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Studies on evaluation of Poondi Lake and its impact on bioindicator fish oreochromis niloticus

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***Abstract*---**Poondi Lake, the major reservoir that supplies water for the metropolitan city Chennai should be devoid of pollution. Even though, the water is treated in prior to the supply, it is mandatory to check the quality of freshwater ecosystem in a periodic manner. Hence, this study is conducted to assess the water quality of Poondi Lake. The results suggest that even though there is presence of Gram-negative rods and Gram-positive cocci in the water and sediment levels, the colony count were under permissible levels. This ensures that the quality of water is safe and potable for humans. The proximate analysis, relative organ weight index, biomarker analysis, enzyme and protein analysis in the bio-indicator species *Oreochromis niloticus* confirms that the freshwater ecosystem of Poondi Lake reservoir is of good quality and the impact of pollution is less. But further analysis at the molecular level will depict the actual scenario of pollution in long term effect. To conclude, periodic assessment of fresh water ecosystem should be done to evaluate and prevent the pollution of freshwater. Even though pollution has an impact on the macromolecules, evaluating at genetic levels gives us a clear picture on the effect of pollution on fresh water biota.

***Keywords*---**Poondi lake, *Oreochromis niloticus,* water quality, biomarker, proximate analysis.

Introduction

Water Pollution

Throughout the world, freshwater environments are experiencing serious threats to both biodiversity and ecosystem stability (Suski and Cooke, 2006) and many strategies have been proposed to solve this crisis (Cowx, 2002). Stress caused by anthropogenic environmental degradation due to urbanization, construction of dams, abstraction of water for irrigation and power generation, and pollution in the last few decades had many negative effects on freshwater fish genetic biodiversity, particularly in rivers. This was coupled with irreversible genetic changes in natural populations by introductions of exotic species and diseases. Freshwater fishes are the most threatened group of vertebrates on earth after amphibians (Bruton, 1995; Duncan and Lockwood, 2001) and the global extinction rate of fishes is believed to be in excess of higher vertebrates (Bruton, 1995; Sisk *et al.*, 1994). Sewage is the biggest source of pollutant of fresh water when discharged into them. Sewage when discharged untreated into the river is enormously unhealthy. The striking consequence is a substantial and immediate drop in the amount of dissolved oxygen in the water. This happens because organic matter stimulates decomposers especially bacteria which break down suspended solids in the sewage. As they respire, the decomposers use up dissolved oxygen (O_2) and the Biological Oxygen Demand (BOD) reduces. The flora and fauna of the rivers experience change and reduction in number due to death by suffocation (Tudge, 1991).

Pollution Assessment

Growing human population and industrialization have led to the pollution of most aquatic ecosystems and consequent deterioration in environmental water quality. Biomarkers for water pollution are early diagnostic tools for biological effect measurement and environmental quality assessment (Cajaraville *et al.,* 2000). Biomarker is defined as a change in biological response that differs from molecular to organism level (Depledge *et al.*, 1995). Fish have been widely documented as useful indicators of environmental water quality because of their differential sensitivity to pollution (Das and Chakrabarty, 2007). Tilapia (*Oreochromis niloticus*) is a fresh water fish that is hardy, prolific, fast growing tropical fish that is farmed mainly in Africa and Asia. Tilapia is beneficial to human beings as they make up a major part of the human diet and provide humans with as much of needed proteins as in meat (Ghorbani and Mirakabad, 2010). Hence, this species is widely used in bio monitoring of the water quality.

Materials and Methods

Study area

Poondi reservoir or Sathiya Moorthy Sagar Anai is located in Poondi. This lake located across Kosasthalai river in Thiruvallur district of Tamilnadu state. Its act as the important water source for Chennai city which is 60 km away. There is an initiation of fish cage culture by the State Fisheries Department. Lakes form an integral part of the River system. They are unique habitats of freshwater that store large amounts of water and trap suspended sediments delivered by rivers.

