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Screening The Bacterial Isolates From Rice Rhizosphere Soil For Lead Bioremediation

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Abstract

Heavy metal contamination of industrial effluent by lead (Pb), Nickel (Ni) chromium (Cr) presents major risks to human health and the environment. This study aims to screen the bacterial isolates (available at P2BL, Osmania University) from rice rhizosphere for tolerance to heavy metals such as lead, nickel, chromium. The strains that were identified previously as *Mesobacillus subterraneus* GHM58, *Pseudomonas migulae* GHK43, *Microbacterium hydrocarbonoxydans* GHQ55, *Brevundimonas nasdae* GHM62, were isolated from rice rhizosphere. The bacterial isolate's growth and lead tolerance were monitored at concentrations of 125 ppm, 250 ppm, and 500 ppm of lead. Growth measurements were taken at 8, 16, 24, and 32 hours. The bacteria strain GHM58, GHQ55. and showed the strongest resistance to lead, using minimum inhibitory concentration (MIC) tests to tolerate doses as high as 500 ppm/L key functional groups involved in metal biosorption, including hydroxyl, carboxyl, phosphate, and amide, were identified by FTIR analysis of the supernatants were collected after 24 and 48 hours. These results demonstrate bacterial strains. and remarkable lead tolerance and demonstrate its potential for use in bioremediation.

Keywords: Heavy metals, lead, bioremediation, Industrial effluents, UV-visible spectrophotometer, FTIR.

1.Introduction

The extensive use of non-biodegradable and toxic metals like chromium (Cr), lead (Pb), and nickel (Ni) in mining, tanning, battery manufacturing, and electroplating has made heavy metal pollution a serious worldwide environmental concern. According to (Singh et al., 2024), traditional clean up techniques are typically costly and have the potential to produce secondary contaminants, notwithstanding their effectiveness. On the other hand, microbial bioremediation uses the inherent detoxifying properties of microorganisms (Zhang et al., 2025) to provide a sustainable and environmentally beneficial alternative (Jabborova et al., 2025).

According to (Mnif et al., 2023), microbes have developed a variety of resistance mechanisms that allow them to survive in settings contaminated with metals, such as biosorption, bioaccumulation, enzymatic transformation, and efflux systems. Bacteria with a high tolerance and capacity for biosorption of Cr, Pb, and Ni include *Pseudomonas*, *Bacillus*, and *Enterobacter*. Chromate-reducing bacteria, for example, change the poisonous Cr (VI) into the less mobile Cr(III), and fungal species such as *Penicillium expansum* use functional groups in extracellular polymeric compounds to biosorb (Adeniran et al., 2024). Similarly, negatively charged cell wall components and EPS are used by Pb-tolerant bacteria to chelate or precipitate Pb(II) (Moeini et al., 2024).

Nickel-resistant strains that can immobilize the metal by ion exchange and siderophore synthesis have been found in nickel-contaminated areas. Along with providing integrated solutions for heavy metal detoxification, recent research also highlights the improved remediation efficiency attained through microbe biochar pairings (Binbin et al., 2025).

2.Material and Methods

2.1. Sample collection

All chemicals and media used for the isolation and identification of lead-tolerant bacteria were of analytical grade. The following reagents were used without further modification: Lead Acetate Trihydrate ($C_4H_6Pb.3H_2O$), Nutrient Broth, and Agar Agar (HiMedia); Nickel (II) Sulfate Heptahydrate ($NiSO_4.7H_2O$) (Merck); and Potassium Dichromate ($K_2Cr_2O_7$) (Fisher Scientific). Bacterial isolates were obtained from the (available at P2BL, Osmania Univeristy) from rice rhizosphere. The identified isolates included strains such as GHM58, GHK43, GHQ55 and GHM62 (Humera et al., 2024)

2.2. Minimum inhibitory concentration (MIC)

To assess their minimum inhibitory concentration (MIC), the bacterial strains were exposed to metal stress using Lead Acetate Trihydrate ($C_4H_6Pb.3H_2O$), Nickel (II) Sulfate Heptahydrate ($NiSO_4.7H_2O$), and Potassium Dichromate ($K_2Cr_2O_7$). Sterilized nutrient agar plates was supplemented with 500 ppm of each metal salt and allowed to solidify. Bacterial cultures were then spot-inoculated onto the metal-amended agar plates and incubated at 37°C. Out of the

four strains tested, three bacterial strains (GHM58, GHQ55 and GHK62) demonstrated notable metal tolerance (Table.3.1.) and were selected for further investigation.

