AN ANALYSIS OF THE BACTERIAL POPULATION AND IDENTIFICATION OF HUMAN PATHOGEN PATTERNS IN SOIL SEDIMENTS AND WATER SAMPLES FROM THE PECHIPARAI DAM OF KANYAKUMARI DISTRICT

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Abstract

The necessary component for all life to survive sustainably on earth and every living depends on water sources for daily activity, survival, and existence. This potential source is actually becoming contaminated as a result of industrialization, and modernization of the human population causes microbial discharges and the excretion of dangerous substances. This condition causes ecological habitations to decline. This inquiry looked at the bacterial populations in soil sediment samples, water, and pathogenic (SLP and PLS) fish parts accumulation in the Pechiparai reservoir. The outcome data revealed theabundance of bacterial population about 194 ± 4 CFU/dilution of average in pre-monsoon season of soilsedimentsamplesand water (197± 2 CFU/dilution in average) over other seasons (On-monsoon and Postmonsoon). Duringmonsoonthe waterbacterialconsistency wasrefreshinghence the aquaticecosystem is balanced with both micro and macroscopic level organisms. Higher bacterial inhabitants was recorded in the surface ofNile tilapia (*Oreochromisniloticus*) and the isolated colonies werestudied for the identification pathogens (SLSand PLS). Differential media (Macconkey), haemolyticand biochemical characterization data revealed the presence of *Bacillus sps* and *Staphylococcus sps*. Further coliforms were enumerated in both water and soil sediments on three seasons. Among others, monsoon season exhibited predominant presence ofcoliforms around 60coloniesinwatersamplesofpre-monsoon season.

Keywords: Industrialization, chemical and microbial contaminants,SLS (*Staphylococcus* like sps), PLS (*Pseudomonas* like sps), colony forming unit (CFU), Nile tilapia(*Oreochromisniloticus*), coliforms and *Basillussps*, *Staphylococcussps* and monsoon seasons.

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Introduction

Water is a plentiful, essential, and life-giving element on earth. Freshwater is also a vital resource for industrial, agricultural, and human consumption purposes (Hahn, 2006). The main cause of water pollution in India is the country's growing population, which has resulted in deforestation and excessive air, water, and water pollution. Point sources, such as

garbage from industry and people, as well as pesticides and fertilisers used on farms, are what create water pollution, according to Amarsinghe and Sharma (2009). There is a significant amount of water in the Kanyakumari district, which is made up of numerous rivers, streams, ponds, and marshes.The uncontrolled urban expansion, deforestation, large-scale sandmining, mushroom stoves, bricks, coconut fibre, and other residential, agricultural, and industrial wastesdischarged into numerous reservoirs in this district are now causing progressive ecological degradation.ThePechiparai reservoir, which was created between 1897 and 1906 by the European engineer MininAlexander, has a catchment area of 207.19 km^2. Based on this observation, five divisions of diverse phytoplankton (104species), and four distinct groupings of zooplankton, have been identified (30species). (65.16%), followed by Dinophyceae (9.62%), Euglenophyceae (9.62%), Cyanophyceae(10.44%), and Chlorophyceae(10.32%)(4.45%). Rotifers were the most prevalent zooplankton category, accounting for 35.40 percent of all samples, followed by crustaceans (30.21 percent), protozoa(21.17%), and cnidarians (21.17%) (13.21%). According to Vidhy.V.and Radhakrishnan Nair, phytoplankton predominated in this biotope (2012). Different bacterial species are found in freshwatercan be impacted by the water's quality. Physical and chemical characteristics of freshwater can have animpactonthediversityandabundanceofmicrobialorganisms.Differentmicrobesreactdifferentlyunder certain circumstances. While some can tolerate a variety of situations, others are either sensitive toor intolerant to them (Raibole and Singh, 2011). As a result, rivers as an aquatic ecosystem must be ingood condition, and one of the most crucial steps to restoring river health is to research and comprehendthe makeup and activities of the microbial population (Zhang *et al*., 2012). Freshwater microorganismsof various sorts should be primarily evaluated, and those bacteria deemed to be significant microbesshould be watched (Velimirov*et al*., 2011). Additionally, it is important to regulate aesthetic aspects of water quality, suchasodour and excessive bacterial growth (Prest. Emmanuelle*et al*., 2016).

