2024 SICASH
SLIIT INTERNATIONAL CONFERENCE ON ADVANCEMENTS IN SCIENCES & HUMANITIES

Isolation of Cd Removal Bacteria from Beira Lake, Colombo, Sri Lanka and Evaluation of their Capacity in Cadmium Removal

T. Thilakarathne *[1], S.P. C. Lankika [2], S. Mirihana [3]

[1,2,3] *Sri Lanka Institute of Information Technology, Malabe, Sri Lanka*

Email address of the corresponding author -*thanushithilakaratne98@gmail.com

Abstract

Cadmium (Cd), a heavy metal, is reported to be exceeding its consumable levels in the environment due to several anthropogenic activities. Cd contamination thus challenges the ecosystems and human health due to its persistence and toxicity. It is confirmed that bioaccumulation of Cd leads to cancers and osteoporosis. Hence, concerning their eco-friendliness and cost-effectiveness, urgent remediation methods are needed for clearing the issue of Cd-contaminated water and soil. The present study deals with three Cd-resistant bacterial strains isolated from Beira Lake, Sri Lanka; and named as TTCB, TTGB, and TTPB. The isolated strains are found to have performed remarkably effectively with the Cd removal efficiencies of 97.4%, 97.5%, and 97.5%, respectively, as determined by an atomic absorption spectrophotometric assay. Their highest effectiveness was found at temperatures ranging from 30°C to 40°C and in a pH range of 6 to 7. Moreover, the isolated strains indicated higher Minimal Inhibitory Concentrations (1200 – 2000 ppm), confirming a strong tolerance to Cd. It is highlighted that the MIC levels of the isolated strains are remarkable in comparison with the model organisms as Bacillus cereus ATCC 10876 and Pseudomonas aeruginosa ATCC (300 – 700 ppm). The resulting potentiality of the isolated strains has suggested a promising bioremediation application for Cd- contaminated environments, offering a valuable solution to address this pressing environmental issue.

Keywords: Cadmium; Beira Lake; Anthropogenic activities; Bioaccumulation

Introduction

Heavy metal pollution is a pressing environmental issue due to its adverse effects on ecosystems and human health. Cd is one of the most toxic heavy metals, primarily released into the environment through industrial processes such as mining, smelting, and battery manufacturing. Its persistence and bioaccumulation in food chains pose significant health risks, including kidney damage, bone demineralization, and carcinogenic effects. Therefore, finding effective methods for Cd removal from contaminated environments is of paramount importance. [3,4] Addressing heavy metal pollution in Sri Lanka through conventional physical and chemical methods poses significant challenges. These methods often require advanced machinery, specialized expertise, and substantial financial investment, all of which are scarce to the South Asian region. The high cost and complexity associated with physical and chemical remediation techniques hinder their widespread adoption, making them impractical for large-scale environmental cleanup

https://doi.org/10.54389/HQAS8740

efforts. Bioremediation is a method that uses micro-organisms to degrade or detoxify pollutants and offers a viable and cost-effective alternative. Certain bacteria have evolved as mechanisms to resist and detoxify heavy metals, making them potential candidates for bioremediation. This approach leverages natural processes and requires fewer resources, making it particularly suitable for regions with limited infrastructure and financial constraints, such as Sri Lanka.

This study focuses on the isolation and characterization of Cd-resistant bacteria from Beira Lake, a polluted water body in Sri Lanka. Beira Lake has historically been subjected to various forms of pollution, making it an ideal site for isolating potential bio-remediators. By tapping into the native microbial diversity, we aim to develop sustainable and locally applicable solutions for Cd pollution.

This research aims to isolate, identify, and evaluate the Cd removal capabilities of bacterial strains from Beira Lake. The isolated strains TTCB, TTGB, and TTPB, from the current study exhibited remarkable Cd removal efficiencies, achieving over 95% removal. This paper presents a detailed analysis of the growth kinetics of these strains under varying Cd concentrations and their efficiency in Cd removal. By understanding the mechanisms these bacteria employ to tolerate and remove Cd, we hope to contribute to developing effective bioremediation strategies for heavy metal pollution. The insights gained from this study could also have broader applications in the bioremediation of other heavy metals and enhance the natural bioremediation applications of industrial wastewater treatment processes, providing Sri Lanka with practical and sustainable solutions to its environmental challenges.