The area of the reservoir is about 121/2 square miles. The total volume of the Water capacity of the reservoir is 3,231 million cubic feet (91.5×106 m 3; 74,200 acresq ft). and primary inflow is 34,887 cusbic/sec, primary outflow is 36484 cubic/sec. Nowadays the water volume and the aquatic species are decreasing because of the un maintenance of the lake by the management. Hence this place has been selected.

Sample Collection

Water and Sediment samples were collected from Poondi reservoir in sterile reagent bottles and it was transported to the laboratory and kept in room temperature until microbial analyses were performed. Fresh fish samples were collected from the fisherman at Poondi reservoir in the month of May, June and July. Collected fishes were transported to the laboratory in a thermo flask with ice on the same day. The samples were preserved in deep freezer until further analyseswere performed.

Proximate analysis (AOAC, 1987)Estimation of Moisture

At first, the initial weight of the samples was taken. Then samples were dried in an oven atabout 105^0C for about 8 to 10 hours until a constant weight was reached and cooled in a desiccator and weight again .Then the samples were minced in an electric grinder. The percentage of moisture content was calculated by the following equation:
Percentage (%) of moisture = (Weight loses/Original weight of sample) × 100

Estimation of Ash

The ash content of a sample is the residue left after ashing in a muffle furnace at about 550^0C - 600^0C till the residue become white. The percent of ash was calculated as follows:

Percentage (%) of ash = (Weight of ash / Weight of Sample) × 100

Data were analyzed by using SPSS.11 statistical Programme with five per cent level ofsignificance.

Molecular Technique

SDS-PAGE electrophoresis method was used to determine the protein composition and molecular mass. The SDS-PAGE was performed according to the method described by Laemmli (1970). The gel containing protein bands can be viewed under UV- illuminator.

Estimation of Biochemical Assay
Estimation of carbohydrate (Dubois et al.,1959)

The carbohydrate content of the homogenized fish tissues were estimated by using dubois etal.,method. The OD values are absorbed at 490 nm. Glucose was taken as standard.

Estimation of protein (Bradford method)

The protein content of the homogenized fish tissues were estimated by using Bradford method. The OD values are absorbed at 595 nm. BSA was taken as standard.

Estimation of lipid (Ehrlich method)

The lipid content of the homogenized fish tissues were estimated by using Ehrlich method. The
OD values are absorbed at 533 nm. Cholesterol was taken as standard.

Enzymatic Assay

Protease (Kunitz method)

The protease content of the homogenized fish tissues were estimated by using Kunitz method. The OD values are absorbed at 578 nm. Tyrosine was taken as standard.

Lipase (Vadehra& Harmon Method)

The lipase content of the homogenized fish tissues were titrated by using Vadehra& Harmonmethod. Olive oil was taken as substrate.

Microbial analysis and Gram Staining

The TPC (Total plate count) observed in water and sediment using spread plate method. From the different colonies were taken and smeared in glass slide to identify the gram negative, gram positive bacteria using gram staining method. Which is followed by Gram's method.

Result and Discussion

Study Area

Poondi Reservoir (later named as Sathyamoorthy Sagar) was constructed in 1944 across the Kosathalaiyar River or Kotralai River in Thiruvallur district with a capacity of 2573 Mcft and placed in service for intercepting and storing Kosathalaiyar River water. Surplus water flows down the river which is again intercepted by Tamaraipakkam Anicut and diverted to Sholavaram Lake and Puzhal lake. There are several industries around the Poondi reservoir such as DMC, DUCK, TATA and etc., Recent years the reservoir is polluted by increased human, industrial waste and the water flow is also reduced hence the impact of pollution had exceed resulting in increase in mortality of fishes. Figure 1 shows the dead Tilapia fishes found at Poondi Lake reservoir. This may be due to the decrease in water flow and increased pollution rate.

