“STUDY ON THE WATER QUALITY PARAMETERS AND THE PRESENCE OF *Escherichia coli* IN VEMBANAD LAKE”

Project Work By

SHEKHA FATHIMA

AB20Z00011

Under the guidance of

DR.MEERA JAN ABRAHAM,

Associate Professor,Department of Zoology,

St.Teresa’s College (Autonomous), Ernakulam

Kochi-683011

Submitted to St.Teresa’s College (Autonomous) Ernakulam

Affiliated to Mahatma Gandhi University, Kottayam in partial fulfillment of requirement for the degree of Bachelor in Science in Zoology

2022-2023

CERTIFICATE

This is to certify that the project entitled "STUDY ON THE WATER QUALITY PARAMETERS AND THE PRESENCE OF *Escherichia coli* IN VEMBANAD LAKE" submitted in partial fulfilment of the requirement for the award of the degree of Bachelor of Science in Zoology to the Department of Zoology, St.Teresa's College affiliated to M.G University, Kerala, done by Ms.SHEKHA FATHIMA Reg. No: AB20ZOO011 is an authentic work carried out by her at Enfys Lifesciences Pvt. Ltd, Kochi under my guidance and supervision during the period of Nov 2022-Dec 2022. The matter embodied in this dissertation has not formed the basis for the award of any Degree/Diploma/ Associateship /Fellowship to the best of my knowledge and belief.

Dr.Jasmin C
Director (Research)
Enfys Lifesciences Pvt Ltd

CERTIFICATE

This is to certify that the project entitled "STUDY ON THE WATER QUALITY PARAMETERS AND THE PRESENCE OF *Escherichia coli* IN VEMBANAD LAKE" submitted by Ms.SHEKHA FATHIMA , Reg no-AB20ZOO011 in partial fulfillment of the requirement of Bachelor of Science of science in Zoology to the Department of Zoology, St.Teresa's College affiliated to Mahathma Gandhi University ,Kottayam is a bonafide work under my guidance and supervision and to my her best knowledge, this is her best effort.

Dr.Meera Jan Abraham	Dr.Soja Louis
Associate Professor	Head of the Department
Department of Zoology	Department of Zoology
St.Teresa's College (Autonommous)	St.Teresa's College
Ernakulam	Ernakulam

EXAMINERS

1.

2

DECLARATION

I hereby declare that project work titled " STUDY ON THE WATER QUALITY PARAMETERS AND PRESENCE OF *Escherichia coli* IN VEMBANADU LAKE" submitted to St. Teresa's College (Autonomous), Ernakulam affiliated to Mahatma Gandhi University, Kottayam in the partial fulfillment of the requirements of Bachelor of Science degree in Zoology, is a record of original project work done by me under the guidance and supervision of Dr. Meera Jan Abraham, Associate Professor, Department of Zoology, St. Teresa's College (Autonomous), Ernakulam.

Name:SHEKHA FATHIMA

Reg.No:AB20ZOO011

Signature

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ABSTRACT

The alarming rate of anthropogenic activities remains one of the main causes of poor water quality in rivers , lakes , etc.The Vembanad lake is under heavy pressure from domestic waste , industrial pollution and eutrophication etc .

The objective of the study is to analyze the water quality parameters and determine the presence of *Escherichia coli* in the Nadakkavu region of Vembanad lake.The procedure involved sample collection from different stations and was tested for MPN(Most Probable Number), SPM (Suspended Particulate Matter),Turbidity,and DNA analysis including onsite results over Salinity , pH , Temperature and Secchi depth.

The average pH of the study is found to be 6.7.The slight acidity is due to untreated effluent discharge, addition of chemical pollutants from agricultural waste.The average of the salinity is 4.1.There is a huge difference seen in turbidity marking highest in 2 stations -Nikarthil and Pullukattu.The average of the Dissolved oxygen is found to be 5.8mg/L. Fluctuations in pH, Salinity , Turbidity ,SPN , MPN were observed due to inundation , variation in daily tides , algal bloom , eutrophication etc .DNA study shows the presence of E coli in all the station which indicates that water is contaminated with feces. The result embodies the need for monitoring the lake system to preserve the water quality and for assessing the level of pollution.

INTRODUCTION

The wetlands are metabolically active ecosystem components which influence the nutrient loadings and serve as regulators of pelagic productivity(Meera et al.,2010).
Vembanad is one of the largest brackish, humid tropical wetland ecosystems (09°00-10°40'N and 76°00'-77°30'E)on the southwest coast of India, fed by 10 rivers. It spreads over the districts of Alappuzha, Kottayam,Ernakulam and Thrissur of Kerala.The lake has freshwater dominant southern zone and saltwater dominant northern zone, both separated by a bund Thaneermukkom.

It has unique characteristics of physiography, geography, climate, hydrology, landuse,flora and fauna(Mani et.al,2013).The major activities that take place here are fishing, tourism, agriculture, boat racing etc. The lake is renowned for its live clam resources and subfossil deposits , as a habitat for the threatened Spot-Billed Pelican *Pelicanus philippensis*,besides possessing a high diversity of finfish and shellfish.The declining biological integrity and fishery production in the aquatic environment lead to biodiversity loss, fish stock reduction and eventually affect the livelihood of fishers(Krishnan et.al,2020).

Escherichia coli (abbreviated as *E. coli*) are bacteria found in the environment, foods, and intestines of people and animals. *E. coli* are a large and diverse group of bacteria.*E-coli* is one of the bacteria which is cosmopolitan in distribution. It belongs to the family Enterobacteriaceae and order Enterobacteriales. It is Gram negative, facultative anaerobic, rodshaped bacteria which indicates the presence of Fecal coliform.They are generally motile by peritrichous flagella . Most bacteria in this family grow well and are most metabolically active at 25-35°C having both respiratory and fermentative metabolism (Saadi et.al.,2017).

Objective : To study the water quality parameters and presence of *Escherichia coli* in Vembanad

REVIEW OF LITERATURE

The study was conducted with an aim of observing physicochemical characteristics of Vembanad lake to know the health status in terms of water quality of the lake .Temperature , Salinity , Dissolved Oxygen and pH are the most important variables influencing the abundance of organisms in the estuarine environment(Krishnan et.al,2020).Among this the most important physical features of the estuarine system, the wind generated and tidal currents causing the circulation of the water form the master factor which play an important role in making these waters a highly complex environment. It is the water circulation patterns which govern factors like distribution of other chemical components of the water in addition to salt content, physical properties such as temperature, suspended matter and biological populations, especially of those species with planktonic stages in their life history. It must be emphasised that all the above factors play complex interactive roles in determining the distribution, survival and growth of young fishes and prawns in the lake. The mechanisms involved and the extent to which changes in these factors influence the productivity of the fishery resources remain to be fully elucidated. However, it is only reasonable to assume that any interference which will upset the balance of the natural equilibrium prevailing in this dynamic ecosystem will have its own adverse effect on the resources.The pollution caused by the discharge of partially treated or untreated wastes from the factories, sewage and chemicals from agricultural operations (both fertilizers and pesticides) finding their way into the water bodies are the main factors contributing to the water pollution problems in these waters. In recent years there have been a number of reports about fish kills due to water pollution in the backwaters around Cochin, which included the Periyar river.Overexploitation of resources and a range of interventions such as salinity barriers, flood water drains and industrial complexes in the drainage basins are found to have severe impact on the ecological status of the wetland leading to its degradation.Since it supports more than half of the population of Kerala, consequences of any destruction caused will be far reaching on the society.

