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Study on the role of *Pseudomonas aeruginosa* in bioremediation of lead (Pb) and cadmium (Cd)

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Abstract
Due to their persistence and toxicity, heavy metal pollution, particularly lead (Pb) and Cadmium (Cd) pollution, presents severe environmental threats. Microbial remediation had been known as an effective strategy to remove and detoxify heavy metals from soil, water, sediments etc. This study aims to quantify the bioremediating capabilities of *Pseudomonas aeruginosa*.
The bacterium was cultivated and subjected to 100 ppm and 50 ppm respectively and the effective of bioremediation was calculated using UV-Spectrophotometer which measures the decline in metal ion concentration over time.
The finding shows a significant decline in Pb and Cd level, indicating that *Pseudomonas aeruginosa* possesses an exception bioaccumulation and biosorptive property and is useful as a bioremediating agent which can help in removal of heavy metals in contaminated sites

Keywords: *Pseudomonas aeruginosa*, bioremediation, heavy metal, bioaccumulation, biosorptive

Introduction
Microorganisms in nature have the ability to recycle and degrade accumulated heavy metals, reducing their toxicity. These microbes were identified as bacteria, fungus, and algae [1]. Any procedure that employs microbes or their enzymes to restore the environment that has been contaminated to its initial state is known as bioremediation. An alternative to traditional techniques for cleaning up metal-polluted soils is bioremediation technology [2,3]. Heavy metal contamination is caused by pollutants that enter the water supply through soils, ground water systems, and rains from the atmosphere, as well as directly by effluent outfalls from businesses, refineries, and waste treatment facilities [4]. Because of their severe toxicity, non-biodegradability, and bioavailability, lead (Pb) and cadmium (Cd) have regarded as the most toxic metals and cause adverse effects on human health, including neurological disorders, kidney damage, and carcinogenic potential [5,6]. *Pseudomonas aeruginosa*, a gram-negative bacterium has been widely used for its property to tolerate and detoxify heavy metals. This bacterium secrets extracellular polymeric substances (EPS), which enhances its ability to bind and immobilize heavy metals reducing their bioavailability in the environment [1,7].
This study aims to evaluate the potential of *P. aeruginosa* in bioremediation of Pb and Cd at concentration of 100 ppm and 50 ppm, using UV spectrophotometer to measure metal removal efficiency. The use of spectrophotometer provides a precise and quantitative analysis of metal ions concentration before and after bacterial treatment, allowing for an accurate assessment of the bioremediation process.

Materials and Methods
Sample Collection
The study was conducted using soil sample collected from Sharda Hospital, which is a potential site for heavy metal contamination due to medical and laboratory waste discharge. The samples were collected under sterile polythene bag and stored in 4°C refrigerator.

Isolation and Screening of Bacteria
The soil sample was serially diluted with distilled water from 10^{-1} to 10^{-5} and each dilution was pour onto a freshly prepared Luria-Bertani agar plates and was kept for incubation at 37°C for 24 hours in an inverted position.
Following incubation, well grown colonies from plate 10^{-3} and 10^{-4} were picked and further by purified by streaking on Citrimide agar media (selective media for *P. aeruginosa*). The plates were incubated for 24 hours at 37°C.

Preparation of synthetic feed solution
1 M of Lead Nitrate [$Pb(NO_3)_2$] and Cadmium Sulphate ($CdSO_4$) was synthesized as a stock solution.
For 1000 ppm (initial stock solution), 1.6g of $Pb(NO_3)_2$ and 1.8g of $CdSO_4$ was dissolved in 50 ml of deionized water. For 100 ppm, 5 mL of each stock solution was dissolved in 45 mL deionized water and for 50 ppm; 5 mL of 100 ppm solution was dissolved in 45 mL deionized water.

Assay for bioremediation activity
5ml of 50 ppm and 100 ppm of heavy metals in were added to four flask freshly prepared Luria-Betani broth 45ml each in which the bacteria were also inoculated and incubated for 24 hours at 37°C. The concentration of the metal was kept at 5 mL/45 mL of the medium maintaining a total volume of 50ml per flask. The flasks were agitated at 180 rpm for 24, 36 and 48 hours followed by centrifugation at each time interval and the optical densities (OD) were observed.

Result
The current investigation's findings provided consistent evidence in support of the goals and objectives. These findings demonstrated that different ion concentration had impact on the amount of heavy metals absorption by *Pseudomonas aeruginosa* bacterial species.

The absorption of metals by *Pseudomonas aeruginosa* at different concentrations

The uptake profile of *P. aeruginosa* was examined in relation to the incubation period in the presence of heavy metals. In the presence of Cadmium, at both 50 and 100 ppm, *Pseudomonas aeruginosa* exhibited little growth at 24 hours, indicating lag phase. From 24 to 48 hours, it displayed an exponential growth phase.

At the concentration of 50 ppm of Lead, *Pseudomonas aeruginosa* displayed exhibited little growth at 24 hours, indicating lag phase. From 24 to 48 hours, it displayed an exponential growth phase however after 48 hours in 100 ppm concentration; we observed the decline in bacterial population.