2.4. Growth of the bacteria in nutrient broth amended with Lead

A growth curve experiment was used to analyze the bacterial growth pattern in the presence of lead. The nutrient broth of 15 ml was placed into 50-ml boiling tubes, sterilized, and lead was added to the broth at concentrations of 125, 250, and 500 ppm. The actively growing bacterial culture were inoculated with 1%

(v/v). All tubes were appropriately labelled and incubated at 37°C in a shaking incubator (150 rpm). Bacterial growth was recorded by measuring optical density at 600 nm (OD_{600}) at 8-hour intervals, including at 8, 16, 24, and 32 hours.

2.4. FTIR analysis

Fourier Transform Infrared (FTIR) spectroscopy was performed to identify functional groups present in the bacterial biomass at 24 and 48 hours. The supernatant obtained from the lead treatment experiment was submitted to CFRD, Osmania university for FTIR analysis. FTIR is a valuable technique for detecting functional groups in bacterial cells, providing insights into their potential metal-binding capabilities (Muhammad et, al., 2023). These analyses contribute to understanding the interaction between bacterial strains and lead ions, as well as their uptake mechanisms from the environment. These temporal changes in FTIR profiles suggest dynamic modifications in bacterial surface chemistry in response to lead stress. Functional groups such as hydroxyl, carboxyl, and amine moieties are known to play critical roles in biosorption and bioaccumulation, facilitating metal ion complexation and detoxification (Jianlong et al., 2009).

3.Results

The bacterial isolates from rice rhizosphere strains tolerance to Heavy metals such as Pb, Cr and Ni are Shown in the Table 3.1.

Table.3.1. Screening of bacteria available from P2BL and isolates from rice rhizosphere soil

S No	Bacterial Isolate/ Strain	500 ppm of heavy metal		
		Pb	**Cr**	**Ni**
1	*M. subterraneus strain* GHM58	+ + +	+	-
2	*P. migulae strain* GHK43	+ ++	+ +	-
3	*M. hydrocarbonoxydans strain* GHQ55	+ + +	+	+
4	*B. nasdae* strain GHM62	+++	+	-

The heavy metal tolerance at 500 ppm for different bacterial strains are shown in Fig.1.2 & 3.

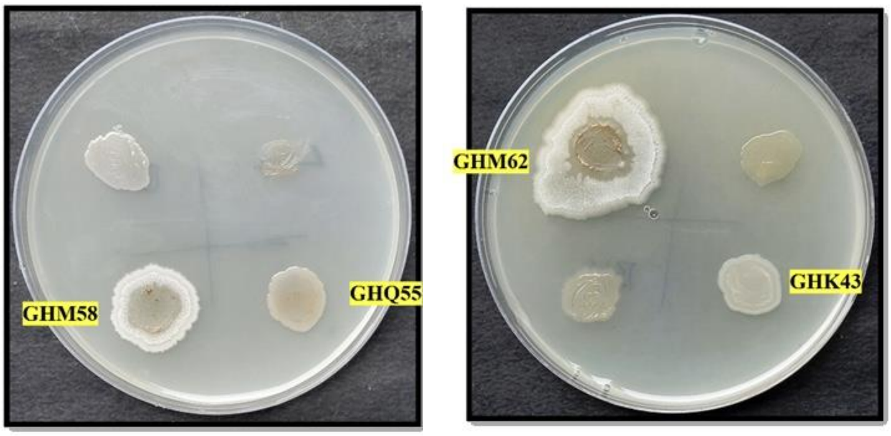


Fig.1. Heavy metal tolerance at 500 ppm of Lead (Pb) for different bacterial strains

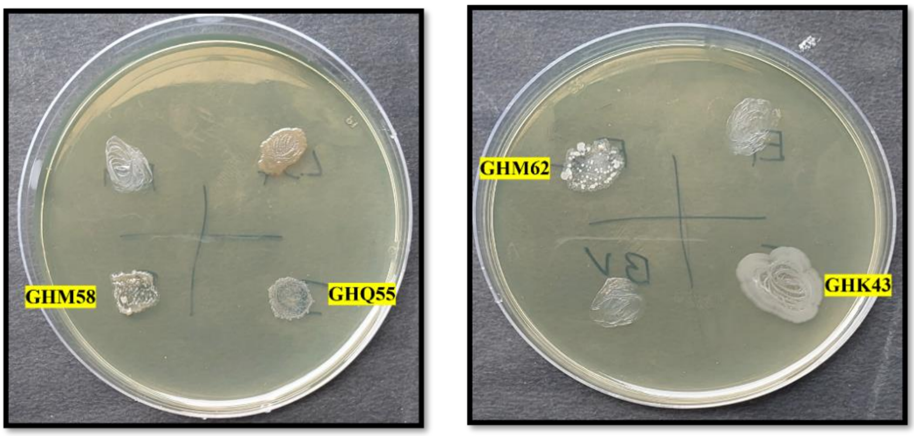


Fig.2. Heavy metal tolerance at 500 ppm of Chromium (Cr) for different bacterial strains

