

Original Research Article

Physicochemical and Microbiological Analysis of Ikogosi Warm Water Spring

ABSTRACT

Introduction: Water is one of the indispensable resources for the continued existence of all living things including man.

Aims: This study investigated the bacterial diversity in Ikogosi warm spring in Ekiti State, Nigeria.

Methodology: Water samples were taken for analyses from Ikogosi warm spring and analyses were made of the hot stream, cold stream, and meeting point region. Twenty isolates were characterized by morphological, biochemical, physiological characteristics and thermophilic screening was carried out.

Results: The results revealed that electrical conductivity was 215.050 $\mu\text{mS/cm}$, (cold); 320.500 $\mu\text{mS/cm}$ (warm), and 305 $\mu\text{mS/cm}$ (meeting point), temperature was close (24.5°C , cold to 37°C , warm). pH, total dissolved solids and chlorine were all far below the WHO guideline values. Average ranges of the heavy metals in water samples were: 1.080, 0.424 and 0.343 ppm iron (Fe) in hot, cold and meeting point samples respectively, 0.010, 0.006 and 0.004 ppm lead (Pb) in hot, cold and meeting point samples respectively; and 0.006, 0.010 and 0.019 ppm chromium (Cr) in hot, cold and meeting point samples respectively. Nickel (Ni), Arsenic (As) and Cadmium (Cd) were not detected. Bacteria majorly found in water samples were *Bacillus* spp., *Escherichia coli*, *Klebsiella* spp. and *Staphylococcus* spp. Out of the twenty (20) isolates screened at high temperature range of 45°C to 65°C , all grew at 45°C and 50°C while nine (9) grew at 55°C . Only two isolates were able to survive at 60°C , while none grew at 65°C .

Conclusion: These isolates can be in the production of thermostable enzymes which can be used in biotechnology.

Keywords: Bacteria, Ikogosi, Physicochemical, Thermophilic, Warm spring

1. INTRODUCTION

Warm springs are unique natural environments for thermophilic microorganisms. In the last decades, thermal environments and thermophiles have gained interest due to their scientific and biotechnological importance. For instance, studying of thermophiles is necessary for better understanding of the origin of life as many scientists believe that life might have arisen in high temperature, and in the evolution of life, there is evidence for thermophilic ancestors [1]. With respect to the biotechnological applications of thermophiles, the representative example in this field is the aerobic thermophilic bacterium *Thermus aquaticus*, which was isolated several decades ago from Yellowstone National Park [2]. *T. aquaticus* has become

Comment [1]: Natural

Comment [2]: Twenty Isolate analyses is justified? Is it representative?

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a source of Taq polymerase which has led for the development of polymerase chain reaction (PCR) [3].

Warm springs are produced by the emergence of geo-thermally heated ground water in volcanically active regions [1]. Warm springs are found throughout the world but they are more concentrated in certain regions in the world; for instance Ikogosi Warm Spring in Ekiti State, Southwestern Nigeria. Warm springs vary widely in their temperature, chemical composition, and pH [4].

Nigeria is among the countries known for having warm spring with varying physicochemical properties. The well-known Nigeria warm spring is Ikogosi warm spring located in the located at Ikogosi, a town in [Ekiti State](#), southwestern Nigeria. Water temperature in Ikogosi warm spring reaches 70°C at the source and 37°C at the confluence [5]. From a geological point of view, the warm spring is related to the Dead Sea Rift, where several springs discharge warm water originating from the Lower Cretaceous Sandstone [6].

The microbial diversity of Ikogosi warm spring was assessed by using culture-dependent methods and results had shown that Ikogosi warm spring is populated by microorganism belonging mainly to the domain of fungi and more precisely to the genus *Aspergillus* [7]. Lately, the isolation and characterization of thermophilic bacterial species belonging to the genera *Geobacillus* and *Anoxybacillus* were documented from Ma'in and Afra hot springs [5]. However, there has not been any record of isolation of bacteria from Ikogosi warm spring.

Data from the aforementioned studies confirm that applying enrichment and isolation approach results in the isolation of limited number of species belonging to the bacterial genus *Bacillus* or *Bacillus*-related species. However, it must be noted that most thermophilic microorganisms in warm springs are generally unculturable [8]. Subsequently, the microbial diversity using culture-dependent methods seems to be underestimated in Ikogosi warm spring. The main purpose of this study is to carry out a physiochemical examination and microbiological analysis of Ikogosi warm spring.

