2. For sources of
	water supply that will require complete
	treatment (coagulation, sedimentation, filtration
	and disinfection) in order to meet the NSDW
Class B	Recreational Water Class 1. For primarily
	contact recreation such as bathing, swimming,
	skin diving, etc. (particularly those designated
	for tourism purpose.)
Class C	a. Fishery water for the propagation and
	growth of fish and other aquatic









WATER QUALITY CRITERIA FOR FRESH WATERS

Table 1 – Water Quality Criteria for Conventional and Other Pollutants Contributing to Aesthetics and Oxygen Demand for Fresh Waters ^(a)

PARAMETER	UNIT	CLASS	CLASS	CLASS	CLASS C	CLASS
Color	PCU	15	50	(c)	(c)	(c)
Temperature ^(d) (max. rise in deg. Celcius)	°C rise		3	3	3	3
pH (range)		6.5 - 8.5	6.5 - 8.5	6.5 - 8.5	6.5 - 8.5	6.0 - 9.0
Dissolved Oxygen ^(e) (Minimum)	% satn mg/L	70 5.0	70 5.0	70 5.0	60 5.0	40 3.0
5-Day 20°C BOD	mg/L	1	5	5	7(10)	10(15)
Total Suspended Solids	mg/L	25	50	(1)	(g)	(h)
Total Dissolved Solids	mg/L	500 (0	1,000 0	-	-	1,000 ()
Surfactants (MBAS)	mg/L	nil	0.2(0.5)	0.3(0.5)	0.5	
Oil/Grease (Petroleum Ether Extracts)	mg/L	nil	1	1	2	5
Nitrate as Nitrogen	mg/L	1.0	10	nr	10 0	
Phosphate as Phosphorus	mg/L	nil	0.1%	0.2 ^(k)	0.4(k)	-

* Phosphate as P concentration should not exceed an average of 0.05 mg/L nor a

maximum of 0.1 mg/L





Method 3010A 1/5

(Preparation and Instrument Parameters-USEPA)

METHOD 3010A

ACID DIGESTION OF AQUEOUS SAMPLES AND EXTRACTS FOR TOTAL METALS FOR ANALYSIS BY FLAA OR ICP SPECTROSCOPY

1.0 SCOPE AND APPLICATION

1.1 This digestion procedure is used for the preparation of aqueous samples, EP and mobility-procedure extracts, and wastes that contain suspended solids for analysis, by flame atomic absorption spectroscopy (FLAA) or inductively coupled argon plasma spectroscopy (ICP). The procedure is used to determine total metals.

1.2 $\,$ Samples prepared by Method 3010 may be analyzed by FLAA or ICP for the following:

Aluminum
*Arsenic
Barium
Beryllium
Cadmium
Calcium
Chromium
Cobalt
Copper
Iron
Lead

Magnesium Manganese Molybdenum Nickel Potassium *Selenium Sodium Thallium Vanadium Zinc

* Analysis by ICP

NOTE: See Method 7760 for the digestion and FLAA analysis of Silver.

1.3 This digestion procedure is not suitable for samples which will be analyzed by graphite furnace atomic absorption spectroscopy because hydrochloric acid can cause interferences during furnace atomization. Consult Method 3020A for samples requiring graphite furnace analysis.

2.0 SUMMARY OF METHOD

2.1 A mixture of nitric acid and the material to be analyzed is refluxed in a covered Griffin beaker. This step is repeated with additional portions of nitric acid until the digestate is light in color or until its color has stabilized. After the digestate has been brought to a low volume, it is refluxed with hydrochloric acid and brought up to volume. If sample should go to dryness, it must be discarded and the sample reprepared.

3.0 INTERFERENCES

3.1 Interferences are discussed in the referring analytical method.

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(Preparation and Instrument Parameters-USEPA)

4.0 APPARATUS AND MATERIALS

- 4.1 Griffin beakers 150-mL or equivalent.
- 4.2 Watch glasses Ribbed and plain or equivalent.
- 4.3 Qualitative filter paper or centrifugation equipment.
- 4.4 Graduated cylinder or equivalent 100mL.
- 4.5 Funnel or equivalent.

