Analysis of physio-chemical parameters and scanning foldscope in the bacterial characterization isolated from river Nambul, Manipur, North-East India

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Abstract

The present study was carried out to analyse microbial growth and water quality based on seasonal distribution pattern of physio-chemical parameters of the river Nambul flowing in heart of Imphal city during the year 2018. A total of eight parameters were monitored for a period of one year to assess the standard of water quality of river Nambul with reference to its pollution status. It was observed that AT found higher in rainy, low in winter; WT high ranged 26.9 ± 0.24^0C in rainy, low ranged 18.34 ± 2.08^0C in winter ; pH high in the rainy (6.92 ± 0.40), low in winter (5.76 ± 0.30); DO (6.28 ± 0.18 mg/l) high in winter, low in rainy (4.16 ± 0.82 mg/l); BOD ranged maximum in summer , minimum in rainy; Hardness value high in summer (112.28 ± 1.09 mg/l), low in winter (79.65 ± 11.02 mg/l). The Chlorine and Phosphorous content were found to be maximum in winter season. The collected water sample was done serial dilution upto 10 $^{-9}$ and isolated with Nutrient Agar. A total of eight isolates were performed biochemical tests and characterized along with Gram's staining technique. The bacterial isolates were reported that the nature of colonies (bacilli, coccus); Gram nature (Gram positive / Gram negative). Significant seasonal variations of the different parameters, microbial growth were observed and the study has a great value in terms of river ecosystem as well as water quality in different seasons. Furthermore, there is need for implementing conservation measures and generating awareness among the people of Manipur towards the Nambul river which provides an integral part of the social, economic and cultural life.

Keywords : Sewage , Nambul , Bacteria , Physio-chemical properties, Loktak lake.

I.Introduction

Water is most essential and precious for all living organisms. An adequate, safe and accessible supply must be available to all. Water provides sustenance to both plants and animals and constitute the habitat for aquatic organisms and takes importance of agriculture and industrial needs (Prasad and Gaur,1992). Due to rapid increase in world population, urbanization and industrialization, the area of polluted water is expanding day by day and lead to depletion of water quality (Khan *et.al.,*2012). An increasing use of chemicals in agriculture may lead the various form of pollution of water bodies (Tripathi and Pandey,1990). Water quality has measured directly with aquatic productivity (Shrestha and Kazama, 2007).

Water pollution is one of the serious problems to humankind. In Manipur, Nambul river is the most polluted river flowing through the heart of the city. The sewage and other waste materials from Ima market , industries in the Imphal town are drained into Nambul river. Manipur's Nambul river's role in the context of maintaining the climatic conditions of Manipur can not be ignored as it directly falls into the Loktak Lake – the largest fresh water lake in North - East India , which plays a vital role in controlling the ecological balance of the region. The pollution of Loktak Lake is also being increased day by day due to the use of chemical fertilizers, pesticides and fungicides by the farmers, cultivators and people around the Lake. Therefore, assessment of physio-chemical properties of the water of Nambul is not only enough for analyzing the suitability of various purposes,but also for protecting the Loktak Lake. Based on the importance of the fresh water body towards human livelihood , aquatic biodiversity, aquaculture ,agriculture assessment of water quality index is very much required.

Depending on the location, river Nambul may contain an array of substances either in solution form or in solid. The different types of substances include both organic and inorganic, toxic chemicals and many more components. Even though sewage contains a lot of waste materials, the micro-organisms will grow by utilizing the organic and in-organic waste. These types of microbes will have a specific type of characteristic compared to bacteria growing in other environments. (Ishak *et. al.,* 2001; Sharpe,2003). Nambul river is a location where different types of waste materials from different sources were merged and hence it may act as a suitable good medium for the proliferation of numerous micro-organisms. These micro-organisms will develop novel mechanisms for utilizing the waste substrates and can become a better source for studying their novel mechanisms,novel enzymes and novel bioactive compounds. Therefore, it is important to study this type of bacterial genus are present in the polluted river Nambul.

The present study is aimed to analyse physio-chemical properties, bacterial genus from river Nambul, Imphal, Manipur State.

II.Materials and methods

2.1. Study site

Nambul is situated in Imphal, Manipur State, India. Its geographical co-ordinates are 23.80^0N to 25.68^0N latitude and 93.03^0E to 94.78^0E longitude with 62.70Kms length originate from Kangchup Hill ranges in the western side at an elevation of 1830 m above mean sea level.

