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Optimization of Cadmium Biosorption by Cadmium Tolerant Bacteria Isolated from Industrial Effluents

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Abstract

Environmental pollution with cadmium (Cd^{+2}), presents serious health hazards as well as environmental issues. The main objective of this study was to check the optimum physiological conditions for Cd^{+2} biosorption by the bacteria isolated from industrial effluents. Minimum inhibitory concentration (MIC) of Cd^{+2} for the bacterial isolates was determined for the screening of the bacteria. Out of all the bacterial isolates, RNLI S1 had the highest MIC value equal to 600 µg/ml. RNMD S3 and RNLI S2 showed the same MIC values equal to 550 µg/ml, which was the second highest value. The MIC values of the bacterial isolates RNMD S4 and RNMD S5 were 500 and 450 µg/ml, respectively. RNLI S6 has the lowest MIC value among all the bacterial isolates which was equal to 400 µg/ml. Three bacterial isolates RNLI S1, RNMD S2, and RNLI S3 were selected for further experiments based on their MIC values. The optimization of physiological parameters such as temperature and pH for cadmium biosorption ability of these bacteria was carried out. Cadmium biosorption potential of RNMD S2 (92.04%) and RNLI S3 (90.81%) was maximum at 45℃, whereas RNLI S1 (88.38%) showed maximum biosorption at 37℃. Optimum pH for maximum cadmium biosorption was 9 for RNMD S2 (80.65%) and RNLI S3 (79.16%) and for RNLI S1 (80.31%) it was found to be 7. The findings of this study highlight the potential of these bacterial isolates, RNLI S1 (Pseudomonas sp.), RNMD S2 (Micrococcus sp.) and RNLI S3 (Acinetobacter sp.) in the application of cadmium bioremediation at various contaminated sites.

Keywords: *Heavy metal contamination, Cadmium Pollution, Industrial Effluents*

Introduction

Heavy metal contamination is highly hazardous to human well-being and the environment because of its poisonous nature. Heavy metal-induced environmental contamination is the main cause for worry. Their concentration has reached a harmful level as a result of widespread human-induced activities which include mineral extraction, farming practices and the removal of waste from industries. There is a wide distribution of heavy metals in the environment. They have classified them into two

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categories: necessary and non-necessary. "Heavy metals" are a family of metals and metalloids having an atomic density more than 5 g cm^{-3} (Kim *et al.,* 2019).

Numerous health issues, including immune system dysfunction, neurological system damage, psychiatric issues, infertility, and reproductive failure, can be brought on by cadmium. Therefore, getting rid of it is crucial to prevent issues with human health and environmental degradation. Bioremediation is the most affordable and environmentally benign method of treating this pollution. Cadmium and its derivatives are considered carcinogenic to humans by the International Agency for Research on Cancer (IARC) (IARC 1993 and 2009). Human exposure to cadmium is primarily caused by eating contaminated food and inhaling fumes from metal industry workers, according to IPCS 1992 and WHO 2010. Respiratory enzymes are readily bound by cadmium, which can lead to cancer (Chen *et al.*, 2021).

There are significant levels of heavy metals and other pollutants in industrial effluents (Al-Ansari *et al.,* 2021). These waste waters seriously harm water resources if they get into rivers and other bodies of water. Cadmium is the most hazardous metal among all, even at very low concentrations. The most important human sources of Cd (II) emission are extraction, smelting, Ni-Cd (II) batteries, metal plating, steel anticorrosive, polymer stabilizers, solar power cells, dyes, cigarette smoking, phosphate fertilizers, and manures (Schaefer *et al.,* 2020).

By using live organisms, such as bacteria, plants, or their enzymes, bioremediation is a natural and sustainable way to remove toxins from contaminated environments. Compared to conventional methods, it provides a more sustainable and economical cleanup solution. Utilizing the unique physiological and biochemical characteristics of plants or the innate metabolic abilities of microorganisms, bioremediation techniques can alter or degrade a wide range of pollutants, such as organic compounds, heavy metals, and petroleum hydrocarbons. Microbial bioremediation is the process of using specific bacteria, fungi, or algae that have the ability to break down or immobilize toxins by enzymatic breakdown, reduction, oxidation, or sequestration. These bacteria may be found naturally in the environment or they may be put on purpose to aid in the cleanup process (Singh *et al.,* 2020).

