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# A Study on the Bioaccumulation Potential of Two Bacteria Isolated from Chettipunyam Lake and Thirukachur Lake water samples of Chennai city

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**KEYWORDS**

Metal chelation, Bioremediation, Bacterial consortium, *Priestia megaterium* and *Bacillus cereus.*

**ABSTRACT**

**Aim:** The present study was aimed to isolate and investigate the bioaccumulation potential of two bacteria isolated form Chettipunyam Lake and Thirukachur Lake, in Chennai City.

Methods: Isolates were identified using standard 16srRNA sequencing and DNA amplification methods. Sample water was collected and physio-chemical characteristics was tested as per APHA standards. Evaluation of bacterial strains for the removal of heavy metals (Zinc, Iron and Copper) from lake water samples was determined using standard methods.

Findings: Two significant metal chelating bacterial cultures isolated from Chettipunyam Lake and Thirukachur Lake was identified as *Priestia megaterium* CL3 and *Bacillus cereus* CL4 16S respectively. Physico-chemical parameters tested as per APHA standards showed that, all three metals, Zn, Fe and Pb was found approximately 1050-1450 folds higher than that of permissible level in both lake water samples. The obtained percentage loss by standard strains when compared to the percentage loss efficiency by the consortium isolates showed almost similar level in percentage loss of maximum up to 89.9% after 60 hours; and up to 92.6% after 120hours.

Conclusion: The findings revealed the potential ability of bacterial isolates for better metal accumulation efficiency from any type of water or waste water samples. As a future study, the treated or metal accumulated water shall be used for basic agricultural activity in small fields where water is not needed in more quantity.

## INTRODUCTION

Due to population growth, industrial development activities such as mining, metal smelting, textile production, burning of fossil fuels, use of fertilisers and pesticides in agriculture, manufacturing of batteries and other metal products in industries, sewage sludge disposal, and municipal waste disposal resulted in large amounts of waste water being released into the environment and aquatic areas (Chibuike *et al*., 2014). Only 30% of waste water in India is treated before it is released into the environment. The majority of the time, untreated industrial effluent water enters water systems such rivers, lakes, groundwater, and coastal waters directly, leading to substantial water pollution. The problem cannot be solved by any of the treatment procedures that have been reported in the scientific literature because pollutants can move from one phase to another (Thapliyal *et al*., 2011).

Based on this heavy metal pollution in the ecosystem, different methods were employed to treat the contaminated sources. Different methods like chemical, physical and biological methods were used for the treatment process (Siddiquee*et al*., 2015). Among the different methods, microbial degradation or bioaccumulation of heavy metals were well studied by many researchers. According to Lee et al. (1998), the most typical biological therapy entails the degradation of pollution into harmless forms via microbial activities.

During bioremediation, microorganisms in contaminated areas take up heavy metal ions. The process in heavy metal compounds that have a strong electronegative group, such as carboxyl, phosphate groups, as well as nitrogen-containing groups, removes the primary chemical functional groups. These groups are essential for binding with hazardous metals at particular or general binding sites

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on the cell membrane of microorganisms, which then allows the metals to be absorbed into the cellular structure of the germs (Timmis*et al*., 1994). The benefit of this method is that the metal ions can be retrieved, stabilised, or buried after being physically removed from the environment, depending on what is thought to be most appropriate. Since the process does not require active cell metabolisms, the biological components in it can be either living or dead cells (Gadd, 2000).

Verma *et al*., (2020) mentioned in their review that microorganisms play a vital role in the removal of heavy metals. In another study Ramasamy and Kamludeen Banu(2007) highlighted that microbes can able to decontaminate heavy metals by chemical precipitation and volatilization. Jyoti and Harsh (2017) significantly pointed out that, microbial cells have the ability to convert heavy metals from one oxidation state to another state, which decreases the toxicity of metal components.

In our present study, our objective is to isolate two bacteria from the lake sites and compare its metal chelating ability with standard bacterial strains (*Dechloromonasaromatica* and *Pseudomonas aeruginosa*). This study was therefore carried out to evaluate the individual and synergistic potential of heavy metal tolerant bacterial strains *Dechloromonasaromatica* and *Pseudomonas aeruginosa* to bioremediate heavy metals like zinc, iron and lead. In parallel, isolates (*Priestia megaterium* and *Bacillus cereus*) from the soil sample collected from two different lakes (Chettipunyam Lake and Thirukachur Lake) were also compared with metal accumulating efficiency of standard strains (*Dechloromonasaromatica* and *Pseudomonas aeruginosa*).

## MATERIALS AND METHODS

### Study area and sample collection

Chennai is a metropolitan city and capital of Tamil Nadu with numerous industries present in the outskirts. Lake water samples were collected from two different locations around Chennai city at Chettipunyam Lake (12.757°N, 79.988°E), and Thirukachur Lake (12.773°N, 79.996°E). All samples were collected in separate sterile screw cap bottle (500mL). Parameters like pH, DO, and temperature were tested during sample collection and recorded (APHA, 1992). All the samples collected were brought to laboratory and stored under refrigeration condition for further physico-chemical characterization.