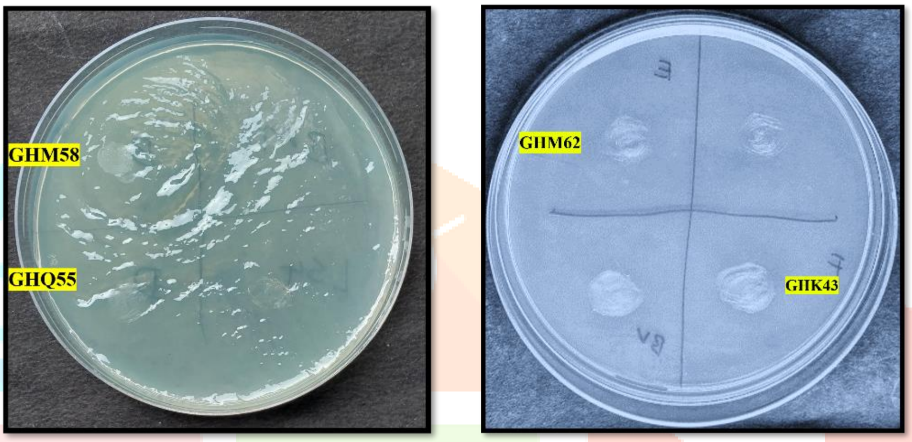


Fig.3. Heavy metal tolerance at 500 ppm of Nickel (Ni) for different bacterial strain

3.1. Minimum inhibitory concentrations (MICs)

The strains GHM58, GHK43, GHQ55 and GHM62, showed significant resistance up to 500 ppm to Pb ($C_4H_6Pb.3H_2O$), and growth can be visualized on plates (Fig.1). GHK43 strain showed moderate resistance up to 500 ppm to Cr ($K_2Cr_2O_7$) plates (Fig.2). No growth was observed on the Ni ($NiSO_4.7H_2O$) plates (Fig.3). The bacterial strains GHM58, GHQ55 was capable to tolerate 2000 ppm to Pb ($C_4H_6Pb.3H_2O$).

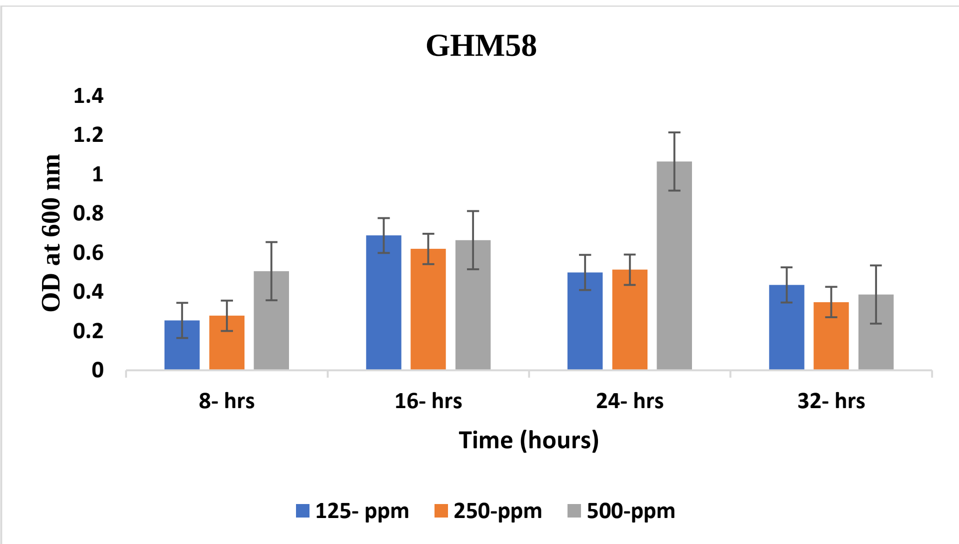


Figure.4. Growth of *M. subterraneus* in nutrient broth amended with Lead

The bacterial strain GHM58 exhibited the maximum growth at 500 ppm, reaching OD_{600} ~1.1 at 24 hours, showing good lead tolerance. At 125 ppm, growth was moderate, but 250 ppm indicated slower growth and an earlier decline (Fig. 4).