Escheichia coli has worldwide distribution and is gram –negative , facultative anaerobic bacteria and is motile by means of peritrichous flagella.One of the most notable features of *E.coli* is broad diversity of disease causing genotypes.A total of 9 samples were collected and each sample was inoculated in lactose broth/agar slant.The presence of gas production indicates the presence of coliform bacteria. For confirmation, colony was inoculated into peptone water broth and incubated at 37°C for 24 hours in a shaker incubator. After incubation, few drops of Kovac' reagent was added. Presence of red coloured ring indicates a positive result .

METHODOLOGY

EXPERIMENT 1

Salinity ,pH and Temperature

Salinity, Temperature and pH was measured using salinometer,pH meter and Thermometer respectively.

EXPERIMENT 2

The Secchi depth is measured using standard protocols:
The white Secchi disk is lowered into the water and the depths at which it disappears and reappears are recorded. The Secchi depth is computed by averaging these two depth measurements.It may be feasible to view the depth of disk disappearance and reappearance directly from the measuring tape at the water surface. If this is not feasible, the distance from the hand-held device (casing) to the water surface (DO) can be measured, as well as the total distance (TO) from the device to the depth at which the disk disappears and reappears. The Secchi depth can then be computed by subtracting DO from TO
Noteif the latter is done, it is essential to measure DO accurately and to keep DO constant throughout the measurement (eg, by maintaining arm at 90 degrees).

For accurate Secchi depth readings, the observer should avoid sun glint regions and shadows, ideally conduct the measurement closer to mid-day (or at the very least record the time and location which can be used to compute sun angle), allow their eyes to adapt near to the Secchi depth, write down sky conditions, and repeat measurements to improve precision.

The disk must sink vertically though the water for accurate Secchi depth readings. The weight of the mini-Secchi (100 g) should be sufficient for vertical deployment from a fixed platform in waters with low current speed. However, in stronger currents, or in

cases where the platform may be moving (e.g, from a boat) extra weight will be required to avoid the disk sinking at an angle.

The colour of the water is measured by looking at the colour of the Secchi disk at roughly half the Secchi depth, matching it to the closest colour on the colour scale and noting the corresponding number.

Once the disk is at half the Secchi depth, it is relatively straight-forward to turn the hand holding the device so that your palm is facing up and the scale is visible and you can see the disk .After operating the device, the mini-Secchi should be cleaned with
fresh water and stored in a dry location. To remove any dirt from the white disk a little washing liquid may be used.

EXPERIMENT 3

Enumeration of total coliform

The MPN method is a well-established and fully documented method of estimating the number of viable microorganisms in environments with extremely low level of health indicators. It estimate a microbial population size based on a process-related attribute. Common examples include growth, enzyme action, or catalytic chemistry. The MPN method involves taking the original solution or sample, and subdividing it by orders of magnitude (frequently 10x or 2x), and assessing presence/absence in multiple subdivisions. The degree of dilution at which absence begins to appear indicates that the items have been diluted so much that there are many subsamples in which none appear. A suite of replicates at any given concentration allow finer resolution, to use the number of positive and negative samples to estimate the original concentration within the appropriate order of magnitude. The samples are incubated and mostly assessed by eye or colour detection (use of specific reagents). The major weakness of MPN methods is the need for large numbers of replicates at the appropriate dilution to narrow the confidence intervals.

Enumeration of total coliform count

The coliform group comprise all aerobic and facultative anaerobic Gram negative non spore forming rod shaped bacteria that ferment lactose with gas and acid formation within 48h at 37 °C. The coliform examination is performed by adding measured volumes of sample to multiple dilution tubes containing appropriate medium and incubating them for 48h at 37 °C, and the coliform counts are computed from number of tubes showing gas and acid production. Durham's tubes are inserted into each tube to monitor gas production. Brilliant green lactose broth (BGLB is confirmative test for coliform. Bile salts and Brilliant green almost completely inhibit the growth of other lactose fermenting bacteria like clostridium.

Materials Required

Lactose broth (HiMedia)

Brilliant Green Lactose Bile Broth

McCartney tube

Pipettes

Durham's tube

Procedure

1. Prepared 30 ml of Lactose broth in double strength (double strength is prepared so as to reduce the effect of dilution that occurs on addition of large volume of sample) and 60 ml in single strength
2. Dispensed the double strength medium into three tubes (10 ml in each tube) and single strength into 6 tubes (10 ml in each tube)
3. Durham tube was inserted into each tube, care was taken to avoid air bubbles
4. Autoclaved at 15lbs pressure for 15min
5. Inoculated 10 ml sample into double strength LC broth
6. Inoculated 3 tubes of single strength with 1 ml and other 3 tubes with 0.1 ml sample
7. Incubated all tubes at 37 °C for 48 h and document turbidity and gas production
8. MPN values were computed from the number of positive tubes.

For confirmation

1. BGLB was dispersed into McCartney tube (10 ml in each tube) and autoclaved
2. A loopful of culture from positive tubes (turbidity with gas production) was inoculated into sterilized BGLB tubes and incubated at 37+0.5 °C 48 h
3. Formation of gas production in BGLB was confirmed as positive test for coliform
4. MPN values were computed from the number of positive tubes .

EXPERIMENT 4

Enumeration of fecal coliform

The organism most commonly used as indicator of fecal pollution is the coliform group as a whole and particularly Escherichia coli, which can produce gas from lactose at 44.5 °C with gas production. E.coli is a Gram negative rod belongs to the family Enterobacteriacee and lives in the intestine of warm blooded animals. They are released in to the environment along with the feces in large number and are considered as an indicator of sewage pollution of water bodies. Enumeration of E. coli in water samples are widely done by MPN method. The various media employed in the identification of E. coli are lactose broth, EC broth and MacConkey agar. Lactose broth is an enriching medium whereas EC broth and MacConkey agar are selective media. The selective nature is due to the bile salt present in the medium, which inhibits Gram positive forms but more important is the temperature of incubation (44.5 °C) at which only E. coli can produce gas and acid from lactose.