### Analysis of physico-chemical characteristics of lake water samples

Different physico-chemical parameters analyzed for the collected lake water samples were pH, total dissolved solids (TDS), biochemical oxygen demand (BOD), total alkalinity as $CaCO_3$, dissolved oxygen, total suspended solids, chemical oxygen demand (COD), turbidity, total chlorides, volatile solids, acidity, and heavy metals such as Zn, Fe, Pb. All the parameters were tested using the standard protocols as per APHA (1988).

### Isolation of metal accumulating bacteria from the lake water samples

Soil samples were collected fromChettipunyam Lake and Thirukachur Lake(from depth of approximately 2 metre) in sterilized polyethylene bags and stored at 4ºC until examination. The soil sample was serially diluted upto $10^{-5}$ dilutions and plated on Nutrient Agar plates. About 1ml of diluted sample was spread onto the surface of Nutrient Agar medium and incubated at 37ºC for 24 h. Single colony was picked spread on the Nutrient Agar plates and subcultured in nutrient broth and again cultured on plates in order to obtain pure cultures. Pure bacterial strain were obtained after successive transfer of a single colony on Nutrient Agar plates and incubated for 24 h at 37ºC temperature.

### Identification of isolates using 16srRNA sequencing

Identification of isolated bacterial strains was done using 16S rDNA sequencing and blast analysis method. The genomic DNA was isolated by usingCTAB and Lysozyme method given for gene sequencing in Ribogen laboratory, Chennai.This purified DNA was used in PCR for amplification of 16S rDNA gene. Theuniversalprimer used for amplification of the bacterial 16S rDNA are presented in Table-1 for Lake Isolate – A ($LI_A$) from Chettipunyam Lake and Table-2 for Lake Isolate – B ($LI_B$) from Thirukachur Lake.

**Table-1: Forward and Reverse primers for Lake Isolate – A ($LI_A$)**

| | |
|---|---|
| P1F | 5′–*C*GGGATCCAGAGTTTGATCCTGGTCAGAACGAACGCT–3′ |
| P6R | 5′– CGGGATCCTACGGCTACCTTGTTACGACTTCACCCC–3′ |

*(Priestia megaterium)*

**Table-2: Forward and Reverse primers for Lake Isolate – B ($LI_B$)**

| | |
|---|---|
| P8F | 5’- AGAGTTTGATCCTGGCTCAG -3’ |
| 1492R | 5’- AAGTCGTAACAAGGTAACC -3’ |

*(Bacillus cereus)*

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The PCR mixtures were prepared in 50µl volumes containing 0.5µM of primer, Tag PCR Master Mix (Qiagen), and 1µl of the extracted DNA. DNA amplification was performed in a thermal cycler (Eppendorf) with an initial denaturation for 2min at 94°C, followed by 30 cycles of denaturation (0.5min at 94°C), annealing (1min at 50°C), and extension (1min at 72°C), plus a final extension for 10min at 72°C. The anticipated product of approximately 1,500 and 1,300bp was isolated after 1% agarose gel electrophoresis of the amplified mixture using a gel extraction kit. Amplicons was sequenced by dye termination method where done in OcimumBiosolutions Laboratory, Netherlands. The sequence was compared with similar 16S rDNA sequences retrieved from the DNA databases by using the BLAST search and the Evolutional relationship was studied in MEGA 4 software.

**Bioremediation of lake water samples with heavy metal ions**

The lake water samples with a mixture of all metal ions were tested for removal with the consortium of *D. aromatic* and *P. aeruginosa* strains. To 1L of lake water sample, nutrient broth compositions for 1L were added to the lake water samples in order to meet the nutrient requirements for the organisms to work. A known amount of biomass (25mL of *D. aromatic* and 25mL of *P. aeruginosa* seed cultures at a biomass concentration of 0.5g $L^{-1}$) was used to inoculate and incubated at desired conditions of temperature and shaking as mentioned in the previous section. Similar experimental set up was done for the isolates (consortia comprising 2.5mL of *Priestia megaterium*and 2.5mL of *Bacillus cereus* seed cultures) to compare the metal accumulation efficacy between the standard strains and isolates. The loss in heavy metal ions (zinc, iron, and lead) from each lake water sample was noted at regular time intervals.

**Analysis of metal ion concentrations**

Samples from each test flask were drawn at 60$^{th}$ hour after inoculation to record the residual metal concentration and also after 120$^{th}$ hour to record the final residual concentration using atomic absorption spectrophotometer. The samples were subjected to centrifugation at 8000 g at 4°C for 10 minutes, and the supernatant obtained was used for measuring the metal ion concentrations. The iron, lead, and zinc contents in the supernatant was determined using Atomic Absorption Spectrophotometer as per APHA standards. The heavy metal loss was calculated by subtracting the final heavy metal level (residual level after 60 and 120 hours) from the initial heavy metal level (residual level at 0 hour).