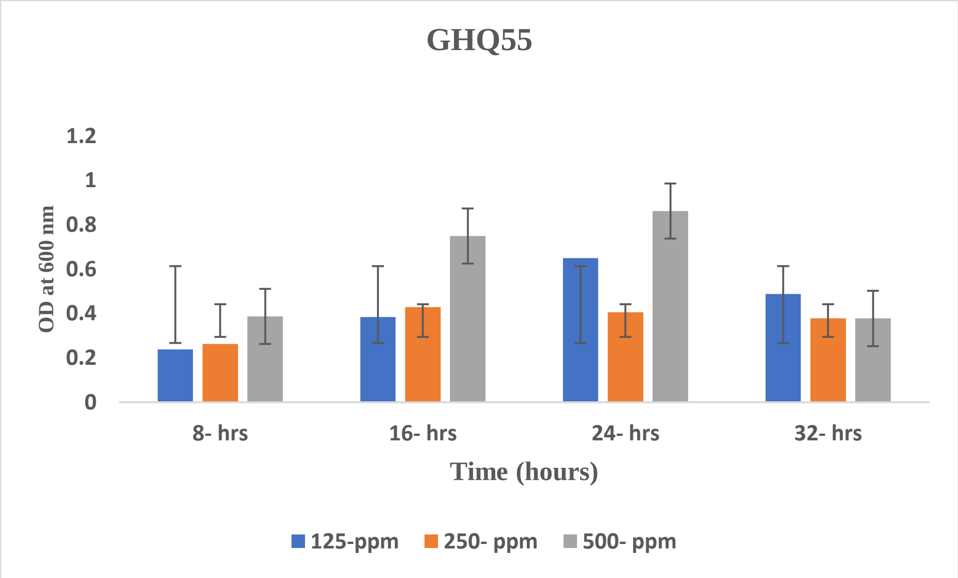


Figure.5. Growth of *M. hydrocarbonoxydans* in nutrient broth amended with Lead

The bacterial strain GHQ55 in Lead-amended broth (OD_{600}) at 125 ppm is gradual increased, max OD (~0.65) at 24 h, and moderate fall at 32 h (~0.5) observed. At 250 ppm, growth is slow at around 0.45 after 16-24 hours. At 500 ppm, the growth occurs, with a peak OD (~0.9) at 24 h and a droped (~0.4) at 32 hours (Fig. 5).

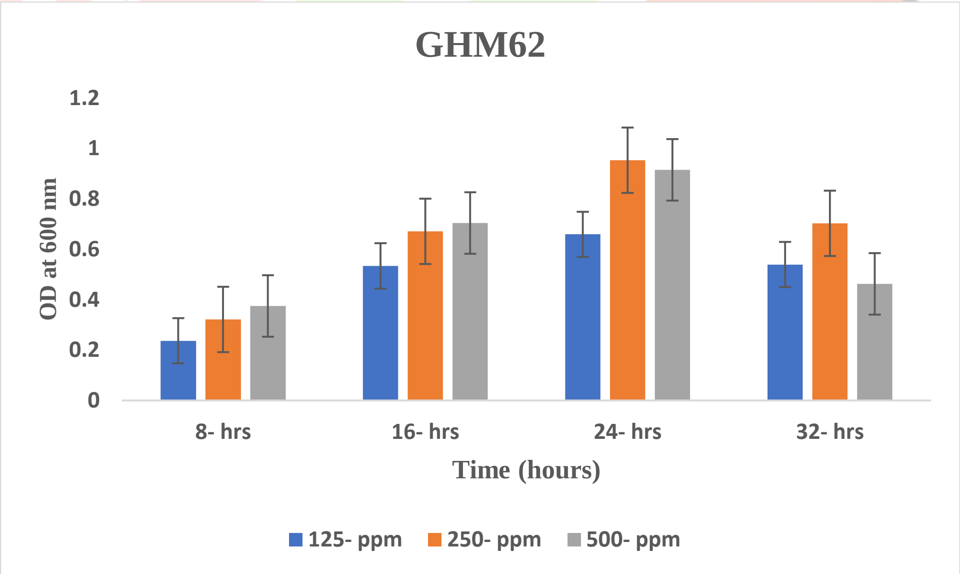


Figure.6. Growth of *B. nasdae* in nutrient broth amended with Lead

The bacterial strain GHM62 in Lead-amended broth (OD_{600}) at 125 ppm growth from ~0.25 (8 h) to ~0.65 (24 h), with a little decrease at 32 h (~0.55) obsevred. The increased the growth 250 ppm, peaking at 24 hours (~0.95) and maintaining a moderately high OD (~0.7) at 32 hours. 500 ppm showed the growth, peaking at ~0.9 at 24 hours, then falling to ~0.5 at 32 hours (Fig. 6).

