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Bioremediation of Heavy Metal Using *Burkholderia cenocepacia* and Optimization of Heavy Metal RemediationSN Praveena Yadavalli¹, Sreenivasulu Kamma^{1*} and Debasish Sahoo²¹ Department of Biotechnology, KL University Vaddeswaram, Andhra Pradesh, India.² Senior Scientist, BioInnoval Lifescience Pvt. Ltd. Bengaluru, India.

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Abstract

Rapid industrialization has resulted in widespread metal contamination of ecosystems, creating a need for effective biological remediation strategies. This study investigated the metal tolerance and biosorption characteristics of *Burkholderia cenocepacia* isolated from heavy metal-contaminated soil. The strain demonstrated effective removal of different metal ions, including As^{3+} , Pb^{2+} , Zn^{2+} , and Cr^{6+} , from multi-metal solutions. Minimum inhibitory concentration (MIC) assays revealed variations in metal tolerance, indicating strain-specific responses to metal stress. The strain showed strong metal tolerance capacity; however, the simultaneous presence of multiple metal ions resulted in competitive binding and reduced metal removal, suggesting inhibition of metal adsorption onto extracellular polymeric substances (EPS). FTIR analysis confirmed the involvement of bacterial functional groups in metal binding and revealed the appearance of new peaks following metal exposure, indicating possible EPS production and associated changes in cellular surface chemistry. EDX analysis further demonstrated metal deposition on bacterial surfaces and within cytoplasmic regions, together with morphological changes and distinct metal adsorption patterns. XRD and EDX analyses confirmed intracellular metal accumulation, highlighting the complexity of microbial-metal interactions. Differences in metal distribution patterns suggested species-specific accumulation mechanisms influenced by metal availability and environmental conditions. The findings emphasize the importance of selecting suitable bacterial strains for specific metal containing effluents, particularly where competitive interactions may limit simultaneous metal uptake. The broad metal tolerance and removal capacity of *B. cenocepacia* indicate its potential application in heavy metal remediation through bioreactors or in situ treatment systems.

Keyword: Heavy Metal, Fourier-transform infrared analysis, Bioremediation, Optimization, Remediation, *Burkholderia cenocepacia*.

1. Introduction

The increasing industrialization in recent decades has led to the extensive discharge of heavy metals into the environment, creating severe ecological and public health concerns. Heavy metals such as lead (Pb), cadmium (Cd), chromium (Cr), arsenic (As), and mercury (Hg) are toxic, non-biodegradable, and interfere with biological processes, and infiltrate the food chain [1]. Soil contaminated by heavy metals occurs throughout various anthropogenic activities, including smelting, mining, wastewater irrigation, and the improper disposal of agricultural and industrial waste. Heavy metal pollution in soil not only compromises crop productivity and soil fertility but also poses severe risks to human health. Hence, the remediation of heavy metal-contaminated soil is a critical environmental challenge that demands sustainable and cost-effective solutions [2]. According to WHO guidelines, the permissible limits of heavy metals in soil are As^{3+} - 20 mg/kg, Pb^{2+} - 100 mg/kg, Zn^{2+} - 300 mg/kg, and Cr^{6+} - 100 mg/kg.

Effective remediation methods of heavy metal-contaminated soils, such as landfilling, excavation, and chemical stabilization, are frequently expensive, environmentally intrusive, and less competent for large-scale

applications [3,4]. Bioremediation has emerged as a potential alternative, refers to the use of biological agents such as plants, microorganisms to detoxify environmental pollutants. This eco-friendly approach capitalizes on natural processes to restore soil strength while minimizing unfavorable environmental impacts [5].

Microbial bioremediation, involves fungi, bacteria, and algae, is one of the most broadly considered methods for heavy metal remediation metals [6]. Microorganisms can interrelate with heavy metals through various processes such as bioaccumulation, biosorption, biomineralization, and enzymatic transformation [7]. These mechanisms permit microorganisms to either sequester heavy metals in less toxic forms or remove them from contaminated environments [8]. Similarly, phytoremediation employs plants to degrade, extract, and stabilize heavy metals from the soil. Certain hyperaccumulator plants have shown extraordinary potential in absorbing heavy metals in their tissues, building them appropriate candidates for remediation [9]. Although, the application of bioremediation faces numerous challenges, such as the time-intensive nature of biological processes, the toxicity of heavy metals to bioremediating agents, and the heterogeneity of contaminated soils [10]. To overcome these limits, optimization techniques have been developed to improve the competence and consistency of bioremediation strategies [11]. Optimization involves the selection of healthy microbial strains or plant species, and the manipulation of environmental parameters such as temperature, pH, and nutrient availability [12].