Material Required

Lactose broth

EC broth (HiMedia)

Mccartney tube

Pipettes

Durham tubes

Procedure:

1. Prepared 30 ml of Lactose broth in double strength (double strength is prepared so as to reduce the effect of dilution that occurs on addition of large volume of sample) and 60 ml in single strength

2. Dispensed the double strength medium into three tubes (10 ml in each tube) and single strength into 6 tubes (10 ml in each tube)

3. Durham's tube was inserted into each tube, care was taken to avoid air bubbles.

4. Autoclaved at 15lbs pressure for 15min

5. Inoculated 10 ml sample into double strength Lactose broth.

6. Inoculated 3 tubes of single strength with 1 ml and other 3 tubes with o.1 ml sample

. 7. The tubes were incubated at 44.5 °C for 48 h instead of 37°C.

8. EC broth was dispersed into McCartney tube (10 ml in each tube) and autoclaved.

9. A loop ful of culture from positive tubes (turbidity with gas production) was inoculated into EC tubes and incubated at 44.5 °C for 48 hrs.

10. Formation of gas production in EC was confirmed as positive test for fecal coliform

EXPERIMENT 5

Isolation and Purification of Bacteria (Streak plate)

The streak plate method is a rapid qualitative isolation method, commonly used for isolation of discrete colonies initially require that the number of organisms in the inoculum be reduced. It is essentially a dilution technique that involves spreading a loopful of culture over the surface of an agar plate. The resulting diminution of the population size ensures that, following inoculation, individual cells will be sufficiently far apart on the surface of the agar medium to effect a separation of the different species present. In the streaking procedure, a sterile loop is used to obtain uncontaminated microbial culture. The process is called "picking colonies" when it is done from an agar plate with isolated colonies and is transferred to a new agar plate using a sterile loop. The inoculating loop or needle is then streaked over an agar surface. On the initial region of the streak, many microorganisms are deposited resulting in confluent growth or the growth of culture over the entire surface of the streaked area. The loop is sterilized by heating the loop in the blue flame of the Bunsen burner, between streaking different sections, or zones and thus lesser microorganisms are deposited as the streaking progresses. The streaking process will dilute out the sample that was placed in the initial region of the agar surface

Material Required

Inoculation loop

Agar plates (LB agar)

Procedure

1. Sterilized the inoculating loop in the Bunsen burner by putting the loop into the flame until it was red hot. Allowed it to cool.
2. Chose an isolated colony from the agar plate culture and spread it over the first quadrant (approximately 1/4 of the plate) using close parallel streaks. Immediately streaked the inoculating loop very gently over a quarter of the plate using a back and forth motion.
3. Flamed the loop again and allowed it to cool. Going back to the edge of area 1 that you just streaked, extended the streaks into the second quarter of the plate.
4. Flamed the loop again and allowed it to cool. Going back to the area that you just streaked, extended the streaks into the third quarter of the plate.
5. Flamed the loop again and allowed it to cool. Going back to the area that you just streaked, extended the streaks into the centre fourth of the plate.
6. Flamed your loop once more.

EXPERIMENT 6

Transferring and stocking of Bacteria (plate to slant)

Pure colonies isolated from a streak plate will be sub-cultured on to an agar slant.

Material Required

Inoculation loop

Agar slants (LB agar)

Procedure

1. Sterilized the inoculating loop in the Bunsen burner by putting the loop into the flame until it was red hot. Allowed it to cool.

2. Chose an isolated colony from the agar plate and spread it on the agar slant. For that removed the cotton plug using the little finger of the hand holding the loop, passed the mouth of the tube quickly through the flame, then inserted the loop into the tube.

3. With the end of the loop touching the agar surface near the bottom, moved it back and forth slightly as you gradually pulled it up toward the top of the slant. Removed it, again passed the mouth of the tube through a flame (optional), and replaced the cap.

EXPERIMENT 7

Agarose Gel electrophoresis

Principle of Agarose gel Electrophoresis

DNA in solution has a net negative charge due to its phosphate backbone (at the pH used during electrophoresis). Therefore, molecules with different lengths can be separated upon electrophoresis through a porous matrix. Agarose was used as a matrix for fragments from 100 base pairs through entire genomes of several megabases. Acrylamide was used for sequencing of DNA and separation of short stretches of DNA (oligonucleotides). Ethidium bromide (EtBr) was usually added to visualize the DNA when the gel is exposed to UV light.

Gel preparation

- Here we prepared 1% agarose gel
- Weighed down 1.2g of Agarose and transfered to a clean 250ml beaker.
- Measured 120ml of 1x TAE buffer and added to the agarose weighed and mixed gently.
- Boiled the mixture in microwave oven for ~ 1 min 40seconds (option: beverages) minutes (in between, took out the beaker and mixed by swirling and ensured that the agarose is dissolved nicely. If not, you could see minute transparent lens like particles in the liquid.) Be cautious while handing the hot beaker. The solution could become 'superheated' and foam over when agitated
- When the agarose was cooled enough (hand warm or place it at 60°C) Added 5ul of Ethidium Bromide (stock: 10mg/ml) and Mixed well by swirling
- Poured the molten agarose in to the gel casting tray with comb inserted

- Left it undisturbed (to set) for minimum 30 minutes at RT
- Removed the gel comb carefully and stored at 4°C in a zip lock cover

Gel loading

- Took out a gel, and placed it on the platform in the gel tank
- Filled the gel tank with 1x TAE buffer (2-3mm above gel surface) Slightly moved the gel inside the buffer to remove any air bubbles trapped inside the wells
- Arranged tip box, loading micropipettes, Samples to load, DNA markers, and a vessel to discard the used tips (optional: parafilm and Dye)
- Wrote down the order at which samples were loaded and then arranged the samples in the rack according to this order before loading; kept the tubes in the rack in the same order *
- Loaded 2ul of the marker and 5-10ul of PC product
- (If you are loading genomic DNA /plasmid DNA then you have to mix the samples with dye)
- Added 2ul each of dye on parafilm according to the number of samples.
- Then added 5ul of each sample to the dye and mix well.
- Then loaded the sample+dye mix to the destined well
- Connected the electrodes to the gel tank, then connected the system to the power
- A voltage of 1V/cm gives better separation and a voltage of 10V/cm givesfaster separation (cm = distance between the electrodes, not gel length).
- Switched on the power; set the voltage at 80 (for a mini gel this is enough): confirmed the current flow in the tank (look for bubbles leaving out from the base of the electrodes)
- Closed the lid of the gel tank (to reduce evaporation loss)
- Allowed the run for 30-45 minutes (set a timer/alarm)
- Switched off the power, after seeing that the tracking dye has reached the desired distance in the gel
- Carefully transferred the gel in to a clean tray, Washed the gel in running water (to avoid salt precipitation on the gel imager platform)