Material and Method

Sample collection: The water samples were collected from three spots of Beira Lake, Colombo. The samples were collected in sterilized, labeled glass bottles with well-closed lids. The temperature of the samples in collecting was present in 27-30 ℃, and pH was present in 6.8-7.25. (Spot 01 – 6.88, Spot 02-7.25, Spot 03- 6.79).

Isolation and characterization

Three water samples were filtered into sterile glass beakers, and six nutrient agar plates were prepared. Each plate contained 500 ppm of Cadmium (Cd2+). Samples (500 µL) were spread on the agar plates and incubated for 72 hours at 37℃. The serial dilution plating method was used for isolation. Serial dilutions (10-1 to 10-9) were prepared for each spot sample. Twenty- seven nutrient agar plates with 25 ppm cadmium were prepared. After 48 hours of incubation at 37℃, selected single colonies were re-streaked on new plates and incubated for 24 hours and the process was repeated two times. Finally, three plates with isolated colonies were named as TTPB, TTGB, and TTCB.

Determining Minimal Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) of cadmium was measured using broth and agar plate methods. A cadmium concentration series ranging from 0 to 4000 ppm was prepared. The plates were streaked with isolated bacterial strains and incubated for 24 hours at 37℃. Simultaneously, LB broth (10 mL) was placed in sterile plastic tubes, and Cadmium was added to create the concentration series. Isolated strains and two model cultures (Bacillus cereus ATCC 10876 and Pseudomonas aeruginosa ATCC 27853) were inoculated into the tubes, those were incubated for 72 hours at 37℃ in a rotary shaker at 150 rpm. Absorbances were measured every 24 hours at 600 nm using a portable spectrophotometer. The MIC for each strain was identified as the concentration at which absorbance reached zero in comparison to the control.

Determining growth ability in different temperature conditions: Isolated strains and two model strains were inoculated into sterile LB broth tubes. Tubes were sealed and incubated for 24 hours at varying temperatures (20, 30, 40, 50, and 60℃). Absorbances

were measured after 24 hours using a Vernier GoDirect SpectroVis Plus spectrophotometer at 600 nm.

Determining growth ability in different pH conditions: The pH of LB broth was adjusted using 1M HCl and 1M NaOH. The flasks were sterilized at 121°C for 15 minutes. Isolated strains were inoculated into flasks and those were incubated for 24 hours at 37°C. Absorbances were measured after 24 hours using a portable spectrophotometer at 600 nm.

Cd Removal Assay

Three isolated and two model strains were inoculated into the broth. Five flasks contained Cd concentrations of 500 ppm for TTPB, TTCB, and TTGB strains, and 300 ppm for the model strains. Two control flasks with 300 ppm and 500 ppm Cd had no inoculum. The flasks were incubated for seven days at 37°C in a rotary shaker at 150 rpm. After incubation, The Cd content in the cell-free supernatants was measured using atomic absorption spectrometry at 228.8 nm.

Results were compared with controls to calculate the heavy metal removal capacity (%) using the formula:

Percentage of heavy metal utilized = (Heavy metal utilized /Heavy metal added to the LB broth) × 100

Heavy metal Utilized(ppm) = Heavy metal added to the LB broth (ppm) - Heavy metal at the end of the incubation period (ppm) (Marzan et al., 2017)

Results and Discussion

1. Primary screening

Filtered samples from three spots were cultured on nutrient agar plates with and without Cd. For primary screening, nutrient agar media with and without 500 ppm Cd were prepared. After 72 hours of incubation using the spread plate method, the results showed higher bacterial growth on non-Cd plates and fewer colonies on Cd plates. This indicates that all three spots contain bacterial strains capable of tolerating Cd, providing observable proof of Cd-tolerant bacteria in Beira Lake, Colombo, Sri Lanka.