Numerous investigations have shown that bioremediation is commonly regarded as one of the most successful and efficient methods for removing pollutants from the environment and restoring contaminated sites for future beneficial use (Ahemad & Malik, 2011). Numerous microorganism-based bioremediation techniques have shown promise in the breakdown and detoxification of a range of heavy metals, including cobalt, nickel, cadmium, chromium, lead, and arsenic (Gupta *et al.*, 2012).

The goal of this work was to separate bacteria resistant to cadmium from industrial effluents in order to optimize physiological conditions, including pH and temperature, for the lab-scale cadmium biosorption process.

Materials and Methods

Sample Collection

Samples of wastewater were gathered from two locations on Rohi Nala Road in Lahore, Punjab. A leather factory and a main drain. Each sample's temperature and pH were recorded on-site when it was collected.

Isolation and Purification of Cadmium Resistant Bacteria

50 µl of each wastewater sample was used to inoculate nutrient agar plates supplemented with cadmium chloride ($CdCl_2$) in order to isolate cadmium-resistant bacteria. The plates were incubated for twenty-four hours at 37°C. Purified isolates were obtained by sub culturing distinct colonies.

Determination of Minimum Inhibitory Concentration (MIC) for Cadmium (Cd^{+2})

The broth dilution method was used to calculate the minimum inhibitory concentration (MIC) of cadmium (Cd^{+2}) for every bacterial isolate. Bacterial cultures were inoculated overnight in nutrient broth tubes with different concentrations of Cd^{+2} (50–1000 µg/ml) and incubated for 24 hours at 37°C and 120 rpm. The lowest concentration at which there was no discernible turbidity was known as the MIC.

Biochemical and Physiological Characterization of Selected Bacterial Isolates

Selected isolates were subjected to standard biochemical tests. Following a 24-hour incubation period in nutrient broth, bacterial growth was evaluated using OD600 measurements at various pH values (5, 7, 9) and temperatures (4°C, 37°C, 45°C) in order to perform physiological characterization.

Optimization of Physiological Parameters for Cd^{+2} Biosorption

Effect of pH

100 µg/ml Cd^{+2} was added to nutrient broth that had been adjusted to pH 5, 7, and 9 in order to cultivate bacterial cultures. The cultures were centrifuged following a 24-hour incubation period at 37°C and 120 rpm. Atomic absorption spectroscopy (AAS) was used to estimate the amount of cadmium in the supernatants, which were kept at 4°C.

Effect of Temperature

The cultures were incubated in broth supplemented with Cd^{+2} (100 µg/ml) at 25°C, 37°C, and 45°C. Samples were centrifuged following a 24-hour incubation period, and the supernatants were kept at 4°C for AAS-based cadmium quantification.

Optimization of Different Physiological Parameters for Cd^{+2} Biosorption

Two physiological factors were optimized for the cadmium biosorption by bacterial isolates.

Impact of pH and Temperature on Cadmium (Cd^{+2}) Biosorption by Bacterial Isolates

The purpose of this study was to assess how environmental variables, particularly pH and temperature, affected the ability of bacterial isolates derived from industrial effluents to biosorb cadmium (Cd^{+2}). Bacterial cultures were cultivated in nutrient broth that had been adjusted to pH 5, 7, and 9, each supplemented with 100 µg/ml Cd^{+2}, in order to ascertain the ideal pH for Cd^{+2} biosorption. The cultures were shaken at 120 rpm for 24 hours at 37°C. The supernatant was then collected by centrifugation for cadmium analysis using atomic absorption spectroscopy (AAS). The bacterial cultures were incubated at 25°C, 37°C, and 45°C under the same experimental conditions in order to evaluate the impact of temperature on biosorption (Parmar et al., 2020).

Statistical Analysis

The effect of temperature and pH on growth and Cd^{2+} removal for each isolate was assessed using a one-way ANOVA and Tukey's HSD post-hoc test. Statistical significance was accepted at $p < 0.05$.

Results

Physicochemical characteristics of wastewater

Table 1 shows the physicochemical characteristics of the wastewater samples, such as pH and temperature were recorded at the time of sample collection. Sample RNMD showed a pH and temperature equal to 9 and 45℃ respectively, whereas, the sample RNLI showed a pH and temperature equal to 7 and 37℃.