2. MATERIAL AND METHODS

2.1 Study area

The small town of Ikogosi-Ekiti in Ekiti State in Western Nigeria is situated between lofty, steep-sided and heavily wooded, north-south trending hills about 27.4 km east of Ilesha (Osun State) , and about 10.5 km southeast of Efon Alaaye (also in Ekiti State). It is located just north of the 7°35'N latitude and slightly west of the 5°00' E longitude. The elevation of the general area is between 457.0m - 487.5m.

Comment [4]: Please add the map of studied area

The Ikogosi Warm Spring is located about 1.61 km west of Ikogosi town. A Rest Camp has been built near the spring by the Baptist Mission. The mission has developed the spring for domestic use and constructed a swimming pool for recreational purposes. At Ikogosi Warm Spring, warm and cold springs ooze out of hills from different sources, flow side by side and meet-the first of such occurrence in the whole world. The natural quiet environment is left untouched for eco-tourism appeal.

2.2 Collection of sample

Standard method of sample collection was used for collecting the water samples. One litre of each samples were collected in clean white plastic bottles from three points of the warm spring. The sampling points are: (a) The oozing point of warm spring (b) The oozing point of

Comment [5]: Standard method describe by whom, mention this

the cold spring, (c) The meeting point (the confluence) of the warm and cold spring. The temperature and pH values of the water samples were taken immediately at the site using a thermometer (calibrated in °C) and pH meter respectively.

2.3 Physicochemical analysis

Physiochemical analysis was carried out on the water samples collected. These analysis covers pH, total dissolved solids (TDS) (mg/l), Electrical conductivity ($\mu\text{S}/\text{cm}$) and chlorides (mg/l). Analysis of the heavy metals for Fe (ppm), Pb (ppm), Ni (ppm), As (ppm), Cr (ppm), Cd (ppm) was carried out.

2.4 Bacteriological analysis

Nutrient agar was prepared with sterile water according to the manufacturer's description and autoclaved at 121°C for 15mins. The sterilized plates were inoculated with 1ml of each sample of water from each point using pour plate method. The agar was poured and allowed to solidify then inverted and incubated at 37°C for 24hours.

Eosin methylene blue (EMB) agar was prepared by weighing according to manufacturer's description (36gm in 1000ml distilled water). The agar was autoclaved at 121°C for 15mins for sterility and 1ml of each water sample was introduced to the plates. The media was allowed to cool to about 45°C before pouring in Petri-dishes. Each plate inverted after cooling to prevent steam formation. The plates were incubated at 37°C for 24hours.

MacConkey agar was prepared under aseptic condition, autoclaved to sterilize at 121°C for 15mins. 1ml of each water sample was introduced into the plate by pour plate method. The sterilized agar was poured into the plates and allowed to cool and solidify. This is followed by incubation at 37°C for 24hours.

Mannitol salt agar was prepared (111gm in 1000ml of sterile water) and sterilized using autoclave at 121°C for 15mins. 1ml of the sample was put in the plate and the sterilized agar poured into the plate then swirled to mix equally. It was allowed to solidify and incubated.

2.5 Biochemical identification of isolates

The bacterial isolates were tentatively identified by means of morphological characteristics, cellular and biochemical tests. Morphological characteristics were observed for each bacterial colony after 24 hours of growth. The colony of each isolate on the nutrient agar media were observed for identification of shape, appearance and colour, colony size, margin and emulsification. The biochemical tests carried out include; catalase test, indole test, methyl red, voges proskauer, citrate and oxidase. The isolates were identified using Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

Comment [6]: Have you done PCR?

2.6 Thermophilic Screening

Isolates from the water samples were sub-cultured on nutrient agar plates and incubated at 37°C for 24hours. The temperature was then increased at interval to 65°C.

3. RESULTS AND DISCUSSION

3.1 Results

Figure 1 shows the TDS for Hot water is 196.150 mg/l, 121.350 mg/l for the Cold water and 174.400 mg/l for the Meeting point. Significant difference exists between the values with Hot water having the highest value. Electrical conductivity for Hot water is 320.500 $\mu\text{S}/\text{cm}$, 215.050 $\mu\text{S}/\text{cm}$ for the Cold water and 305.100 $\mu\text{S}/\text{cm}$ for the Meeting point. pH of the samples collected for the study are arranged from pH 7.075, 6.860, and 7.140 for Hot, Cold and Meeting point water respectively.