Materials and Methodology Study Area

One of the water storage reservoir in Tamil Nadu, ispechiparai reservoir situated close to the village of Pechiparai, in the Kanyakumari District, 43 kilometres(27miles) from Nagercoil. The reservoir was created by the Jincy Dam, which was built about 1.6 km below where the streams Kallar,Chittar, and Kuttiyar met the Kodayar River. The reservoir is 207.19 km2 (80 square miles) in size, and its depth is 14.63 metres (48.0ft). Water from the reservoir is used by city people, farmers, and plants forirrigation and drinking.

SampleCollectionandPreparation

The samples were collected in three different seasons' worth of water and soil sediment samples were taken a septically from the Pechiparai water reservoir using polythene bottles (Pre,On, and Post Monsoon). After being delivered to the analytical laboratory, the sediment samples were shed dry for physicochemical and heavy metal examination.

Analysis of bacterial population

The protocol for enumeration of bacterial population in three seasons (Pre,On and Post)monsoons at three different sites of Pechiparai Dam was followed by Colle and Miles (1989). Stocksolutions of soil sediments and water samples (Site-I, II and III) of three seasons were prepared bymeasuring 1 ml (1ml) in9ml of sterile water for water samples and0.1g in 10 mL distilled water. Anine (10^{-1} to 10^{9}) of serial dilutions were made from stock sample solution. This was done to reduce thebacterial population to obtain isolated strains. Later, 0.1ml of each of respective samples were thenpipette out from the 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} dilution were inoculated onto the surface of respective Petridishes containing 20ml of a solidified and sterile Nutrient Agar, and then spread evenly with sterile glass spreader and the plates were marked properly. The plates were then incubated for 24hr at37°C(including the control plates). Counting of thebacterialcolonies wasdone using the StuartDigitalcolony counter (Abba *et al*., 2008). The developed microbial colonies were counted and calculated ascolony forming units per millilitre or gram (cfu/ml or cfu/g) of each sample respectively (Eleyowo*et al*.,2016).

No.Of cell perml of sample(CFU/ml)= No.ofcoloniescounted
Volume plated XDilution factor

Analysis of bacterial population in Niletilapia Fish

Aselectedpechiparifishspecies,Niletilapia(*Oreochromisniloticus*)(Linnaeus,1758),wascapturedfromPechiparaiDamandbroughtlivetothelaboratoryforbacterialenumeration.Dissectingthe fish to separate thegut and surface sectionsyielded the sample tissue extracts. The portionswerethen cleaned in autoclaved distilled water and softly crushed for use as inoculums using tissue extract.Stock solutions of Fish tissue samples of Gut and Surface were prepared by measuring 1 ml of tissueextract (1ml) in 9ml of sterile water. A nine (10-1 to 109) of serial dilutions were made from stocksample solution. This was done to reduce the bacterial population to obtain isolated strains. Later, 0.1mlof each of respective samples were then pipette out from the 10-5, 10-6, 10-7 and 10-8 dilution wereinoculated onto the surface of respective Petri dishes containing 20ml of a solidified and sterile NutrientAgar, and then spread evenly with sterile glass spreader and the plates were marked properly. The plateswerethen incubatedfor24 hr at 37°C.

Isolation of Heamolyticstrains from Selected water and soil samples of Pechiparai Dam

The blood agar plates were prepared in an appropriate way to isolate heamolytic strains. Thepreparedbloodagarmediumwassterilisedusingamoistheatsterilisationmethodinanautoclave at121 °C for 20 minutes. Cooled at room temperature, 5% sheep blood was withdrawn and added into themedia aseptically. Then slight mixing was done to disassociate the blood in the media. The media waspoured into sterilized petriplates and left for solidification. After solidification, the sample inoculumswere streaked on petriplates with control organism such as *Staphyloccussps*and*Pseudomonas sps*. Theinoculated plates were kept in incubator at 37 ºC for 24 hrs. End of the incubation, the heamolyticstrainswere identified by determining the zone formed around the colonies indicated the strains possessedheamolyticactivitycompared with positive control strains.