Table 1: *Physicochemical Characteristics of Wastewater*

Sample	Sample Code	Location	pH of Sample	Temperature
1	Rohi Nala Main Drain (RNMD)	Rohi Nala road Lahore Punjab	9	45℃
2	Rohi Nala Leather Industry (RNLI)		7	37℃

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Isolation, purification and characterization of bacterial isolates

Six distinct bacterial isolates exhibiting resistance to cadmium were selected based on morphological variations of the bacterial colonies. Three of the bacterial isolates were selected from the leather industry's wastewater sample and three were picked from the main drain's wastewater sample. The data was showing in table 2.

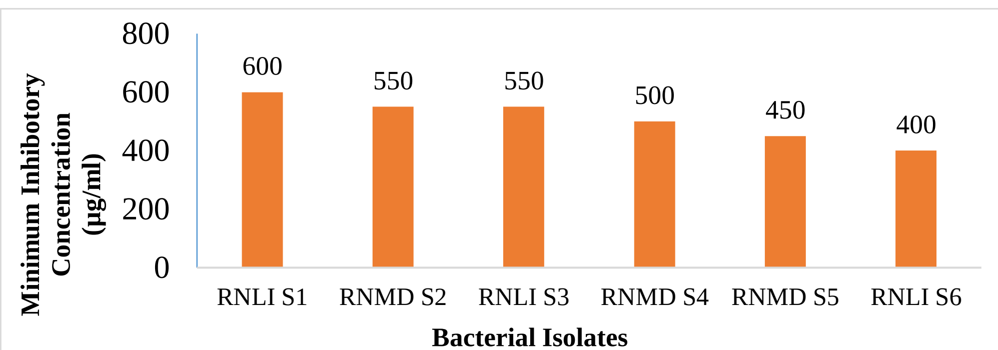
Table 2: *Number and Codes for Bacterial Isolates*

Sample Codes	Isolates	Codes for Isolates
RNMD	3	RNMD S2, RNMD S4, RNMD S5
RNLI	3	RNLI S1, RNLI S3, RNLI S6

Screening of the Bacterial Isolates through Minimum Inhibitory Concentration (MIC) for Cd^{+2}

Figure 1 illustrates minimal inhibitory concentration values of Cd^{+2} for each bacterial isolate. Every bacterial isolate had a distinct MIC value. Out of all the bacterial isolates, RNLI S1 showed the highest MIC value, *i.e.,* 600 µg/ml. RNLI S3 and RNMD S2 showed the same MIC values equal to 550 µg/ml, which is the second highest value. The MIC values of the bacterial isolates RNMD S4 and RNMD S5 were 500 and 450 µg/ml, respectively. RNLI S6 has the lowest MIC value of all the bacterial isolates which was equal to 400 µg/ml. Three bacterial isolates, RNLI S1, RNMD S2, and RNLI S3 were selected for further investigation based on their MIC values since they exhibited the higher MIC values for Cd^{+2} (Figure 1).

Figure. 1: *Minimum Inhibitory concentration of Bacterial Isolates for Cadmium (Cd+2)*



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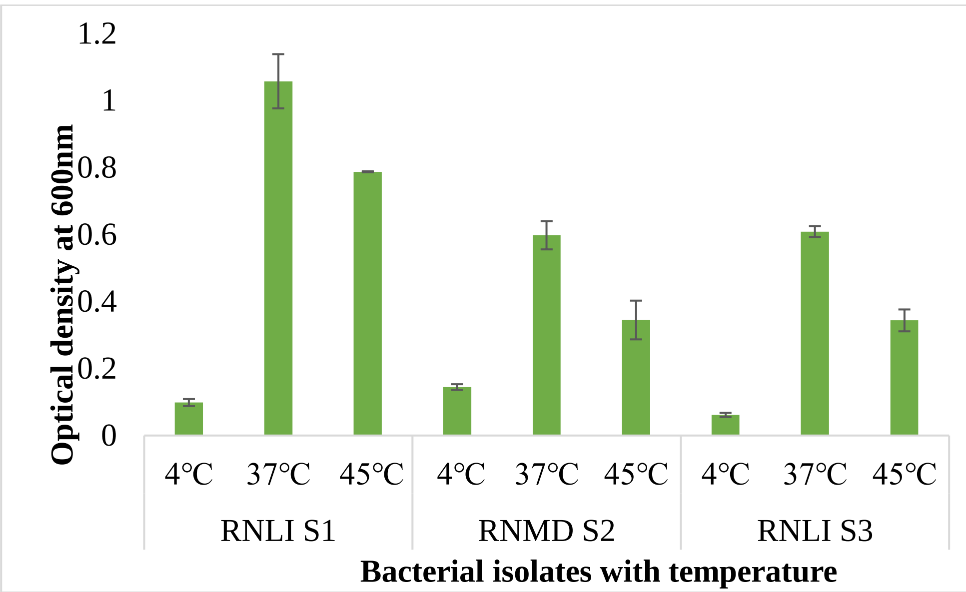
Optimization of Different Physiological Parameters for the Growth of Bacterial Isolates