REAGENT PREPARATION

Preparation OF 50 x TAE buffer

- Tris base (0.4M)
- Glacial acetic acid (0.2M)
- Na2 EDTA.2H20 (20mM)

  - 242g

  - 57.1 ml

  - 37.2g
- Adjust pH to 7.7- 8.0; make up to 1 liter and Autoclave

Preparation of 1 TAE buffer (for gel preparation and gel run)

- 50x TAE -10ml
- Distilled water - 490ml
- Mix well; No need to autoclave

Ethidium Bromide stock solution

- 1-10 mg/ml in H20;
- Store at 4°C in a dark bottle

Gel loading buffers

They serve three purposes in DNA electrophoresis:

- Increase the density of the sample: This ensures that the DNA will drop evenly into the well
- Add color to the sample: Simplifies loading
- Add mobility dyes: The dyes migrate in an electric field towards the anode at predictable rates. This enables one to monitor the electrophoretic process. At least five loading buffers are commonly used for agarose gel electrophoresis. They are prepared as six-fold concentrated solutions. Alkaline loading buffer is used when performing alkaline gel electrophoresis.

10X TBE Stock

- Tris Base - 108.0g

- Boric acid - 55.0g
- 0.5M EDTA (pH 8.0) - 40 ml
- Make up volume to 1 liter and Autoclave
- Storage Temperature 4°C

Agarose related remarks:

- Agarose loses its gel strength noticeably 5 years after production.
- Avoid agents that disrupt hydrogen bond formation (e.g. urea), since that will decrease melting temperature, gelling temperature and gelstrength.
- Make sure that all agarose is dissolved before pouring the gel. A non-homogenous gel (in agarose concentration) will result in strange shaped bands.
- Don't pour the agarose solution when >60°C. The gelling surface may bend or even crack.

PCR PROTOCOL

- Used TAKARA EmeraldAmp® GT PCR Master Mix (#RR310A).
- Provided with aliquots of mastermix, sterile water, MC12, BSA etc for work.
- Used gloves while handing PC reagents.
- Labeled the tubes

PCR MIX (1X PREPARATION)

- Template
- Forward primer
- Reverse primer
- BSA/MgcI2/DMSO
- Master mix
- MilliQ

PCR conditions

- 95°C for 2 minutes
- 94°C for 30 seconds

- 52/55°C for 30 seconds
- 72°C for 45 seconds
- 72°C for 5 minutes. (35 cycles)
- Store the samples at-20°C

EXPERIMENT 8

Suspended Particulate Matter

1litre water was collected and carried to the lab. The samples were stored at 4^0C until processed. The water samples were mixed well and passed through 0.45 micron filter paper (Millipore; HAWP04700) and the filter paper was placed carefully inside sterile, pre-weighed glass petri plates. The volume that passed through the filter paper were noted down for finalcalculations. The plates were then kept inside hot air oven set at 60^0C for 24-48hrs. Later, the filter paper was weighed and noted the vales. This was repeated under concordant values where obtained. If the samples are highly turbid, then the volume of sample passing through the filter paper may vary. In such situations, we will be filtering water samples until the filter paper get clogged.

Suspended Particulate matter (mg/L) = Final weight of the filter paper (mg) x1000 / Volume of water filtered

<u>DNA isolation</u>

For DNA work, 1Litre of water was collected, carried to Lab and stored at 4^0C until processed. The DNA samples were processed with in 24hrs of collection. The samples were filtered through 0.22micron mixed cellulose esters filter paper (Merck GSWP04700). The volume that passed through the filter paper were noted down for final calculations. After completing the filtration, the filter paper was folded in to two, and transferred to sterile pouches made up of aluminium foil and kept inside properly labeled small size zip lock pouches and stored at -20^0C until DNA isolation. If the samples are highly turbid, then the volume of sample passing through the filter paper may vary. In such situations, we will be filtering water samples until the filter paper get clogged.

DNA isolation was done from the filter paper using Macherey-Nagel NucleoSpin Soil (cat # 740780.250) Kit with few modifications. Briefly, half of the filter paper was carefully cut out with the help of a sterile scissors. Then the filter paper was minced in to very small pieces on a sterile surface. These pieces were transferred to a labeled sterile microcentrifuge tubes and 2-3 sterile glass beads (1-2mm) were added to the tubes then700µl of Buffer SL1 and 150 µl of Enhancer SX were added, mixed thoroughly by vortexing for 45 seconds. To this tube, 20 µl of Proteinase K (20mg/ml) and 10 µl of lysozyme was added and mixed gently and the tube was incubated at 55°C for 3hrs. After the incubation period, the tube was centrifuged at 11,000 x g for 2 min and the supernatant was carefully transferred to a fresh tube. To this tube, 150 µl of Buffer SL3 was added and vortexed for 5 seconds and then the tube was incubated at on ice for 4 min. The tube was spun at 11,000 x g for 2 min and the clear supernatant was passed through spin column for Inhibitor removal. The collected flow through was treated with 250 µl of Buffer SB ; mixed by vortexing for 5 seconds and then transferred to spin column assembly for DNA capture. The membrane with DNA was washed four times ; 1st time with Buffer SB (500 µl) and then with Buffer SW1 (500 µl) and 3rd and 4th wash with buffer SW2(650µl).A Final spin at 11,000 x g for 2 min was given to dry the spin column; then the collection tube was replaced with a fresh sterile Microcentrifuge tube. The DNA on the membrane was eluted with 60 µl of Buffer SE. The quality of DNA was checked by agarose gel electrophoresis. DNA was diluted appropriately and used as template for PCR.

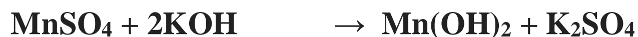
EXPERIMENT 9

DISSOLVED OXYG

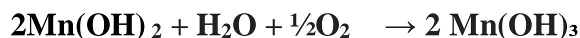
AIM : To estimate the amount of oxygen dissolved in water using Winkler's method .

PRINCIPLE : In Winkler's method , nascent oxygen (O) released from water is used to liberate iodine from potassium iodide and this iodine is estimated using standard sodium thiosulphate ($Na_2S_2O_3$) . When manganese sulphate ($MnSO_4$) is added to the sample of water followed by strong alkaline potassium iodide , manganese hydroxide (**$Mn(OH)_2$**) will be formed. This combines with dissolved oxygen in water to form manganic hydroxide (**$Mn(OH)_3$** . This on acidification with concentrated sulphuric acid in the presence of alkaline potassium iodide

releases iodine equivalent to the amount of oxygen used in this reaction. The amount of iodine liberated is estimated by titrating the sample solution against 0.01N sodium thiosulphate.



$$MnSO_4 + 2KOH \rightarrow Mn(OH)_2 + K_2SO_4$$



$$2Mn(OH)_2 + H_2O + \tfrac{1}{2}O_2 \rightarrow 2\,Mn(OH)_3$$

$$2\,Mn(OH)_3 + 2KI + 3H_2SO_4 \rightarrow 2MnSO_4 + K_2SO_4 + 6\,H_2O + I_2$$

$$2Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2NaI$$

REQUIREMENTS : Manganese sulphate , alkaline potassium iodide, conc. sulphuric acid , sodium thiosulphate , fresh starch solution (as indicator) , burette , pipette , conical flasks , measuring cylinders , beakers , reagent bottles , oxygen estimation bottles with stopper and samples of water .

