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"Innovative Excellence for Tomorrow"

EXTENDED ABSTRACTS

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VAVUNIYA CAMPUS OF THE UNIVERSITY OF JAFFNA
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QUALITY ANALYSIS OF THE WATER SUPPLIED FOR PUBLIC FROM COLLECTOR WELLS

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Abstract

The study was aimed to determine the water quality of the collector wells in the Vallipuram area of the northern Sri Lanka and to determine the suitability of the water for domestic utilization. Because of the infection of well water by iron and coliform bacterial species in 2016, public water supply was temporarily stopped and after a complete sterilization and clean-up process using chlorination and integrated control methods only, the water supply restarted after six months. Since then, there had been no steps made to check the quality of the drinking water supplied to the public from these wells. Water samples (36) were collected from the four major water supply collector wells in both top, middle and bottom randomly in triplicates during the rainy season of 2018. Samples were analyzed for pH, Electrical conductivity (EC), Turbidity, Total iron, Colour, Coliform test and Bio chemical tests such as Indole production test, Methyl red test,Voges-Proskauer test, Oxidase test and Catalase test. The results were compared with WHO and SLS standards and it was concluded that all the water samples from the collector wells tested, did not have any contaminants thus the quality of those samples was good for drinking.Based on the biochemical and molecular analysis,the bacterial species presented in some water samples was identified as *Bacillus thuringiensis.* Exactly similar conclusion was reached when the same research study was repeated after 6 months from the earlier study and the values obtained for the physico chemical factors tested were not significantly different with that of the previous study. Therefore, water in all the collector wells could be used for domestic and sustainable agricultural purposes continuously in the future too.

Keywords: Collector wells,*E. coli*,physico-chemical, pH, salinity, water quality,

Introduction

Water plays an essential role in human life. Although statistics, the World Health Organization reports that approximately 35% of people were without access to safe drinking water (WHO, 2004). Ground water is the most important source of supply for drinking, irrigation and industrial purposes. Increasing population and its necessities have led to the deterioration of surface and sub-surface water (Basavaraj et al., 2015). Water is polluted on all the surfaces of earth. All metabolic and physiological activities and life processes of aquatic organisms are generally influenced by such polluted waste and hence, it is essential to analyze the physic-chemical characteristics of the drinking water (WHO, 2004). People in Jaffna Peninsula, depend mainly on ground water for their drinking and other domestic purposes as other water sources such as waterfalls and rivers are not available and fresh water ponds and rainfall are not sufficient. ManalkaduSand Dunes- Manalkadu village is a miniversion of a desert. Right at the town of Point Pedro begins the Manalkadu Sand dunes. The sparsely populated coastal stretch is punctured with isolated villages centeredaround avillage well. The acres ofsand dunesare also found covered with thick bush while beyond the dunes one can spot one of the most beautiful beaches of the Northern Province. Sand

dune, sand dune: Sand dunes are Jaffna lagoon of the northern Sri Lanka is surrounded by the densely populated Jaffna peninsula containing Palmyra palms, coconut plantations, and rice paddies. There are numerous fishing villages and some salt pans (Kapilan, 2015). The Jaffna lagoon is a shallow water body and has extensive mudflats, sea grass beds and some mangroves. Underground water quality of the coastal area of the lagoon is continuously degrading due to fishing related activities and dumping of garbage without proper management. The coastal areais widelyused for the fishing purposes and for small scale production of salt. Unoccupied land scare used as points todump garbage improperly. Most of the wells that are closer to sea are not used for publicconsumption becauseof thesalty nature (R Kapilan, 2015). Because of the infection of well water by iron and coliform bacterial species in 2016, public water supply was temporarily stopped and after a complete sterilization and clean-up process using chlorination and integrated control methods only, the water supply restarted after six months. Since then, there had been no steps made to check the quality of the drinking water supplied to the public from these wells. It was decided to analyses its ground water so that some remedies for improvement could be possible and sampling locations. However there is no recent scientific water quality analysis conducted for the collector wells to determine the water quality and to identify the potential pollutants. Vallipuram area was selected as an ideal place of the above mentioned situation. Therefore this study was aimed at determining the water quality of the collector wells in the Vallipuram area of the northern Sri Lanka and to determine the suitability of the water for domestic utilization.