Impact of Temperature

Three distinct temperature values 4°C, 37°C, and 45°C were selected in order to examine the impact of temperature on the development of bacterial isolates. According to the findings, the three bacterial isolates RNLI S1, RNMD S2, and RNLI S3, all displayed maximum growth at 37°C, which is the optimum temperature for their growth. The bacterial isolates showed minimal growth at 4°C (Figure 2).

Figure. 2: *Growth of Bacterial Isolates at Different Temperature (4°C, 37°C and 45°C) to check the Optimum pH for the Bacterial Isolates*



Impact of pH

The growth of bacteria was examined at three distinct pH values such as 5, 7, and 9, in order to determine the ideal pH values for the optimal growth of bacterial isolates. The optimal temperature of 37°C was maintained for 24 hours after which the optical density of each bacterial culture was measured at 600 nm. The findings showed that the optimal pH value for bacterial isolate RNLI S1 and RNMD S2 was maximum at pH 9 whereas RNLI S3 showed maximum growth at pH 7 (Figure 3).

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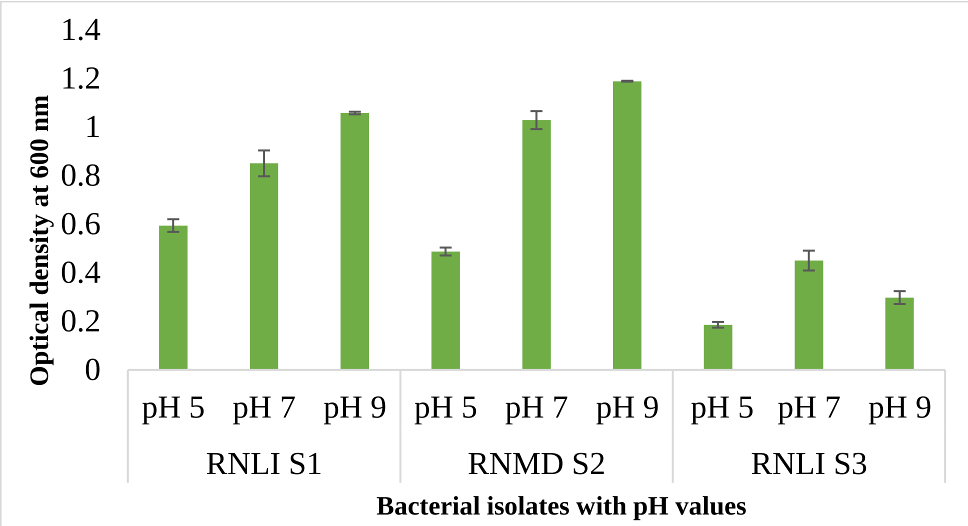
Figure. 3: *Growth of Bacterial Isolates at different pH (5, 7 and 9) to check the Optimum pH for the Bacterial Isolates*



Optimization of Different Physiological Parameters (pH and temperature) for Cd^{+2} Biosorption

Impact of Temperature on Cd^{+2} Biosorption

All of the bacterial isolates' ability for Cd^{+2} biosorption was examined at three distinct temperatures like 25°C, 37°C and 45°C. Based on the findings, RNLI S1 absorbed 84.93, 88.38, and 86.51 µg/ml Cd^{+2} at temperature 25°C, 37°C, and 45°C respectively. At 25°C, 37°C, and 45°C, the bacterial isolate RNMD S2 absorbed 84.97µg/ml, 88.04µg/ml, and 92.04 µg/ml, while RNLI S3 absorbed 86.37µg/ml, 88.09µg/ml, and 90.81 µg/ml respectively. According to the results, RNLI S1 showed maximum Cd^{+2} biosorption at 37°C, whereas, RNMD S2 and RNLI S3 showed maximum biosorption at 45°C (Figure 4).