The objective of this research is to examine the bioremediation for heavy metal-contaminated soils and examines optimization strategies to enhance process. The goal of this research is to create a thorough framework for sustainable soil restoration by investigating the roles of microbial populations and environmental conditions. In addition to advancing our scientific knowledge of heavy metal remediation, the study's conclusions will help businesses, environmental organizations, and legislators implement environmentally beneficial methods of reducing soil contamination.

2. Material and methods

2.1 Isolation, Screening and Identification of Bacteria

The heavy metal tolerant bacteria were isolated from coal mine soil sample by serial dilution technique on nutrient agar medium and bacteria were screened on minimal salt agar medium with 0.1% concentration of sodium arsenite (As^{3+}), lead nitrate (Pb^{2+}), zinc sulfate (Zn^{2+}), or potassium dichromate (Cr^{6+}) [13]. The selected isolates were identified through Biochemical test followed by Bergey's Manual and 16s rRNA sequence [14].

2.2 16s rRNA sequence determination

The genomic DNA of the bacterial strain was extracted after it was cultured for the entire night in nutrient media. The following universal bacterial 16S rDNA primers were used to amp up the bacterial 16S rDNA from the isolated genomic DNA: forward primer (5' AGAGTTTGATCMTGGCTCAG 3') and reverse primer (5' TACGGYTACCTTGTTACGACTT 3'). Using pipettes set aside especially for this use, PCR reactions were set up in a clean room to detect the bacterial species. The PCR reactions were carried out in 20 μl volumes using 1 $\times$ PCR buffer containing 0.16 mM dNTP and 2 μl of the genomic DNA sample. Combine 0.75 U of Taq DNA polymerase (MBI, Fermentas, Lithuania) with 20 pmol of forward and reverse primers. Two drops of mineral oil were placed on top of the mixtures. The PCR conditions were as follows: an initial denaturation at 95°C for 6 minutes, 40 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 1 minute, and extension at 72°C for 1 minute. The amplification was performed in a thermal cycler (Eppendorf Mastercycler 4, 230 V/50-60 Hz). A final extension was performed at 72°C for 10 min. PCR products were analyzed using 2% agarose gel electrophoresis. The gel was run at 100 volts for 45 minutes at room temperature, and the sizes of the amplified 16S rDNA segments utilizing primers were assessed using molecular weight markers NEX-GEN DNA Ladder (100 bp). Ethidium bromide staining was applied to the PCR products, which were then displayed on a Zenith Gel Documentation System (Model No. Gel.LUMINAX-312). Sanger's Method was then used to sequence the samples (ABI 3730xl DNA Analyzer).

2.3 Sequence alignment and data analyses

BioEdit, an online pairwise alignment tool, was used to align the forward and reverse sequences that were acquired. E value a $<1 \times 10^{-5}$ and maximum hits (99 or 100%) with a species in the NCBI reference database were taken into consideration when identifying the query sequences. MEGA X was utilized for phylogenetic tree analysis using the Neighbour Joining (NJ) method in addition to BLAST.

2.4 Optimization

The selected bacteria were used to optimize highest degradation of heavy metals. Optimization effect such as concentration of metals, temperature, pH, carbon source, nitrogen source of heavy metals were optimized to achieve highest degradation [15].

2.5 Fourier transform infrared spectrometric analysis

Fourier transform infrared spectrometric (FTIR) was used to identify the functional groups presence on the cell surface which was responsible for the adsorption of heavy metals. The absorbance of the IR spectrum was recorded within 500 to 4000 cm^{-1} using a FTIR (Thermo fisher, Nicolet iS20) [16].

2.6 X-ray Diffraction

An Advanced Bruker AX D8 diffractometer (Shimadzu, XRD7000) , equipped with nickel-filtered Cu $K\alpha$ radiation ($\lambda = 1.542 \text{ \AA}$) at 40 kV and 40 mA, was used to examine the diffraction patterns of the cellulosic materials at room temperature in order to study the crystalline nature of extracted cellulose. The samples were characterized by XRD analysis. 0.5 seconds each step was the scan speed [17,18].

2.7 EDX Analysis

Energy dispersive X-ray spectroscopy (JEOL, JED2300) was carried out to examine the metals effect of the bacterial cells (*Burkholderia cenocepacia*) grown without metal stress and bacterial cells grown with metals. Samples were fixed in 2.5% glutaraldehyde in Sorensen phosphate buffer (pH 7.2), dehydrated with increasing solutions of ethanol (30, 50, 70, 80, 90, 95, and 100%) and HDMS. Carbon tape was used to secure the prepared samples to the sample holder. The samples were sputter coated with carbon to enhance electron conduction.