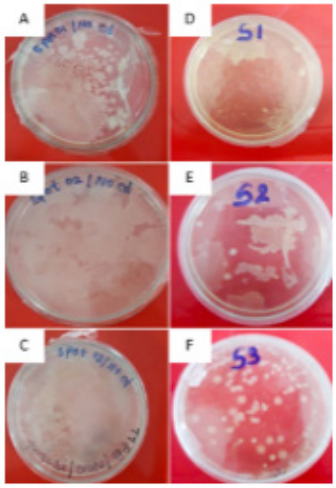

Figure 1.
Comparison of Bacteria cultures from 3 spots A,B and C have no Cd in the culture media and E,F and G contain 500 ppm Cd in the culture media

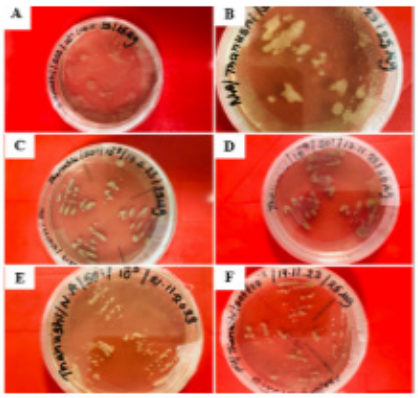

Figure 2. Serial dilution plate images as an example from spot 01

Isolation by serial dilution

Serial dilution was performed on nutrient agar plates containing 25 ppm Cd to reduce bacterial concentrations to manageable levels. This step is crucial for isolating single colonies, particularly from Beira Lake, a highly polluted aquatic source in Sri Lanka with diverse microorganisms due to various anthropogenic pollutants. Isolating individual colonies for further study would be challenging without dilution. The selected isolated colonies, named TTPB (purple), TTGB (green), and TTCB (creamy), were isolated through the streak plate method. The process involved re-streaking single

colonies on nutrient agar plates and incubating them for 48 hours each time, repeated twice to ensure purity. The isolated strains are considered Cd removal bacteria as they demonstrated the ability to grow on plates containing 25 ppm Cd. This concentration is significantly higher than the natural Cd levels in the Earth's crust which range from 3-8 ppm (American Institute of Physics).

Figure 3. Isolated Strains which are labeled as TTPB, TTGB & TTCB respectively

3. Determine the MIC of isolated strains and Model cultures

Table 01. Values of MICs in isolated strains and model strains

Name of the bacterial Strain	MIC of the certain strain against the Cd
Bacillus cereus ATCC 10876	400-500
Pseudomonas aeruginosa ATCC 27853	600-700
TTCB	1700-2000
TTGB	1200-1500
TTPB	2000-2500

The minimal inhibitory concentration (MIC) is crucial in microbiology for determining the effectiveness of antimicrobial agents. For heavy metals like Cd, MIC assesses the concentration at which bacterial growth is inhibited. [5,6,7] Determining the MIC for Cadmium in bacterial strains such as Pseudomonas aeruginosa and Bacillus cereus, known for their resistance, is vital for understanding their susceptibility to this heavy metal. The typical approach involves preparing Cadmium dilutions, inoculating them with bacterial cultures, and identifying the lowest concentration that inhibits visible growth after incubation. This information helps evaluate microbial survival in Cadmium-contaminated environments and develop strategies to mitigate its impact on health and the environment. According to these observations, all isolated strains can tolerate more Cd levels in their growth media than those used in the two model cultures. Therefore, isolated strains are more suitable to develop a Cd bioremediation than the model cultures.

4. Determine the optimum pH and temperature range

All five bacterial cultures exhibit optimal growth between 20°C and 40°C, which is typical for most bacterial strains, although some can thrive outside this range. Higher temperatures can increase heavy metal toxicity to bacteria by enhancing metal uptake, leading to higher intracellular concentrations and more severe toxic effects. Temperature directly impacts bacterial metabolic rates, influencing metal detoxification and resistance processes. Proteins involved in detoxification, like metallothionein, and metal transporters can be denatured at high temperatures, reducing detoxification capacity and slowing growth. Additionally, high temperatures can increase bacterial cell membrane fluidity, affecting metal uptake and membrane integrity. The isolated strains and model cultures grow well within the 20°C to 40°C range, matching the temperature range of polluted aquatic environments in Sri Lanka, which simplifies the application of heavy metal bioremediation techniques without the need for specific temperature control. [10]The pH of the environment significantly affects bacterial strains exposed to heavy metal-containing media.