**RESULTS AND DISCUSSION**

**Isolation of metal accumulating bacteria from the lake water samples**

Soil samples collected from Chettipunyam Lake and Thirukachur Lakewas serially diluted and plated. After incubation, the shape and colour of the colonies were noted. Circular, white and cream coloured; and smooth surfaced colonies were selected. One single colony from each type was picked and sub-cultured onto Nutrient agar plates. In Fig. 1 the selected pure culture isolates were presented.

**Fig. 1: Lake Isolates**

Left side: Lake Isolate – A ($LI_A$) from Chettipunyam Lake
Right side: Lake Isolate – B ($LI_B$) from Thirukachur Lake

**Identification of isolates using 16srRNA sequencing**

Lake isolate – A ($LI_A$) and Lake isolate – B ($LI_B$) isolated from the respectiveChettipunyam Lake and Thirukachur Lakes were identified using sequence analysis of the 16S rRNA gene. The method has been reported as fast and precise technique to recognize the phylogenetic position of bacteria. Full-length 16S rDNA of strain were sequenced and used to create phylogenetic development tree. The 16S rDNA of bacterial isolates, $LI_A$ and $LI_B$ was amplified with primers P1F and P6R; and P8F and 1492R

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respectively. The PCR amplification products were detected by 0.8% agarose gel electrophoresis in ultraviolet (UV) light. The length of fragment is about 1500bp of purified PCR product. Comparative analysis of the sequences with already available database showed that the strain, $LI_A$ was closer to the genus *Priestia megaterium* CL-3. And another strain, $LI_B$ was found closer to the genus*Bacillus cereus*CL4 16S. The constructed phylogenetic tree has been presented in Fig. 2 and Fig. 3. The obtained DNA sequence for each isolate was presented in Fig. 2A and Fig. 3B.

