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Evaluation of tolerance and reduction capabilities of some bacterial isolates to lead

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Abstract

Lead contamination from mining activities poses significant environmental and health risks, necessitating effective remediation strategies. This study evaluates the lead tolerance and reduction capabilities of five bacterial isolates—*Pseudomonas aeruginosa* strain A3, *Bacillus paraflexus* strain rnr13, *Providentia stuartii* strain MF1, *Pantoea agglomerans*, and *Commamonas thioxydans* strain MA1, sourced from lead-contaminated effluents in Ikwo L.G.A, Ebonyi State, Nigeria. Effluent samples were collected from two mine sites and a discharge point, and bacterial tolerance to lead concentrations (100–300 mg/L) was assessed using Luria Bertani broth. Lead reduction was investigated under varying pH (2.5 and 5.2) and temperature (30°C, 37°C, and 45°C) conditions, with residual lead quantified via a colorimetric Dithizone method. Results revealed that *P. aeruginosa* A3, *B. paraflexus* rnr13, and *P. stuartii* MF1 exhibited increased growth with higher lead concentrations and time, while *P. agglomerans* and *C. thioxydans* MA1 showed reduced tolerance. Lead reduction was significantly enhanced at pH 5.2 and 45°C, with *P. aeruginosa* A3 and *B. paraflexus* rnr13 demonstrating superior reduction capacities. Statistical analysis using ANOVA confirmed the significant influence of pH and temperature on lead reduction ($p < 0.05$). These findings underscore the potential of indigenous lead-tolerant bacteria for bioremediation of contaminated mine effluents, advocating for their use in consortium-based strategies to mitigate heavy metal pollution under optimized environmental conditions.

Keywords: Lead tolerance; Bacterial isolates; Bioremediation; Environmental factors; Mine effluent treatment

Introduction

Bacteria exhibit heavy metal resistance and reduction mechanisms that enable survival in metal-rich environments (Pal *et al*. 2022). Both plants and microbes have developed strategies to mitigate metal toxicity by neutralizing or converting metals into less harmful forms, thus lowering environmental lead concentrations (Ghori *et al*. 2019). Through metabolism-dependent and -independent processes, living and dead biomass, along with cellular products like polysaccharides, facilitate metal accumulation, removal, and reduction (Hansda *et al*. 2016).

Microbes utilize several mechanisms to reduce heavy metals, including biotransformation, metal oxidations, methylation, metal organic complexation, extracellular and intracellular metal sequestration, exclusion by permeable membrane, and production of metal chelators and extracellular polymetric substances (Prabhakaran *et al*. 2016). Bacteria, being ubiquitous and abundant, have evolved these mechanisms to tolerate and mitigate the effects of heavy metals, exhibiting a range of metabolism-dependent and metabolism-independent processes for metal uptake and accumulation (Nanda *et al.* 2019).

The direct use of microorganisms with unique catabolic potential and/or their products, such as enzymes and biosurfactants, has been identified as a novel approach to enhancing remediation efficiency (Eze *et al*. 2023; Mishra *et al*. 2021). The application of biotechnology in controlling and reducing metal pollution has gained significant attention in recent years, proving relevant in the field of metal pollution and control (Mani and Kumar, 2014).

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In developing countries, where crude mineral excavation practices prevail, mining industries discharge heaps of wastes and ponds containing high levels of heavy metals, posing a major environmental and health concern (Shengo, 2021; Jayakumar *et al*. 2021). To meet regulatory safe discharge standards, it is essential to remove toxic metals from effluents before release into the environment (Carolin *et al.* 2017). Research studies have demonstrated that using microorganisms with unique catabolic potential or their products, instead of chemical oxidants used by miners, is a more environmentally friendly approach to waste treatment (Puyol *et al*. 2017). This method, known as bioremediation, can be achieved using indigenous microorganisms, which are a mixture of beneficial microbes naturally present in a specific area (Kumar and Gopal, 2015).

This research aims to evaluate the tolerance and reduction capabilities of bacterial isolates to lead, with the potential to treat effluents from lead mining industries before discharge into the environment. The potential of these indigenous organisms may be harnessed in the future to clean up mining effluents before discharge into soil or water bodies, thereby reducing lead pollution and its toxic effects on soil and water biota (Begum *et al*. 2022). An effluent treatment facility within the industry would be more efficient and beneficial than treating large volumes of water bodies (Gadipelly *et al*. 2014; Rajasulochana and Preethy, 2016).

