BIOSORPTION OF MERCURY, LEAD AND CADMIUM FROM LANDFILL LEACHATE USING *BACILLUS SUBTILIS*

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ABSTRACT

This study investigates the efficacy of *Bacillus subtilis* for biosorption of mercury, lead, and cadmium in landfill leachate. *Bacillus subtilis* was introduced into landfill leachate in varying amounts and placed in controlled and natural environments for three weeks. Results showed that *Bacillus subtilis* was able to degrade Cd by 9.00 to 18.10%, Pb by 2.80 to 25.00%, and Hg by 88.20 to 90.30% when placed in an incubator. When kept at room temperature, biosorption efficiency for Cd ranged from 36.30 to 54.10%, Pb ranged from 2.80 to 25.00%, and Hg ranged from 90.30 to 91.0%. *Bacillus subtilis* demonstrated higher efficacy in degrading mercury compared to lead and cadmium at room temperature. Overall, the study suggests that *Bacillus subtilis* has potential for bioremediation of metal contamination in the environment. *[African Journal of Chemical Education—AJCE 15(1), January 2025]*

INTRODUCTION

Cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, and zinc exhibit inherent mutagenic and carcinogenic properties, further intensifying the global health problem they pose. The careless release of heavy metals into soil and rivers exacerbates this issue, as these metals linger and continue to exert adverse effects on both ecosystems and human health [1]. One of the most pressing environmental challenges confronting the global community revolves around effective waste management. This multifaceted issue encompasses the improper treatment and disposal of wastewater, which, when left untreated, unleashes a cascade of adverse effects on human health, living organisms, and the broader environment. Within wastewater, a host of toxic chemicals lurk, posing a significant threat to both terrestrial and aquatic ecosystems [2]. The accumulation of toxic metals in various environmental compartments, particularly in urban areas, has become a pressing concern due to the substantial increase in industrial waste discharge into soil and water. This influx of toxic metals poses a significant threat to ecosystems and public health. Toxic metals persist in the environment through processes such as leaching and plant absorption, leading to long-lasting contamination [3]. Furthermore, the use of untreated wastewater for agricultural purposes, such as irrigation, introduces an insidious threat to soil health [4]. Lead, cadmium, and arsenic present significant health risks to both human and wildlife populations, highlighting the urgent environmental concern around the discharge of such leachate. The current techniques for removing heavy metals from landfill leachate are not only costly but also energy-intensive, making them unsuitable for use in rural locations with limited infrastructure and resources [5]. Therefore, there is an urgent need for the creation of long-term, practical, and cost-effective methods for the treatment of heavy metal-containing landfill leachate. In addition to reducing the immediate environmental risks brought on by heavy metal contamination, these solutions should also be practical and available for usage in a variety of geographical situations, including remote rural locations with limited resources [6].

Several remediation methods are employed, including chemical precipitation, oxidation or reduction, filtration, ion-exchange, reverse osmosis, membrane technology, evaporation, and electrochemical treatment [7]. However, it's important to note that many of these methods become less effective when dealing with toxic metal concentrations below 100 mg/L. As the environmental and health risks associated with toxic metals persist, ongoing research and innovative approaches to remediation are essential to mitigate the impact and protect both ecosystems and human well-being [8]. The responsible management and reduction of toxic metal emissions from industrial processes are key steps in addressing this global challenge and ensuring a healthier, cleaner environment for future generations [9].

Microbial remediation has earned its reputation as an effective, environmentally friendly, and cost-efficient approach to combating contamination. With its widespread acceptance, adaptability to various settings, safety profile, and ecological advantages, it has become a valuable tool in our efforts to safeguard and restore the health of our environment [10]. This technology promotes the restoration of ecosystems affected by contamination. It not only removes or reduces pollutants but can also enhance the overall health of the environment, ensuring that it remains sustainable for future generations.