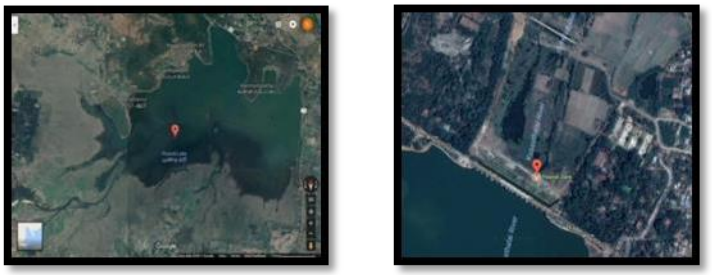


Figure 1. Map and location of sampling site, Poondi Lake, Tiruvallur District, India

Water and Sediment Microbial Load Analysis

Without microbe the life cannot exist in the earth. But the excess in the microbial load in fresh water ecosystem leads to infectious diseases. Hence monitoring the microbial flora is necessaryto determine the quality of fresh water. The water and sediment sample was serial diluted and cultured in the plate by pour plate method.

The total plate count (TPC) observed in water and sediment samples obtained from Poondi Lake were represented in figures 2 and 4.

A: Sample Water stock

B: Sample Water 10^{-1} dilution

C: Sample Water 10^{-3} dilution

Table 1: Colony count of water sample

Sl. No	Sample	CFU/ml
1	Water stock	200 ± 2.34
2	Water 10^{-1} dilution	4 ± 0.24
3	Water 10^{-3} dilution	0.2 ± 0.016

Figure 2. Microbial load analysis of the water sample collected from Poondi Lake, Tiruvallur District, India by Pour Plate method

The TPC values obtained at stock water sample is 200 cfu/0.1ml and it is 0.2 cfu/ 0.1ml at the dilution of 10^{-3}. The TPC values of sediment is comparatively higher than the water sample *i.e.*, 400 cfu/0.1ml in sediment stock solution. The decrease in microbial load of water sample compared to sediment may be due to the flow of water. The water sample consists of both Gram negative rods and Gram positive cocci microbial flora which is depicted in figure 3, mostly the rods are in chains and the cocci are in clusters whereas the figure 3 depicts the sediment microbial flora which consists of only Gram positive cocci. This shows that Poondi lake is

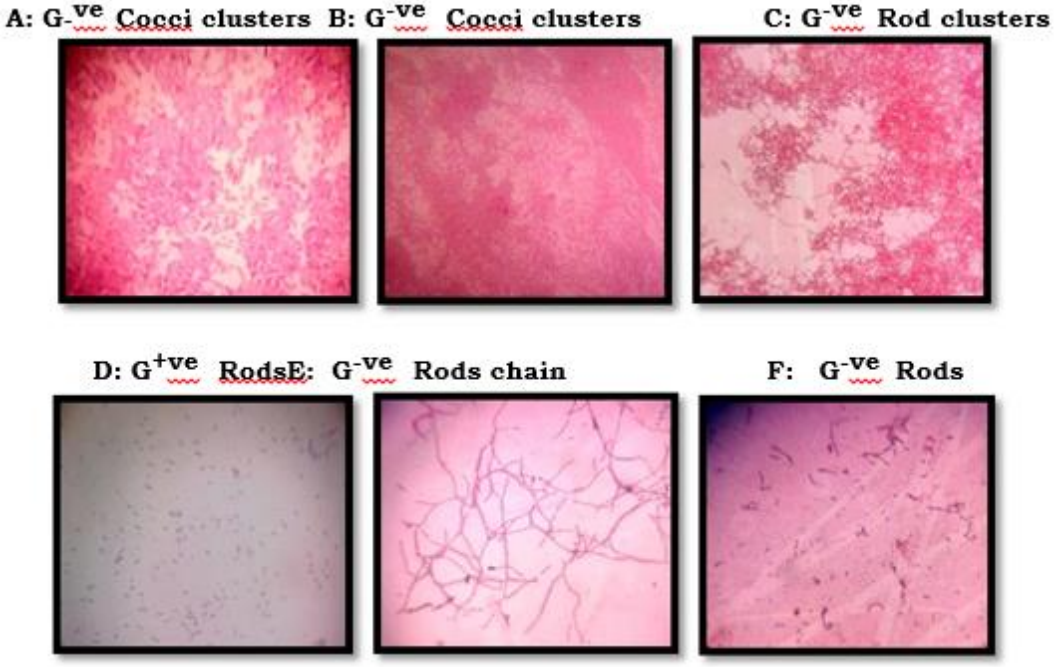
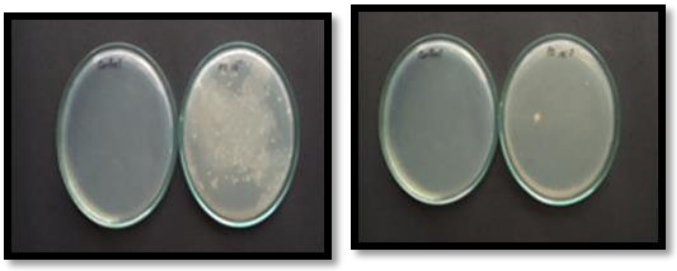