**Fig. 4: Phylogenetic tree – *Priestia megaterium*($LI_C$)**

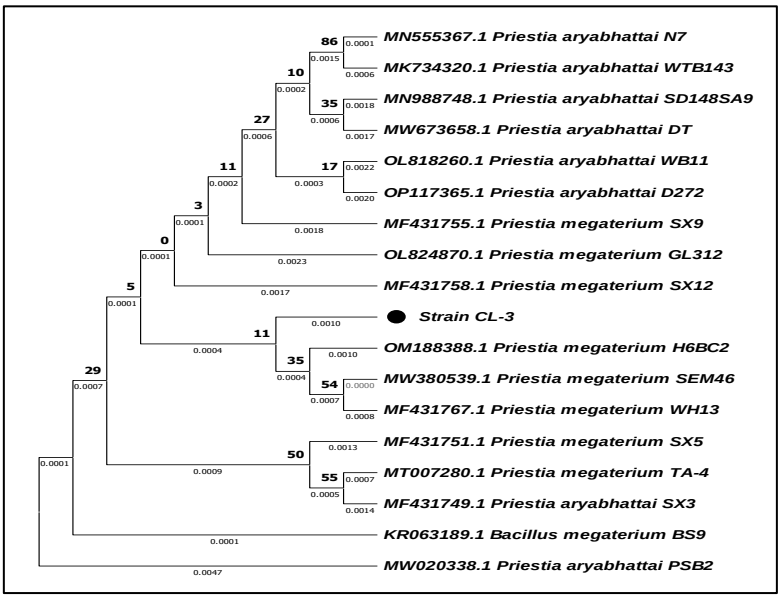


**Fig. 4B: Amplified DNA sequence of *Priestia megaterium*($LI_C$)**

GGTGCGGCGTGCTATACTGCAGTCGAGCGACTGA
TTAGAAGCTTGCTTCTATGACGTTAGCGGCGGACG
GGTGAGTAACACGTGGGCAACCTGCCTGTAAGAC
TGGGATAACTTCGGGAAACCGAAGCTAATACCGG
ATAGGATCTTCTCCTTCATGGGAGATGATTGAAAG
ATGGTTTCGGCTATCACTTACAGATGGGCCCGCGG
TGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAA
GGCAACGATGCATAGCCGACCTGAGAGGGTGATC
GGCCACACTGGGACTGAGACACGGCCCAGACTCC
TACGGGAGGCAGCAGTAGGGAATCTTCCGCAATG
GACGAAAGTCTGACGGAGCAACGCCGCGTGAGTG
ATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAG
GGAAGAACAAGTACGAGAGTAACTGCTTGTACCT
TGACGGTACCTAACCAGAAAGCCACGGCTAACTA
CGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAA
GCGTTATCCGGAATTATTGGGCGTAAAGCGCGCG
CAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACG
GCTCAACCGTGGAGGGTCATTGGAAACTGGGGAA
CTTGAGTGCAGAAGAGAAAAGCGGAATTCCACGT
GTAGCGGTGAAATGCGTAGAGATGTGGAGGAACA
CCAGTGGCGAAGGCGGCTTTTTGGTCTGTAACTGA
CGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGG
ATTAGATACCCTGGTAGTCCACGCCGTAAACGATG
AGTGCTAAGTGTGTTAGAGGGTTTCCGCCCTTTAG
TGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGG
AGTACGGTCGCAAGACTGAAACTCAAAGGAATTG
ACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTT
TAATTCGAAGCAACGCGAAGAACCTTACCAGGTC
TTGACATCCTCTGACAACTCTAGAGATAGAGCGTT
CCCCTTCGGGGGACAGAGTGACAGGTGGTGCATG
GTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTA
AGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTG
CCAGCATTTAGTTGGGCACTCTAAGGTGACTGCCG
GTGACAAACCGGAGGAAGGTGGGGATGACGTCAA
ATCATCATGCCCCTTATGACCTGGGCTACACACGT
GCTACAATGGATGGTACAAAGGGCTGCAAGACCG
CGAGGTCAAGCCAATCCCATAAAACCATTCTCAGT
TCGGATTGTAGGCTGCAACTCGCCTACATGAAGCT
GGAATCGCTAGTAATCGCGGATCAGCATGCCGCG
GTGAATACGTTCCCGGGCCTTGTACACACCGCCCG
TCACACCACGAGAGTTTGTAACACCCGAAGTCGG
TGGAGTAACCGTAAGGAGCTAGCCGCCTAAGTGA
CAGAGGCTT

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**Fig. 5: Phylogenetic tree – *Bacillus cereus* ($LI_D$)**

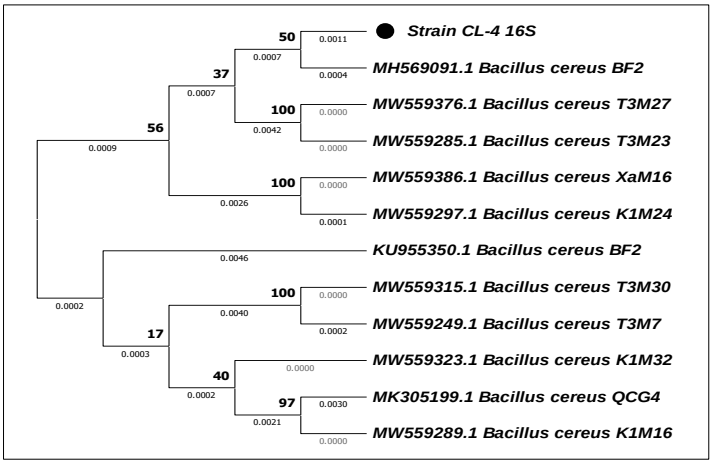


**Fig. 5B: Amplified DNA sequence of *Bacillus cereus* ($LI_D$)**

GGCGGTTGAAATAAAAAATTTGAGGTGGCTGCTATAATGCAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATG
AAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGG
GCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGAC
CCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGAT
CGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGA
AAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGAAGAACAAG
TGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCG
GTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATG
TGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAAT
TCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAAGCGACTTTCTGGTCTGTAACT
GACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGT
GCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCG
CAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGC
GAAGAACCTTACCAGGTCTTGACATCCTCTGCCAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAG
GTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTT
AGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAA
ATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGG
TGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTA
GTAATCGCGATCAGCTGCTTCA

**Physico-chemical characteristics of lake water samples**

The physico-chemical characteristics of two different lake water samples collected from Chettipunyam and Thirukachur lake sites were presented in Table-1. All the physico-chemical parameters were found within the permissible level as per APHA standards in all the four lake water samples collected. However, variation in metal types and its contents were significantly found among the types of lake water samples tested. A higher concentration of lead approximately 1050-1450 folds higher than that of permissible level was found in Thirukachur Lake followed by Chettipunyam Lake. The zinc ion concentrations in Chettipunyam and Thirukachur lakes were found higher with 32.5mg $L^{-1}$ and 30.5mg $L^{-1}$ respectively; which was ~1700-1725 folds higher than the permissible levels. Similar trend in iron concentrations in Thirukachur and Chettipunyam lakes were found higher with 24.5mg $L^{-1}$ and 21.5mg $L^{-1}$ respectively. The variation and the types of metal contents found among the two sources may be due to different in discharge contents in the lake. The industries and other physical activities around these lake sources would also influence the metal and its types and contents,

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etc. Based on the obtained results of heavy metal levels in the water samples collected, known concentration of metal salts of zinc, iron and lead was prepared and the metal degrading efficiency of bacteria was investigated. The degradation studies of individual bacteria and consortia for each metal salts were determined. In the next section the degradation of each metal salts expressed in terms of percentage was presented.