The solubility and toxicity of heavy metals depend on pH, as metals can form different chemical compounds at various pH levels. For example, metals may form less bioavailable precipitates at high pH and be more soluble and toxic at low pH. Extreme pH levels can disrupt bacterial cell membranes,

increasing permeability and heavy metal uptake. Bacteria produce extracellular polymeric substances (EPS) that bind heavy metals, and the binding capacity of EPS is influenced by pH. The activity of metal detoxification enzymes is also pH-dependent, with extreme pH levels potentially denaturing these enzymes and reducing their effectiveness. pH directly impacts bacterial growth rates, with most bacteria having an optimal pH range for growth.[1,2,9] In this research, water samples from three spots in Beira Lake, with initial pH values of 6.88, 7.25, and 6.97, were used. All isolated strains showed optimal growth in the pH 6-7 range, eliminating the need for specific pH adjustments in media.

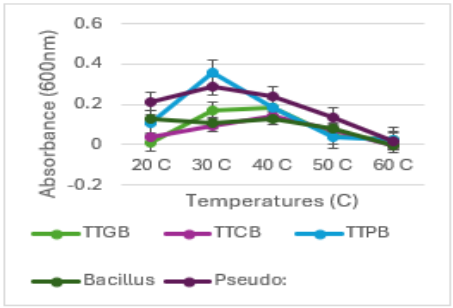

Figure 4. Effect of temperature on bacterial growth

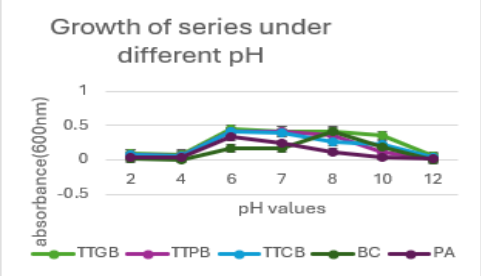

Figure 5. Effect of pH on bacterial growth

5. Cd removal ability

According to Atomic AbsorptionvSpectroscopy (AAS) analysis, isolated bacterial strains exhibit superior Cd removal abilities compared to model cultures. Microbes can remove Cd through various mechanisms, including intracellular accumulation, adsorption onto cell surfaces or extracellular polymeric substances (EPS), conversion of soluble Cd into insoluble forms like Cd sulfide or Cd phosphate, enzymatic transformation, and conversion into volatile forms. These processes reduce Cd concentration, bioavailability, and toxicity in the environment. Gene expression analysis, proteomics, and metabolomics are recommended for a detailed understanding of these Cd removal mechanisms. The AAS analysis results show that isolated strains TTCB, TTPB, and TTGB have Cd removal abilities of 97.4%, 97.5%, and 97.5%, respectively, after 7 days of incubation. In comparison, Pseudomonas aeruginosa ATCC 27853 and Bacillus cereus ATCC 10876 show Cd removal abilities of 95.8% and 95.6%, respectively. Thus, the isolated strains are more effective at removing Cd than the model cultures.

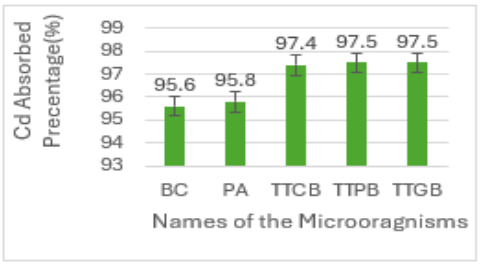

Figure 6. Results of AAS analysis

Conclusion

This research successfully isolated Cd removal bacterial strains from the polluted waters of Beira Lake, Sri Lanka. The primary conclusion of this study is that specific bacterial strains, notably Pseudomonas aeruginosa ATCC 27853 and Bacillus cereus ATCC 10876 and the isolated strains TTCB, TTGB, and TTPB, exhibited remarkable Cd removal capabilities, achieving removal percentages of 95.6%, 95.8%, 97.4%, 97.5%, and 97.5% respectively. These findings underscore the potential of these bacteria in bioremediation applications aimed at mitigating heavy metal contamination in aquatic environments.