Comment [7]: change the unit where you mentioned mg/l into mgL-1

Comment [8]: pH of hot water should be high. Check this.

Comment [9]: pH of hot water should be maximum. check this

Figure 2 shows the concentrations of heavy metals, Fe in the water samples ranged from 0.343 ppm in Meeting point water to 1.080 ppm in Hot water. Cr ranged from 0.006-0.019 ppm. Ni, As and Cd were not detected in all the samples. The highest value was recorded in Hot water (0.010 mg/l) and the lowest value was also recorded at the Meeting point (0.004 ppm).

Table 1 shows the cultural characteristics of the colony isolate from hot origin all the colonies form circular, there elevation appear convex and flat while the colour were pink, creamy and greenish metallic. The number of colonies on each plate ranges from 6 to 48. The highest number of colonies was recorded in sample H10 which contain (48) while the least is observed in H1. The colony characteristics of cold origin are circular and rhizoid the form of the colony, there elevation are flat and convex while the colour are whitish and creamy. The total number of colony per plate ranges from 3 – 93cfu/mL the highest colonies were observed in sample C3 while the least was in sample C2. The colony characteristic of meeting point origin shows that the form of each colony is circular, there elevation are convex respectively while the colour are purple, pink and creamy. The total number of colony per plate ranges from 2 – 60cfu/mL the highest colonies were observed in sample MP4 while the least was in sample MP1.

Table 2 show the biochemical characteristic of the isolate from hot region, this organism isolated are gram positive and gram negative bacteria, in the table it contain catalase, coagulase and sugar fermentation test. The probable organisms isolated were *Staphylococcus aureus* spp., *Klebsiella* spp., *Bacillus* spp., and *E. coli*. The biochemical characteristic of the isolate from cold origin showed grams gram positive and gram negative bacteria, in the table it contain catalase, coagulase and sugar fermentation test. The probable organisms isolated were *Bacillus* spp., *Klebsiella* spp., and *Staphylococcus* spp. The biochemical characteristic of the isolate from meeting point sample displayed grams gram positive and gram negative bacteria, in the table it contain catalase, coagulase and sugar fermentation test. The probable organisms isolated were *Bacillus* spp., *Klebsiella* spp., and *Staphylococcus* spp.

Comment [10]: Is it repeated?

Table 3 shows the thermophilic screening of hot, cold and meeting point of Ikogosi water sample, the screening were carried out at different temperature 45°C, 50°C, 55°C, 60°C and 65°C. as recorded the 45°C and 50°C the thermophile were present in all the sample analyzed, at 55°C the thermophile were present only in sample (H1, H2, H3, H4, H6, C2, C4, MP3 and MP6, at 60°C it was present only in sample H1 and H4 while at 65°C it was absent all through.

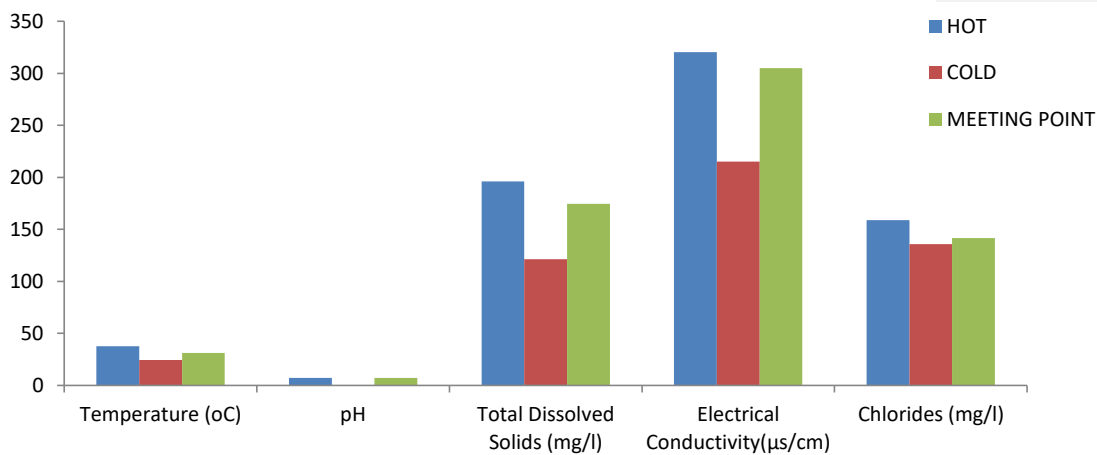


Figure 1: Physiochemical parameter of water (hot, cold and meeting point)