Identification of Heamolytic strains from Water and Soil samplesColonyCharacterization

The media was prepared in the appropriate volume for each isolate and sterilized by autoclavingat 121 °C for 20 minutes. The sterile medium was allowed to cool at room temperature and poured intosterilepetridishes. Following solidification, the shortlisted isolates were quadrant streaked and incubatedat 37 °C for 24 hours. after incubation the morphology were assessed for the identification of strains.Macconkey agar medium was used as a selective medium to find *staphylococcus* and *Pseudomonas*likeorganisms (SLO and PSLO). The isolated colonies were stained using Gram's Staining Solutions for thedeterminationofthe cell membranetypeand shapeofthebacteria.

Biochemical identificationCitrateutilizationtest

Bacterial colonies are picked up from a straight wire and inoculated into the slope of Simmon'scitrate agar and incubated overnight at 37 °C. 2. If the organism has the ability to utilise citrate, themedium changes its colour from green to blue. If the colour of the medium changes to blue, it is citratepositive.

Nitrate ReductionTest

Inoculate then itratebroths with bacterial suspension. In cubate the tubes at the optimal temperature of 30°C or 37°C for 24 hours. After incubation, look for N_2 gas first before adding reagents.Add 6-8 drops of nitrite reagent A and add 6-8 drops of nitrite reagent B. Within a minute or less,observe for the reaction (color development). If no colour develops, add zinc powder. Observe for atleast3 minutes for are dcolour to develop after theaddition of zinc.

Catalase Test SlideMethod

Usealooporsterile wooden stick to transfer a small amount of colony growth on to the surfaceof a clean, dry glass slide. Place a drop of 3% H_2O_2 on the glass slide. Observe the evolution of oxygenbubbles.

Methylred(MR)test

The bacterium to be tested was inoculated into glucose phosphate broth, which contains glucose and a phosphate buffer, and incubated at 37oC for 48hours. Over the 48hours, the mixed-acid producing organism must produce sufficient acid to overcome the phosphate buffer and remain acid. The pH of the medium is tested by the addition of 5

drops of MR reagent. The development of red istakenas apositive. MR-negative organisms produce yellow.

Vogesproskauer(VP)test

Bacterium to be tested is inoculated into glucose phosphate broth and incubated for at least 48hours.0.6mlofalpha-naphtholisadded to the test brothand shaken .0.2ml of 40% KOH is added to the broth and shaken. The tube is allowed to stand for 15minutes.Theappearance of red wine is taken as a positive test. The negative tubes must be held for one hour since maximum colour development occurs within one hour after the addition of reagents.

Detection of Coli forms from water and soil Sediments of Pechiparaidam

The samples were prepared as serial dilutions in sterile distilled water as dilutions 10^{-4}, 10-5, and10^{-6}. Eos in methylene blue agar was prepared as per the volume required and auto claved at 121°Cfor20 min. The media wasallowed to cool down and plated on sterile petriplates. After solidification, 0.1ml was inoculated on EMB agarplates using the spread plate technique. Inoculated plates were incubated at 37°C for 24 hours. After incubation, observe green metallic sheen coloured colonies for coli form detection.

Results and Discussion

Analysis of bacterial population in water, soil sediments and fish samples

The bacterial population of study samples from the pre, on, and post monsoon seasons wereassessed using the conventional spread plating technique followed by serial dilutions. After incubation,the plates were counted for distinct bacterial colonies and expressed as CFU (colony forming units). Thehigher CFU of pre-monsoon seasons of water site-I showed 69 X 10^6 CFU from site-I, 48 X 10^8 fromsite-II, and 42 X 10^8 CFU from site-III from pechiparai water samples. Regarding soil sediments, theCFU of site-I was 62 X 10^5, 61 X 10^5 for site-II, and 63 X 10^7 CFU were observed in pre-monsoonseasons. During the on-mansoon season, the CFU of pechiparai water samples showed the abundance ofbacterialpopulationas32X10^6,whilesite-IIwas29X10^5and49X10^8CFU**(Figure:1andTable: 1)**. While in soil sediments, site-I exposed 7 X 10^5 CFU, 14 X 10^5 from site-II, and 4 X 10^5. In the post-monsoon season, the bacterial populations decreased as 7 X 10^6 from site-I, 6 X 10^8 was CFU of site-II,and 6 X 10^6 colonies from site-III of pechiparai water samples. whereas in soil sediments, higher CFUwas observed in site-I at 10 X 10^8, though site-II showed 6 X 10^5 and 9 X 10^6 CFU were enumeratedfromsite-IIIrespectively**(Figure: 2 and Table:2)**.