Materials and Methods

Study area

This study was conducted in the Vallipuram costal area between August and September in 2018 and February and March in 2019.Collector wells are located in sand dunes. The location is rich in water and there are less hardness issues, less population density in the area so that anthropogenic is also very less.

Reagents and Chemicals

All the chemicals used in this study were of analytical grade and the standard methods were for the examination of water and its components (APHA, 1989).

Sampling method

Samples were collected and analysis was done by following “Standard method of analysis of water" from nine different spots of the each collector wells. Precautionary measures were adopted to minimize cross contamination of samples. Water samples were collected in sterilized screw-capped Durant bottles of one liter capacity and analyzed in laboratory for their biological and physic-chemical parameters. This was done carefully to avoid contact between the Walls of well, thus avoiding contamination of samples. Samples were labeled as collector well 1, 2, 3 and 4.

Water analysis

The collector wells were operated before the collection of the water samples. After the samples were collected, sample containers were kept in ice box and carried to Water board, Jaffna and later used for the physic-chemical analysis based on SLS and APHA guidelines The water quality parameter estimation and calibration of equipments were done using standard methods and techniques.

pH, Electrical conductivity, Turbidity, Total iron concentration and Colour

The pH of the water samples was measured by using digital pH meter. Electro conductivity was measured by the conductometry. Turbidity was measured by using Naphelo turbidity meter. Water samples were analyzed for total iron concentration. Total Iron was measured by the photometry (spectrophotometer) - Ferro Ver Method and used ferrous iron reagent powder pillows.The color of the samples was determined by the Hazen colour disk and lovibond comparator.

Physico-Chemical analysis

The various physico-chemical parameters were examined using the Standard Methods for the Examination of Water (APHA 1987)

Coliform test

After the chemical Analysis in the water board, the water samples were taken to the Department of Botany laboratory, University of Jaffna for the Coliform test and Bio chemical analysis. 10 ml of water from each samples were used to perform standard coliform test (Barrow and Feltham 1993, Fisher, 1975,Theivandrarajah, 1990).

Bio chemical analysis

Bacterial strains that were isolated from the water sample was subjected to variety of morphological and biochemical tests, as described in Bergey's Manual of Systematic Bacteriology (Bergey et al., 2001) and other methods (Barrow and Feltham 1993, Fisher, 1975, Theivandrarajah, 1990). Shape and arrangement of endospore were observed. under oil-immersion microscope after gram staining.Production of acid from different carbohydrates such asglucose, xylose and mannose were tested. Productionof urease, hemolysis of blood agar, indole test, nitratereduction test, decomposition of tyrosine, hydrolysis ofstarch, citrate utilization test and Voges-Proskauer (VP)test were done on the selected strain [34]. Growth of theselected strain was tested at 5, 15, 25, 35, 40, 45, 50,55 and 60°C at pH 9.0, and 100 rpm. Effect of differentconcentrations of NaCl on the growth of the selectedstrain was tested.

Characterization of the strain by molecular means

DNA was extracted from bacterial isolates by KIT method in Botany laboratory, and it was sent to Gene Tech to molecular analysis. Pure culture of the selected bacterial strain was grown overnight on Nutrient Broth and the DNA extraction was done using kit (QIAGEN Inc.Mississauga, ON, Canada) by Cell Lysis method and 16S rDNA was amplified by

Thermocyclerusing the primers, Forward: 5' AGAGTTTGATCCTGGCTCAG 3', Reverse: 5'TACCTTGTTACGACTT 3'. The amplified 16S rDNAPCR product was sequenced using automated sequencer. The Sequence Similarity Search was done for the 16S rDNA sequence using online search tool BLAST (http://www.ncbi.nlm.nih.gov/blast/). The unknown organism was identified using the maximum aligned sequence through BLAST.

Statistical analysis

Statistical analyses were performed using R2.15.3 (R Development Core Team, 2010). The data were analyzed using ANOVA. Determination of significant differences at $p \leq 0.05$ was estimated by performing Tukey's multiple comparison test.