1. Manganese sulphate solution : (480 gm $MnSO_4 . 4H_2O$ or 400gm $MnSO_4 . 2\,H_2O$ or 364gm $MnSO_4 . H_2O$ in 1 litre distilled water)
2. Alkaline iodide solution : (KOH - 700gm and KI - 150gm in 1 litre distilled water)
3. Sodium thiosulphate solution - 0.1N $Na_2S_2O_3$ (24.82gm $Na_2S_2O_3 . 5\,H_2O$ in 1 litre distilled water)

Dilute this stock solution 10 times (1ml to 10 ml) to get 0.01 N solution .

PROCEDURE 1. FIXATION OF WATER SAMPLES

Water sample is taken in a bucket and a 250 ml reagent bottle is immersed in it with minimum disturbance. Taking care to avoid any air bubbles inside it, close the bottle with the stopper under water. Take out the bottle and remove the stopper. To the water sample in the bottle add 1 ml of manganese sulphate solution followed by 1 ml alkaline potassium iodide solution . Add the reagents well below the surface by keeping the tips of the pipette near the bottom and gradually pulling them upwards. Close the bottle tightly with the stopper and avoid air bubbles inside. Shake the sample very well in order to complete precipitation inside. Leave the mixture undisturbed for sometime so that the precipitate settles down completely.

2. TITRATION OF WATER SAMPLE

Add 2 ml of conc. H_2SO_4 carefully to the precipitate. Close the bottle with the stopper and mix the contents very well to dissolve the precipitate completely .

From the straw coloured solution thus formed, pipette out 20 mlinto a clean conical flask . Titrate against 0.01 N sodium thiosulphate solution from the burette . When the solution turns pale yellow add a few drops of freshly prepared starch solution as indicator. Continue the titration till

the disappearance of the blue colour. Note down the burette reading and repeat the titration for concordant values.

RESULT

Amount of dissolved oxygen in the water sample = ……….. mg/L

EXPERIMENT 10

TURBIDITY

Measuring turbidity requires taking a sample from the source and using an instrument to analyze it. Ensure the turbidimeter has been recently calibrated using turbidity calibration standards and take a minimum of two readings. In most modern turbidimeters, a sample is obtained, added to a vial and placed in the instrument. The fixed light beam is then shone in the direction of the sample to measure how much light is transmitted and how much is scattered, by photodetectors set 90-degrees to the sample.

EXPERIMENT 11

CHLOROPHYLL

Chlorophyll is extracted in 80% acetone and the absorbances at 663m and 645m are read in a spectrophotometer. Using the absorption coefficients, the amount of chlorophyll is calculated.

OBSERVATION AND RESULT

Table 1: pH,Temperature and Salinity of samples taken from Nadakkavu region of Vembanad lake

SAMPLING STATION	Ph	Temperature	Salinity
Thottungal	7	38.2	4
Chaliyath	6.5	38.3	5
Chauka	6	39.05	6
Ameda	7	37.22	4
Kalezhuthu	7	37.5	5
Panchayathukadavu	7	38.44	5
Pullukattu	7	37.88	1
Kannampilly	6.5	32.2	4
Nikarthil	7	37	3

Table 2 :Secchi depth and colour of water samples of Nadakkavu region of Vembanad lake

SAMPLING STATION	DO Distance from observer to water	TD Total distance ie. Distance from observer to depth at which disk disappears/reappears	Secchi depth	Secchi colour code	Latitude	Longitude
Thottungal	1.67	2.64	0.97	20	10.1024°N	76.3512°E
Chaliyath	1.36	1.68	0.32	19	10.1024°N	76.3512°E
Chauka	1.78	1.93	0.15	19	10.1108°N	76.3487°E
Ameda	1.6	1.75	0.15	19	10.1108°N	76.3487°E
Kalezhuthu	1.77	1.85	0.08	20	10.1108°N	76.3487°E
Panchayathukadavu	1.74	2.06	0.32	20	9.8984°N	76.3621°E
Pullukattu	1.92	2.16	0.24	16	9.8984°N	76.3621°E
Kannampilly	1.21	2.16	0.95	18	9.8984°N	76.3621°E
Nikarthil	1.52	1.95	0.43	20	9.8984°N	76.3621°E

Table 3 :Estimation of MPN for detection of coliform bacteria of water samples of Nadakkavu region of Vembanad lake

SAMPLING STATION	Strength of lactose broth	No: of tubes	Gas production	MPN
Thottungal	DSLB +10ml	3	3	2.4
	SSLB+1ml	3	3	
	SSLB+.1ml	3	0	
Chaliyath	DSLB +10ml	3	3	>24
	SSLB+1ml	3	3	
	SSLB+.1ml	3	3	
Chauka	DSLB +10ml	3	3	11
	SSLB+1ml	3	3	
	SSLB+.1ml	3	2	
Ameda	DSLB +10ml	3	3	>24
	SSLB+1ml	3	3	
	SSLB+.1ml	3	3	
Kalezhuthu	DSLB +10ml	3	3	1.5
	SSLB+1ml	3	2	
	SSLB+.1ml	3	1	
Panchayathukadavu	DSLB +10ml	3	3	>24
	SSLB+1ml	3	3	
	SSLB+.1ml	3	3	
Pullukattu	DSLB +10ml	3	3	>24
	SSLB+1ml	3	3	
	SSLB+.1ml	3	3	
Kannampilly	DSLB +10ml	3	3	>24
	SSLB+1ml	3	3	
	SSLB+.1ml	3	3	
Nikarthil	DSLB +10ml	3	3	>24
	SSLB+1ml	3	3	
	SSLB+.1ml	3	3	

Table 4 :Estimation of MPN for detection of fecal coliform bacteria in water samples of Nadakkavu region of Vembanad lake