**TABLE-1: PHYSICO-CHEMICAL CHARACTERISTICS OF LAKE WATER COLLECTED FROM DIFFERENT LAKES SURROUNDING TO CHENNAI CITY**

| Parameters | Permissible Levels | Chettipunyam Lake | Thirukachur Lake |
|---|---|---|---|
| Biochemical Oxygen Demand (mg $L^{-1}$) | 30 | 18.5 | 18.5 |
| pH | 5.0 - 7.0 | 6.8 | 6.8 |
| Total dissolved solids (mg $L^{-1}$) | 1500 | 334 | 334 |
| Total Alkalinity As $CaCO_3$ (mg $L^{-1}$) | 200 | 108 | 108 |
| Dissolved Oxygen (mg $L^{-1}$) | 30 | 20.8 | 20.8 |
| Total Suspended Solids (mg $L^{-1}$) | 1000 | 156 | 156 |
| Chloride as Cl (mg $L^{-1}$) | 250 | 86 | 86 |
| Chemical Oxygen Demand (mg $L^{-1}$) | 250 | 105 | 105 |
| Turbidity (NTU) | 5 NTU | 3 NTU | 3 NTU |
| Volatile Solids (mg $L^{-1}$) | 1000 | 168 | 168 |
| Acidity (pH) | pH ≤7.0 | 6.8 | 6.8 |
| Zinc (mg $L^{-1}$) | <0.005 | 30.5 | 32.5 |
| Iron (mg $L^{-1}$) | <0.01 | 21.5 | 24.5 |
| Lead (mg $L^{-1}$) | 0.007 | 12.5 | 13.5 |

**Evaluation of bacterial strains for removal of heavy metal ions lead, zinc, and iron from Lake Water samples**

Evaluation of the bacterial consortium (*D. aromatica* + *P. aeruginosa*) for the removal of heavy metal ions from lake water samples (Chettipunyam Lake and Thirukachur lake), was presented in Table-2. All three types of metal ions (Zn, Fe and Pb) was reduced maximum up to 90.5% after 60 hours; and up to 94.5% after 120hours.

Almost similar percentage loss was found evident for the second set of experiment where each metals were exposed to microbial consortium of the lake soil isolates (*Priestia megaterium* and *Bacillus cereus*). Evaluation of this bacterial consortium for the removal of heavy metal ions from lake water samples (Chettipunyam Lake and Thirukachur lake), was also presented in Table-2. The obtained percentage loss by standard strains when compared to the percentage loss efficiency by the consortium isolates showed almost similar level in percentage loss of maximum up to 89.9% after 60 hours; and up to 92.6% after 120hours. Reduction in metal concentration for each type of metals from $0^{th}$ hour to $120^{th}$ hour by the bacterial consortium was significantly evident from the Table for standard and isolated species respectively.

**TABLE-2: EVALUATION OF BACTERIAL STRAINS FOR REMOVAL OF HEAVY METAL IONS LEAD, ZINC, AND IRON FROM LAKE WATER SAMPLES**

| Lake/Microbial consortia | Metal ions | 0 h (mg $L^{-1}$) | Percentage Loss % | |
|---|---|---|---|---|
| | | | 60 h | 120 h |
| **Chettipunyam Lake (*D. aromatica* + *P. aeruginosa*)** | Zinc | 30.5 | 88.5 | 91.8 |
| | Iron | 21.5 | 87.5 | 91.0 |
| | Lead | 12.5 | 88.0 | 91.5 |
| **Chettipunyam Lake (*Priestia megaterium* + *Bacillus cereus*)** | Zinc | 31.6 | 87.3 | 91.3 |
| | Iron | 23.3 | 86.9 | 90.6 |
| | Lead | 15.6 | 87.3 | 90.3 |
| **Thirukachur Lake** | Zinc | 32.5 | 90.5 | 92.0 |

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| | | | | |
|---|---|---|---|---|
| **(***D. aromatica* + *P. aeruginosa***)** | Iron | 24.5 | 90.5 | 93.0 |
| | Lead | 13.5 | 90.0 | 94.5 |
| **Thirukachur Lake (***Priestia megaterium* + *Bacillus cereus***)** | Zinc | 33.9 | 89.3 | 91.9 |
| | Iron | 25.6 | 88.3 | 92.6 |
| | Lead | 14.3 | 89.9 | 89.6 |

Two bacterial isolates from Chettipunyam Lake and Thirukachur Lake was isolated and identified. So as to decide the connection between isolated bacterial strains and identified *Priestia megaterium* and *Bacillus cereus*, the phylogenetic dependent on halfway 16S rRNA was constructed. The highest sequence similarity of Chettipunyam Lake isolate ($LI_A$) was found evident with species, *Priestia megaterium* CL-3 and the sequence similarity of Thirukachur Lake isolate ($LI_B$) was close to species,*Bacillus cereus*CL4 16S. Phylogeny based on Clustal X clearly indicates that $LI_A$ and $LI_B$ strains are *Priestia megaterium*and*Bacillus cereus*. The 16S rDNA sequences were submitted in the GenBank under the accession number OQ740739for *Priestia megaterium*and OQ743415for *Bacillus cereus*respectively.