2.2. Collection of sample

The water samples were collected in seasonal intervals within Imphal area of Nambul river. The sample was collected in a sterile bottle, brought to laboratory and stored in clean place. 1 ml of water sample was used for isolating the bacteria through serial dilution and agar plate culture technique.

2.3. Physio-chemical Parameters

Air temperature (AT) and Water temperature (WT) were measured using mercury thermometer, PH was measured using PH meter, Dissolved Oxygen (DO) was estimated following Winkler's method and Biochemical Oxygen Demand(BOD) by Titration methods after 5 days of incubation at 20^0C and titration of initial and final DO. Hardness,Total chlorine content were obtained by standard titration methods. For the determination of phosphate methods of Trivedi and Goel(1986) was followed.

2.4. Isolation of bacteria from river Nambul

The collected water sample was taken and it is serially diluted with distilled water .The serial dilution was done upto10^{-9}. An aliquot of 100μl of sample was collected from each dilution and were spread on Nutrient agar plate. The plates were incubated at 37^0C for 24 hrs. After successful growth of micro-organisms, the pure colonies of bacteria were sub-cultured on Nutrient agar plates, incubated at 37^0C to achieve vigorous growth and preserved in 20% glycerol vials at - 80^0C.

2.4.1. Characterization of bacterial culture

2.4.2. Morphological studies

2.4.2.1. Gram staining of bacteria

On the basis of cell wall composition to differentiate the bacteria whether it belongs to Gram positive or Gram negative, Gram's staining was done(Aneja,2003).

2.4.2.2. Shape, Size, Colour and Nature of Colonies

Morphological characteristics such as shape,size,colour and nature of colonies were studied by microscopic foldscope observation.The colonies may fall either round (cocci) or rod (bacillus) shape. The colour of the colony was observed under foldscope.

2.4.3. Biochemical tests

Biochemical tests were carried out for identification of unknown bacteria which includes amylase test, catalase test, citrate test, indole test, methyl red test and voges prokaur test. These tests were performed according to standard methods(Pacarynuk and Danyk,2004; Collins *et.al.,*1989).

III. Results and Discussion

The seasonal variations of physio-chemical properties of river Nambul is as shown in Table 1.

Table 1:- Seasonal variations of physio-chemical properties of river Nambul.

Parameters	Summer	Rainy	Winter
AT(^{0}C)	24.86 ± 1.92	27.83 ± 0.26	17.82 ± 0.31
WT(^{0}C)	24.72 ± 2.17	26.9 ± 0.24	18.34 ± 2.08
pH	6.89 ± 0.23	6.92 ± 0.40	5.76 ± 0.30
DO (mg/l)	4.38 ± 0.92	4.16 ± 0.82	6.28 ± 0.18
BOD (mg/l)	8.16 ± 1.28	5.14 ± 0.71	8.09 ± 1.28
Hardness (mg/l)	112.28 ± 1.09	105 ± 12.18	79.65 ± 11.02
Chlorine (mg/l)	16.4 ± 1.21	18.3 ± 0.3	20.2 ± 1.66
Phospherous (mg/l)	0.58 ± 0.16	1.36 ± 0.8	1.55 ± 0.33

3.1. Air temperature

Temperature is one of the most significant factor in an Aquatic environment. Changes in the air temperature naturally affect the water temperature. (Kumar , 1997). AT was found to be higher in rainy (27.83 ± 0.26^0C) and low in winter (17.82 ± 0.31^0C).

3.2. Water temperature

Water temperature is one of the most important parameters which control inborn physical qualities of water. Seasonally, the average maximum mean value was recorded as 26.9 $\pm$ 0.24 ^{0}C in summer and miniimum as 18.34 $\pm$ 2.08 ^{0}C in winter. The summer temperature was always above the winter temperature due to lack of sunlight (Sinha and Biswas, 2011).

3.3. pH

The pH is an important factor in determining the productivity of an ecosystem(Singh *et.al.*,2009). It also gives the intensity of acidic or basic nature of water.Changes in the pH value of watermay be the result of various biological activities (Gupta *et.al.*,1996).The pH value of Nambul showed that the highest average mean value was recorded as 6.92 $\pm$ 0.40 during rainy season and lowest 5.76 $\pm$ 0.30 during winter season. This may be due to the source of contamination and growth of algae , affect the pH level to higher. Most probably the removal of large amount of CO_2 by photosynthetic process by aquatic plants during summer while rainy season there was active decomposition of organic matter (Meetei and Singh , 2011). Jogesh and Misthra (2014) also reported highest pH in summer and lowest in rainy season in river Nambul.