The objectives of the study are to determine the level of lead tolerance by bacterial isolates and evaluate the effects of physical parameters such as pH and temperature on lead reduction

Materials and methods

Study area

The effluent was obtained from lead mine site located in Ikwo L.G.A of Ebonyi State with a long history of lead contamination due to the industrial activities in the surrounding area. It is geographically located on Nigeria's Benue Trough, which is a major mining province with large deposits in the lower and middle Benue regions. The project area is referenced geographically on a point location of Latitude 06O10'29.6' and Longitude 08O08'08.0'. Effluent samples were collected from three distinct locations; two mine sites and a receiving water body at the effluent discharge point.

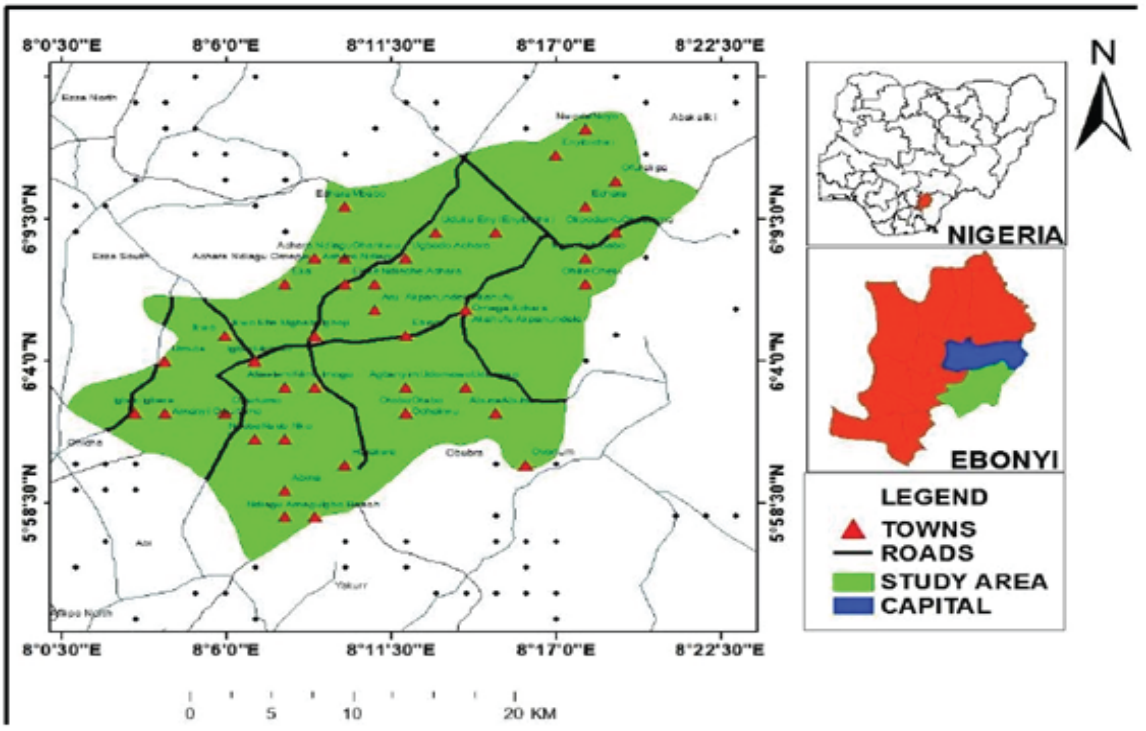

Fig. 1. Map of ebonyi state showing Ikwo LGA the study area

Sample collection

Industrial effluents were aseptically collected using sterile bottles and transported to the Microbiology laboratory of Michael Okpara University of Agriculture, Umudike, and the National Soil, Plant, and Water Laboratory in Umuahia for analysis.

Bacteria confirmation

The bacterial strains *Pantoea agglomerans, Providentia stuartii* MF1, *Pseudomonas aeruginosa* A3, *Commamonas thioxidans strain* MA1*, and Bacillus paraflexus* strain rnr13 were sourced from the Microbiology Laboratory at Michael Okpara University of Agriculture, Umudike, Nigeria. The strains were confirmed using the methods described by Utami *et al*. (2020) and Nwagwu *et al.* (2017).