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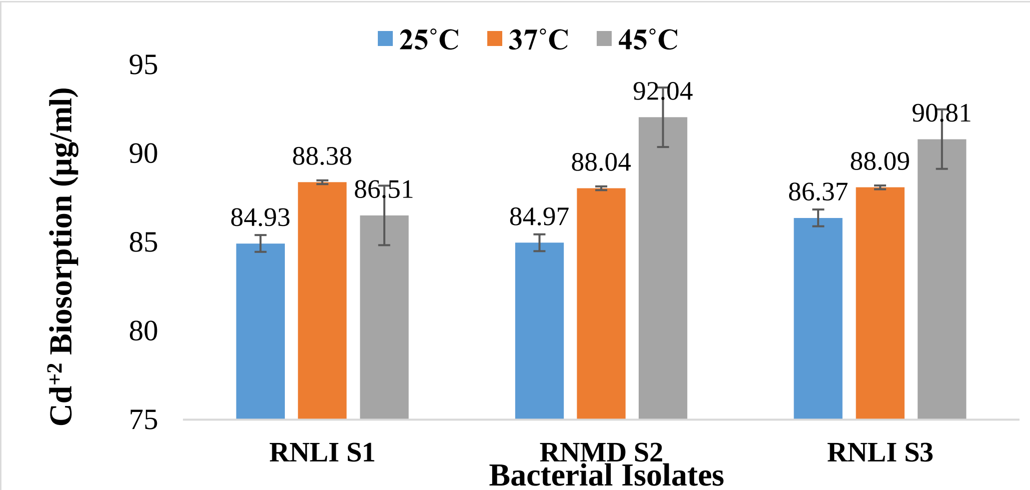
Figure. 4: *Biosorption Potential of Bacterial Isolates for Cadmium (Cd+2) at Different Temperature (25℃, 37℃ and 45℃)*



Impact of pH on cadmium biosorption

At different pH (5, 7, 9) it was investigated how well bacteria could biosorb. The goal of this was to find the ideal pH for bacterial isolates to facilitate the biosorption of cadmium. Atomic absorption spectrophotometry (AAS) was used to measure the amount of Cd^{+2}biosorption following a 24-hour incubation period at the ideal temperature (37°C for RNLI S1, 45°C for RNMD S2 and RNLI S3). The findings showed that at pH 5, 7, and 9, RNLI S1 absorbed 78.75, 80.31, and 76.82 µg/ml of Cd^{2+}, respectively. At pH 5, 7, and 9 the bacterial isolate RNMD S2 absorbed 76.96, 78.84, and 80.65 µg/ml of Cd^{2+}. RNLI S3 demonstrated that at pH 5, 7, and 9 absorptions of Cd^{2+} is equivalent to 75.01, 77.52, and 79.61 µg/ml Cd^{+2} According to the results, RNLI S1 displayed the most Cd^{2+} biosorption at pH 7, whereas RNMD S2 and RNLI S3 displayed the highest Cd^{2+} biosorption at pH 9 (Figure 5).