Table:1. Bacterial population of water samples of three different monsoon seasons of Pechiparai Dam

Bacterial population in water samples as CFU/ dilution in average			
Seasons	**Site-I**	**Site-II**	**Site-III**
Pre-Monsoon	197	179	154
On-Monsoon	88	91	117
Post-Monsoon	21	18	15

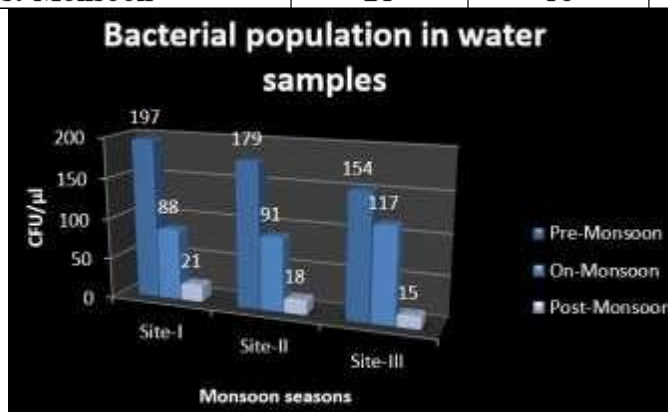


Figure:1. Bacterialpopulationofwatersamplesofthreedifferentmonsoonseasonsof Pechiparai Dam**Table: 2.** Bacterial population in Soil sediment samples of three different monsoon seasons ofPechiparaiDam

BacterialpopulationinSoilsedimentsamplesasCFU/dilutionin average			
Seasons	**Site-I**	**Site-II**	**Site-III**
Pre-Monsoon	177	194	194
On-Monsoon	18	33	14
Post-Monsoon	20	21	29

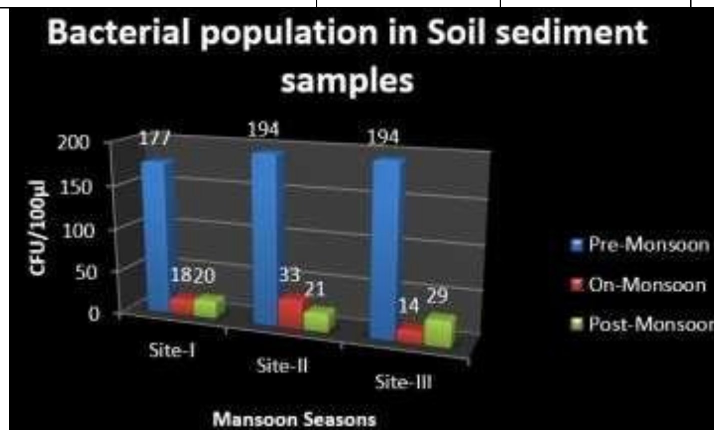


Figure: 2. Bacterial population of Soil sediment samples of three different monsoon seasons of PechiparaiDam

The bacterial population ofgut and surface tissue extracts of Niletilapiafish was caught from Pechiparai dam. The tissue extracts were spread on plates from serial dilution and the colonies werecounted. About 47 X 10^5maximum colonies were enumerated from gut extract, and 42 X 10^6 CFU wereobtainedfrom thesurfacetissueextract of Niletilapiafish**(Figure: 3 andTable:3)**.