As per literature survey, many research works on sample analysis of different lakes in and around Chennai City was carried out. The findings in these research was well supportive in comparison with our findings.

Kiran et al., (2018) reported heavy metal removal from aqueous solution using sodium alginate immobilized sulfate reducing bacteria. During the study, the researchers noticed metal removal of about Cu-96.4%, Cd-92%, Zn-79.8%, Fe-71%, Pb-61.5% and Ni-47.5% using *Desulfovibrio* sp.

Tang et al., (2019) studied the kinetics simulation of Cu and Cd removal and the microbial community adaptation in a periphytic biofilm reactor using microbial consortia containing *Proteobacteria* sp, *Cyanobacteria* sp, *Bacteroidetes* sp, *Firmicutes* sp, *Acidobacteria*sp, *Chlorobium*sp, *Acinobacteria*sp, *Spirochaetes* sp,*Nitrospirae*sp and*Armatimonadetes*sp. The results exhibited that Cu and Cd was removed by the consortia up to 99% and 99.7% respectively.

Kamde et al., (2019) described the integrated bio-oxidation and adsorptive filtration reactor for removal of arsenic from wastewater using *Acidothiobacillusferroxidans.* During the study, the organisms were found capable of reducing the metals upto Cu-100%, Zn-100%, Fe-85%, Ni-90%, As-95%, Co-75% and Cr-100%.

Ibrahim et al., (2019) studied the heavy metal removal using a fixed bed bioreactor packed with a solid supporter inoculated with *Pseudomonas aeruginosa.* During the study, the researchers noticed metal removal of about Cu-100%, Cd-100%, Zn-100%, Fe-62% and Pb-47% using *Pseudomonas aeruginosa*.

Singh et al., (2021) explained the Rermoval of Cd and Ni with enhanced energy generation using biocathode microbial fuel cell using microbial consortia containing *Ochrobactrum*sp, *Halomonas*sp and*Achromobacter* sp. Cadmium was removed up to 87% and Nickel was removed up to 92% by the consortium.

Wei et al., (2019) used microbial consortia containing *Comamonas*sp and *Pseudomonas* sp for the removal of Hg upto 88.9%. The researchers used simultaneous removal of elemental mercury and NO by mercury induced thermophilic community in membrane biofilm reactor.

Giordani et al., (2019) studied the effect of low pH and metal content on microbial community structure in an anaerobic sequencing batch reactor treating acid mine drainage. During the study, the researchers observed different types of metal removal of about Cu-99.3%, Zn-99.4% and Fe-99.9% using microbial consortia containing *Syntrophobacter*sp, *Methanosaeta*sp, *Geobacter*sp, *Anaerolinea*sp, *Longilinea* sp.

Aguilar et al., (2021) removed Ni upto 99% using microbial consortia containing *Kosmotogal*sp, *Ruminococcus*sp and *Clostridium* sp. The researchers studied the removal of nickel (II) from wastewater using a zeolite-packed anaerobic bioreactor.

Fadzli et al., (2021) highlighted the application of electricity generation and heavy metal remediation by utilizing yam (*Dioscoreaalata*) waste in benthic microbial fuel cells. The researchers used *Bacillus* sp, *Klebsiella* sp and *Enterobacter* sp as consortia in removing Cd-88%, Pb-90.14% and Cr-90.34%

Liu et al., (2018) studied the removal of sulfate and heavy metals by sulfate-reducing bacteria. During the study, the researchers noticed metal removal of about Cu-98.5%, Zn-96.3%, Fe-95.2%, Mn-93.8% using *Desulfovibrio* sp.

Shahid et al., (2019) reported the remediation of polluted river water by floating treatment wetlands. During the study, the researchers noticed metal removal of about Fe-72.5%, Pb-40.9%, Ni-70.3%, Cr-77.7% and Mn-83.5% using microbial consortia containing *Bacillus cereus, Aeromonas salmonicida and Pseudomonas gessardii.*

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Tara et al., (2019) used microbial consortia containing *Acinetobacter junii, Rhodococcussp and Pseudomonas indoloxydans* for the removal of Fe-90%, Cd-60%, Cr-90% and Ni-80%. On-site performance of floating treatment wetland macrocosms augmented with dye-degrading bacteria for the remediation of textile industry wastewater.

Yu et al., (2020) explained the removal of heavy metal wastewater in constructed wetlands with resistant microorganisms using *Serratia sp and Pseudomonas sp*. During the study, the organisms were found capable of reducing the metals up-to Cd-99.6% and Zn-94.41%.