Figure 3. Photomicrograph of the microorganism isolated from the water sample collected from Poondi Lake, Tiruvallur District, India.

A: Sample Sediment 10^{-1} dilution B: Sample Sediment 10^{-3} dilution



B: Sample Sediment 10^{-5} dilutionTable 2: Colony count of sediment sample

Sl. No	Sample	CFU/ml
1	Sediment 10^{-1} dilution	400 ± 1.76
2	Sediment 10^{-3} dilution	26 ± 0.24
3	Sediment 10^{-5} dilution	7 ± 0.016

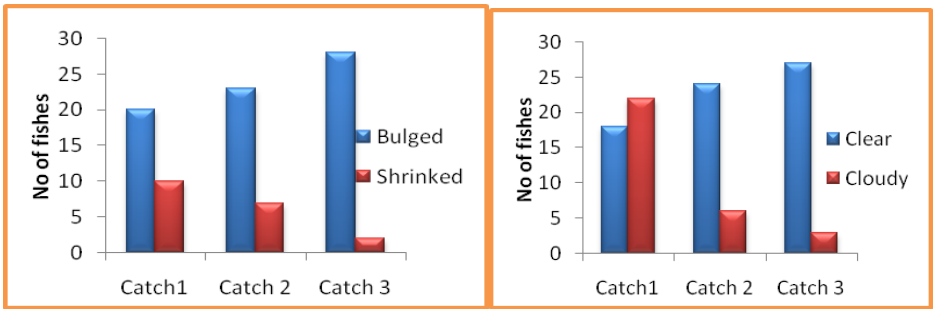
Figure 4 A, B. Microbial load analysis of the sediment sample collected from Poondi Lake, Tiruvallur District, India by Pour Plate method.

contaminated with human and animal faeces, but the colony count is under permissible level and further biochemical characterization is required to identify the organism. (Anusuya Devi, (2016), reported that Poondi lake is free of faecal*E.coli* and Streptococci contamination, but in the current data presence of gram negative and gram positive bacteria were observed this may be due to the sampling period since the sample collection is made in summer season the possibility of increase in microbial flora is more due to reduction in water level.

Proximate Analysis of *Oreochromis niloticus*

Tilapia inhabits fresh water and is found in shallow streams, ponds, rivers and lakes (Chapman and Frank, 1992) and cannot survive in temperate climate. They are fast growing, lean and short- lived, with primarily vegetarian diet thus contains low level of mercury (McCrary *et al.*, 2005). It is low in saturated fat, calories, carbohydrates and sodium, potassium, phosphorus, niacin, selenium, vitamin B12 and is a good protein source (Trewavas, 1983). The physical characteristics of tilapia fishes obtained from Poondi lake at three different catches were represented in figure 5.