Table:3. Bacterialpopulation inTilopiafish(*Oreochromisniloticus*)ofPechiparaiDam

Bacterial PopulationinTilopiafish(*Oreochromisniloticus*)	
Fishpart	**Colonies**
Gut	135
Surface	153

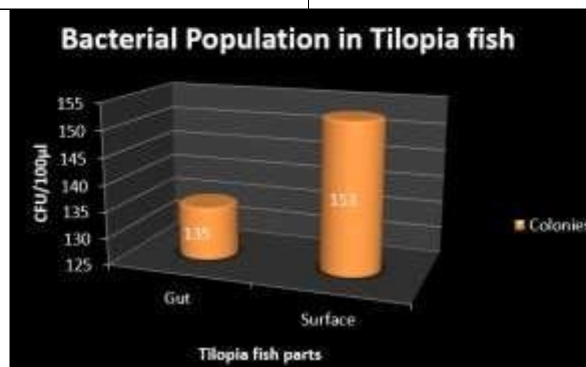


Figure:3. BacterialpopulationofTilopiafishpartsamplesofPechiparaiDam

Isolation of Heamolytic strains

This assessment revealed the presence of heamolytic strains in sheep blood agar plates comparedwith pseudomonas (PLS) and Staphylococcus like species (SLS). The inoculums from water and soilsediments of three different (Pre,

On, and Post monsoon) seasons of three sites (I, II, and III) wereinoculated on blood agar plates and the heamolytic zone observed against positive control strains (PLSand SLS). From soil sediment samples collected pre-monsoon, 4 heamolytic colonies were identifiedfrom site-I, 2 from site-II, and 1 from site-III. While on-monsoon, 4 heamolytic strains from site-I, site-II, and site-III exhibited 3 colonies each, while site-IV contained 1 heamolytic colony in soil sediments.Whereas, after the monsoon, two colonies were found in soil sediments fromall three sites (I, II, andIII). Other hand, of the water samples, 1 colony was observed in both sites-I and II in the pre-monsoon season and no heamolytic colonies were observed from on and post monsoon of all three sites (I, II, andIII) of pechiparai water samples**(Figure: 4)**.

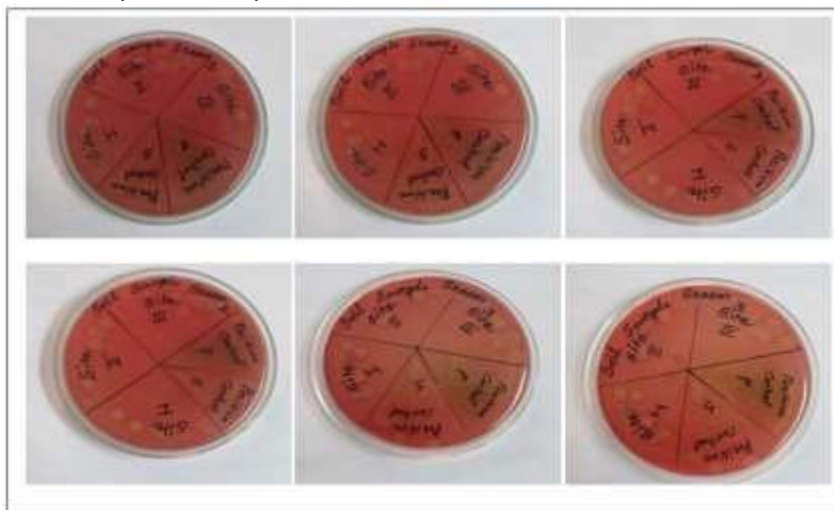


Figure:4. Identification of Heamolytic Colonies from water and soilsedimentsamplesofthreedifferentmonsoon seasons ofPechiparai Dam of Pechiparai Dam

Identification of Heamolyticstrains

Among the identified heamolytic strains from soil sediments and water samples from the pre, on,and post monsoon seasons of Pechiparai water reservoir, burly zone produced strains were furthersubjected for genus identification through straining and biochemical tests. The selected strains from soilsediments from three sites of pre, on, and post monsoon were observed as gram positive (purple) andcocci chain in shape except for colony-1 from site-I of soil sediment of pre monsoon. The biochemical results revealed that the strain confirmation at genus level showed Bacillussps.ie.Thecolony-1from site-I of soil sediment of the pre monsoon season and the other strain were identified as *Staphylococcussps*from colony-2 of thefirst siteof thepostmonsoon(**Table:4 and Figure:5**).