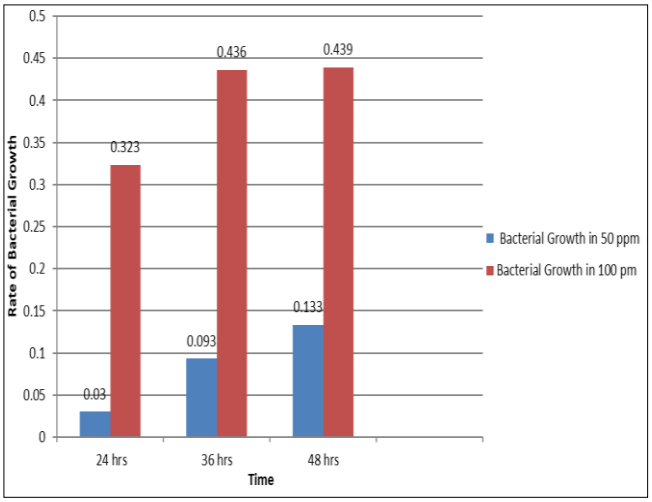
Effect of Cadmium (Cd) Concentration

At 50 ppm, the bacteria grows slowly (O.D 0.030 at 24 hours) and then 0.093 at 36 hours and 0.133 at 48 hours. After 48 hours of incubation, the maximum uptake of cadmium was calculated to be 99.73%.

At 100 ppm Cadmium, the bacteria growth initial O.D was 0.323 at 24 hours, then rises to 0.436 at 36 hours and a practically constant 0.439 at 48 hours. The maximum uptake of cadmium was calculated to be 99.56% after 48 hours.

Table 1: Comparative absorption of cadmium at different concentration and time

Concentration	Incubation time (hours)	O.D at 600nm
50 ppm	24	0.030
	36	0.093
	48	0.133
100 ppm	24	0.323
	36	0.436
	48	0.439



Effect of Lead (Pb) Concentration

Pseudomonas aeruginosa shows maximum uptake of 50 ppm lead was calculated to be 99.06% after 48 hours. Even after the decrease in O.D for 100 ppm at 36 and 48 hours, the uptake was known to be 99.68%.

Table 2: Comparative absorption of lead at different concentration and time

Concentration	Incubation time (hours)	O.D at 600nm
50 ppm	24	0.174
	36	0.218
	48	0.469
100 ppm	24	0.567
	36	0.565
	48	0.319

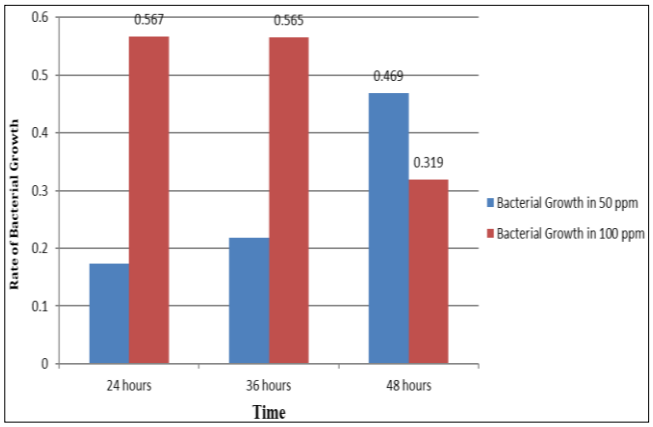


Table 4: comparison of bacterial growth (O.D) at different concentration

Effect of PPM (Parts Per Million) in bacterial growth

The influence of heavy metal content on bacterial development fluctuates with exposure levels. At 100 ppm, bacteria often endure considerable stress, resulting in a prolong lag phase and diminished development owing to toxicity. Nevertheless, some strains, such as *Pseudomonas aeruginosa* may have evolve over time. At 50 ppm, the stress is reduced, allowing a more rapid acclimatization of bacteria resulting in more growth relative to 100 ppm.

Conclusion

This study demonstrated the significant role of *Pseudomonas aeruginosa* in the bioremediation of lead and cadmium, emphasizing its capacity to endure and assimilate heavy metals effectively. The bacterial growth pattern reveals an initial phase at both 50 ppm and 100 ppm concentration, followed by exponential growth phase from 24 to 48 hours, suggesting effective adaptability to metal stress. A decrease in bacterial population was seen after 48 hours at high die (100 ppm), indicating a limit for metal tolerance.

The absorption efficiency of *P. aeruginosa* was very high, achieving cadmium removal rate of 99.73% at 50 ppm and 99.56% at 100 ppm after 48 hours. Lead absorption was 99.06% at 50 ppm and 99.68% ppm. These results validate the potential of P. aeruginosa as a viable option for heavy metals bioremediation, providing an environmentally sustainable and efficient method for mitigating lead and cadmium pollution in the ecosystem.

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