A: Eye **B: Pupil**



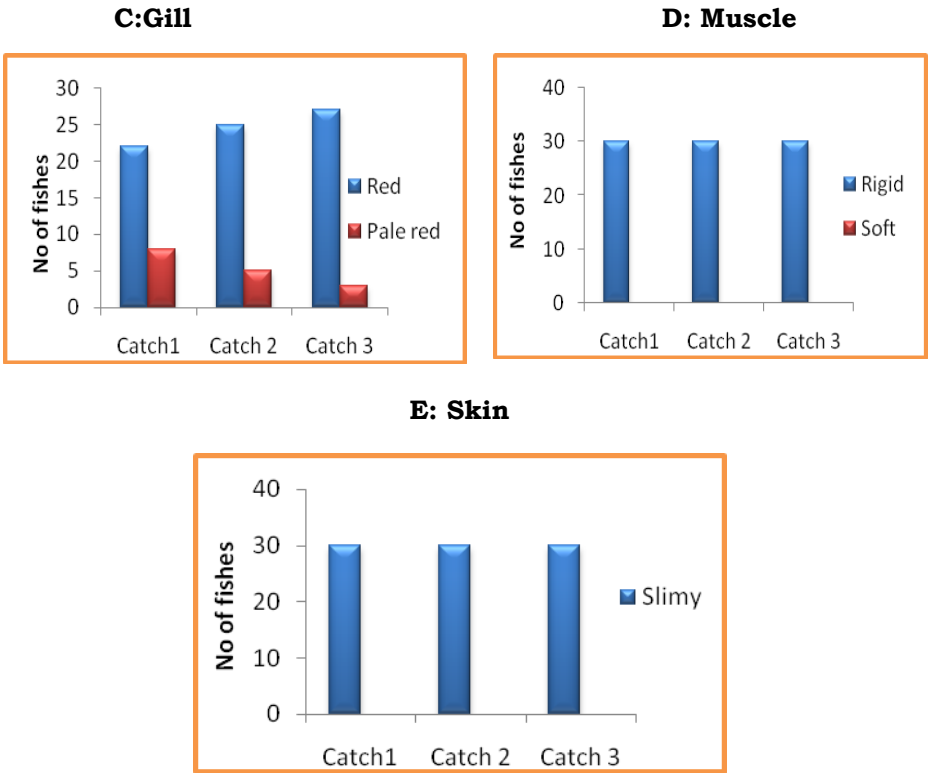
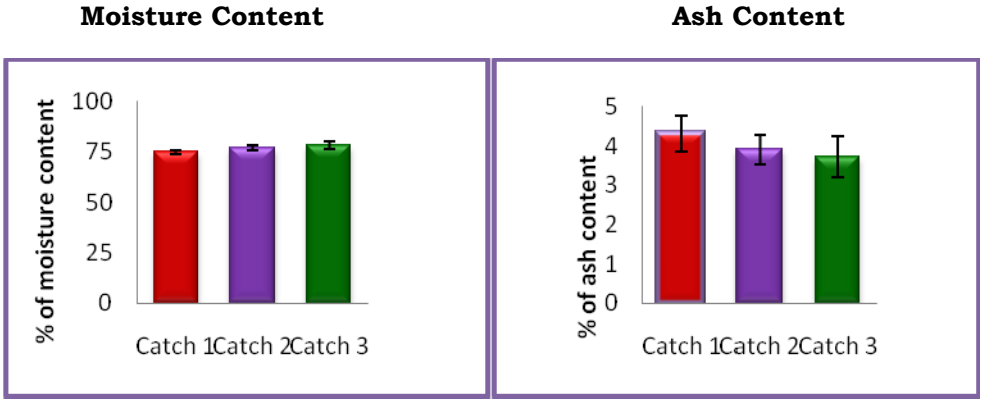


Figure 5. Physical Characterization of bio-indicator species *Oreochromis niloticus* collected from Poondi Lake,Tiruvallur District, India