SAMPLING STATION	Strength of lactose broth	No: of tubes	Gas production	MPN
Thottungal	DSLB +10ml	3	0	<0.03
	SSLB+1ml	3	0	
	SSLB+.1ml	3	0	
Chaliyath	DSLB +10ml	3	0	<0.03
	SSLB+1ml	3	0	
	SSLB+.1ml	3	0	
Chauka	DSLB +10ml	3	1	0.036
	SSLB+1ml	3	0	
	SSLB+.1ml	3	0	
Ameda	DSLB +10ml	3	3	0.23
	SSLB+1ml	3	0	
	SSLB+.1ml	3	0	
Kalezhuthu	DSLB +10ml	3	3	0.23
	SSLB+1ml	3	0	
	SSLB+.1ml	3	0	
Panchayathukadavu	DSLB +10ml	3	2	0.091
	SSLB+1ml	3	0	
	SSLB+.1ml	3	0	
Pullukattu	DSLB +10ml	3	3	0.23
	SSLB+1ml	3	0	
	SSLB+.1ml	3	0	
Kannampilly	DSLB +10ml	3	0	0.23
	SSLB+1ml	3	0	
	SSLB+.1ml	3	0	
Nikarthil	DSLB +10ml	3	3	0.23
	SSLB+1ml	3	0	
	SSLB+.1ml	3	0	

Table 5: Volume of water sample filtered for DNA analysis

SAMPLE NAME	VOLUME OF WATER FILTERED
Thottungal	500ml
Chaliyath	500ml
Chauka	300ml
Ameda	300ml
Kalezhuthu	300ml
Panchayathukadavu	400ml
Pullukattu	400ml
Kannampilly	400ml
Nikarthil	155ml

Table 6 :SPM calculation for water samples taken from Nadakkavu region of Vembanad lake

SAMPLE NAME	PETRI WEIGHT {g}	PETRI WEIGHT +FILTER PAPER	VOLUME FILTERED IN ML	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	SPM {wt in mg/volume filtered} x 1000
Thottungal	74.667	0.078	600	74.76	74.760	74.777	74.762	74.761	33.33
Chaliyath	71.551	0.082	750	71.76	71.647	71.648	71.640	71.643	66.67
Chauka	71.741	0.081	500	71.84	71.837	71.853	71.835	71.837	60
Ameda	71.268	0.080	400	71.34	71.344	71.353	71.344	71.342	10
Kalezhuthu	72.169	0.082	400	72.25	72.245	72.251	72.251	72.252	2.5
Panchayathukadavu	73.294	0.085	450	73.38	73.379	73.377	73.380	73.376	4.44
Pullukattu	72.587	0.086	500	72.66	72.675	72.694	72.672	72.665	2
Kannampilly	72.198	0.085	500	72.28	72.284	72.304	72.284	72.283	2
Nikarthil	73.146	0.082	180	72.228	73.229	73.230	73.227	73.230	94.44

Table 7 :Chlorophyll and turbidity of water samples taken from Nadakkavu region of Vembanad lake

SAMPLING STATION	CHLOROPHYLL {ugL}	TURBIDITY {NTU}
Thottungal	1.55	3.1
Chaliyath	5.64	2.9
Chauka	3.06	3.7
Ameda	2.92	2.6
Kalezhuthu	3.16	2.8
Panchayathukadavu	17.75	4
Pullukattu	1.55	10.3
Kannampilly	21.73	4.8
Nikarthil	11.03	12.5

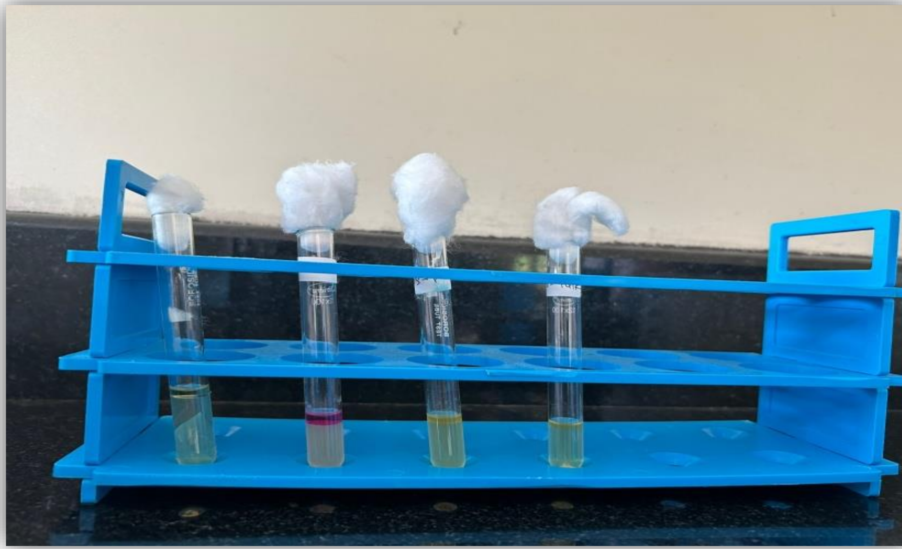


Fig 1 –Indole test

Presence of red ring in the 2 nd tube indicates its Indole positive

Fig 2 –Indole test

Presence of red ring in the 4th tube indicates it is tested positive for Indole test