Tolerance of bacterial Isolates to lead

The ability of bacterial isolates to withstand elevated lead concentrations was evaluated to identify strains suitable for lead reduction studies. Each isolate (5 mL) was inoculated into Luria-Bertani broth supplemented with lead at concentrations ranging from 100 to 300 mg/L. Cultures were incubated at 30°C with continuous shaking at 160 rpm for 5 days. At 24-hour intervals, samples were collected, centrifuged to obtain supernatant, and residual lead levels were quantified using a colorimetric assay with Dithizone working solution, measuring absorbance at 510 nm (Rice *et al*. 2012). Isolates demonstrating robust lead tolerance were selected for subsequent lead reduction experiments.

Lead reduction assay

The capacity of bacterial isolates to reduce lead under varying environmental conditions was assessed, focusing on pH and temperature effects. In a batch experiment, 5 mL of each isolate was inoculated into Luria-Bertani broth supplemented with 100 mg/L lead, adjusted to pH 2.5 or 5.2, and incubated at 30°C, 37°C, or 45°C. Cultures were shaken at 120 rpm for 5 days. At 24-hour intervals, samples were collected, centrifuged, and residual lead in the supernatant was quantified using a colorimetric assay with Dithizone working solution, with absorbance measured at 510 nm (Xiao *et al*. 2017; APHA, 2005).

Statistical analysis

Statistical Analysis of this study was done using descriptive techniques and analysis of variance (ANOVA) was done using the MINITAB software.

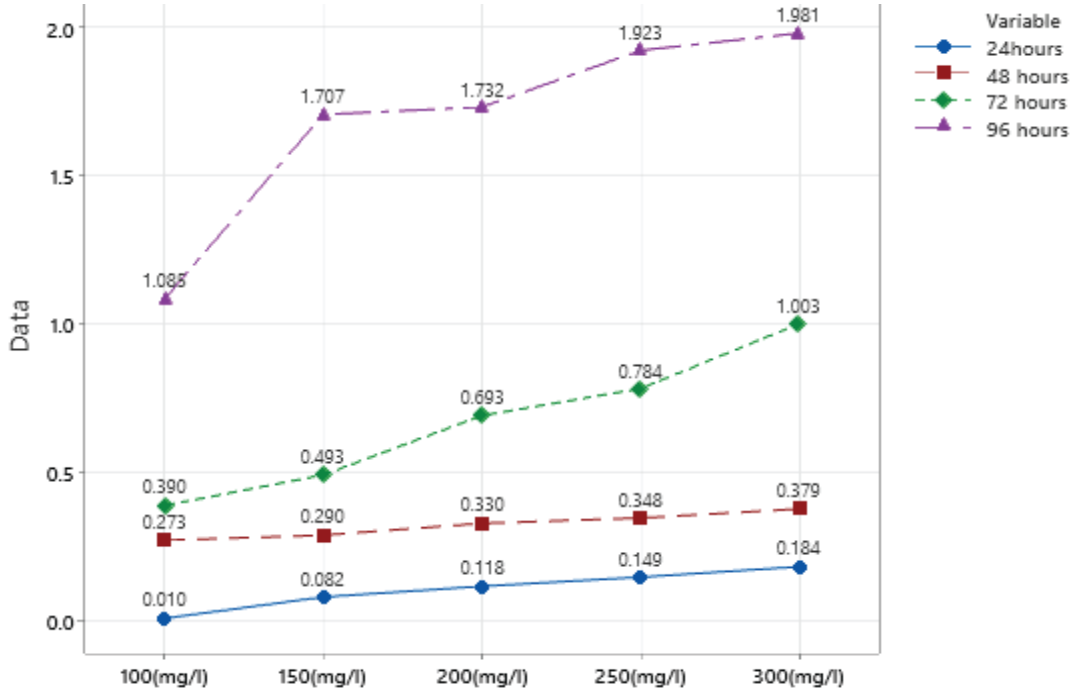

Fig. 2. Tolerance values of MN515055 Pseudomonas aeruginosa strain A3 to different concentrations of lead at 24, 48, 72, and 96 hours. Data are presented as mean ± standard deviation (n = 3)

Results and discussion

Table I presents data on the effect of pH 2.5 on lead (Pb) reduction at 24, 48, 72, and 96 hours. Statistical analysis at a 95% confidence level indicated that pH 2.5 had no significant effect on lead reduction.

Table II displays results for lead (Pb) reduction at pH 5.2, measured at 24, 48, 72, and 96 hours. Statistical analysis at a 95% confidence level showed that pH 5.2 significantly enhanced lead reduction.