4.6 Hot plate or equivalent heating source - adjustable and capable of maintaining a temperature of $90\,{}^-95\,{}^\circ\!\mathrm{C}.$

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 Reagent Water. Reagent water will be interference free. All references to water in the method refer to reagent water unless otherwise specified. Refer to Chapter One for a definition of reagent water.

5.3~ Nitric acid (concentrated), $\rm HNO_3.~$ Acid should be analyzed to determine levels of impurities. If method blank is < MDL, the acid can be used.

5.4~ Hydrochloric acid (1:1), HCl. Prepared from water and hydrochloric acid. Hydrochloric acid should be analyzed to determine level of impurities. If method blank is \leq MDL, the acid can be used.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

 $6.1\,$ All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

6.2 All sample containers must be prewashed with detergents, acids, and water. Plastic and glass containers are both suitable. See Chapter Three, Step 3.1.3, for further information.

6.3 Aqueous wastewaters must be acidified to a pH of < 2 with HNO3.

7.0 PROCEDURE

7.1~ Transfer a 100-mL representative aliquot of the well-mixed sample to a 150-mL Griffin beaker and add 3 mL of concentrated HNO_3. Cover the beaker with

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Method 3010A 3/5

(Preparation and Instrument Parameters-USEPA)

a ribbed watch glass or equivalent. Place the beaker on a hot plate or equivalent heating source and cautiously evaporate to a low volume (5 mL), making certain that the sample does not boil and that no portion of the bottom of the beaker is allowed to go dry. Cool the beaker and add another 3-mL portion of concentrated HNO₃. Cover the beaker with a nonribbed watch glass and return to the hot plate. Increase the temperature of the hot plate so that a gentle reflux action occurs.

NOTE: If a sample is allowed to go to dryness, low recoveries will result. Should this occur, discard the sample and reprepare.

7.2 Continue heating, adding additional acid as necessary, until the digestion is complete (generally indicated when the digestate is light in color or does not change in appearance with continued refluxing). Again, uncover the beaker or use a ribbed watch glass, and evaporate to a low volume (3 mL), not allowing any portion of the bottom of the beaker to go dry. Cool the beaker. Add a small quantity of 1:1 HCl (10 mL/100 mL of final solution), cover the beaker, and reflux for an additional 15 minutes to dissolve any precipitate or residue resulting from evaporation.

7.3 Wash down the beaker walls and watch glass with water and, when necessary, filter or centrifuge the sample to remove silicates and other insoluble material that could clog the nebulizer. Filtration should be done only if there is concern that insoluble materials may clog the nebulizer. This additional step can cause sample contamination unless the filter and filtering apparatus are thoroughly cleaned. Rinse the filter and filter apparatus with dilute nitric acid and discard the rinsate. Filter the sample and adjust the final volume to 100 mL with reagent water and the final acid concentration to 10%. The sample is now ready for analysis.

8.0 QUALITY CONTROL

 $8.1\,$ All quality control measures described in Chapter One should be followed.

8.2 For each analytical batch of samples processed, blanks should be carried throughout the entire sample-preparation and analytical process. These blanks will be useful in determining if samples are being contaminated. Refer to Chapter One for the proper protocol when analyzing blanks.

8.3 Replicate samples should be processed on a routine basis. A replicate sample is a sample brought through the whole sample preparation and analytical process. A replicate sample should be processed with each analytical batch or every 20 samples, whichever is greater. Refer to Chapter One for the proper protocol when analyzing replicates.

8.4 Spiked samples or standard reference materials should be employed to determine accuracy. A spiked sample should be included with each batch of samples processed and whenever a new sample matrix is being analyzed. Refer to Chapter One for the proper protocol when analyzing spikes.

8.5 $\,$ The method of standard addition shall be used for the analysis of all EP extracts and delisting petitions (see Method 7000, Step 8.7). Although not

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Method 3010A 4/5

(Preparation and Instrument Parameters-USEPA)

required, use of the method of standard addition is recommended for any sample that is suspected of having an interference.

- 9.0 METHOD PERFORMANCE
 - 9.1 No data provided.
- 10.0 REFERENCES

 Rohrbough, W.G.: et al. <u>Reagent Chemicals. American Chemical Society</u> <u>Specifications</u>. 7th ed.: American Chemical Society: Washington, DC, 1986.