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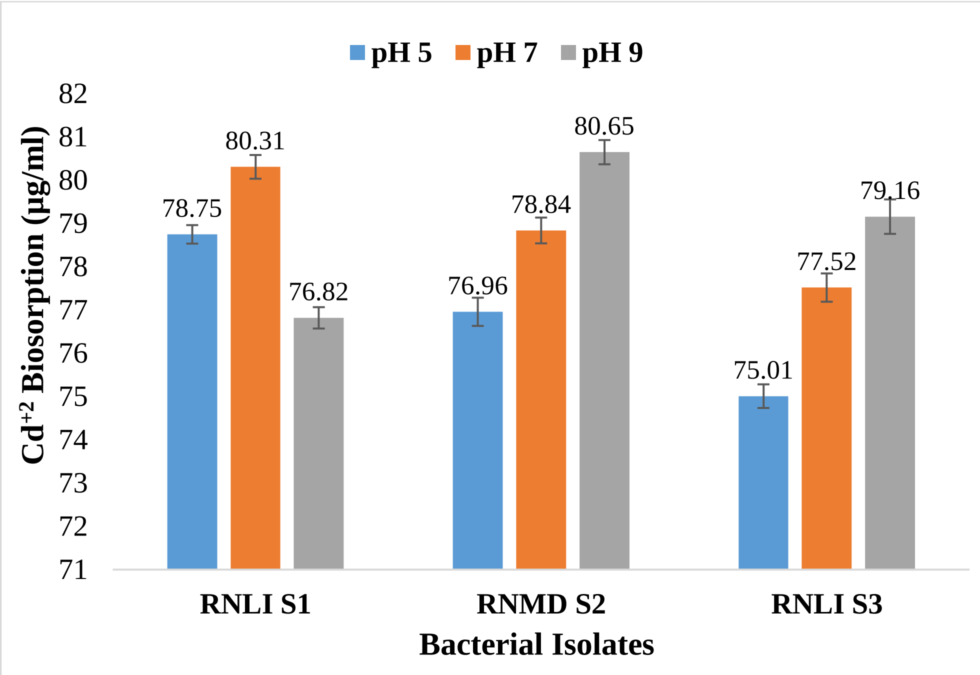
Figure. 5: *Biosorption Potential of Bacterial Isolates for Cadmium (Cd+2) at Different pH (5, 7 and 9)*



Biochemical tests of Bacterial Isolate

Table number 3 shows the results of the biochemical characterization of the bacterial isolates (RNLI S1, RNMD S2 and RNLI S3).

Table 3: *Biochemical Analysis of Isolated Bacteria*

Biochemical Tests	RNLI S1	RNMD S2	RNLI S3
Oxidase	Positive	Positive	Positive
Catalase	Positive	Positive	Positive
MR t	Positive	Positive	Positive
VP	Negative	Negative	Negative
Indole	Positive	Negative	Negative
Citrate utilization	Positive	Negative	Positive
Urease	Positive	Negative	Positive
Hydrogen supplied	Positive	Negative	Negative
Triple sugar iron	Red/yellow	Yellow/yellow	Red/yellow
Gram Staining	Negative	Negative	Negative

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Identification of Bacterial Isolates on the Basis of Biochemical Tests

On the basis of biochemical characterization, the bacterial isolate RNLI S1 belongs to the genus *Pseudomonas*, RNMD S2 belongs to the genus *Acinetobacter* and RNLI S3 belongs to the genus *Klebsiella.*

Discussion

The negative effects of heavy metal pollution on the health of people and plants have made it a worldwide concern. While some of these metals are necessary as micronutrients, when their concentration rises over what is needed, these micronutrients become harmful (Valko *et al.*, 2016). Since the start of the industrial revolution, heavy metal pollution has been acknowledged as a global issue. Heavy metal contamination is highly hazardous to human health and the environment due to its poisonous nature. Their concentration has reached a harmful level as a result of widespread anthropogenic activities such as industrial operations, particularly mining, agricultural processes, and the disposal of industrial waste materials. Without sufficient treatment, the majority of companies release wastewater and its effluent containing hazardous heavy metals into rivers as reported by Cui *et al.* (2013).

Industrial effluents were utilized to extract bacteria resistant to cadmium. The goal of this work was to optimize the biosorption of cadmium (Cd^{+2}), utilizing cadmium resistant bacteria under various physiological parameters, such as temperature and pH in order to determine the ideal circumstances that would improve the biosorption effectiveness. Two types of wastewater samples, one from main drain and one from specifically leather industry, were collected from Rohi Nala Road Lahore, Punjab. Firstly, cadmium resistant bacteria were isolated on nutrient agar plates supplemented with 100 µg/ml of cadmium, six different bacterial isolates demonstrating resistance to cadmium were identified. This adds to our understanding of the biosorption of cadmium by heavy metal-resistant bacteria and validates their eligibility for additional research aimed at enhancing cadmium biosorption reported by Choińska-Pulit, Sobolczyk-Bednarek and Łaba, 2018.