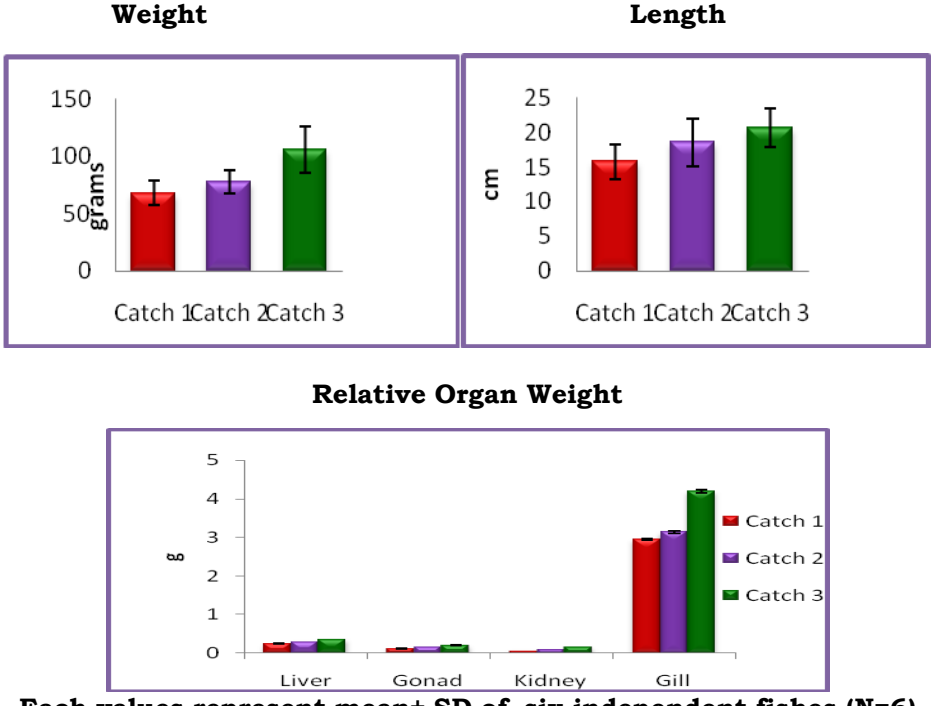
The appearance of eyes, pupil, gill colour, muscle and skin were normal and the except few fishes others were healthy. Totally thirty fishes were analysed for each catch to analyse the physical appearance.

The moisture and ash content of Tilapia fish caught from Poondi Lake were depicted in the figure 6. There is no difference in moisture and ash content of fish during different catches, the moisture content is 75-77% whereas the ash content is 4 – 4.5%, this shows that the fishes were healthy and the mineral content in the fish are at the normal level. The Length and relative organ weight of the Tilapia fishes, were represented in figure 9. The weight of the fishes increased during the third catch, this may be due to the rain on the month of July which would enriched theecosystem of fish and diluted the microbial load and pollutants in the Poondi Lake.



Each values represent mean± SD of six independent fishes (N=6)

Figure 6. Result of proximate analysis of the bio-indicator species *Oreochromis niloticus* collected from Poondi Lake, Tiruvallur District, India.



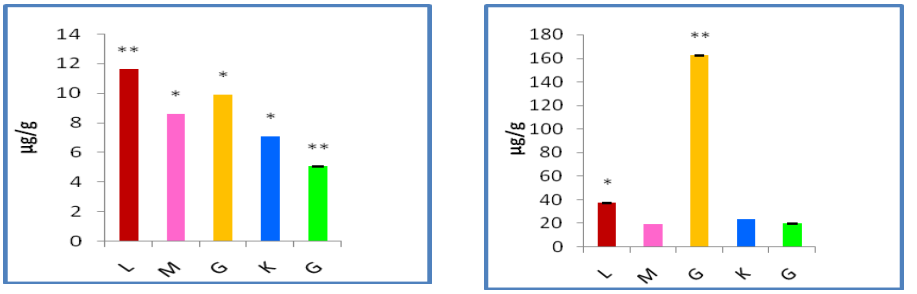
Each values represent mean± SD of six independent fishes (N=6)