Result and Discussion

pH, Electrical conductivity, Turbidity, Total iron concentration and Colour

The pH value of water sample in the study area ranged from 7.0 – 8.0. This shows the pH of the water sample was slightly alkaline. On an average, pH of all the samples was in desirable limits as prescribed for drinking water standards. Lime deposits below the soil, decaying Plant and Animal wastes, seasonal rain might have influenced in the observed pH value. The specific conductivity of water samples in the study varies between 1200 -2500 µ mho /cm. the maximum permissible limit of specific conductivity for drinking water is 3500µ mho /cm. however the average specific conductivity exceeds the permissible limit because of its high values during rainy season. In rainy season, due to the floods and rains water level in the well will increase and this will lead to increase in the amount of electrolytes.The turbidity of the water samples fell in the range of5.5 – 27 NTU. World health organization (WHO) prescribed the highest desirable limit is 5NTU and maximum permissible limit is 25 NTU(Kapilan, 2015). In most of the areas the water was very clear and the value of turbidity present was in the permissible limit. As all the water wells that were used for drinking were kept covered all the time there was a very less chance of substances reaching the water.Iron is one of the most abundant metals in the earth's crust. It is found in natural fresh waters at levels ranging from 0.5 to 50 mg/liter. Iron may also be present in drinking water as a result of the use of iron coagulants or the corrosion of steel and cast iron pipes during water distribution.Color in water is caused by minerals. Color can also be caused by industrial or municipal contaminations. Color is usually only a problem with surface water. But some ground waters containing iron or manganese can also have significant color levels. Color is classified as either true color or apparent color.True color is due to the colloidal organic compounds in the water. Apparent color is caused by colored suspended matter such as clay or iron precipitates in treatment applications.

Coliform count

There were no positive answers for the coliforms. Therefore, there were no coliform bacteria present in any of the water samples collected from Vallipuram wells.

Morphological Characteristics of the bacterial strain

Growth of the bacteria was observed in the agar media. Aloopful of bacteria was isolated on the same solidified medium, a pale yellow coloured growth was observed on the plates after an incubation of 24- 48 hours. Individual separate colonies were obtained. Since only one type of growth was observed hence, it could be concluded that is also possible to obtain the isolate as a pure culture.Gram Staining showed that gram positive rod shaped.

Microscopic Studies and Biochemical tests

Biochemical tests were carried out to confirm the genus of the strain and to identify the species. Since the strain produced O_2 from H_2O_2, it was concluded that the strain is a catalase producer. When tryptophan water medium was inoculated with the isolated strain and mixed with Kovc'sindole reagent, red colour ring was not observed. This indicated that strain cannot utilize tryptophan and produceindole. If the organism produces starch hydrolyzing enzymes it can be hydrolyze starch into mono saccharides. To check whether the organism has utilized starch, after the growth of the organism I_2 has to be added. If there is a blue colour formation, it indicates the production of starch hydrolyzing enzyme (Theivendrarajah, 1990). When the starch-agar medium was inoculated with the strain and I_2/KI was added after 48 hours of incubation, a blue colour was not formed. This indicated that the strain produce starch-hydrolyzing enzymes. Based on the biochemical tests performed, the genus of the strain isolated from the water sample was identified as *Bacillus* sp.

Final confirmation of species of identified strain *Bacillus sp*

Characteristics of the isolated bacterial strain were compared with other Bacterial species. If the character of strain is similar to the known species its score would be 1.if the character is not similar and variable, it would not get any score. Total score was counted, divided by total characteristics and it was multiplied by 100 and presented as a percentage. Based on these morphological findings and biochemical studies, the isolated strain got the highest score of 95% showing similarities with *Bacillus spp*. The strain showed clear characteristics of *Bacillus spp* than the other suspicious bacterial species. As the bacterial strain got the highest score, it was identified as *Bacillus spp*.

Characterization of the strain by molecular means

Based on the culture and morphology studies, the genus of the selected strain (B2) could be *Bacillus* since it showed positive results to the gram staining, spore formation, motility, catalase test and triple sugar iron agar test. The DNA from the isolated strain was isolated and the 16S rDNA was amplified and sequenced. The BLAST analysis of the strain using its 16S rDNA sequence data showed that strain CEL PTK1 had highest homology (100 %) with *Bacillus thuringiensis* (GenBank accession no AE017355).

Conclusion

This study reveals that all the values obtained for the water samples were within the permissible limit in all levels of all the collector wells, according to the WHO and SriLankan standards. The samples did not contain any pathological contaminants. The BLAST analysis of the isolated strain using its 16S rDNA sequence data showed that strain isolated from the wells had highest homology (100 %) with *Bacillus thuringiensis* (GenBank accession no AE017355). A periodical analysis needs to be done to make sure there are no more contaminants in the drinking water sample.

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