3.2. FTIR analysis

Fourier Transform Infrared (FTIR) spectroscopy was employed to investigate functional groups associated with the bacterial culture supernatants of *strain* GHM58, strain GHQ55, strain GHM62 are exposed to Lead ($C_4H_6Pb.3H_2O$) at 500ppm concentration amended in nutrient broth for24 hr and 48 hr. The spectra revealed several peaks corresponding to biomolecular groups potentially involved in metal interaction and sequestration of the lead.

The bacterial strain GHM58 at 24 hours, showed prominent peaks at ~3371 cm^{-1} and ~2933 cm^{-1}, indicating O–H/N–H and C–H stretching vibrations of hydroxyl, amine and aliphatic sidechains of amino acids respectively (Fig.7). The strong absorption at 1639 cm^{-1} reflects the presence of amide I or carboxyl groups, suggesting a role for proteins or acidic amino acids in metal interaction. Peaks at 1381 and 1231 cm^{-1} further support the presence of carboxyl and amine groups involved in biosorption.

a

b

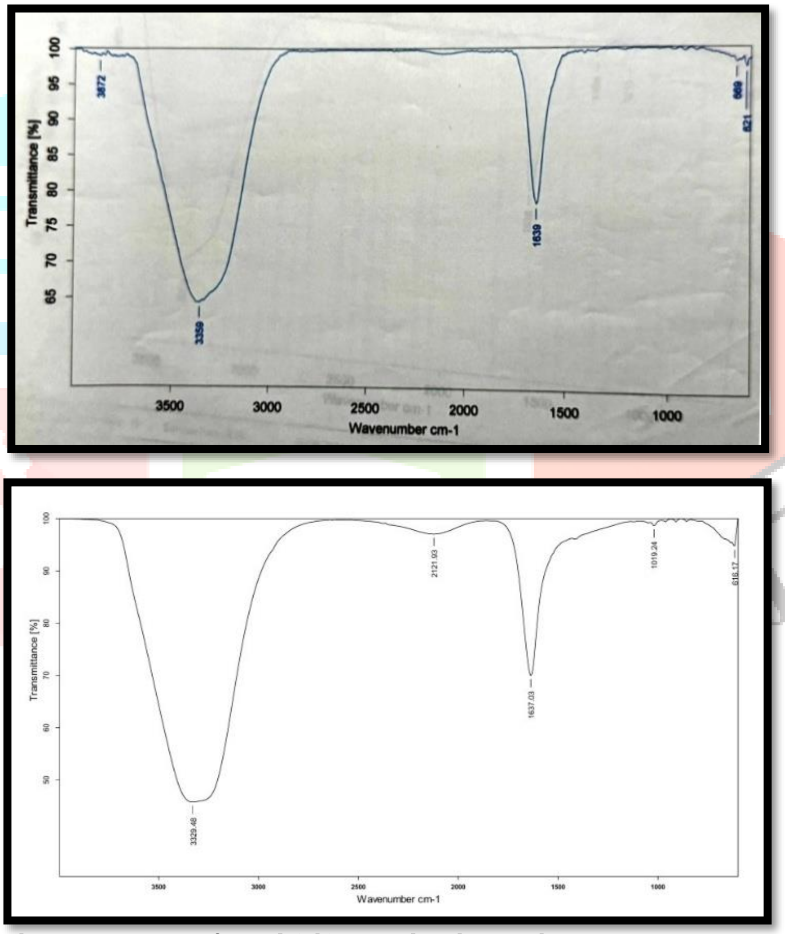
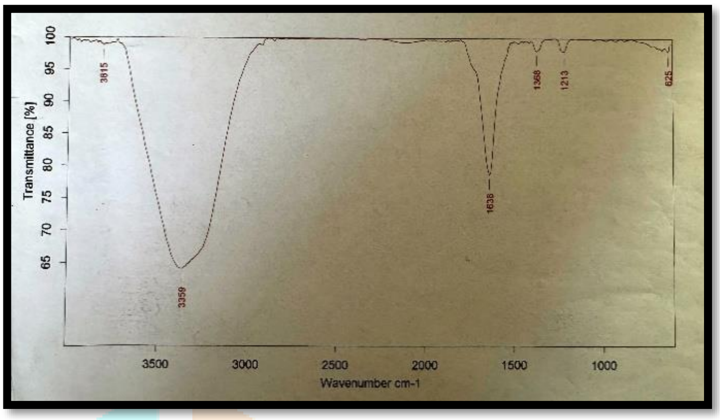