Hussain et al., (2018) studied Treatment of the textile industry effluent in a pilot-scale vertical flow constructed wetland system augmented with bacterial endophytes. The researchers noticed metal removal of about Fe-89%, Cd-72%, Cr-97% and Ni-88% using microbial consortia containing *Bacillus endophyticus, Bacillus pumilus, Microbacteriumarborescens and Pantonea sp.*

Nawaz et al., (2020) reported Bacterial augmented floating treatment wetlands for efficient treatment of synthetic textile dye wastewater. During the study, the researchers noticed metal removal of about Cu-77.5%, Zn-89.7%, Fe-81.0%, Pb-73.3%, Ni-86.9% and Mn-70 %using microbial consortia containing *Acinetobacter junii, Rhodococcussp and Pseudomonas indoloxydans*.

Shahid et al., (2021) highlighted the performance of four macrophytes in bacterial assisted floating treatment wetlands for the removal of trace metals from polluted river water. The researchers used microbial consortia containing *Aeromonas salmonicida, Pseudomonas indoloxydans, Bacillus cereus, Pseudomonas gessardii and Rhodococcussp*as consortia in removing Fe-85.7%, Pb-91.6%, Cr-98.1%, Ni-75.3% and Mn-85.3%.

Akram et al., (2020) removed Cr up-to 88% using microbial consortia containing *Pseudomonas aeruginosa, Onchrobactrumsp and Enterobacter*. The researchers studied the remediation of Cr6+ in bacterial assisted floating wetlands.

Kabeer et al., (2021) described the Role of heavy metal tolerant rhizosphere bacteria in the phytoremediation of Cu and Pb using Eichhornia crassipes (Mart) using microbial consortia containing *Bacillus cereus, Paenibacillus alvei, Aeromonas caviae, Paenibacillustaiwanensis and Achromobacterspanius.* During the study, the organisms were found capable of reducing the metals upto Cu-95% and Pb-93.4%.

Enterobacter species have been registered by Fadzli*et al*. (2021) as a potent species for heavy metal remediation recording high removal efficiency of Pb+2, Cd+2, and Cr+3 as 90.14, 88.00, and 90.34%, respectively, within 30-day incubation (Fadzli*et al*., 2021). *E. cloacae* have been observed as an efficient microbial biosorbent giving a high uptake concentration of Pb+2 (2.3 mmoles) from the initial concentration (7.2 mmol) (Kang *et al*., 2015). In addition, *E. cloacae* have been found to have MIC (1000 ug/ml) with Cr+2 having a mechanism of intracellular accumulation of heavy metal and recording 81% of Cr+2 reduction from the liquid medium after 120-h incubation period (Rahman *et al*., 2015).

Si et al., (2019) studied the Mechanism and performance of trace metal removal by continuous-flow constructed wetlands coupled with a micro-electric field using microbial consortia containing *Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria, Firmicutes, Nitrospirae, Spirochaetes and Cyanobacteria*. The results exhibited that removed by the consortia up to Cu-97.6%, Zn-80.1%, Cd-74%, Ni-69.8% and Co-67.1% respectively.

Mu et al., (2021) reported Removal of Cr (VI) and electricity production by constructed wetland combined with microbial fuel cell (CW-MFC): influence of filler media. During the study, the researchers noticed metal removal of about Cu-99% using microbial consortia containing *Acetoanaerobium and Exiguobacterium.*

Chen *et al*., (2021) studied that, the bacterial strains that were isolated in polluted soil area could not reduce the field metal percentage. By the availability of suitable conditions for bacterial growth, isolated strains were adapted for metal high percentages in the presence of growth factors and nutrition. It is noteworthy that the nature of the clay soil in the drain area does not allow aerobic bacterial growth but allows anaerobic bacteria enumeration.

Wellsbury*et al*. (2002) recognized that small pores restrict bacteria movement and activity, limit nutrient transport, diminish space availability, slow the rate of division, and lead to reduced biodiversity. So, the most species of bacteria isolated in this study were Enterobacter sp.

Kelany et al., (2023) recently studied the bioremediation of industrial wastewater heavy metals using consortium of different *Enterobacter*spp (*Enterobacter kobei* OM144907 SCUF0000311, *Enterobacter cloacae* OM180597 SCUF0000312, and *Enterobacter hormaechei* OM181067 SCUF0000313). During the research, the researchers found that, out of thirty bacterial isolates, only 3 isolates sighted the highest metal resistance activity for $Zn^{+2}$, $Fe^{+2}$, $Pb^{+2}$, $Co^{+2}$, $Mn^{+2}$, $Ni^{+2}$, and $Cd^{+2}$. The minimum tolerance activity (MIC) of heavy metal concentrations against *E. kobei* and *E. cloacae* was 25, 15, and 15 mmol/l for $Ni^{+2}$, $Fe^{+2}$, and $Mn^{+2}$, respectively, and 10 mmol/l for $Zn^{+2}$, $Pb^{+2}$,