Bacillus subtilis is a versatile bacterium with a wide range of applications in science, industry, agriculture, and biotechnology. Its adaptability, ability to form spores, and diverse metabolic capabilities make it a valuable microorganism for research and practical use in various fields. *Bacillus subtilis* possesses innate mechanisms for both tolerating and effectively sequestering heavy metals, demonstrating its potential as an eco-friendly solution for mitigating heavy metal contamination in water systems [11]. Through its innate processes, this bacterium can immobilize hazardous heavy metals, including lead, cadmium, and chromium, by adeptly adsorbing them onto its cell surfaces or even precipitating them intracellularly. This dual-action approach serves to substantially diminish the concentration of heavy metals within contaminated water bodies [12]. The unique ability of *Bacillus subtilis* to serve as a natural adsorbent and a reservoir for heavy metals showcases its promise as a sustainable and environmentally sound strategy for addressing heavy metal pollution concerns in aquatic environments [7]. *Bacillus subtilis* has a natural ability to tolerate and sequester heavy metals. It can immobilize toxic heavy metals such as lead, cadmium, and chromium by adsorbing them onto its cell surface or precipitating them intracellularly. This reduces the concentration of heavy metals in contaminated water bodies [7]. The objective of the study was to investigate the potential use of *Bacillus subtilis* for degrading toxic metals in landfill leachate.

Materials and Methods

Sample Collection

The landfill leachate was collected into a gallon. After collecting the water sample, it was labelled with a sample code number. The collection and processing of the water sample was important in ensuring both the accuracy and reliability of the laboratory results.

Landfill Leachate

The landfill leachate was measured using a measuring cylinder and filtered into a tiny container at a volume of 40 mL. Following labelling, it was sent to the University of Ghana's Ecological Laboratory for analysis. Atomic Absorption Spectrophotometer (GFAAS) was used to measure the concentration of the heavy metals in the land leachate sample, and the corresponding concentrations of 0.231 for Cd, 0.036 for Pb and 0.011 for Hg were recorded.

Determination of pH of the Landfill Leachate

The pH was determined with a pH meter at the Spanish Laboratory at the Nyankpala Campus of the University for Development Studies, Ghana. This measurement was performed by measuring 50 mL of the leachate in conical flask. Before using the pH meter calibrated. The calibration involved the use of known pH buffer solutions to set the meter's reference points. Once prepared, the pH electrode was immersed into the leachate, stirred gently to ensure proper contact, and the reading was awaited until it stabilised. The pH was 7.6.

Inoculation of *Bacillus subtilis* in Contaminated Nutrient Agar of Landfill Leachate

The surface of the agar plates that had previously been contaminated with the landfill leachate was exposed to the *Bacillus subtilis*. This procedure aided in the investigation of the reactions and interactions of the *Bacillus subtilis* with the pollutants in the leachate. Where 3 of the plates were placed in an incubator for 48 hrs at a temperature of 37 ^{o}C and labelled as CE R1, CE R2 and CE R3. The other 3 plates were keep at room temperature at the lab and labelled as ET R1, ET R2 and ET R3.

Calculations for Biosorption Efficiency

For each of the pollutant uptake and the sorbent concentration all at equilibrium was computed using the symbol Q_e [13]. Therefore, the calculation for biosorption efficiency was represented mathematically as;

$$Q_e\ (\%) = \frac{C_i - C_f}{C_i} \times 100$$

Where C_i and C_f are the initial and final concentrations (mg/L) of cadmium, lead and mercury.

RESULTS AND DISCUSSION

Remediation of Cadmium, Lead and Mercury in Landfill Leachate Observe in an Incubator (35 – 37 °C)

The degradation efficacy of *Bacillus subtilis* observed in an incubator for three weeks is presented in table 1. The study observed *Bacillus subtilis* in an incubator for three weeks was able to degrade Cd that ranged from 9.00 to 18.10% (Table 1). The study observed *Bacillus subtilis* in an incubator for three weeks was able to degrade Pb that ranged from 2.80 to 25.00% (Table 1). The study observed *Bacillus subtilis* in an incubator for three weeks was able to degrade Hg that ranged from 88.20 to 90.30% (Table 1). Toxic metals are often found in landfill leachate, posing a significant environmental threat. Landfills generate leachate, a complex mixture of contaminants that can seep into surrounding ecosystems, causing harm to both wildlife and humans [14]. The findings of this study offer a crucial step towards developing more sustainable and effective strategies for mitigating the environmental impact of landfill leachate. The current study further provides valuable insights into the biosorption capabilities of *Bacillus subtilis* for various toxic metals found in landfill leachate under controlled incubation temperatures. This suggests that the effectiveness of bioremediation using *B. subtilis* may vary depending on the specific contaminants present. The higher biosorption efficiency of *B. subtilis* for mercury can likely be attributed to this metal's unique chemical properties and exceptionally high toxicity. *B. subtilis's* enhanced ability to target mercury underscores its potential as a powerful tool for mitigating the harmful effects of mercury contamination.