Figure:7. The FTIR spectra of metal (Pb) treated with *M. subterraneus* GHM58 at 24 hours(a) 48 hours(b)

The bacterial strain GHM58 at after 48 hours, shifts in the peaks were observed at 3283 cm^{-1} replacing the 3371 cm^{-1} band, and the appearance of a sharp metal-ligand-associated peak at 470 cm^{-1}, indicative of enhanced metal complexation over time. The emergence of a strong band at 1115 cm^{-1} may correspond to phosphate or C–N stretching, reflecting possible phosphate-metal interactions or metabolic changes under stress. These findings strongly suggest that functional groups such as hydroxyl, amine, carboxylate, and phosphate moieties in bacterial biomolecules play key roles in lead binding via biosorption and extracellular complexation mechanisms.

a



b

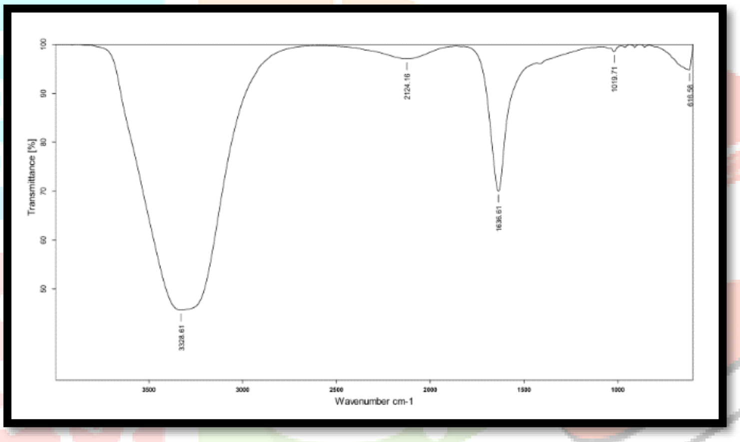


Figure.8. The FTIR spectra of metal (Pb) treated with *M. hydrocarbonoxydans* GHM55 at 24 hours(a) 48 hours(b)

The bacterial strain GHM55 after 24 hours in FTIR spectra (Fig. 8) showed broad absorption bands at 3915 cm^{-1} and 3300 cm^{-1} correspond to –OH and –NH stretching vibrations, indicating the presence of alcohol or amine groups. A strong band at 1633 cm^{-1} was assigned to the amide I or carboxylate groups, suggesting the involvement of proteins and acidic residues in metal binding. Additional peaks at 1250 cm^{-1}, 1213 cm^{-1}, and 624 cm^{-1} were associated with C–N, P=O, and metal–oxygen (M–O) bonds, respectively, indicating the participation of phosphate groups and bacterial exopolysaccharides in lead complexation.

The bacterial strain GHM55 after 48 hours in FTIR spectra showed with slight shifts indicating sustained and perhaps enhanced interaction with lead ions. Peaks at 3285 cm^{-1} (–OH/–NH), 1633 cm^{-1} (C=O/COO^-), and 1427 cm^{-1} (symmetric COO^- stretch) were evident, alongside a distinct band at 474 cm^{-1}, indicative of metal–oxygen bonding. These findings strongly suggest that functional groups such as hydroxyl, amine, carboxylate, and phosphate moieties in bacterial biomolecules play key roles in lead binding via biosorption and extracellular complexation mechanisms.

The bacterial strain GHM62 after 24hr in FTIR showed a broad peak at 3368 cm⁻¹ indicating hydroxyl or amine stretching vibrations, while a peak at 2124 cm⁻¹ may represent C≡C or C≡N groups. Strong bands at 1749 cm⁻¹ and 1639 cm⁻¹ correspond to carbonyl (C=O) and amide linkages, suggesting protein involvement in Pb^{2+} binding. Peaks at 1384, 1213, and 670 cm⁻¹ indicated the presence of carboxyl, phosphate, and metal–oxygen interactions, supporting the role of bacterial biomolecules in biosorption.