3. Result and Discussion

3.1 Isolation and Screening of heavy metal resistant bacteria

Among the numerous microbial isolates screened during this study, one particular isolate stood out due to its significant positive effects in the presence of heavy metals including arsenite (As^{3+}), lead (Pb^{2+}), zinc (Zn^{2+}), and hexavalent chromium (Cr^{6+}). These heavy metals are well known for their toxic impact on the environment and living organisms, making their bioremediation a subject of critical importance. The isolate demonstrated high tolerance and potential detoxification capabilities against these metal ions, which warranted further investigation into its identity and biochemical properties. The decision to prioritize this isolate was driven by its consistent performance in preliminary assays that measured resistance and biotransformation capacities. To accurately identify the microbial species and understand its potential role in bioremediation, a combination of classical biochemical testing and molecular analysis was employed. Initially, biochemical tests helped narrow down the possibilities based on metabolic and enzymatic profiles, giving insights into the genus-level classification. However, to achieve precise species-level identification, 16S ribosomal RNA (rRNA) gene sequencing was conducted. The 16S rRNA gene is a highly conserved genetic marker in prokaryotes and is commonly used for phylogenetic studies and taxonomic classification. Sequencing results revealed that the isolate belonged to the species *Burkholderia cenocepacia*, a bacterium known for its metabolic versatility and environmental resilience. To confirm the presence and size of the amplified 16S rRNA gene, gel electrophoresis was performed. The PCR product was run on an agarose gel, and a DNA ladder, specifically MAGSPIN-21, was used as a molecular size marker. As shown in Figure 1, the band corresponding to the 16S rRNA gene was observed at approximately 1500 base pairs (bp), which aligns with the expected size for this gene. This confirmation was a crucial step in validating the sequencing results ensuring that the correct fragment was amplified for phylogenetic analysis. In addition to sequence confirmation, a phylogenetic tree was constructed to determine the evolutionary relationship of the isolate with other related bacterial species. The resulting tree, shown in Figure 2, illustrated the close genetic proximity of the isolate to other strains of *Burkholderia cepacia*. This tree was generated using multiple sequence alignments and appropriate phylogenetic inference methods such as neighbor-joining or maximum likelihood analysis. The branching patterns and bootstrap values supported the taxonomic placement of the isolate within the *Burkholderia cepacia* complex. The identification of this isolate as *Burkholderia cenocepacia* is particularly notable because members of this species complex have been reported in previous studies to possess various mechanisms for heavy metal resistance and degradation of organic pollutants. These mechanisms include the production of metal-chelating compounds, efflux systems, and enzymatic transformation. Therefore, this isolate

holds promise as a potential candidate for biotechnological applications in the remediation of environments contaminated with toxic heavy metals.

