JIGNASA STUDY PROJECT
ON

“Exploration of defluording bacteria in water sample”

Department of Microbiology

NTR GOVERNMENT DEGREE WOMEN
MAHABUBNAGAR -509001
(B Grade by NAAC with 2.86 CGPA)

[AFFILIATED TO PALAMURU NIVERSITY]
NEAR BUS STAND, MAHABUBNAGAR
2020 - 21

NTR GOVERNMENT DEGREE WOMEN
MAHABUBNAGAR -509001

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Dept of Microbiology with Gratitutbe to

Dr K Padmavati

Principal

CERTIFICATE

This is to certify that the project entitled **"Exploration of defluording bacteria in water sample"** in NTR. Govt. Degree college for women; MBNR has been by the students of B.Sc. Microbiology III year (2020-2021) under the supervision of **Dr K Padmavati** This report incorporates original result of BSc III year students. This work has not been submitted for the award of any other degree or diploma in a part or full prior to this date.

Place: Mahabubnagar

Principal

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This is to certify that the project entitled **"Exploration of defluording bacteria in water sample"** in NTR. Govt. Degree college for women; MBNR has been by the students of B.Sc. Microbiology III year (2020-2021) under the supervision of Dr A. Shiva Shanker. This report incorporates original result of BSc III year students. This work has not been submitted for the award of any other degree or diploma in a part or full prior to this date.

Place: Mahabubnagar

SUPERVISOR

DECLARATION

The results of the study presented in this report entitled **"Exploration of defluording bacteria in water sample"** are original and carried out by us under the guidance of A. Shiva Shanker, Research Lab dept of Microbiology & NTR. Government degree College for women, Palamuru University. All right reserve to Dept of Microbiology, Palamru University due to providing cultures, chemicals and lab equipment.

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We would like to express special thanks to our Mentor A. Shiva Shanker as well as Principal Dr K Padmavati who gave us the golden opportunity & Prof. Pindi Pavan Kumar, Dept of Microbiology Registrar, Palamuru University, provide chemical, culture and allow to utilize lab equipments to do this project on the topic **"Exploration of defluording bacteria in water sample"** which also helped us in doing a lot of research and we came to know about so many new things we are really thankful to them. Secondly we would also like to thank our friends who helped us a lot in finalizing this project within the limited time frame.

CONTENTS

Introduction to Microbiology

A microbes is a microscopic organisms that made up of single cell (unicellular) A microbe is any living thing that spends its life at a size visible sometimes only with a microscope. It is too tiny to be seen with the naked eye. Microbes are the oldest form of life on Earth. Some types have existed for billions of years. They may live as individuals or cluster together in communities. Microbes live in the water you drink, the food you eat, and the air you breathe. Right now, billions of microbes are swimming in your belly and mouth, and crawling on your skin! Don't worry, over 95% of microbes are good for you. Microbes include bacteria, viruses, fungi, algae, and protozoa. Most microorganisms are unicellular but this is not universal since some multicellular organisms are microscopic. Some unicellular protists and bacteria like Thiomargarita namibiensis are macroscopic and visible to the naked eye. However these microbes are man used in several ways.

Applications of Microbes

1) Household Products
   - Curd formation
   - Cheese
   - Bread
2) Industrial Products
   - Fermented Beverages
   - Antibiotics
   - Enzymes and Vitamins

3) Sewage Treatment

4) Biogas Production

5) Biocontrol Agents

Title

“Exploration of defluording bacteria in water sample”

Statement of problems

Fluoride is a mineral in your bones and teeth, Fluoride is found naturally in soil, water, and foods and rocks Fluoride is commonly used in dentistry to strengthen enamel, which is the outer layer of your teeth. Fluoride helps to prevent cavities. It's also added in small amounts to public water supplies This process is called water fluoridation. The Department of Health and Human Services (DHHS) sets the optimal level of fluoride for preventing tooth decay at 0.7 ppm, or 0.7 milligrams (mg) in every liter of water. Excessive exposure to fluoride has been linked to a number of health issues **likw** Dental fluorosis, Skeletal fluorosis, Thyroid problems**,** Neurological problems Other health problems.

Anthropogenic and natural forms of fluorides have become a major problem worldwide in groundwater resources creation of inexpensive materials for removal of fluoride stable form of fluorine is a challenging criterion to provide safe drinking water to public.

The problem is persist

Aim of the project

Isolation and identification of efficient defluoriding bacterial in water sample.

Objectives of the project

- Isolation of fluoride resistance bacteria
- Identification of isolate by Biochemical and 16S rRNA sequence.
- Optimization of bacterial growth by physical factor of Tem, pH and rpm.
- Quantification of fluoride up taken by bacteria through spectrophotometric method.
- Analysis of isolate surface elemental analysis by study by SEM and EDXA analysis.