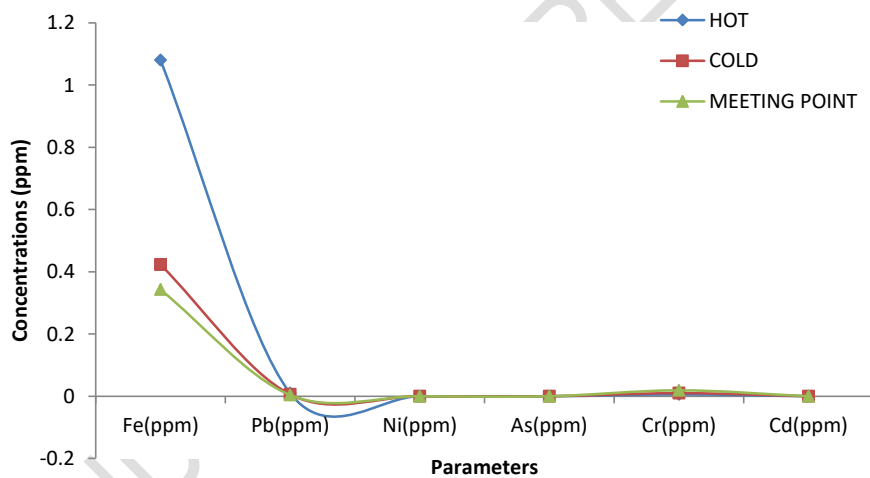


Figure 2: Heavy metal parameters of Ikogosi hot, cold and meeting point water

Table 1 Cultural characteristics and number of colonies for hot origin

Samples	Characteristics			Number of colonies
	Form	Elevation	Colour	
Hot region				
H1	Circular	Convex	Creamy	6
H2	Circular	Flat	Creamy	15
H3	Circular	Convex	Creamy	13

H4	Circular	Convex	Creamy	18
H5	Circular	Convex	Creamy	13
H6	Circular	Convex	Creamy	10
H7	Circular	Convex	Creamy	11
H8	Circular	Convex	Creamy	11
H9	Circular	Convex	Pink	9
H10	Circular	Convex	Creamy	48
H11	Circular	Convex	Greenish metallic	36
Cold region				
C1	Rhizoid	Flat	Whitish	16
C2	Circular	Convex	Creamy	3
C3	Circular	Convex	Creamy	95
C4	Circular	Convex	Pink	18
Melting point region				
MP1	Circular	Convex	Purple	2
MP2	Circular	Convex	Creamy	6
MP3	Circular	Convex	Creamy	3
MP4	Circular	Convex	Pink	60
MP5	Circular	Convex	Creamy	13

Table 2: Biochemical analysis hot origin samples

Sample	Gram rxn	Catalase	Coagulase	Sugar fermentation			Probable organisms
				Lactose	Glucose	Sucrose	
Hot region							
H1	+ Rod	+	-	+	+	+	<i>Bacillus</i> spp.
H2	+ Cocci	+	-	+	+	+	<i>Staphylococcus</i> spp.
H3	+ Cocci	+	-	+	+	+	<i>Staphylococcus</i> spp.
H4	+ Rod	+	-	+	+	+	<i>Bacillus</i> spp.
H6	- Cocci	+	-	+	+	+	<i>Klebsiella</i> spp.
H5	- cocci	+	-	+			<i>Klebsiella</i> spp.
H7	+cocci	+	-	+			<i>Staphylococcus</i> spp.
H8	+ cocci	+	-	+			<i>Staphylococcus</i> spp.
H9	- cocci	+	-	+			<i>Klebsiella</i> spp.
H10	- cocci	+	-	+			<i>Klebsiella</i> spp.
H11	- Rod	+	-	+			<i>Escherichia coli</i>
Cold region							
C1	+ Rod	+	-	+			<i>Bacillus</i> spp.