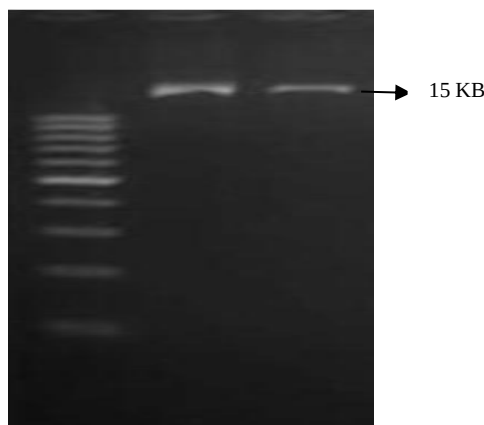


Figure 1 Agarose gel showing single 1500 bp of 16s amplicon

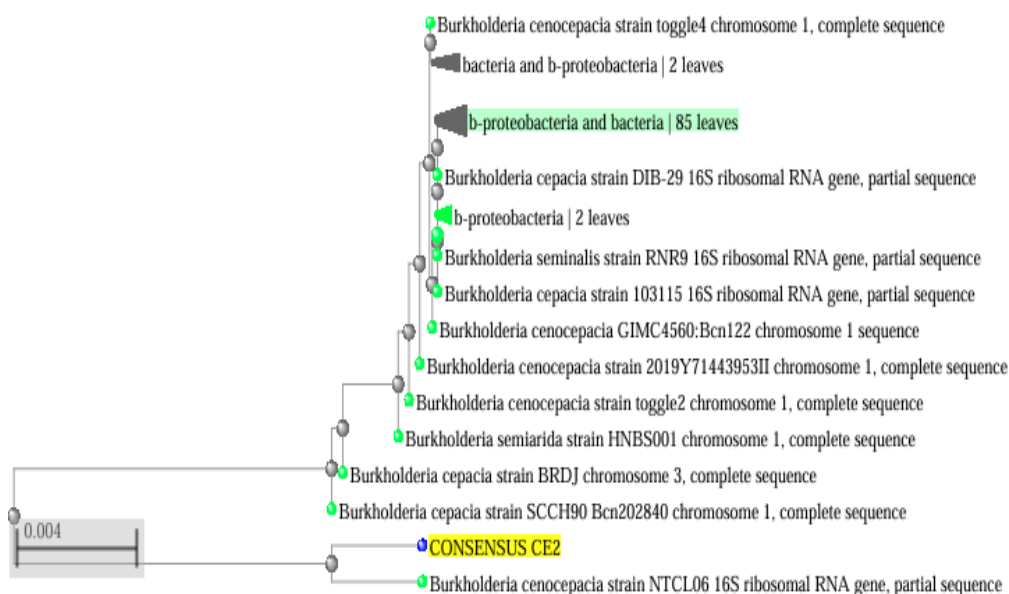


Figure 2 BLAST phylogeny tree

3.2 Optimization parameters

It was observed that the remediation of As^{3+} , Pb^{2+} , Zn^{2+} , or Cr^{6+} was effectively carried out by considering various factors, including the pH levels, metal concentrations, temperature, carbon source, and nitrogen source. Pertaining to pH, a wide range from 2 to 10 was examined. The results highlighted that pH 8 exhibited the highest level of degradation, while pH 2 and pH 10 showed maximum remediation potential as indicated in Fig 3. Notably, remediation rates remained above 65% for pH levels below 7 and peaked at around 72% for samples with a pH of 10. These findings underscore the significance of pH in influencing the efficiency of remediation processes for the specified metal ions, emphasizing the need for further exploration and understanding of these dynamics to optimize remediation strategies.

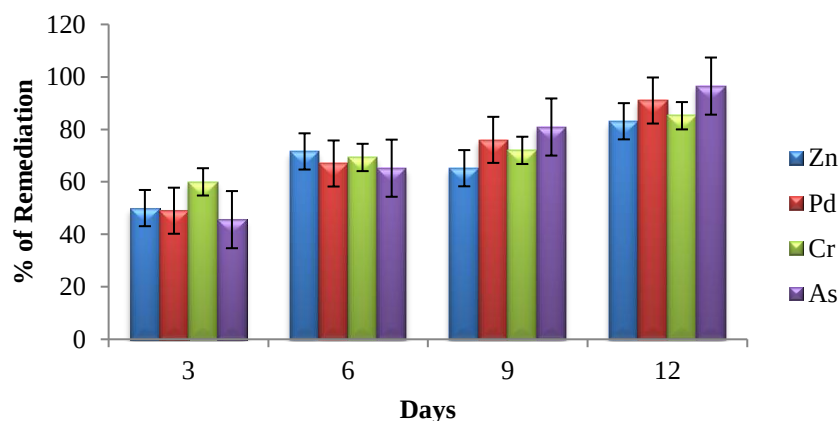


Figure 3 Optimization of As, Pb, Zn, or Cr at pH 8

At various temperatures, the effectiveness of the remediation process was notable. The highest level of remediation, approximately 62%, was observed at 35°C (Fig. 4). In comparison, temperatures below 30°C resulted in a lower remediation rate of around 56.8%, indicating a significant difference in efficacy. In contrast, temperatures exceeding 40°C exhibited a remediation rate similar to the peak at 62%, suggesting a consistent performance at higher thermal conditions. These findings underscore the critical influence of temperature on remediation efficiency, with distinct trends emerging across different temperature ranges.

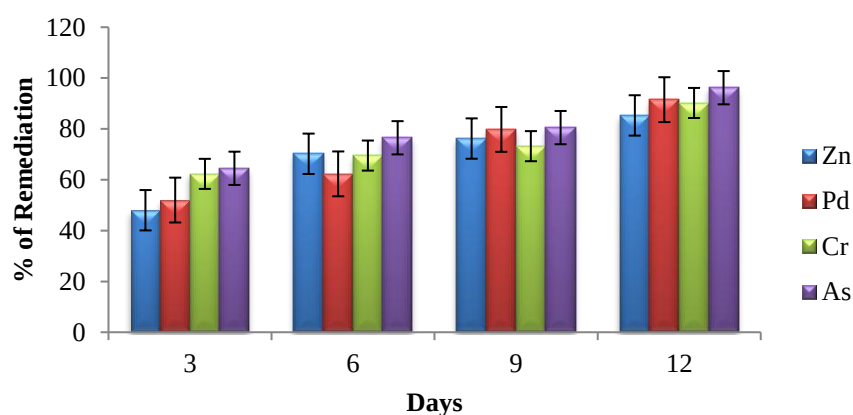


Figure 4 Optimization of As, Pb, Zn, or Cr at 35°C .