Table:4. Biochemical identification of isolated strainsfrom water and soil sediment samples of Pechiparai Dam

Test	Organism 1(I1)(*Bacillussps*)	Organism 2(III1)(*Staphylococcussps*)
Citrate	Positive	Positive
Nitrate	Positive	Positive
TSI	Gulucose– Positive	Gulucose– Positive
	H2S-negative	H2S-negative
Indole	negative	negative

MR	Positive	negative
VP	negative	negative
Catalase	negative	Positive
Urease	negative	negative

Figure:5.Biochemical Identification of HeamolyticColonies

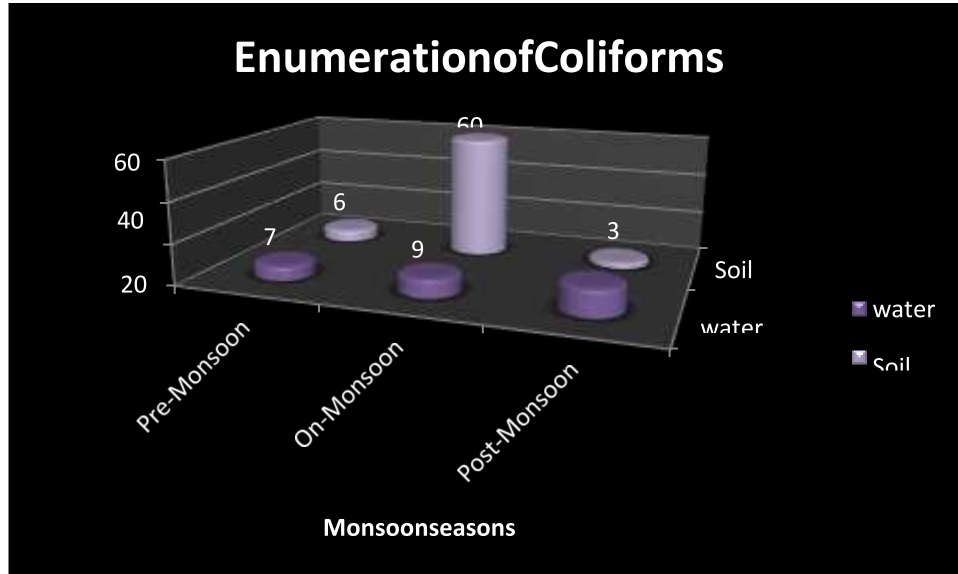
DetectionofColiforms

This was achieved by spread plating the inoculums on EMB agar medium and observing greenmetallic sheen colonies were observed as Escherichia coli coliforms. Table: and figure:show thecoloniesthat werecounted**(Table:5 and Figure:6)**.

Table: 5. Enumeration of Coliforms in water and Soil sediment in three different seasons of PechiparaiDam

Season	**water**	**Soil**
Pre-Monsoon	7	6
On-Monsoon	9	60

Figure:6. Enumeration of Coliforms from water and soilsediments of three sites of three monsoon seasons in Pechiparai Dam



Conclusion

Theenumerationresultsrevealedtheabundanceofbacterialpopulationsinsoilsedimentcollected from sites I, II, and III in the pre monsoon season and, secondarily, water samples from threesites (I, II, and III) in the pre monsoon season. However, the gut microbial ecosystem showed a largerbacterial population than fish surface tissues. Among the enumerated and isolated strains from water andsoil sediment samples, 7 heamolysis strains were identified from pre-monsoon soil sediments, 8 strainsfrom the monsoon season and 6 strains from the post-monsoon season. While from water samples, 2strains possessed heamolytic activity from pre-monsoon samples alone. The observer heamolytic strains were shortlisted by strong zone formation on blood agar plates and characterised morphologically and biochemically *as Bacillussps*(colony-1fromsite-Iofsoilsampleonpremanosoon) and *Staphylococcus sps*(colony-2 from site-I of post monsoon). Above all, the water and soil sediment samples from three different sites showed the presence of quite alarge population of*E.* coliforms.

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