C2	+ Rod	+	-	+	<i>Bacillus</i> spp.
C3	- Cocci	+	-	+	<i>Klebsiella</i> spp.
C4	+ Cocci	+	-	+	<i>Staphylococcus</i> spp.
Melting point region					
MP1	- Cocci	+	-	+	<i>Klebsiella</i> spp.
MP2	+ Cocci	+	-	+	<i>Staphylococcus</i> spp.
MP3	+ Rod	+	-	+	<i>Bacillus</i> spp.
MP4	+ Cocci	+	-	+	<i>Staphylococcus</i> spp.
MP5	- Rod	+	-	+	<i>Escherichia coli</i>
+	=	Positive			
-	=	Negative			

Table 3: Thermophilic screening

Sample	45°C	50°C	55°C	60°C	65°C
H1	+	+	+	+	-
H2	+	+	+	-	-
H3	+	+	+	-	-
H4	+	+	+	+	-
H5	+	+	-	-	-
H6	+	+	+	-	-
MP1	+	+	-	-	-
MP2	+	+	-	-	-
C1	+	+	-	-	-
C2	+	+	+	-	-
H7	+	+	-	-	-
MP3	+	+	+	-	-
H8	+	+	-	-	-
H9	+	+	-	-	-
C3	+	+	-	-	-
MP4	+	+	-	-	-
H10	+	+	-	-	-
H11	+	+	-	-	-
C4	+	+	+	-	-
MP5	+	+	+	-	-
+	=	Positive			
-	=	Negative			

3.1 Discussion

The TDS for Hot water is 196.150 mg/l, 121.350 mg/l for the Cold water and 174.400 mg/l for the Meeting point (Figure 1). Significant difference exists between the values with Hot water having the highest value. All the TDS values are lower than WHO value (500 mg/l) standard for drinking water. Water with high TDS values, usually have no health threats to humans until the value exceed 10,000 mg/l. However, high TDS may produce aesthetically displeasing colour, taste and odour as well as hardening of water [9].

Electrical conductivity for Hot water is 320.500 $\mu\text{S}/\text{cm}$, 215.050 $\mu\text{S}/\text{cm}$ for the Cold water and 305.100 $\mu\text{S}/\text{cm}$ for the Meeting point. Significant difference exists between the values and the WHO value (250 $\mu\text{S}/\text{cm}$). Hot water and meeting point water were above WHO standard for domestic water while only Cold water fall below the permissible limit. The high conductivity values could be due to decomposition and mineralization of organic materials [10].

pH of the samples collected for the study are arranged from pH 7.075, 6.860, and 7.140 for Hot, Cold and Meeting point water respectively. This clearly indicates that all the samples fall within the WHO standard of pH 6.5 – 8.5. Cold water is slightly acidic while the others are basic on the pH scale. The increase in pH of the water samples above the normal background levels may be due to the presence of dissolved carbonate and bicarbonate present in the water, which is known to affect pH of almost all surface waters [10]. Based on these guidelines, the pH of the stream waters would not adversely affect its use for domestic and recreational purposes and the aquatic ecosystem. Water samples in the study had mean Cl concentrations below the WHO acceptable limits for drinking and domestic water that is 250 mg/l. The highest concentration was recorded from Hot water with a value of 158.750 mg/l and the lowest concentration was from Cold water with a value of, 135.900 mg/l. Chloride toxicity has not been observed in human except in special cases of impaired sodium chloride metabolism as reported in congestive heart failure [9]. Little is known about the effect of prolonged intake of large amount of chloride in the diet. The presence of chlorides in high concentrations makes water hard and increases the electrical conductivity. High concentration of chloride can make waters unpalatable and therefore, unfit for drinking [11].

Comment [11]: Justify why pH was not maximum in hot water?

The concentrations of iron (Fe) in the water samples ranged from 0.343 ppm in Meeting point water to 1.080 ppm in Hot water (Figure 2). All the water samples did not exceed the WHO limit of 1.0 ppm except Hot water that is higher than the limit. The presence of iron in drinking water may increase the growth of pathogenic organisms, since most of these organisms need iron to grow. The major effect of the presence of iron in domestic water is aesthetic because of the colour [12]. Therefore, Fe concentration does not currently present any aesthetic problems in all the water sources in this community.