In Yeast extract, the highest levels of lead (Pb^{2+}), chromium (Cr^{6+}), arsenic (As^{3+}), and zinc (Zn^{2+}) remediation were achieved at a concentration of 0.3% in *Burkholderia cenocepacia* according to Fig. 5. Conversely, remediation percentages below 63.5% were observed at varying concentrations of 0.1%, 0.2%, 0.4%, and 0.5%. These findings highlight the significance of the 0.3% concentration in promoting efficient remediation of As^{3+} , Pb^{2+} , Zn^{2+} , or Cr^{6+} by *Burkholderia cenocepacia* in Yeast extract. Particularly, the trend of decreasing remediation efficacy as concentrations deviated from 0.3% underscores the delicate balance required for optimal remediation performance. Overall, these results emphasize the importance of careful concentration selection in bioremediation studies, as even slight deviations can significantly impact remediation efficiency and efficacy, as evidenced by the varying remediation percentages recorded across different concentration levels.

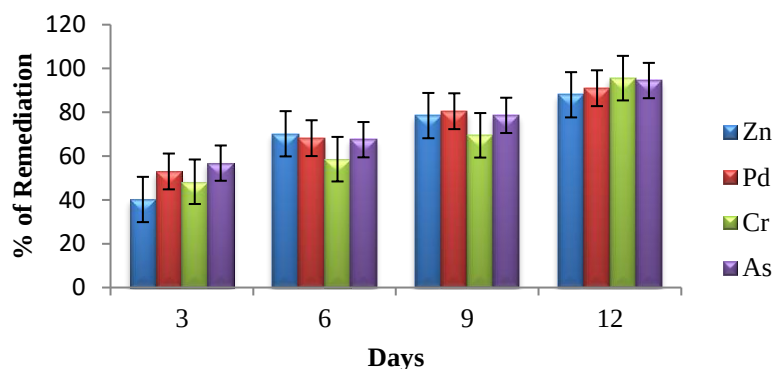


Figure 5 Optimization of As, Pb, Zn, or Cr at 0.3% of yeast extract

In the context of glucose's remediation impact, it is interesting to note that the most significant process of removing pollutants such as As^{3+} , Pb^{2+} , Zn^{2+} , or Cr^{6+} was observed at a concentration of 0.1% within the *Burkholderia cenocepacia* species, as depicted in Fig. 6. This particular concentration level resulted in an impressive remediation rate exceeding 70.3%, showcasing the efficacy of this specific condition. Conversely, the remediation rates dropped below the aforementioned threshold when the concentrations were varied to 0.2%, 0.3%, 0.4%, and 0.5%. This observation reinforces the notion that the remediation performance of *Burkholderia cenocepacia* is notably sensitive to changes in concentration, emphasizing the significance of maintaining the optimal levels for effective remediation. Additionally, these findings underscore the importance of focusing on the fine balance between concentration levels and the resulting remediation rates when utilizing *Burkholderia cenocepacia* for environmental cleanup applications. Understanding these dynamics can provide valuable insights for designing more targeted and efficient remediation strategies in glucose-rich environments contaminated by As^{3+} , Pb^{2+} , Zn^{2+} , or Cr^{6+} .

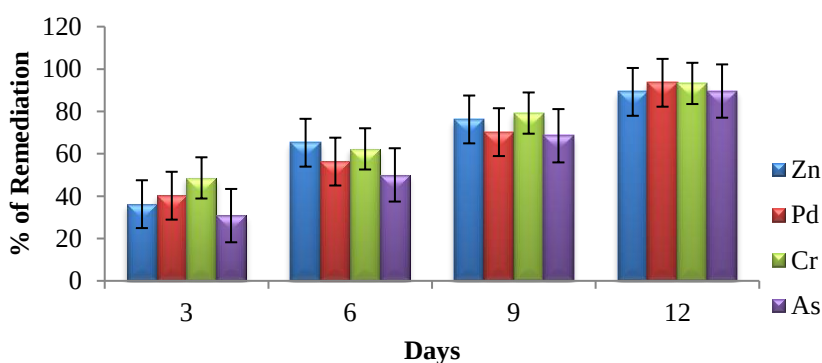


Figure 6 Optimization of As, Pb, Zn, or Cr at 0.1% of Glucose

In the context of the ammonium sulphate, the remediation process of As^{3+} , Pb^{2+} , Zn^{2+} , or Cr^{6+} was specifically investigated. Notably, the concentration of 0.1 was found to be particularly effective when employed in *Burkholderia cenocepacia*, as depicted in Fig. 7. The remediation rate achieved at this specific concentration was notably high, surpassing the 65.3% milestone. However, it is crucial to mention that at other concentrations - 0.2, 0.3, 0.4, and 0.5% - the remediation rates observed were consistently below the 65.3% threshold. This observation signifies the remarkable efficiency of the 0.1 concentration in facilitating the remediation process, showcasing *Burkholderia cenocepacia*'s unique ability to effectively mitigate the presence of these particular elements. The data presented in Graph 5 serves as a visual representation of the significant remediation outcomes achieved, underscoring the importance of precise concentration levels in optimizing remediation efforts. These findings shed light on the potential application of ammonium sulphate in addressing environmental contamination concerns associated with As^{3+} , Pb^{2+} , Zn^{2+} , or Cr^{6+} , highlighting the need for further research to enhance remediation techniques and strategies for optimal environmental management.