On the other hand, lead and cadmium, while still highly toxic, exhibit different chemical behaviours and binding affinities compared to mercury [15]. The observed lower biosorption efficiency for these metals highlights potential limitations in utilising *B. subtilis* as the sole remediation strategy. It suggests that additional microbial species or alternative techniques might be necessary to effectively address lead and cadmium contamination within landfill leachate. Factors such as the specific chemical interactions between the toxic metals and bacterial cell components, as well as potential competitive dynamics among different metal ions, warrant further investigation [16].

Table 1: Remediation of cadmium, lead and mercury in landfill leachate observe in an incubator (35 - 37°C)

Sample ID	Pollutant	Initial Conc.	Final Conc.	(%) Recovery
CE R1	Cd	0.011	0.010	09.00
CE R2	Cd	0.011	0.009	18.10
CE R3	Cd	0.011	0.009	18.10
CE R1	Pb	0.036	0.033	08.30
CE R2	Pb	0.036	0.035	02.80
CE R3	Pb	0.036	0.027	25.00
CE R1	Hg	0.321	0.038	88.20
CE R2	Hg	0.321	0.031	90.30
CE R3	Hg	0.321	0.035	89.21

Remediation of Cadmium, Lead and Mercury in Landfill Leachate Observe at a Room Temperature (25 – 27 °C)

The degradation efficacy of *Bacillus subtilis* observed at a room temperature (25 – 27 °C) for three weeks is presented in table 2. The study observed percentage biosorption of *Bacillus subtilis* at a room temperature (25 – 27 °C) for three weeks for Cd ranged from 36.30 to 54.10% (Table 2). The study observed percentage biosorption of *Bacillus subtilis* at a room temperature (25 – 27 °C) for three weeks for Pb ranged from 2.80 to 25.00% (Table 2). The study observed percentage biosorption of *Bacillus subtilis* at a room temperature (25 – 27 °C) for three weeks for Hg ranged from 90.30 to 91.0% (Table 2). *Bacillus subtilis* exhibits a significantly higher efficacy in degrading mercury compared to lead and cadmium when subjected to ambient room temperature conditions

[17]. This discrepancy in performance underscores the selectivity and specificity of *Bacillus subtilis* in its interactions with different heavy metals, shedding light on its metal-binding and detoxification mechanisms. Mercury, renowned for its toxicity and persistence in the environment, poses substantial challenges in remediation efforts [18]. The heightened effectiveness of *Bacillus subtilis* in degrading mercury presents a promising avenue for addressing mercury contamination in landfill leachate [19]. Given the bacterium's demonstrated proficiency in detoxifying mercury, it emerges as a potentially valuable bioremediation tool in mitigating mercury-related environmental hazards. *Bacillus subtilis* exhibits remarkable efficiency in degrading mercury, its performance in degrading lead and cadmium appears comparatively subdued under identical conditions [20].

Table 2: Remediation of cadmium, lead and mercury in landfill leachate observe at a room temperature (25 – 27 °C)

Sample ID	Pollutant	Initial Conc.	Final Conc.	(%) Recovery
ET R1	Cd	0.011	0.007	36.30
ET R2	Cd	0.011	0.005	54.10
ET R3	Cd	0.011	0.007	36.30
ET R1	Pb	0.036	0.034	05.60
ET R2	Pb	0.036	0.035	02.80
ET R3	Pb	0.036	0.027	25.00
ET R1	Hg	0.321	0.028	91.30
ET R2	Hg	0.321	0.031	90.30
ET R3	Hg	0.321	0.028	91.30

Remediation of Cadmium, Lead and Mercury in Landfill Leachate Observe in an Incubator and Under Room Temperature

Bacillus subtilis displayed superior biosorption efficiency for mercury and cadmium at room temperature compared to its incubation temperature. One mechanism possibly underlying this phenomenon is that physiological changes in *B. subtilis* at different temperatures might alter its metal-binding capabilities [21]. Furthermore, the formation of biofilms, which contribute significantly to heavy metal biosorption processes, could be impacted by temperature variations. Biofilm formation protects bacteria from adverse conditions and may enhance their ability to interact with heavy metals at specific temperatures. Potentially, the binding mechanisms that facilitate lead biosorption are more effective at the bacterium's optimal growth temperature. To gain a deeper understanding of these dynamics, future investigations should delve into the specific molecular-level processes governing biosorption at different temperatures. Elucidating the temperature-dependent alterations in bacterial surface chemistry and the metabolic pathways involved in metal uptake and sequestration will have valuable implications for optimizing bioremediation strategies [22].