2. <u>1985 Annual Book of ASTM Standards</u>, Vol. 11.01; "Standard Specification for Reagent Water"; ASTM: Philadelphia, PA, 1985; D1193-77.

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3010A - 4

Revision 1 July 1992



Method 3010A 5/5

(Preparation and Instrument Parameters-USEPA)

ACID DIGESTION OF AQUEOUS SAMPLES AND EXTRACTS FOR TOTAL

METALS ANALYSIS BY FLAA OR ICP SPECTROSCOPY

METHOD 3010A ACID DIGESTION OF AQUEOUS SAMPLES AND EXTRACTS FOR TOTAL METALS ANALYSIS BY FLAA OR ICP SPECTROSCOPY



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RESULT OF AAS ANALYSIS FOR COPPER AND LEAD

De La Salle University-Dasmariñas College of Science and Computer Studies PHYSICAL SCIENCES DEPARTMENT City of Dasmariñas

CHEMISTRY RESEARCH LABORATORY CERTIFICATE OF ANALYSIS

Name of Client:Michael A. JalandoonDate:May 5, 2018Affiliation and Address:De La Salle University-Dasmariñas, City of Dasmariñas, CaviteContact Number:0907-0503715E-mail Address:mikeljalandoon08@gmail.com

A. Sample Description

Ashed and/or acid digested samples of sediments and fish tissues of Oreochromis niloticus (Tilapia) from Maragondon River were analyzed for copper (Cu) and lead (Pb) content.

B. Summary of Procedure

Flame Atomic Absorption Spectrophotometer (Shimadzu AA-7000) was used for analysis. Calibration curve was prepared according to the instrument's user manual.

C. Results



Figure 1. Calibration Curve for Lead (Pb) Analysis

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De La Salle University - Dasmariñas **GRADUATE STUDIES RESULT OF AAS ANALYSIS FOR COPPER AND LEAD** Abs=0.50490Conc-0.0059833 r=0.9994 А Ь 0.500 s 0.400 0.300 0.200 0.100 0.000-F ++0.000 0.250 0.500 0.750 1.000 Conc (ppm)

Figure 2. Calibration Curve for Copper (Cu) Analysis





 Table 1. Copper (Cu) and Lead (Pb) Content of Sediments and Oreochromis niloticus (Tilapia)

 from Maragondon River (First Sampling)

	Sample ID	Cu:FlameCon t Actual Conc.	Cu:Flame Cont Actual Conc. Unit	Pb:FlameCon t Actual Conc.	Pb:Flame Cont Actual Conc. Unit
1	F-U1	0.0534	ppm	-0.3957	NONE
2	F-U2	0.0435	ppm	-0.4078	NONE
3	F-U3	0.0489	ppm	-0.4095	NONE
4	F-M1	0.0635	ppm	-0.4164	NONE
5	F-M2	0.0483	ppm	-0.4147	NONE
6	F-M3	0.0495	ppm	-0.4199	NONE
7	F-D1	0.0649	ppm	-0.4302	NONE
8	F-D2	0.0523	ppm	-0.4302	NONE
9	F-D3	0.0536	ppm	-0.4302	NONE
10	S-U1	0.1384	ppm	-0.3543	NONE
11	S-U2	0.2729	ppm	-0.3750	NONE
12	S-U3	0.1899	ppm	-0.3302	NONE
13	S-M1	0.1980	ppm	-0.3647	NONE
14	S-M2	0.2044	ppm	-0.3681	NONE
15	S-M3	0.2256	ppm	-0.3612	NONE
16	S-D1	0.1529	ppm	-0.3750	NONE
17	S-D2	0.1582	ppm	-0.3750	NONE
18	S-D3	0.1889	ppm	-0.3647	NONE





Table 2. Copper (Cu) and Lead (Pb) Content of Sediments and Oreochromis niloticus (Tilapia)

 from Maragondon River (Second Sampling)