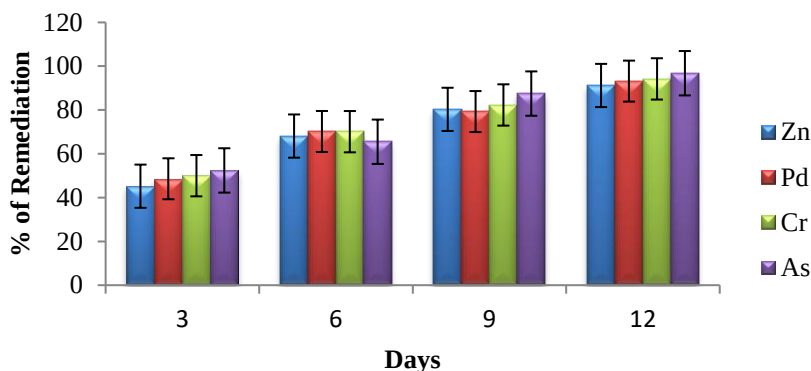


Figure. 7 Optimization of As, Pb, Zn, or Cr at 0.1% Ammonium sulphate

3.3 FTIR Analysis

An in-depth analysis was conducted using Fourier Transform Infrared Spectroscopy (FTIR) to identify the functional groups involved in the biosorption process of heavy metals such as arsenic (As^{3+}), lead (Pb^{2+}), zinc (Zn^{2+}), and chromium (Cr^{6+}). FTIR spectroscopy is a powerful analytical technique widely used to detect functional groups on the surface of biological materials based on their vibrational transitions when exposed to infrared radiation. The biosorption capability of biomass largely depends on the chemical nature and availability of these surface functional groups, which can interact with metal ions through mechanisms such as ion exchange, complexation, and coordination bonding [19, 20]. During the experiment, the biomass samples that had been exposed to metal ions were subjected to FTIR analysis. This allowed for the detection of changes in the spectra, which signify interactions between the functional groups on the biomass and the metal ions. The FTIR spectra revealed multiple absorption bands across a wide range of wavelengths (Figure 8 and Figure 9), each corresponding to specific functional groups. These bands reflect the vibrational energies of chemical bonds, which can shift or change in intensity when metal binding occurs. Prominent among the observed bands was one at approximately 3299.65 cm^{-1} , indicating the presence of bound or free hydroxyl (O–H) groups as well as stretching vibrations associated with amine (N–H) groups. The O–H group, often present in polysaccharides and phenolic compounds, plays a crucial role in biosorption by forming hydrogen bonds or coordinating with metal ions [21]. The N–H stretching in this region can be attributed to the presence of amino or amide groups, which are known to form stable complexes with heavy metal ions, enhancing the biosorption capacity of the biomass [22]. In addition to these groups, FTIR spectra identified several other peaks corresponding to functional groups involved in metal ion binding. These included carboxyl ($-\text{COOH}$) groups, which are known for their high affinity for divalent and trivalent metal ions due to their ability to donate electron pairs [23]. Amide groups ($-\text{CONH}-$) were also observed, likely originating from proteins or peptides present in the biomass. These groups participate in metal coordination through lone pairs on oxygen and nitrogen atoms. Aliphatic ($-\text{CH}_2$) and aromatic ($\text{C}=\text{C}$) structures were detected as well, indicating the presence of lipids or aromatic compounds, which can interact with metals via π -electron systems. Moreover, the spectra showed peaks related to alkenes ($\text{C}=\text{C}$), which may also contribute to biosorption through weak interactions or structural support. The presence and shifts of these functional group peaks before and after metal exposure strongly support the conclusion that these groups were involved in binding metal ions. The variation in band intensities and positions after metal exposure suggests that biosorption occurs primarily through the interaction of these functional groups with metal ions, which alters their vibrational characteristics. This detailed FTIR analysis, therefore, provides critical insights into the biochemical mechanisms underlying the biosorption of As^{3+} , Pb^{2+} , Zn^{2+} , or Cr^{6+} by the selected biomass.