Fig 3

Petri plates containing filter paper for measurement of SPM

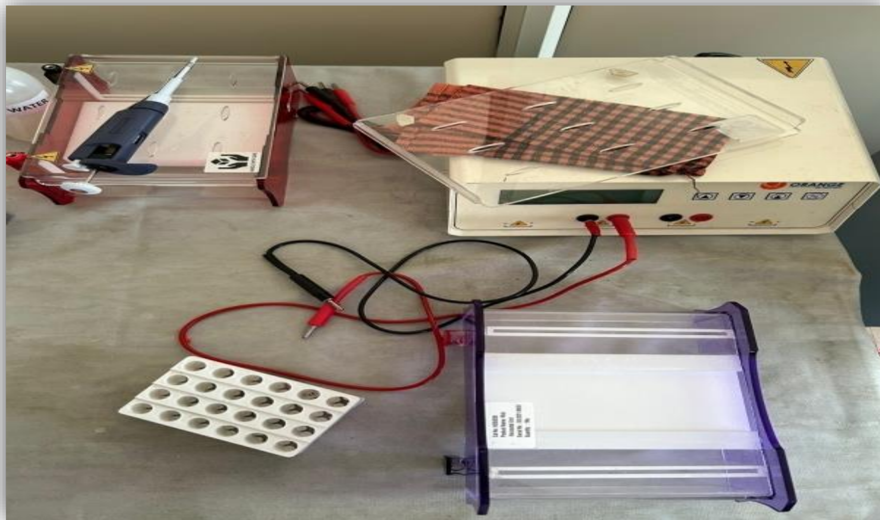


Fig 4

Process of gel loading for Electrophoresis

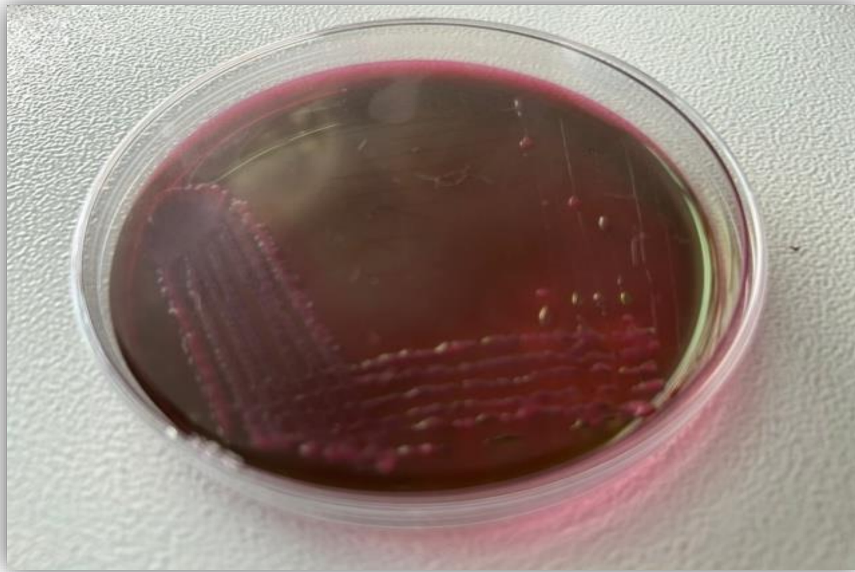


Fig 5

Streak plate with green metallic sheath –indicates the presence of E coli colonies

Fig 6

Tubes showing gas bubbles in MPN estimation

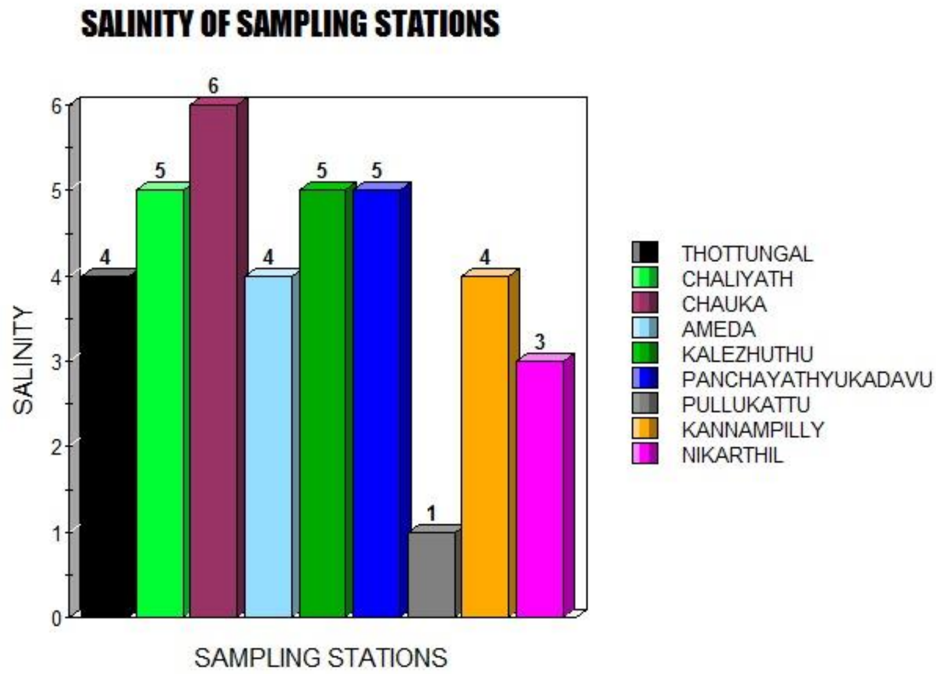


Fig 7

Bar graph representing salinity of water samples taken from Nadakkavu region of Vembanad lake

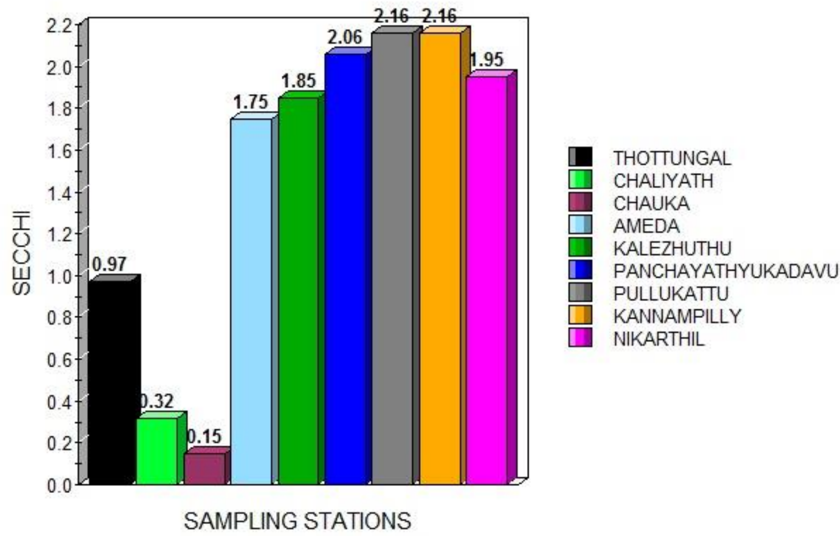


Fig 8

Bar graph representing secchi depth of samples from Nadakkavu region of Vembanad lake

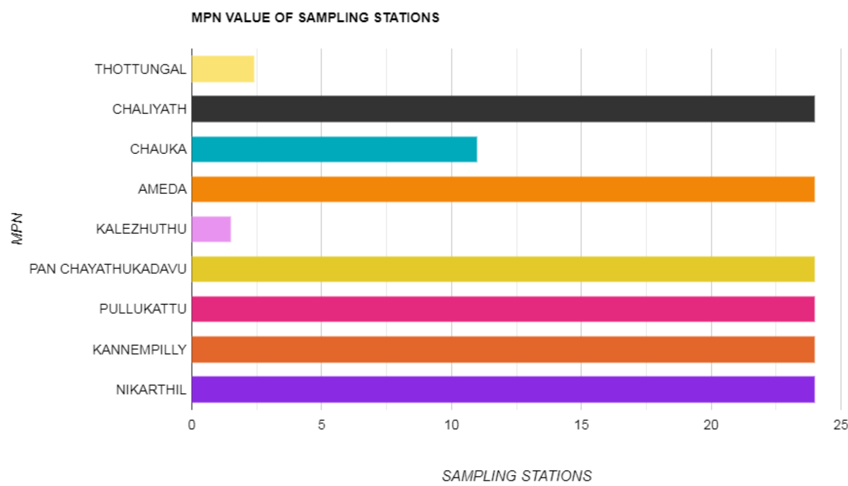


Fig 9

Horizontal bar graph representing MPN value of samples of Nadakkavu region of Vembanad lake

DISCUSSION

E –coli in water is a strong indicator of sewage or animal waste contamination. Sewage and animal waste can contain many types of disease causing organisms. Consumption may result in severe illness, children under 5 years of age and those with compromised immune system and the elderly are very suspectible. In India the rate of deaths due to Escherichia coli (E.Coli) per lakh population stood at 16.1 in 2019.