	Sample ID	Cu:FlameCon t Actual Conc.	Cu:FlameCont Actual Conc. Unit	Pb:FlameCon t Actual Conc.	Pb:FlameCont Actual Conc. Unit
1	F2-U1	-0.6307	NONE	-0.5717	NONE
2	F2-U2	-0.6317	NONE	-0.5734	NONE
3	F2-U3	-0.4775	NONE	-0.5751	NONE
4	F2-M1	-0.6137	NONE	-0.5682	NONE
5	F2-M2	-0.622	NONE	-0.5631	NONE
6	F2-M3	-0.642	NONE	-0.5648	NONE
7	F2-D1	-0.645	NONE	-0.5613	NONE
8	F2-D2	-0.6376	NONE	-0.5613	NONE
9	F2-D3	-0.6486	NONE	-0.5613	NONE
10	S2-U1	-0.3589	NONE	-0.3025	NONE
11	S2-U2	-0.3246	NONE	-0.1404	NONE
12	S2-U3	-0.4189	NONE	-0.306	NONE
13	S2-M1	-0.3466	NONE	-0.3094	NONE
14	S2-M2	-0.5048	NONE	-0.2922	NONE
15	S2-M3	-0.4145	NONE	-0.306	NONE
16	S2-D1	-0.4965	NONE	-0.306	NONE
17	S2-D2	-0.5168	NONE	-0.3112	NONE
18	S2-D3	-0.4635	NONE	-0.3163	NONE



RESULT OF AAS ANALYSIS FOR COPPER AND LEAD

Table 3. Copper (Cu) and Lead (Pb) Content of Sediments and Oreochromis niloticus (Tilapia)from Maragondon River (Third Sampling)

	Sample ID	Pb:FlameCon t Actual Conc.	Pb:Flame Cont Actual Conc. Unit	Cu:FlameCon t Actual Conc.	Cu:Flame Cont Actual Conc. Unit
1	F3-U1	-0.4046	NONE	-0.3698	NONE
2	F3-U2	-0.3966	NONE	-0.3918	NONE
3	F3-U3	-0.4046	NONE	-0.3929	NONE
4	F3-M1	-0.4143	NONE	-0.3808	NONE
5	F3-M2	-0.4079	NONE	-0.3876	NONE
6	F3-M3	-0.4143	NONE	-0.3837	NONE
7	F3-D1	-0.4014	NONE	-0.3812	NONE
8	F3-D2	-0.4014	NONE	-0.1851	NONE
9	F3-D3	-0.3772	NONE	-0.3801	NONE
10	S3-U1	-0.3256	NONE	-0.2338	NONE
11	S3-U2	-0.2821	NONE	-0.0891	NONE
12	S3-U3	-0.2917	NONE	-0.1948	NONE
13	S3-M1	-0.3175	NONE	-0.2487	NONE
14	S3-M2	-0.3175	NONE	-0.1871	NONE
15	S3-M3	-0.3369	NONE	-0.2159	NONE
16	S3-D1	-0.2982	NONE	-0.2536	NONE
17	S3-D2	-0.3111	NONE	-0.2555	NONE
18	S3-D3	-0.3224	NONE	-0.2745	NONE

Certified by:

Date: May 7, 2018

Ms. Jonnacar S. San Sebastian Laboratory Supervisor, BSD



APPENDIX G RAW DATA PHYSICO-CHEMICAL CHARACTERISTICS OF WATER IN MARAGONDON RIVER (1/2)

TEMPERATURE °C			-
Upstream	Midstream	Downstream	Upstream
28.6	26.5	29.0	1.14
28.0	26.0	26.0	0.79
27.0	26.0	26.0	0.68
30.8	28.5	27.5	0.95
28.8	28.4	28.1	0.55
27.8	28.4	28.5	0.35
28.9	29.2	27.8	1.2
28.9	28.8	27.7	0.36
28.8	29.9	29.8	0.62
	(C)) (

TRANSPARENCY (m)				
Upstream	Midstream	Downstream		
1.14	0.73	0.28		
0.79	0.69	0.32		
0.68	0.76	0.26		
0.95	0.75	0.3		
0.55	0.67	0.3		
0.35	0.92	0.17		
1.2	0.58	0.4		
0.36	0.9	0.2		
0.62	0.92	0.15		