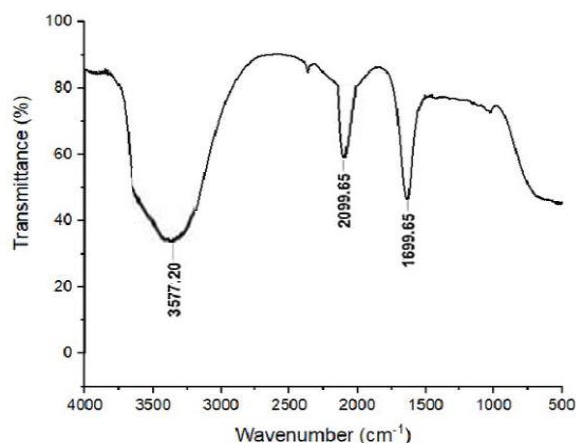


Figure 8 Metal Free Bacterial Sample

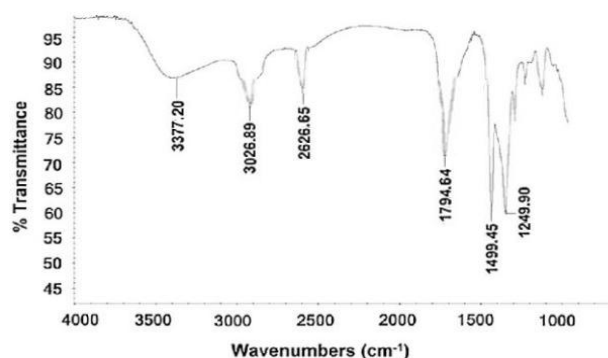


Figure 9 Metal Loaded bacterial Sample

Table 1 Different assigned peaks in FTIR spectra for metal loaded and non metal loaded bacterial sample.

Pick No.	Group frequency	Bond	Assignment
1.	1249.90	CH_2	Alkene
2.	1499.45	C-O stretching	Carbonyl
3.	1699.65	C-O stretching	Carbonyl
4.	1794.64	C=N	Imine and Oxime
5.	2099.65	C=N	Imine and Oxime
6.	2626.65	C-H stretch	Aldehyde
7.	3026.89	N-H	Amine
8.	3377.20	C=O, N-H	Amine
9.	3577.20	C=O	Alcohol

3.4 XRD and EDX Analysis

Figure 10 presents the X-ray diffraction (XRD) profiles obtained from *Burkholderia cenocepacia* samples exposed to heavy metals namely As^{3+} , Pb^{2+} , Zn^{2+} , and Cr^{6+} as well as an untreated control. XRD is a powerful analytical technique used to determine the crystalline structure of materials by measuring the diffraction patterns of X-rays passing through a sample. In biosorption studies, XRD can help identify whether heavy metal ions interact with the microbial surface to form crystalline complexes, precipitates, or biomineralized structures [24].

The XRD pattern of the untreated control sample showed no significant or sharp diffraction peaks, suggesting that the surface of *B. cepacia* cells in their native state lacks any organized crystalline structures that are detectable by XRD. This amorphous profile is typical of biological samples, where the organic nature of cellular components (proteins, lipids, polysaccharides) results in a lack of crystallinity [25].

In contrast, the XRD analysis of *B. cepacia* biomass that had been treated with As^{3+} , Pb^{2+} , Zn^{2+} , or Cr^{6+} revealed the presence of six distinct diffraction peaks, observed at 2θ values of approximately 15.56° , 29.85° , 33.52° , 41.20° , 49.80° , and 55.61° . These peaks were clearly distinguishable and indicate the formation of crystalline phases as a result of interaction between the metal ions and the biosorbent surface. These peaks correspond to

crystallographic planes with Miller indices (hkl) of 100, 111, 200, 220, 311, and 102, respectively. Such indices are typically associated with crystalline structures of metal salts or complexes that may have formed due to biosorption or bioaccumulation processes [26].

To further validate the identity of the crystalline materials formed on the surface of the biomass, the d-spacing values (interplanar distances) were calculated and compared with standard patterns provided by the Joint Committee on Powder Diffraction Standards (JCPDS) database. The computed d-spacing values were approximately 4.21, 3.49, 2.43, 3.69, 3.82, and 3.56 Å. These values demonstrated a close match with those associated with compounds such as zinc oxide sulfate ($\text{ZnSO}_4 \cdot x\text{H}_2\text{O}$), zinc sulfate (ZnSO_4), and various sulfur-based compounds. This matching suggests that Zn^{2+} , in particular, may have undergone chemical transformation or complexation during biosorption, resulting in the formation of detectable crystalline precipitates on the bacterial surface [27].

The appearance of these specific peaks in metal-loaded *B. cenocepacia* cells supports the hypothesis that biosorption may not only involve surface binding via functional groups but could also lead to mineral precipitation or intracellular sequestration of heavy metals, depending on the chemical environment and the redox potential of the system. In biosorption literature, such findings reinforce the multifunctional role of microbes in bioremediation: they can bind, transform, and sometimes even detoxify heavy metals through biologically mediated mineralization processes [28].