Figure 9. Relative organ weight analysis of the bio-indicator species *Oreochromis niloticus* collected from Poondi Lake, Tiruvallur District, India

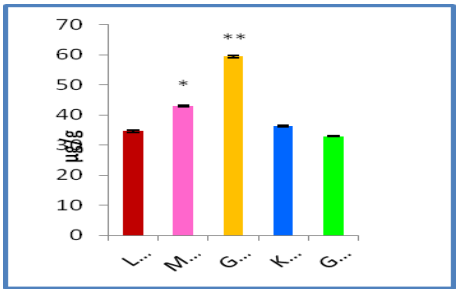
Biochemical Estimation of Various Tissues of *Oreochromis niloticus*

Carbohydrates serve as the instant energy (Fig. 7) source during stress condition hence the total carbohydrate level was estimated in different tissues of bioindicator species. *Oreochromis niloticus* and it is depicted in the figure 7-A.

A: Total Carbohydrate **B: Total Lipid**



C: Total Protein



Each values represent mean± SD of three independent experiments (N=3)

Figure 7. Biomarker estimation of the bio-indicator species *Oreochromis niloticus* collected from Poondi Lake, Tiruvallur District, India

The gonad possess increased carbohydrate levels compared to the organs this may be due to the increase energy demand of the organ. Since after carbohydrate, it's the lipid molecules which are utilized to overcome the stress. Total lipid was formed by the cholesterol, phospholipid, and triglyceride and so the elevation in its components leads to the increase in total lipid hence the total lipid content were assessed and depicted in figure 7-B. The liver consists slightly increased lipid content compared to the other organs. The protein level depicted in figure 7-C shows that the all the organs more or less same amount of protein content which is slightly increased in the gonads. The levels of protein, fat, and ash in fish usually change according to nutrition, living area, fish size, catching season, seasonal and sexual variations as well as environmental difference in the levels of biomarkers in the organs of *Oreochromis niloticus.*

Enzyme Estimation of Various Tissues of *Oreochromis niloticus*:

Protease is any enzyme that performs proteolysis; protein catabolism by hydrolysis of peptide bonds. They are involved in a multitude of physiological reactions from simple digestion of food proteins to highly regulated cascades (*e.g.*,

the blood-clotting cascade, the complement system, apoptosis pathways, and the invertebrate prophenoloxidase-activating cascade). Proteases can either break specific peptide bonds (limited proteolysis), depending on the amino acid sequence of a protein, or break down a complete peptide to amino acids (unlimited proteolysis). Hence this protease enzyme is estimated in various vital organs of the Tilapia fish and depicted in the figure A, the level protease enzyme is increased in liver and comparatively decreased kidney, this may be increased metabolic rate.