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$Co^{+2}$, and $Cd^{+2}$, while against *E. hormaechei*, it is 15 mmol/l for Ni+2, Fe+2, and Mn+2 and 10 mmol/l for Zn+2, Pb+2, Co+2, and Cd+2. The consortium and solitary application of bacterial isolates towards heavy metal removal at 100%, 200%, and 300% industrial wastewater concentrations were conducted and showed that more than 90% removal of $Zn^{+2}$, $Fe^{+2}$, $Pb^{+2}$, $Mn^{+2}$, $Ni^{+2}$, and $Cd^{+2}$ from a non-concentrated polluted sample (100%) was reported by the three strains. With doubling the polluted sample concentration (200%), the highest removal efficiency for $Zn^{+2}$, $Pb^{+2}$, $Mn^{+2}$, $Ni^{+2}$and $Cd^{+2}$ was reported by *E. cloacae* as 70. 75, 66, 65, and 57%, respectively. Removal efficiency after increasing the polluted sample concentration to 300% showed that *E. cloacae* removed above 45% of all tested heavy metals except $Pb^{+2}$. Ultimately, *E. cloacae* exposed the highest efficiency with recommendations for heavy metals removal under higher concentrations.

Abdollahi*et al*. (2020) have reported that *E. cloacae* had MIC 3000 ug/ml and 50 ug/ml against Pb+2 and Cd+2 with accumulation capacity 45ug Pb+2/ml and 30ug Cd+2/ ml. Also, Ghosh *et al*. (2022) have reported that *E. cloacae* expressed a high potency of tolerance towards high concentrations of Cd+2 (4000 µg/ml), Pb+2 (3312 µg/ml), and $As^{+3}$ (1500 µg/ml), where the removal efficiency of Cd+2 was recorded 72.11%. With a few reports on the capability of *E. hormaechei* (SCUF0000313) and *E. kobei* (SCUF0000311) to bioremediate heavy metals, Heidari*et al*. (2020) have found that *E. hormaechei* exposed a high efficiency of uptake towards Ni+2 than Pb+2 and Cd+2.

Heavy metal removal in nutrient medium and in lake water samples (Chettipunyam Lake and Thirukachur Lake) by the microbial consortium was found significantly at higher levels. As per literature survey, it has been reported that, different types of mechanisms are involved in removing the metal content or its toxicity level by the microbes. The process are named as bioaccumulation, biosorption, biostimulation, bioremediation, biodegradation, immobilization, etc. Most common mechanisms involved in metal removal are reported here as per literature survey.

Bioaccumulation is the process where, the uptake mechanisms of metal into the bacterial cell have been studied to be through processes as passive diffusion, facilitated diffusion and active transport (Spain and Alm, 2013). Malik, (2004) earlier, supported that, microbial cells through interactions can adsorb or absorb the heavy metals onto the binding sites on the cellular surface. Mosa et al., (2016) reported that, during an active uptake, the heavy metal ions pass across the plasma membrane into the cytoplasm during which the cell is influenced by physical, chemical, and biological processes including intracellular and extracellular processes. The surface structures of bacteria play a vital role in how the bacteria interacts with the surrounding environment. Depending on the type of bacterial cell wall types (Gram-Negative and Gram-Positive bacteria vary in teichoic acid percentage), the bioaccumulation of heavy metals into their cell membrane differs. Guine et al., (2003) stated that the cell walls of the gram negative bacteria contain lipopolysaccharide, phospholipids and peptidoglycan layers, those of the gram-positive bacteria have minor amounts of teichoic acid usually present along with the peptidoglycan in several layers, the latter forming as much as 90% of the cell wall. The organism capable of bioaccumulation must be able to transform the metal, changing it from a toxic to a harmless form even when tolerating the high concentrations of one metal or a combination of metals are gets exposed (Fomina et al., 2014). We expect this similar metal removal process in our study by the microbial consortium (*Priestia megaterium*)and(*Bacillus cereus*). However, the exact process need to be studied in future using different parameters and optimizations.

## CONCLUSION

Two significant metal chelating bacterial cultures were isolated from Chettipunyam Lake and Thirukachur Lake, in Chennai City. According to the method of 16srRNA sequencing and phylogenetic classification, the isolates were identified as *Priestia megaterium* and *Bacillus cereus*. Physico-chemical parameters tested as per APHA standards showed that, all three metals, Zn, Fe and Pb was found approximately 1050-1450 folds higher than that of permissible level in both lake water samples. The obtained percentage loss by standard strains when compared to the percentage loss efficiency by the consortium isolates showed almost similar level in percentage loss of maximum up to 89.9% after 60 hours; and up to 92.6% after 120hours. The findings revealed the potential ability of bacterial isolates for better metal accumulation efficiency from any type of water or waste water samples. As a future study, the treated or metal accumulated water shall be used for basic agricultural activity in small fields where water is not needed in more quantity.

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