The growth experiments conducted under varying Cd concentrations revealed that all isolated strains

demonstrated significant resilience and growth even at higher Cd concentrations, highlighting their potential for effective bioremediation in environments with varying levels of Cd pollution. Among the isolated strains, TTCB, TTGB, and TTPB showed the highest tolerance and Cd-degrading efficiency, making them promising candidates for future bioremediation strategies. Furthermore, the study established that the identified bacteria not only remove Cd but also produce secondary metabolites, including purple pigments, which could have additional industrial applications. This dual functionality enhances the value of these bacteria in environmental and industrial biotechnology.

Finally, this research contributes valuable data to the field of environmental microbiology, particularly in the context of heavy metal bioremediation. The methodologies and findings presented can serve as a foundation for further research and practical applications in cleaning up Cd-contaminated sites. Future studies should focus on the genetic mechanisms underlying Cd degradation in these strains and explore large-scale bioremediation trials in various polluted environments.

Acknowledgment

The authors would like to thank the Sri Lanka Institute of Information Technology and the Institute of Chemistry, Sri Lanka for the financial support and resources of this study. The guidance of Ms. Chathurangi Patirana, Mr. Shanitha Mirihana & Mr. Sehan Jayasinghe are also hereby acknowledged.

References

Bang, S.-W., Clark, D. S. and Keasling, J. D. (2000a) 'Engineering hydrogen sulfide production and cadmium removal by expression of the thiosulfate reductase gene.

Bang, S.-W., Clark, D. S., Keasling, J. D. (2000b) 'Cadmium, lead, and zinc removal by expression of the thiosulfate reductase gene from Salmonella typhimurium in Escherichia coli'. Biotechnol Lett, 22:1331-1335.

Faroon O, Ashizawa A, Wright S, et al. Toxicological Profile for Cadmium. Atlanta (GA): Agency for Toxic Substances and Disease Registry (US); 2012 Sep. 1, PUBLIC 113 HEALTH STATEMENT. Available from: https://www.ncbi.nlm.nih.gov/books/NBK158840

Faroon O, Ashizawa A, Wright S, et al. Toxicological Profile for Cadmium. Atlanta (GA): Agency for Toxic Substances and Disease Registry (US); 2012 Sep. 2, RELEVANCE TO PUBLIC HEALTH. Available from: https://www.ncbi.nlm.nih.gov/books/NBK158837/

Jebril, N., Boden, R. & Braungardt, C. (2021a) The effect of pH, calcium, phosphate and humic acid on cadmium availability and speciation in artificial groundwater. J. Phys.: Conf. Ser. 1879 02202.

Jebril, N., Boden, R. & Braungardt, C. (2021b) 'The isolation and identification of cadmium-resistant Brevibacillus agri C15', J. Phys.: Conf. Ser. 022015:1-15 115.

Jebril, N., Boden, R., & Braungardt, C. (2022). Cadmium resistant bacteria mediated cadmium removal: a systematic review on resistance, mechanism and bioremediation approaches. IOP Conference Series: Earth and Environmental Science, 1002(1). https://doi.org/10.1088/1755-1315/1002/1/012006

Marzan, L. W., Hossain, M., Mina, S. A., Akter, Y., & Chowdhury, A. M. A. (2017). Isolation and biochemical characterization of heavy-metal resistant bacteria from tannery effluent in Chittagong city, Bangladesh: Bioremediation viewpoint. The Egyptian Journal of Aquatic Research, 43(1), 65-74.

Volesky, B., & Naja, G. (2007). Biosorption technology: starting up an enterprise. International Journal of Technology Transfer and

Commercialisation, 6(2/3/4), 196. https://doi.org/10.1504/IJTTC.2007.01780

Xiao, X., Han, X., Wang, L.-G., Long, F., Ma, X.-L., Xu, C.-C., Ma, X.-B., Wang, C.-X. & Liu, Z.-Y. (2020) 'Anaerobically photoreductive degradation by CdS nanocrystal: Biofabrication process and bioelectron-driven reaction coupled with Shewanella oneidensis MR-1'. Biochem Eng J, 154:107466.