Effectiveness of *Bacillus subtilis* in Removing Toxic Metals from Landfill Leachate

The efficacy of *Bacillus subtilis* in degrading heavy metals such as cadmium, lead, and mercury within landfill leachate is a crucial focus of this research [23]. *Bacillus subtilis* demonstrates significant effectiveness in the degradation process across varying temperatures, including both incubating and ambient conditions [24]. This suggests a promising potential for the practical application of *Bacillus subtilis* in diverse environmental settings, offering insights into its utility under different temperature regimes [25].

Moreover, the results underscore the importance of considering environmental factors such as temperature in evaluating the performance of *Bacillus subtilis* in heavy metal degradation [26]. Specifically, the research highlights the enhanced efficacy of *Bacillus* subtilis at elevated temperatures, suggesting potential optimization strategies for its application in waste treatment scenarios. These findings contribute to a deeper understanding of the mechanisms underlying *Bacillus subtilis*-mediated degradation processes and their applicability in real-world environmental remediation efforts. Furthermore, exploring the underlying mechanisms by which *B. subtilis* facilitates the degradation of these heavy metals would provide valuable insights into its potential for broader applications. Investigating the specific metabolic pathways or enzymatic processes involved in this degradation could pave the way for further optimization and refinement of this bioremediation strategy [27]. The findings reveal that *B. subtilis* exhibits greater efficiency in degrading these metals at incubation temperatures. However, the research also demonstrates its capacity to function at room temperature, suggesting potential real-world applications under diverse environmental conditions.

Implication of the Current Research to Chemistry Education among undergrads

This study has significant implications for chemistry education among undergraduate students. The research highlights the worth of interdisciplinary approaches in addressing environmental pollution. The combination of microbiology, chemistry, and environmental science offer students a deeper understanding of the complex interactions between microorganisms, pollutants, and the environment [28].

The study also underscores the need for hands-on, inquiry-based learning experiences in chemistry education. The engagement with real-world environmental issues in the likes of heavy metal pollution allows students to develop practical skills in laboratory techniques, data analysis, and critical thinking [29]. Furthermore, the use of microorganisms like *Bacillus subtilis* as biosorbents can illustrate key concepts in chemistry, such as adsorption, desorption, and equilibrium. In a broader perspective, the current research has implications for the development of chemistry curricula that emphasize sustainability, environmental responsibility, and social relevance. By incorporating case studies and examples from environmental chemistry, educators can inspire students to pursue careers in fields related to environmental sustainability [30]. The study's focus on biosorption as a low-cost, eco-friendly solution to heavy metal pollution can also promote student engagement with real-world problems.

CONCLUSION

B. subtilis can effectively degrade cadmium, lead, and mercury, with a particularly high efficacy for mercury degradation. Notably, the bacterium's biosorption performance for mercury and cadmium was even greater at ambient room temperature, highlighting its potential for cost-effective and adaptable applications. The effectiveness of *Bacillus subtilis* at room temperature suggests its practical applicability in real-world scenarios. Recommendations for field applications should take into account the feasibility and advantages of employing *Bacillus subtilis* under ambient conditions, as this could simplify remediation processes and reduce energy requirements.

The results of this research suggest that further exploration of *Bacillus subtilis*-based remediation strategies is warranted. Future studies should focus on optimizing biosorption conditions, investigating the long-term sustainability of this approach in diverse environmental settings, and potentially integrating *B. subtilis* with other bioremediation techniques. These efforts hold the promise of developing innovative and environmentally sound solutions to address the critical challenge of heavy metal contamination from landfill leachate.

Moreso, this study offers a rich opportunity for interdisciplinary learning, hands-on experimentation, and socially relevant education in chemistry for undergraduate studies. The integration of this research into chemistry education fosters a deeper appreciation for the role of chemistry in addressing environmental challenges and promote the development of skilled, environmentally conscious undergrads.

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Conflict of Interest

The authors declare no conflict of interest.

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