The average pH of the study is found to be 6.7 (Table 1).From the earlier studies (2007-17) the average ranged between 7-7.1.But there was a decrease in ph ie, Increase in acidity during the year 2018-19 which was due to frequent floods. Inundation of saline water etc. pH ranged between 6.5-7 in the 9 sampling stations .The pH values in the study area remained within the permissible limits during the course of sampling (Krishnan et.al,2020).

Variance was noticed from average salinity of 1.5 ppt during the year 2007, 0.4-0.6 during the year 2016-17 to 4.1 ppt during the year of 2022.This variation can be explained on the basis of daily tides , velocity and flow of water , evaporation .But during the course period of 2018-2019 flood , there was a sudden hike in salinity -11.94. Flood led to abnormal water level, destruction of aquatic plants and invertebrates , adverse effects on irrigation and domestic purpose of water .This variation in salinity was also linked to irregular rainfall.As a result , useful microbes got destroyed by inactivating its biological process. The raised saline content enhanced the growth of fecal coliforms in the water(Varma et.al,2002).

Highest salinity of 6 ppt is found in Chauka region and lowest of 1 is observed in Pullukattu.It is due to dumping of garbage as well as discharge of untreated effluents. Decreased level of salinity cannot be underestimated as decline in coliform level, rather can be understood as initiation of fecal contamination

Turbidity is yet another factor that increases the chlorophyll and dissolved oxygen in water . Data collected prior to flood indicate that the turbidity was around 1-2 NTU. But the data

collected after the flood was around the range of 2-12NTU. The highest turbidity of 12.5 and 10.3 NTU occurred in Nikarthil and Pullukattu region and lowest at Ameda region (Table 7).

Turbidity is a crucial indicator that is related to chlorophyll content and dissolved oxygen in water. Highest chlorophyll lead to higher turbidity and murky water. As the chlorophyll level increases it initiates the growth of green plants and by the utilisation of oxygen causes respiratory defects to aquatic organisms. As the inhabitants of water dies off, there is deposition of sediments and debris. These may accumulate a lot of sewage waste in water that hinder the light to penetrate towards bottom region. Irregular heavy rainfall and rainstorm, increased velocity of water also increase chlorophyll level. After the recently occurred flood the region of Nikarthil had more dominance of water hyacinth and aquatic invertebrates. The roots of the aquatic weed named *Eichhornia crassipes* is able to absorb the water and other substrates in the water and it also secreates a bio oil 'pyrolytic oil' that cause the pyrolysis of water molecules and lead to rise in pH. Population increase and tourism in the region is also an indirect factor to turbulent water(Masago et.al,2020). The region of Ameda had less accumulation of plants and abundance of aquatic organisms , indicates that the presence of fecal colliforms are in limited number.Turbidity is indirectly indicate the presence of fecal colliforms in water bodies. It may harm aquatic inhabitants, dredge spawn beds and affect gill functions.

The Most Probable Number is an indicator to presence of coliform bacteria. The presence of coliform bacteria is confirmed by gas bubbles formed in Durgham's tube .Gas bubbles in 5 tubes crossed 24 (Chaliyath, Ameda,Panchayathukadavu, Nikarthil, Kannempilly) (Table 3,4). The presence of fecal coliform bacteria was further confirmed in 4 water samples by indole test.

Suspended Particulate Matter (SPM) is another factor that contribute to turbulent water. The concentration of SPM during lockdown period decreased by 15.9% on average compared with pre-lockdown period. The SPM quantified for April 2020 is the lowest for 11 out of 20 zones of Vembanad lake. When compared with preceding years, the percentage decrease in SPM for April 2020 is up to 34% from the previous minima.(Yunus et.al,2020).

As compared to the previous years its concentration rate have been increased in 2023. The highest SPM value is at Nikarthil region approximately 94.44 Mu and that of lower at Pullukattu and Kannempally approximately 2 Mu (Table 6).These are fine solids or liquids that are distributed in the air as a result of activities such as combustion process, Industrial operation or natural processes like volcanic eruption. The major chemical components are Lead, Nickel and Arsenic. These may cause lung damage and respiratory problems.

Within 3 years the industrialisation and globalisation have improved. Major source of primary particles are domestic burning, power plant, resuspension of road and construction dust. These suspended particles are indirectly related to turbidity, thereby reduces light penetration ability of water, affect its quality. Due to insufficient amount of light photosynthesis get blocked and the oxygen concentration get reduced in the aquatic ecosystems cause the death and decay of organisms within the environment. It cause environmental damage by changing the nutrient balance in coastal water and large river basins

The average of Dissolved oxygen is found to be 5.8mg/L . A DO greater than 5mg/L in water is required for sustaining aquatic fauna .High dissolved oxygen was found due to suitable phytoplankton in water and minimum was found due to evaporation. Presence of organic waste in water may overload a natural system causing serious depletion of oxygen supply that in turn leads to fish kills .(Krishnan.et.al,2020)

DNA analysis is one indicator of presence of E.coli in Vembanad lake. The estimated amount of bacteria in water were increased during 2019 and 2023. The rate was at normal during the lockdown period as the community had maintained complete hygiene and reduction in the number of bacteria.

Based on the analysis of E.coli in sampling stations under study the number of E.coli primer is abundant in Chaliyath and Kalezhathu. There were even more number of DNA strands in Pullukattu, Chauka , Kannampilly,Thottunkal, Ameda and Nikarthil. The confirmation of E.coli in water indicates recent fecal contamination.

CONCLUSION

The study was conducted with an aim of observing physicochemical characteristics of Vembanad lake to know the health status in terms of water quality of the lake .Due to human intervention and ever increasing pollution , Vembanad lake and its associated wetlands are facing an acute environmental and ecological crisis. Globally 2 million people use water contaminated with feces for their daily use. Microbial contamination of drinking-water as a result of contamination with feces poses the greatest risk to drinking-water safety. The DNA analysis showed the presence of E.coli in all water samples from 9 different stations. This indicates that water is contaminated with feces. There is decline in fishery, degradation in water quality , intensive sedimentation etc .Therefore it is imperative to take a holistic approach for the conservation and management of wetland system . The management strategies recommended for biodiversity conservation includes controlling pollution ,improvement of water quality by solid and liquid waste management and regular monitoring .

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