Thus, the results derived from the XRD analysis provide strong evidence for the successful biosorption and probable biotransformation of As^{3+} , Pb^{2+} , Zn^{2+} , and Cr^{6+} ions by *B. cenocepacia*, showcasing its potential for use in environmental cleanup applications involving toxic heavy metals.

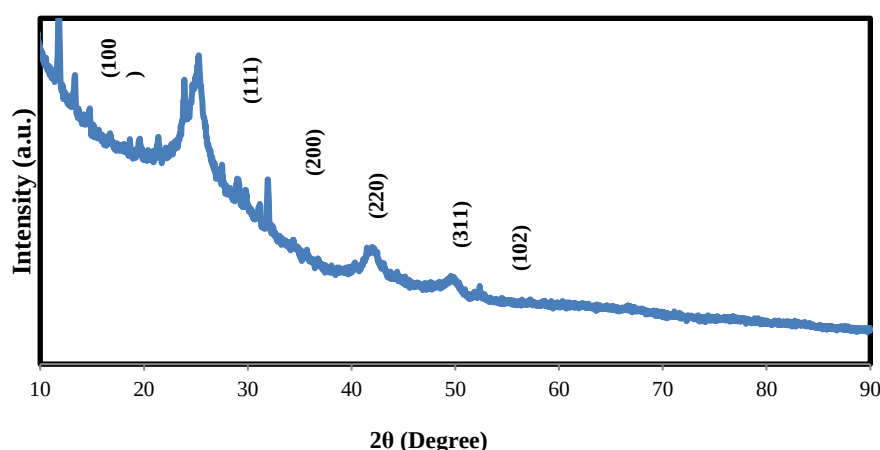


Figure 10 XRD analysis of bacterial Sample

The presence of adsorbed metals on the cell surfaces of *Burkholderia cenocepacia* was confirmed by the energy-dispersive X-ray spectroscopy (EDX) investigation, which can be shown in Figure 11. As shown in Figure 5, the quantified results, which illustrate the distribution of the four metals on the cell surfaces of *Burkholderia cenocepacia*, are provided. Among these four metals, Cr^{6+} was the most prevalent on the surface of the cell of *Burkholderia cenocepacia*, followed by As^{3+} , Pb^{2+} , and Zn^{2+} .

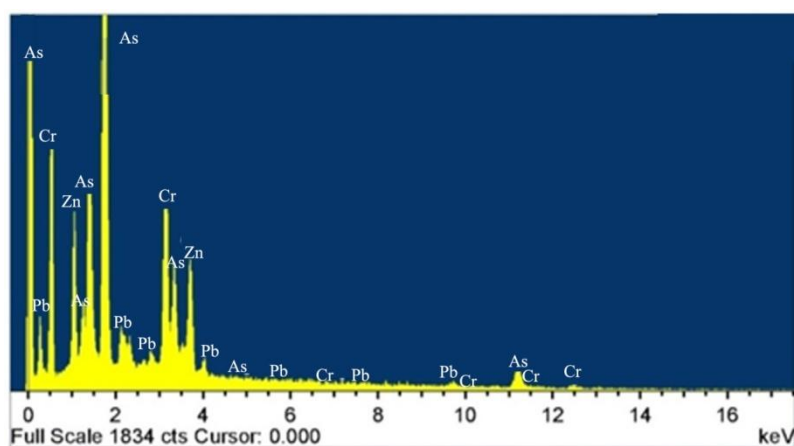


Figure 11 EDX analysis bacterial Sample

4. Conclusion

The bacterial strain in the study effectively removed various metal cations. The strain was suited for possible use in metal remediation in bioreactors or in situ applications since it demonstrated the ability to remove heavy metals and to flourish throughout a broad range of metal concentrations. Because strains have more and easier access to carboxyl and amide functional groups which are essential for metal binding they have shown effective metal removal. This study demonstrated that when several metals are present in an aqueous solution at the same time, the extracellular polymeric substance (EPS) may block metal adsorption, which lowers total metal uptake. The interactions between metal ions and functional groups on the strain's surface were validated by FTIR, XRD, and EDX techniques. When taken as a whole, these results improve our knowledge of the bacterial strain's capacity to remove metals and adapt. They highlight the potential of these strains for use in treating metal-contaminated effluents for environmental remediation. The study also highlights how important it is to customize strain selection to the unique metal composition of effluent environments in order to improve the efficacy and efficiency of metal removal techniques.

5. Author Contributions

SN Praveena Yadavalli: Investigation, Formal analysis, Data curation. Sreenivasulu Kamma: Writing – review & editing, Validation, Formal analysis, Data curation. Debasish Sahoo: Writing – review & editing, Writing – original draft, Visualization, Supervision, Conceptualization.

6. Conflict of Interest

The authors have declared that no conflict of interest exists.

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