SALINITY				
Upstream	Midstream	Downstream		
0.1	0.1	0.1		
0.1	0.1	0.1		
0.1	0.1	0.1		
0.1	0.1	0.1		
0.1	0.1	0.1		
0.1	0.1	0.1		
0.1	0.1	0.1		
0.1	0.1	0.1		
0.1	0.1	0.1		

	рН				
Upstream	Midstream	Downstream			
7.9	7.4	7.0			
7.3	7.4	7.2			
7.5	7.4	7.1			
7.8	7.6	6.9			
7.9	7.6	7.3			
7.8	7.6	7.3			
7.5	7.2	6.9			
7.5	7.3	6.9			
7.5	7.3	7.0			



RAW DATA PHYSICO-CHEMICAL CHARACTERISTICS OF WATER IN MARAGONDON RIVER (2/2)

TOTAL DISSOLVED SOLIDS (mg/L)				
Upstream	Midstream	Downstream		
88.1	187.0	82.3		
93.3	90.0	81.3		
85.0	90.0	90.0		
90.0	190.9	80.1		
90.9	122.4	80.4		
92.0	90.8	102.3		
90.7	96.3	81.7		
90.9	89.7	161.1		
90.6	190.1	161.1		

DISSOLVED OXYGEN				
Upstream	Midstream	Downstream		
7.4	7.8	7.6		
7.8	7.9	7.6		
7.5	7.8	7.5		
8.0	8.2	7.9		
8.4	8.2	7.7		
7.6	7.9	7.6		
7.7	7.4	4.5		
7.6	7.2	6.0		
7.8	7.5	6.3		

NITRATES (mg/L)		
Upstream	Midstream	Downstream
1.4	1.8	1.7
2.2	2.0	0.4
1.9	2.2	2.0
2.2	1.9	0.8
1.5	1.6	2.4
2.4	2.1	2.4
1.5	2.0	0.3
1.8	2.5	1.9
4.9	0.9	1.6

 (\cap)

PHOSPHATES (mg/L)		
Upstream	Midstream	Downstream
1.6	1.4	0.4
1.4	1.6	0.5
1.6	1.4	0.2
1.7	1.3	0.2
1.5	1.3	0.2
1.6	1.2	0.2
1.5	1.5	0.6
1.7	1.4	0.4
1.7	1.4	0.4



APPENDIX H

Taxonomic Classification of Tilapia

Kingdom:	Animalia
Phylum:	Chordata
Class:	Actinopterygii
Order:	Perciformes
Family:	Cichlidae
Genus:	Oreochromis
Species:	Oreochromis niloticus



Morphometrics Characteristics of O. niloticus (tilapia) Fish

- A. Total length
- B. Head length
- C. Body depth
- D. Snout length
- E. Eye diameter
- F. Caudal peduncle length



APPENDIX I

CORRELATION BETWEEN PHYSICO-CHEMICAL PARAMETERS AND

LEAD AND COPPER CONCENTRATION ON SEDIMENTS (1/6)

Pb Sediments	Transparency
-0.3541	0.87
-0.2477	0.62
-0.3011	0.73
-0.3654	0.73
-0.3031	0.78
-0.3243	0.8
-0.3679	0.29
-0.314	0.26
-0.3113	0.25

/	Cu Sediments	Transparency
	0.2004	0.87
	-0.3674	0.62
	-0.1726	0.73
	0.2093	0.73
	-0.422	0.78
	-0.2172	0.8
	0.1667	0.29
	-0.4934	0.26
	-0.2612	0.25
		10 M

Pb Sediments	Temperature
-0.3541	27.87
-0.2477	29.13
-0.3011	28.87
-0.3654	26.17
-0.3031	28.43
-0.3243	29.3
-0.3679	26
-0.314	28.03
-0.3113	28.43

(C)

Cu Sediments	Temperature
0.2004	27.87
-0.3674	29.13
-0.1726	28.87
0.2093	26.17
-0.422	28.43
-0.2172	29.3
0.1667	26
-0.4934	28.03
-0.2612	28.43



CORRELATION BETWEEN PHYSICO-CHEMICAL PARAMETERS AND

LEAD AND COPPER CONCENTRATION ON SEDIMENTS (2/6)