Review of literature

Various processes have been reported for fluoride removal, viz. electrodialysis (Jayarathne et al., 2014), reverse osmosis (Sehn, 2008), nanofiltration, adsorption (Halder et al., 2015), biosorption

(Mukherjee and Halder, 2016), Nalgonda technique (Mukherjee and Halder, 2016), ion exchange (Halder et al., 2015). These processes have advantages like non-requirement of media, higher uptake capability, easy availability of chemicals etc. But the major drawbacks associated with these processes are high initial cost, water desalination, higher waste generation, excessive water and electricity consumption, problem of waste disposal etc (Mukherjee and Halder, 2016). These physico-chemical methods are also reportedly ineffective or expensive when the contaminant concentration is very low. Although the removal capability of microorganisms is comparatively lower than that of the materials used for defluoridation in the aforementioned processes, yet bioremediation might be a viable way of fluoride removal. Some of the advantages associated with bioremediation over other processes are simple operation, cost-effectiveness, lesser energy requirements, lower generation of sludge etc (Mukherjee et al., 2017).

Research and methodology

Bacterial culture: *Escherichia Coli*, *Acinetobacter* sp., *Pseudomonas* sp., *Klebsiella* sp., *Enterobacter* sp., *Serratia* sp., *Bacillus* sp., all bacterial culture was isolated from ground and surface water bacteria provided by department of microbiology palamuru University. After that all bacterial screening for fluoride resistance at different concentration by adding fluoride to culture media contain 20, 40, 60, 80 and 100mg concentration of LB.

Identified fluoride resistance bacteria: Presence of colony on high concentration fluoride contain plate, colony marked and sub culture into fresh plate for further study.

Optimization of culture conditions: Optimization study was conducted by varying pH and temperature. The pH sensitivity of bacterial isolates were determined at different pH by adjusting the pH (0.1 HCl and 0.1 NaOH was used to adjust the pH) of the culture broth dissolved in buffer to pH range of 5-10.

Temperature is a vital parameter which helps in understanding the ability of the bacteria to survive at greater and lower temperatures. These were incubated at different temperature intervals (20 ^{0}C, 25 ^{0}C, 30 ^{0}C, 35 ^{0}C and 40 ^{0}C) at 100 rpm for 48hours. Bacterial growth was recorded at 540 nm after 24 h and then centrifuged at 5000 rpm for 15 min. The supernatant was used for analysing the residual fluoride concentration

Determination of fluoride removal activity of isolates:

In order to determine the fluoride degrading capability, the isolated strains were inoculated in 250-mL conical flasks in triplicates containing culture broth with 100 mg/L fluoride concentration and incubated at 35 ^{0}C on a rotary shaker at 100 rpm. Samples were taken from these flasks and analysed for fluoride concentration and growth. Ten millilitres of sample was taken at a time for fluoride analysis and centrifuged at 5000 rpm for 15 min, and the supernatant was subjected to fluoride concentration analysis using SPADNS colorimetric method.

Conclusions

Removal of fluoride from drinking water by microorganisms is interest in recent years. In this present research an attempt was made to remove fluoride from drinking water by using living micro organisms viz. *Escherichia Coli*, *Acinetobacter* sp., *Pseudomonas* sp., *Klebsiella* sp., *Enterobacter* sp., *Serratia* sp., *Bacillus* sp., collected from drinking water resources of Mahabubnagar district, Telangana State, India. Above six strains of bacterial isolates employing culture broth containing 100 mg/L of fluoride to evaluate their efficiency in reducing fluoride concentration in water. Batch optimization study was conducted to screen the efficiency of potential bacteria for the removal of fluoride. SPADNS colorimetric method was adopted to measure fluoride concentration in broth media. Results revealed that only *Acenetobacter* sp., was found to be efficient with the fluoride removal percentage of 58 after 10 hours of incubation period at 35 ^{0}C and the pH of 7.5. It was concluded that there was no proper defluorination activity found after 10hours.

We observed the 58 percentae of after10 hours of incubation period at

References:

Jayarathne, A., Weerasooriya, R., Chandrajith, R., 2014. A rapid method for the removal of fluoride in contaminated groundwater using natural crystalline apatite: a laboratory and field study. Environ Earth Sci. http://dx.doi.org/ 10.1007/s12665-014-3998-7.

Sehn, P., 2008. Fluoride removal with extra low energy reverse osmosis membranes: three years of large scale field experience in Finland. Desalination 223 (1e3), 73e84.

Halder, G.N., Sinha, S., Dhawane, S., 2015. Defluoridation of wastewater using powdered activated carbon developed from Eichhornia crassipes stem: optimization by response surface methodology. Desal Water Treat 56, 953e766.

Mukherjee, S., Halder, G., 2016. Assessment of fluoride uptake performance of raw biomass and activated biochar of Colocasia esculenta stem: optimization through response surface methodology. Environ. Progr. Sustain. Energy 35 (5), 1305e1316.

Halder, G.N., Sinha, S., Dhawane, S., 2015. Defluoridation of wastewater using powdered activated carbon developed from Eichhornia crassipes stem: optimization by response surface methodology. Desal Water Treat 56, 953e766.

Mukherjee, S., Yadav, V., Mondal, M., Banerjee, S., Halder, G., 2017. Characterization of fluoride-resistant bacterium Acinetobacter sp. RH5 towards assessment of its water defluoridation capability. Appl Water Sci 7 (4), 1923e1930.