3.4. Dissolved Oxygen (DO)

DO is one of the important parameters of water which directly affects the survival and distribution of flora and fauna in an ecosystem. DO level fluctuate seasonally and also with the altitude . Cold water holds more oxygen than warm water. Water holds less oxygen at higher altitude. DO was found to be higher in winter season (6.28 ± 0.18 mg/l) and low in rainy season (4.16 ± 0.82 mg/l). The high value of DO during the winter may be due to growth of large quantity of aquatic plants and low value in oxygen in the rainy may be due to the utilization of oxygen for metabolic activities by the increasing growth of bacteria (Pandey, *et.al.*, 1999) . Singh,*et.al.*, (2013) also observed highest value of DO in winter season and lowest in rainy season.

3.5. Biological Oxygen Demand (BOD)

BOD is a good index of organic pollution. The BOD fluctuation ranges is shown (Table I). If the amount of organic matter in sewage is more, the more oxygen will be utilized by bacteria to degrade it thereby increasing biological oxygen demand. The mean value concentration is maximum in summer and minimum in rainy season. The maximum values of BOD in summer may be due to high pollutant load drained from Ima Market,Industries in Imphal Town. Pathak, *et.al.*(2012) noted higher values of BOD in rainy compared to summer season.

3.6. Hardness

Hardness of water is important in determining the suitability of water for domestic and industrial uses. The relative amounts of calcium and magnesium hardness, carbonate and non-carbonate hardness present in water are the factors while determining the most economical type of softening process. Determination of hardness serve as a basis for routine control of softening process. The total hardness values ranged shown in Table I . Total hardness mean value is found as maximum as concentration of 112.28 $\pm$ 1.09 mg/l during summer and minimum value 79.65 ± 11.02 mg/l during winter season. Total hardness of river Nambul increases along the downstream. Hardness values of water sample are not drinking use.(Rajkumar, *et.al.*,2016).

3.7. Chlorine

Chlorine content in water gives a rough estimate of salinity. The portable water is chlorinated to make the water free from micro-organisms. Chloride occur widely in waste water and are usually associated with Na^+ . Presence of Chloride above usual background concentration in a water source is also used as indicator of pollution of domestic sewage. The mean concentration of chlorine was found to be higher in winter and low in summer (Table I). This may be due to highly discharge of industrial waste.

3.8. Phospherous

Phospherous is an important index in ecological studies and often regarded as limiting element in water eco-system (Hecky and Kilhan , 1988). The mean value of phosphorous was found as maximum in winter(1.55 ± 0.33 mg/l) and minimum in summer (0.58 ± 0.16 mg/l). If untreated , phosphorous polluted water is consumed; symptoms like shortness of breath can be used. Lastly, such polluted water which is drained into the Loktak Lake will play a pivotal role in the eutrophication of the largest fresh water lake in the North East region.(Sharat ,*et.al.*, 2010).

3.9. Bacterial isolation

In the present study the bacterial colonies were isolated and characterized from river Nambul, Imphal, Manipur.

3.10. Cultural characteristic of bacterial isolates

Morphological characteristic of bacteria such as shape, size, colour and nature of colonies were determined with selected Nutrient Agar medium during rainy season. In addition all the isolates were tested by Gram's staining to check whether they belongs to Gram positive or Gram negative.The details of the morphological characteristic are shown in Table 2. Some of the microscopic foldscope observation are also shown (Fig. A and B) .The bacterial isolate NSW_1,NSM_3,NSW_7 were found Gram positive coccus; NSW_2,NSW_6 are Gram negative coccus; NSW_4,NSW_5 are Gram positive bacilli; NSW_8 is Gram negative bacilli. The isolate NSW_1,NSW_3 tentatively identified as it belongs to genus *Staphylococcus*. The strains of this genus were responsible for skin infections and may cause blisters, abscesses, redness and swelling in the infected area. The isolate NSW_6 may belongs to the genus *Streptococcus*.

Streptococcus are responsible for skin infection, strep throat. Isolate NSW_4, NSW_5 may belongs to genus Bacillus and strains may cause gastrointestinal track infections in ruminants and humans. Many of the isolates belonging to genus bacillus are known to produce industrially importance enzymes such as proteases, amylases, lipases and many more (Mahesh , *et.al*., 2007).