Pb Sediments	Dissolved Oxygen
-0.3541	7.56
-0.2477	7.99
-0.3011	7.7
-0.3654	7.82
-0.3031	8.11
-0.3243	7.4
-0.3679	7.56
-0.314	7.72
-0.3113	5.59

Cu Sediments	Dissolved Oxygen
0.2004	7.56
-0.3674	7.99
-0.1726	7.7
0.2093	7.82
-0.422	8.11
-0.2172	7.4
0.1667	7.56
-0.4934	7.72
-0.2612	5.59

Pb Sediments	рН
-0.3541	7.56
-0.2477	7.8
-0.3011	7.5
-0.3654	7.39
-0.3031	7.57
-0.3243	7.28
-0.3679	7.1
-0.314	7.16
-0.3113	6.92

Cu Sediments	рН
0.2004	7.56
-0.3674	7.8
-0.1726	7.5
0.2093	7.39
-0.422	7.57
-0.2172	7.28
0.1667	7.1
-0.4934	7.16
-0.2612	6.92



CORRELATION BETWEEN PHYSICO-CHEMICAL PARAMETERS AND

LEAD AND COPPER CONCENTRATION ON SEDIMENTS (3/6)

Pb Sediments	Salinity
-0.3541	0.1
-0.2477	0.1
-0.3011	0.1
-0.3654	0.1
-0.3031	0.1
-0.3243	0.1
-0.3679	0.1
-0.314	0.1
-0.3113	0.1

Cu Sediments	Salinity
0.2004	0.1
-0.3674	0.1
-0.1726	0.1
0.2093	0.1
-0.422	0.1
-0.2172	0.1
0.1667	0.1
-0.4934	0.1
-0.2612	0.1

Pb Sediments	TDS
-0.3541	88.78
-0.2477	90.97
-0.3011	90.76
-0.3654	122.33
-0.3031	134.72
-0.3243	125.36
-0.3679	84.51
-0.314	87.61
-0.3113	134.66

and the second se	
Cu Sediments	TDS
0.2004	88.78
-0.3674	90.97
-0.1726	90.76
0.2093	122.33
-0.422	134.72
-0.2172	125.36
0.1667	84.51
-0.4934	87.61
-0.2612	134.66



CORRELATION BETWEEN PHYSICO-CHEMICAL PARAMETERS AND

LEAD AND COPPER CONCENTRATION ON SEDIMENTS (4/6)

Pb Sediments	Nitrates
-0.3541	1.83
-0.2477	2.03
-0.3011	2.73
-0.3654	2
-0.3031	1.87
-0.3243	1.8
-0.3679	1.37
-0.314	1.87
-0.3113	1.27

Cu Sediments	Nitrates
0.2004	1.83
-0.3674	2.03
-0.1726	2.73
0.2093	2
-0.422	1.87
-0.2172	1.8
0.1667	1.37
-0.4934	1.87
-0.2612	1.27

Pb Sediments	Phosphates
-0.3541	1.52
-0.2477	1.59
-0.3011	1.62
-0.3654	1.46
-0.3031	1.3
-0.3243	1.41
-0.3679	0.37
-0.314	0.2
-0.3113	0.45

Cu Sediments	Phosphates
0.2004	1.52
-0.3674	1.59
-0.1726	1.62
0.2093	1.46
-0.422	1.3
-0.2172	1.41
0.1667	0.37
-0.4934	0.2
-0.2612	0.45



CORRELATION BETWEEN PHYSICO-CHEMICAL PARAMETERS AND

LEAD AND COPPER CONCENTRATION ON SEDIMENTS (5/6)

Pb Sediments	Air temperature
-0.3541	31.67
-0.2477	34.17
-0.3011	35
-0.3654	31.33
-0.3031	33.4
-0.3243	35
-0.3679	31
-0.314	32
-0.3113	34

Cu Sediments	Air temperature
0.2004	31.67
-0.3674	34.17
-0.1726	35
0.2093	31.33
-0.422	33.4
-0.2172	35
0.1667	31
-0.4934	32
-0.2612	34

Pb Sediments	Flow rate
-0.3541	450.22
-0.2477	414.67
-0.3011	394.89
-0.3654	440.67
-0.3031	116.33
-0.3243	115.78
-0.3679	0
-0.314	0
-0.3113	0