Chromium (Cr) ranged from 0.006-0.019 ppm. Nickel (Ni), Arsenic (As) and Cadmium (Cd) were not detected in all the samples. The water samples within the study area were characterized by lead concentrations. The highest value was recorded in Hot water (0.010 mg/l) and the lowest value was also recorded at the Meeting point (0.004 ppm), they are lower than the WHO value (0.01 ppm) except Hot water which have the same value with the WHO recommended level of 0.01 ppm. At levels higher than 0.01 ppm, possible neurological damage in foetus and young children may occur [13].

Comment [12]:
Please add the reason behind the change of the physico-chemical parameters in different situations? mechanism of changing.

The isolation of bacteria was successfully performed and bacterial isolates; there were bacterial isolates, which lives on the hot, cold and warm water of Ikogosi. Screening isolates of thermophilic bacteria originating from 3 stations (hot, cold and meeting point), yielded four isolates that survived at a constant temperature of 45 °C, 50 °C, 55 °C, 60 °C. The obtained isolate was successfully purified and kept stable growing at a constant temperature of 45 °C. Each contains bacteria capable of living at a constant temperature of 45 °C, for 48 hours.

Thus, the decrease in temperature in the laboratory compared with the in situ water temperatures does not kill the bacteria present in the hot cold and warm water samples. Bacteria are capable of forming endospores at ambient temperatures that do not support. Bacteria were successfully isolated with nutrient agar medium. All isolates obtained were identified and characterized by morphology, microscopic, and biochemical tests as show in

the result obtained. From the test results, it is suspected that the isolates obtained are genus *Bacillus* spp., *Escherichia coli*, *Staphylococcus* spp., and *Klebsiella* spp. has the characteristic as shown in table 2.

The discovery of gram negative isolates in the water samples may be associated with environmental conditions. Gram-negative bacteria require relatively simpler nutrients compared to gram-positive. This means the ability of this group of bacteria to grow in a higher environment than gram positive. The bacterial isolates obtained from Ikogosi water sample, were able to grow at a temperature range of 45 °C - 60 °C.

The result of fermentation test of glucose, lactose and sucrose all isolates showed positive result (table 2). This was indicated by the color change of fermentation medium of glucose, lactose and sucrose from red to yellow during incubation for 24 hours, and 48hours respectively. According to Harley [14], the yellow color of the media was caused by the phenol solution that forms acid in the carbohydrate media. The color change was caused by isolates capability to use glucose, lactose and sucrose as the carbon source.

The activity of the optimum bacterial isolates of thermophilic at temperature distribution of 45 °C – 60 °C was also reported by Muharni [15] and Indawarti [16], respectively in bacteria isolated in hot springs.

Based on the result of study, there were 4 isolate bacteria, *Escherichia coli*, *Staphylococcus* sp, *Bacillus* spp. and *Klebsiella* spp. Characterization of thermophilic bacterial isolates macroscopically showed different properties, whereas microscopic observation showed *bacillus*, rod and coccus shaped bacterial cells as well as Gram positive and negative bacteria. Observation of physiological test of all isolates grew at 45 °C – 60 °C. This study contributes to the development of conservation strategies and provides information on the characteristic diversity of bacteria as a potential enzyme producer from Ikogosi Hot Spring. The novelty of this research is the determination the bacteria diversity characterization and identification of bacteria isolate based on macroscopic observation, microscopic, physiological test, biochemical test and optimization of its growth in level of temperature.

4. CONCLUSION

The bacterial diversity in Ikogosi warm spring may be of public health significance as carriers of disease-causing organisms or because they produce toxins. It is desirable that these free-living organisms should be absent. Guideline values are not available yet for free-living organisms in water. Knowledge of the identity and abundance of organisms in raw water supplies is valuable in water resource management.

The bacterial diversity in Ikogosi warm spring could further be analyzed phenotypically and enzymatically analysis to indicate the presence of thermophilic bacteria subspecies. Furthermore, the results of this study can be exploited further for production of biotechnological important and industrially thermostable enzymes. This study widens the opportunities for further research to be conducted to explore more the immense significance of these isolates, where there is lack of intensive studies regarding these bacterial.

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Comment [13]:

Give the precautionary measures to uplift the physico-chemical parameters in rationale to WHO standard

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