Table 2: - Morphological characteristic of isolates of Nambul Sewage Water (NSW) during rainy season.

| Isolate number	Characterization of colony		Cell features	
	Colour	**Nature of colony**	**Gram nature**	**Shape**
NSW_1	White	Irregular,clusters,shiny	Gram positive	Staphylococcus
NSW_2	Cream	Round shaped, shiny	Gram negative	Monococcus
NSW_3	Cream	Round shaped,shiny	Gram positive	Staphylococcus
NSW_4	Golden Yellow	Rod shaped	Gram positive	Bacilli
NSW_5	White	Rod shaped,chain ,shiny	Gram positive	Bacilli
NSW_6	Cream	Round shaped, transparent	Gram negative	Streptococcus
NSW_7	White	Round shaped,shiny	Gram positive	Monococcus
NSW_8	Cream	Rod shaped	Gram negative	Bacilli
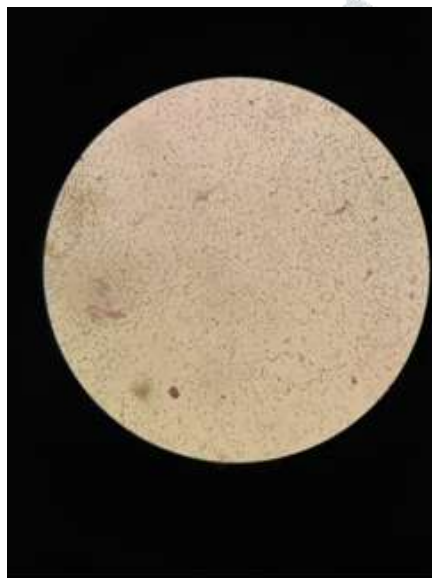
Light microscope   Foldscope

Fig : A. Round shape Gram negative coccus isolated from NSW_2.
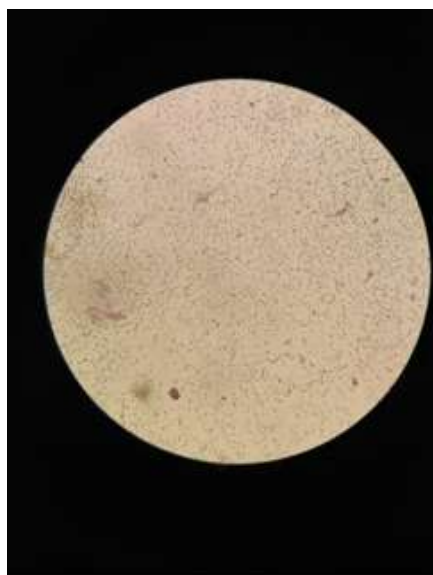
Light microscope   Foldscope

Fig: B. Rod shape Gram negative bacilli isolated from NSW_8.

3.11. Biochemical characteristics tests

Different biochemical tests were carried out for the eight isolates to know their biochemical characteristics and are shown in Table3.

Table 3: Biochemical characteristics of Nambul Sewage Water (NSW) isolates during rainy season.

Name of the test	NSW_1	NSW_2	NSW_3	NSW_4	NSW_5	NSW_6	NSW_7	NSW_8
AMT	+	-	-	+	+	-	+	-
CAT	+	-	+	+	+	+	-	+
CIT	+	+	+	+	-	-	-	-
IT	+	-	+	+	-	+	-	-
MRT	+	+	+	+	+	-	+	-
VPT	+	-	+	+	-	+	-	-

Note:- (+) = positive, (-) = negative, AMT – Amylase Test, CAT- Catalase Test, CIT- Citrate Test, IT – Indole Test, MRT –Methyl Red Test, VPT – Voges Proskauer Test.

The NSW_1, NSW_4 were found positive results in all biochemical assays whereas NSW_8 was found negative result in all biochemical tests except catalase test.

IV.Conclusion

The seasonal variation pattern of different parameters were found to be influenced by different environment factors in river Nambul . Nambul Sewage Water which is a collection of different contaminants along with organic and inorganic materials will become a good source of bacterial growth. These bacteria may be beneficial or harmful to the mankind. So, purification of sewage water at several stages is necessary for good health of mankind. Ultimately, Nambul rivers discharge waste material into the Loktak Lake, the pollution of this river will also affects the Loktak Lake.Thus, Nambul river needs proper treatment to conserve this water body from future contaminants and pollution.

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