Cu Sediments	Flow rate
0.2004	450.22
-0.3674	414.67
-0.1726	394.89
0.2093	440.67
-0.422	116.33
-0.2172	115.78
0.1667	0
-0.4934	0
-0.2612	0



CORRELATION BETWEEN PHYSICO-CHEMICAL PARAMETERS AND

LEAD AND COPPER CONCENTRATION ON SEDIMENTS (6/6)

Pb Sediments	Depth
-0.3541	0.78
-0.2477	0.96
-0.3011	0.99
-0.3654	1.43
-0.3031	1.83
-0.3243	1.35
-0.3679	0.38
-0.314	0.39
-0.3113	0.35

Cu SedimentsDepth0.20040.78	
0.2004 0.78	
-0.3674 0.96	
-0.1726 0.99	
0.2093 1.43	
-0.422 1.83	
-0.2172 1.35	
0.1667 0.38	
-0.4934 0.39	
-0.2612 0.35	



APPENDIX J



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14 December 2017

Ms. ELVIE ESTRADA MPDC Maragondon, Cavite

Dear Ms. Estrada

The undersigned is a student of De La Salle University- Dasmariñas and is currently doing a research study entitled " Physico-Chemical Assessment of Water and Heavy Metal Analysis of Sediments and *Oreochromis niloticus* (TILAPIA) in Maragondon, River Philippines as part of requirements in Master of Science Major in Biology.

In this connection, I would like to ask permission from your office to allow me to conduct water analysis in Maragondon River during the months of January to March 201**9**.

Thank you.

Respectfully

Mr. MICHAEL A JALANDOON MS Biology DLSU-Dasmariñas

Endorsed MERCURIO Dr. A

Thesi Adviser DLSU-Dasmariñas

Noted Dr. JOHNN Dean

DLSU Dasmariñas

10/14/70 M

DLSU-D creating possibilities





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14 December 2017

Ms. TERESITA NUESTRO MENRO Maragondon, Cavite

Dear Ms. Nuestro,

The undersigned is a student of De La Salle University- Dasmariñas and is currently doing a research study entitled "Physico-Chemical Assessment of Water and Heavy Metal Analysis of Sediments and *Oreochromis niloticus* (TILAPIA) in Maragondon, River Philippines as part of requirements in Master of Science Major in Biology.

CAVITE 4115 PHILIPPINES

In this connection, I would like to ask permission from your office to allow me to conduct water analysis in Maragondon River during the months of January to March 2018.

Thank you.

Respectfully

Mr. MICHAEL ALANDOON MS Biology DLSU-Dasmariñas

Endorsed b

Dr. A Thesis Adviser DLSU-Dasmariñas

Not Dr. JOHN CHING

Dr. JOHNJIY AFCHING Dean DLSU Dasmariñas

read

J-D creating possibilities



APPENDIX K

PHOTODOCUMENTATION

Measurement of the Physico-chemical Parameters in Maragondon River











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Morphometric Characteristics of Oreochromis niloticus (Tilapia)



Morphometrics of Tilapia were gathered with body pointing to the left using ruler.



APPENDIX L

Preparation of Fish Samples for Heavy Metal Analysis











A. Weighing B. fish fillet C. tissue were finely chopped D. weighing of tissue E. samples were placed in furnace for ashing F. tissues were turned into white ash G. acid digestion H. samples in hot plate I. filter J. digested solution



Preparation of Sediment Samples for Heavy Metal Analysis





A. Sediment samples B. sediments were oven dried C. sieving of sediments D. weighing (5grams) E. acid digestion in hot plate F. samples were filtered G. digested solution



ATOMIC ABSORPTION SPECTROPHOTOMETRY ANALYSIS



Testing for the heavy metals in sediments and tissues of Tilapia



Michael A. Jalandoon is a regular permanent teacher of Pag-asa National High School since SY 2011 to the present. He finished his elementary education at Dr. Jose P. Rizal Elementary School and secondary education at the Sisters of Mary School Boystown at Silang, Cavite. He earned his Bachelor of Science in Education major in Biology in 2004 at Philippine Normal University Manila.