a

b

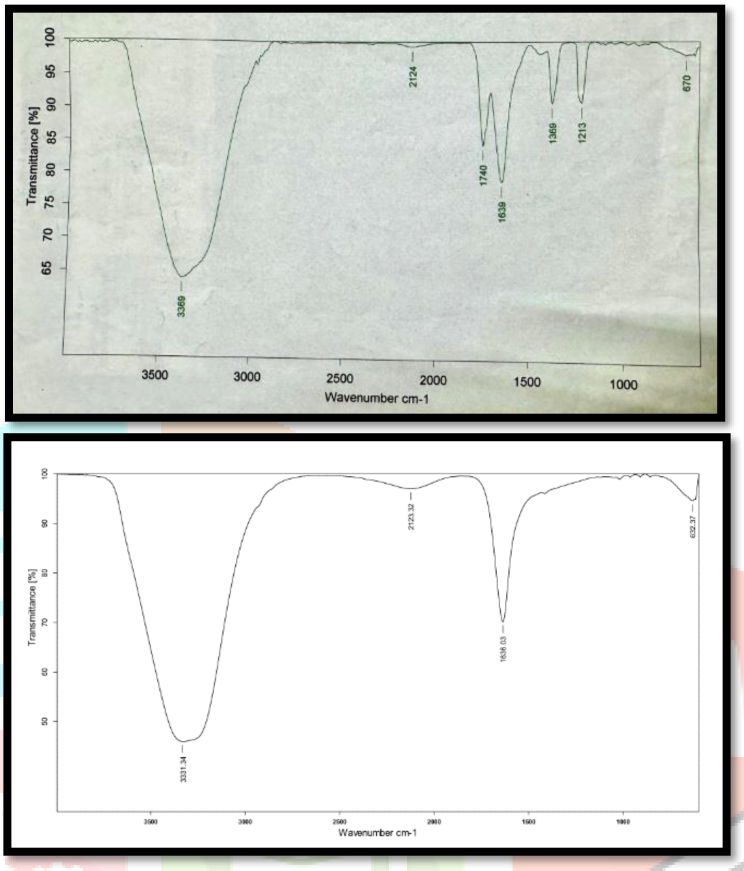


Figure.9. The FTIR spectra of metal (Pb) treated with *B. nasdae* GHM62 at 24 hours(a) 48 hours(b)

The bacterial strain GHM62 after 48 hr in FTIR showed persistent peaks at 3283 cm⁻¹ (O–H/N–H stretching), 2923 cm⁻¹ (C–H stretching), and 1633 cm⁻¹ (amide I or C=O stretching), indicating continuous involvement of proteins and polysaccharides in lead ion interaction. A new band at 474 cm⁻¹ suggested potential Pb–O bonding, (Fig. 9) highlighting increased metal complexation over time. These shifts confirm the dynamic role of bacterial biomolecules in Pb^{2+} biosorption.

FTIR spectra revealed significant functional group interactions involved in Pb^{2+} biosorption across all three cultures. Broad peaks in the range of 3280–3368 cm⁻¹ indicated O–H and N–H stretching, characteristic of hydroxyl and amine groups. Peaks between 1633–1650 cm⁻¹ corresponded to amide I (C=O), suggesting protein involvement. Additional peaks at 1384 cm⁻¹ (C–H bending) and 1213 cm⁻¹ (P=O stretching) reflected the role of lipids and phosphates. Notably, new peaks near 470–670 cm⁻¹ in 48-hour samples signified Pb–O bonding, indicating active metal complexation. The intensification or shift of peaks over time highlighted increased microbial interaction with lead ions.

4. Discussion

The tolerance and biosorption behavior of bacterial *strain* GHM58, strain GHQ55, and strain GHM62 under heavy metal stress particularly lead (Pb^{2+}) were evident from both MIC and growth profile assessments. All three cultures exhibited substantial resistance to Pb, with visible growth observed up to 500 ppm Pb ($C_4H_6Pb.3H_2O$) on solid media (Fig.1). Notably, the *strain* GHM58 and strain GHQ55 demonstrated even higher tolerance, sustaining growth at concentrations up to 2000 ppm, indicating intrinsic resistance mechanisms, possibly mediated by extracellular sequestration or efflux systems (Jianlong et al., 2009). In contrast, the strain GHM62 showed moderate tolerance to Cr ($K_2Cr_2O_7$), also sustaining growth up to 500 ppm (Fig.2), but none of the cultures showed growth on $NiSO_4{\cdot}7H_2O$ plates, suggesting limited or absent resistance mechanisms for Ni^{2+} (Fig.3).