Using the broth dilution method, the minimum inhibitory concentration of the bacterial isolates (RNLI S1, RNMD S2, RNLI S3, RNMD S4, RNMD S5, RNLI S6) was found in order to estimate the highest level of cadmium (Cd^{+2}) resistance for these bacteria. Both liquid and solid media can be used to calculate MIC, however liquid media have greater MIC values than solid media. This is due to the fact that bacteria are better able to access metal in liquid media than in solid media (Basu and Paul, 1999). Every bacterial isolate had a distinct minimum inhibitory concentration (MIC). Out of all the bacterial isolates, RNLI S1 had the highest MIC value, at 600 µg/ml. RNMD S3 and RNLI S2 showed the same MIC values equal to 550 µg/ml, which is the second highest value. The MIC values of the bacterial isolates RNMD S4 and RNMD S5 were 500 and 450 µg/ml, respectively. RNLI S6 has the lowest MIC value of all the bacterial isolates, equals to 400 µg/ml. Three bacterial isolates RNLI S1, RNMD S2, and RNLI S3 were chosen for further investigation based on their MIC values since they exhibited the strongest resistance to Cd^{+2} as reported by Abbas et al., 2016.

It is clear from comparing these results with earlier research that different bacterial species showed differing levels of cadmium resistance. For instance, a study reported that *Bacillus* sp. exhibited a strong resistance pattern to cadmium (1800 µg/ml). The MIC values of *Pseudomonas* sp. (Ps/P-4) and *Klebsellia* sp. (K/G-1) against cadmium were likewise found to be high, at 1800 µg/ml and 1600 µg/ml, respectively. *Staphylococcus sp.* (St/ P -5) and *Proteus sp.* (Pr/G-2) exhibited 800 µg/ml MIC value by Nath *et al.*, 2012. Moreover, another study demonstrated that *B. cereus* and *E. coli* exhibited very low heavy metal resistance to cadmium which is 50 µg/ml. The two strain BG and BS showed high level resistance to cadmium which is 1200 µg/ml and 2000 µg/ml (Hu Qing *et al.*, 2007).

Optimizing various physiological parameters, such as pH and temperature, for the growth of bacteria was an additional aspect of this investigation. An important field of study that advances the development of biotechnological applications is the optimization of bacterial growth conditions. Researchers may unleash the full potential of bacteria and use their skills for a variety of advantageous goals by finding out the optimal growing circumstances for these organisms. Temperature is essential for development and survival of bacteria. Three distinct temperature values (4°C, 37°C and 45°C) were investigated in our study for their impact on the growth of the bacterial isolates. Notably, all the three bacterial isolates, RNLI S1, RNMD S2 and RNLI S3 exhibited optimal growth at 37℃, which is the standard temperature preference for mesophilic bacteria. The limited enzymatic activity and slower metabolic processes at lower temperatures can be the cause of a slowdown that was seen at 4°C. In a prior study on temperature-dependent growth patterns in bacterial species, B. cereus CS1 was found to be capable of surviving at temperatures as low as 10°C, although its optimal growth temperature was 40°C (Wu *et al.*, 2019).

As a result, our study's results, which highlight how sensitive bacteria are to temperature, are consistent with what is already known. At various pH values (5, 7 and 9), the impact of pH on the growth of the bacterial isolates was also investigated. All of the bacterial isolates, including RNLI S1 and RNMD S2, showed maximum growth at pH 9 after a 24-hour incubation period at the ideal temperature of 37°C, but RNLI S3 showed maximum growth at pH 7. A pH difference from the ideal range can interfere with cellular functions such as protein stability, enzymatic activities, and

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food uptake, which can impact bacterial growth (Sezonov *et al.*, 2007). Our results are corroborated by earlier research that found pH-dependent growth patterns in 43 different species of bacteria by Sun *et al.*, 2021.

Conclusion

The study showed that the bacterial isolates RNLI S1, RNMD S2, and RNLI S3 have strong biosorption capacities and are resistant to cadmium. Whereas RNMD S2 and RNLI S3 functioned best at 45°C and pH 9, RNLI S1 demonstrated the highest cadmium biosorption at 37°C and pH 7. The two isolates with the greatest potential for cadmium biosorption were RNMD S2 and RNLI S3. According to these results, these bacterial isolates may be useful and environmentally benign agents for the bioremediation of environments contaminated with cadmium.

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