A: Protease level **B: Lipase level**

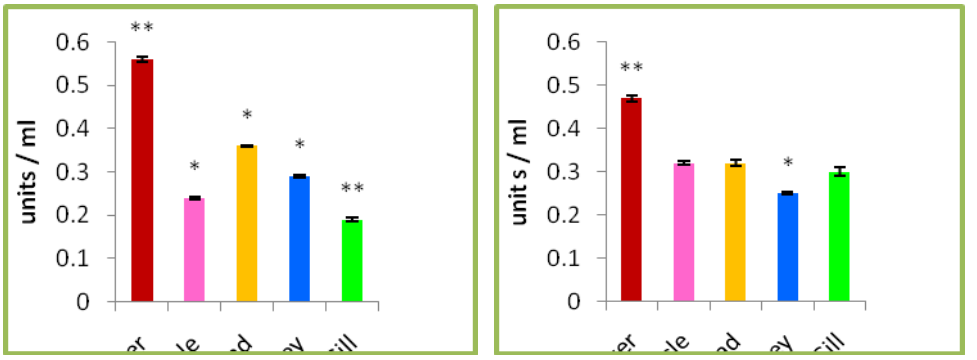
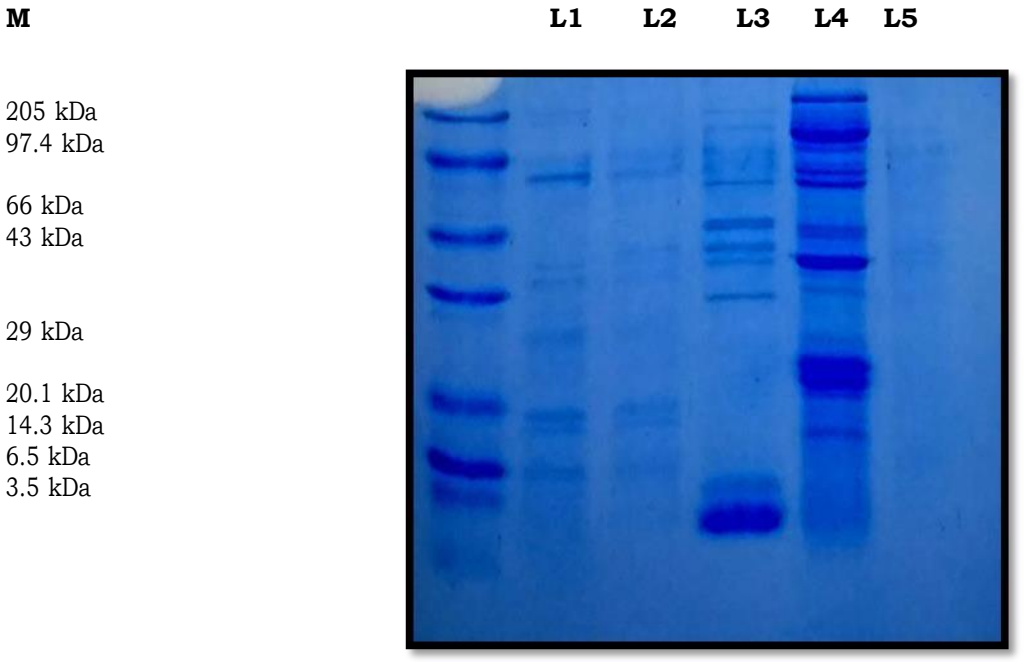


Figure 8. Enzyme analysis of the bio-indicator species Oreochromis niloticus collected from Poondi Lake, Tiruvallur District, India B: Lipase level

Lipase is Any enzyme that catalyzes the hydrolysis of fats (Svendsen, 2000). Lipases are a subclass of the esterase's. Lipases perform essential roles in the digestion, transport and processing of dietary lipids. The levels of lipase enzyme were shown in the figure 8-B, both the levels of lipids and lipase enzyme were increased in the liver compared to the other organs this may be due the lipoprotein metabolism.

Protein Profile (SDS-PAGE) of Selected Organs of *Oreochromis niloticus*:

Gel electrophoresis is a separation technique which is often used to separate large molecules such as protein or nucleic acids which may have molecular masses in the range from thousands to millions of Daltons. The separation in electrophoresis is based on two characteristics of the molecules being separated electrical charge and size. Quantitative evaluation of protein levels can be accomplished with protein profiling, which shows us unique expression patterns. The electrophoretic pattern profile of five vital organs of *Oreochromis niloticus* were depicted in the figure 9. The Gonad protein sample shows dense protein bands with various molecular weight, whereas the gill, liver and kidney shows less dense protein bands. The muscle protein sample showed very less dense bands. The expression of protein varied among the organs but the impact of pollution on the protein profiling will be confirmed with the further analysis of protein expression for specific stress response proteins.



Conclusion

To conclude, the assessment of Poondi lake water during the summer season, where there is scarcity for water in which contamination may have a greater impact on public health. Since Chennai is the city which mainly depends on the Poondi reservoirs for their water source, the presence of both gram-positive cocci and gram-negative rods in the samples confirms the contamination. Even though there is no changes at the macromolecular level of fishes, the mortality and the decrease in gonadal size infers there is some abnormalities at genetic level which requires further investigations to prevent serious health hazards to the society.

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