Growth kinetics under various Pb concentrations further substantiated differential tolerance and adaptation strategies among the *strain* GHM58 exhibited concentration-dependent growth, with lag phases extending to 8 hours for all treatments. At 500 ppm, OD_{600} values remained low initially but increased sharply (~1.2) by 24 hours, indicating a delayed but robust exponential phase, possibly due to an initial stress response followed by physiological acclimation. In contrast, 125 ppm and 250 ppm treatments peaked earlier (~0.65–0.70 at 16 hours) and then declined, suggesting transient adaptation followed by nutrient limitation or toxicity (Gupta et al., 2008) (Jabborova et al., 2025).

The strain GHQ55 also demonstrated strong tolerance at 500 ppm, with early signs of adaptation evident by 8 hours and the highest OD (~0.9) observed at 24 hours. This aligns with studies suggesting that bacterial populations can enter active metabolic states even under metal-induced oxidative stress. Interestingly, the strain GHM62's growth was most stable at 250 ppm, where OD values remained high (~0.95 at 24 hours and ~0.7 at 32 hours), while growth at 500 ppm peaked earlier and declined by 32 hours (~0.45), suggesting possible late-phase toxicity or exhaustion of detoxification capacity.

In line with the observed growth patterns under lead stress, FTIR spectral analysis clearly demonstrated the role of functional groups in the biosorption of Pb2+ by bacterial strains. The presence of hydroxyl, amine, and protein-associated groups in metal binding was demonstrated by the detection of distinctive peaks in all cultures that corresponded to O–H and N–H stretching vibrations (3280–3368 cm^{-1}), amide I bands (1633–1650 cm^{-1}), and C–H bending (~1384 cm^{-1}). These categories are frequently linked to biomolecules found on the surface of bacteria, including proteins, lipids, and extracellular polymeric substances (EPS), which aid in the sequestration of metals via complexation and ion exchange (K Vijayaraghavan et al., 2008).

In both the 24- and 48-hour FTIR spectra, phosphate-related vibrations were noticeable, especially P=O stretching, which was seen at about 1213–1250 cm^{-1}. According to Liu et al. (2010), these peaks are indicative of the active binding of Pb2+ by phosphate-rich biomolecules, which may be sourced from EPS or cell wall components. Additionally, metal–oxygen (Pb–O) stretching vibrations are responsible for the appearance of discrete bands between 470 and 670 cm^{-1} at 48 hours, particularly close to 474 cm^{-1} in GHQ58 and 624 cm^{-1} in GHQ55. According to (Firinca et al., 2023), these bands corroborate the idea of gradual biosorption over time by showing signs of Pb2+ complexation and stability.

At 24 hours, strain GHQ58's FTIR spectra had notable peaks at 3915, 3300, 1633, 1250, and 624 cm^{-1}. At 48 hours, however, changes were noted to 3285, 2342, 1633, 1427, and 474 cm^{-1}. These spectral characteristics verify that hydroxyl, amine, carbonyl, and phosphate groups are still involved in the Pb2+ interaction. The presence of proteinaceous residues is indicated by the significant peak at 1633 cm^{-1}, which is associated with amide I or carboxylate stretching. Similarly, substantial absorption bands at 3915 and 3300 cm^{-1} in GHQ55 correspond to –OH and –NH groups. The bands at 1250 cm^{-1} (C–N), 1213 cm^{-1} (P=O), and 624 cm^{-1} (M–O) provide additional evidence that bacterial extracellular polysaccharides (EPS) and biomolecules containing phosphate are involved in metal binding (Fig. 8) (K Vijayaraghavan et al., 2008).

An explanation of time-dependent metal uptake is supported by the temporal shift and intensification of FTIR peaks across time points. In this concept, surface-bound functional groups first interact with Pb2+, then stabilize through complex formation. These results are consistent with earlier research showing that microbial biomass demonstrated improved Pb2+ adsorption through amide, phosphate, and hydroxyl groups (Gupta et al., 2008). A promising contender for bioremediation applications, GHM62's progressive intensification of metal-binding peaks indicates effective biosorption capabilities. The FTIR findings taken together support the bacterial surface chemistry's pivotal involvement in lead biosorption and provide proof of these strains' capacity for bioremediation.

5.Conclusion

Overall, the findings suggest that biosorption mechanisms in the *strain* GHM58, strain GHQ55 and the strain GHM62 are time- and concentration-dependent, involving multiple functional groups with potential coordination to Pb^{2+} ions. These results align with previous studies that highlighted the role of microbial surface chemistry in metal resistance and biosorption. These three strains GHM58, strain GHQ55, GHM62 demonstrated relatively more stable Pb^{2+} uptake at moderate concentrations, indicating its potential for bioremediation under controlled exposure levels.

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