Table III shows the effect of a 30°C temperature on lead (Pb) reduction at 24, 48, 72, and 96 hours. Statistical analysis at a 95% confidence level revealed no significant effect of 30°C on lead reduction.

Table IV reports data on lead (Pb) reduction at 37°C, measured at 24, 48, 72, and 96 hours. Statistical analysis at a 95% confidence level indicated that 37°C had no significant effect on lead reduction.

Table V presents results for lead (Pb) reduction at 45°C, measured at 24, 48, 72, and 96 hours. Statistical analysis at a 95% confidence level demonstrated that 45°C significantly influenced lead reduction.

Figure 1 illustrates the tolerance of *Pseudomonas aeruginosa* strain A3 (MN515055) to varying lead concentrations at 24, 48, 72, and 96-hour intervals. Analytical results provided sufficient evidence to conclude that the growth of *P. aeruginosa* strain A3 increased with higher lead concentrations and over time.

Figure 2 depicts the tolerance of Comamonas thiooxidans strain MA1 (MN294683) to different lead concentrations at 24, 48, 72, and 96-hour intervals. Analytical results confirmed that the growth of *C. thiooxidans* strain MA1 decreased as lead concentrations and incubation time increased.

Figure 3 shows the tolerance of *Pantoea agglomerans* (AM184307) to various lead concentrations at 24, 48, 72, and 96-hour intervals. Analytical results indicated that the growth of *P. agglomerans* decreased with increasing lead concentrations and over time.

Figure 4 presents the tolerance of *Bacillus paraflexus* strain rnr 13 (MT023693) to different lead concentrations at 24, 48, 72, and 96-hour intervals. Analytical results demonstrated that the growth of *B. paraflexus* strain rnr 13 increased with higher lead concentrations and over time.

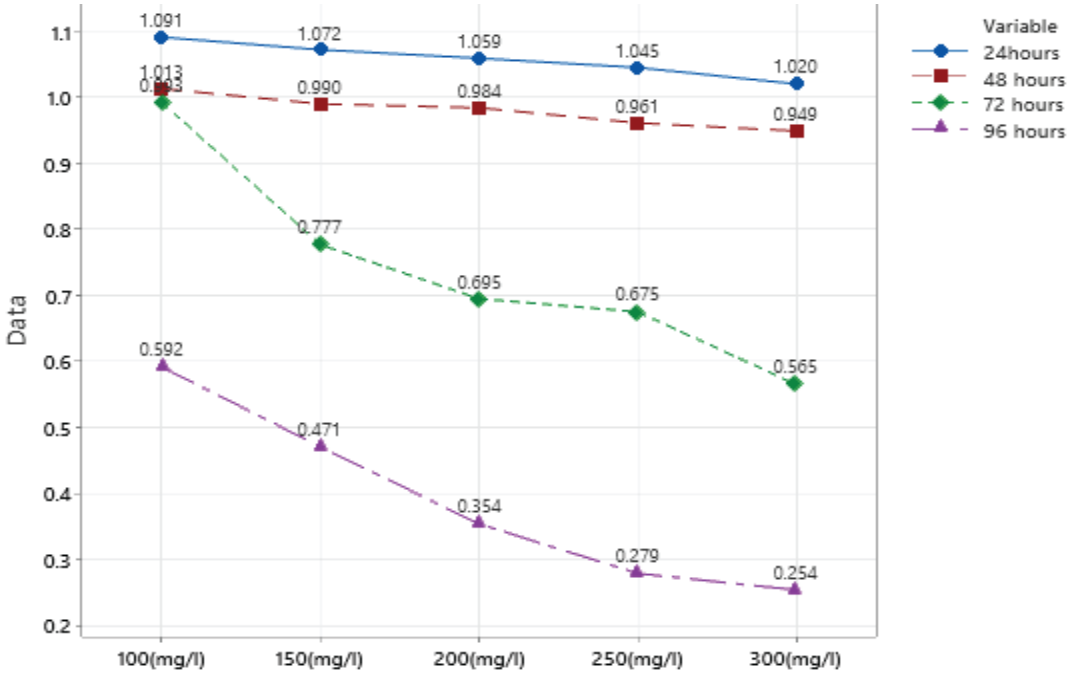

Fig. 3. Tolerance values of MN294683 Commamonas thioxydans strain MA1 to different concentrations of lead at 24, 48, 72 and 96 hours interval. Data are presented as mean ± standard deviation (n = 3)

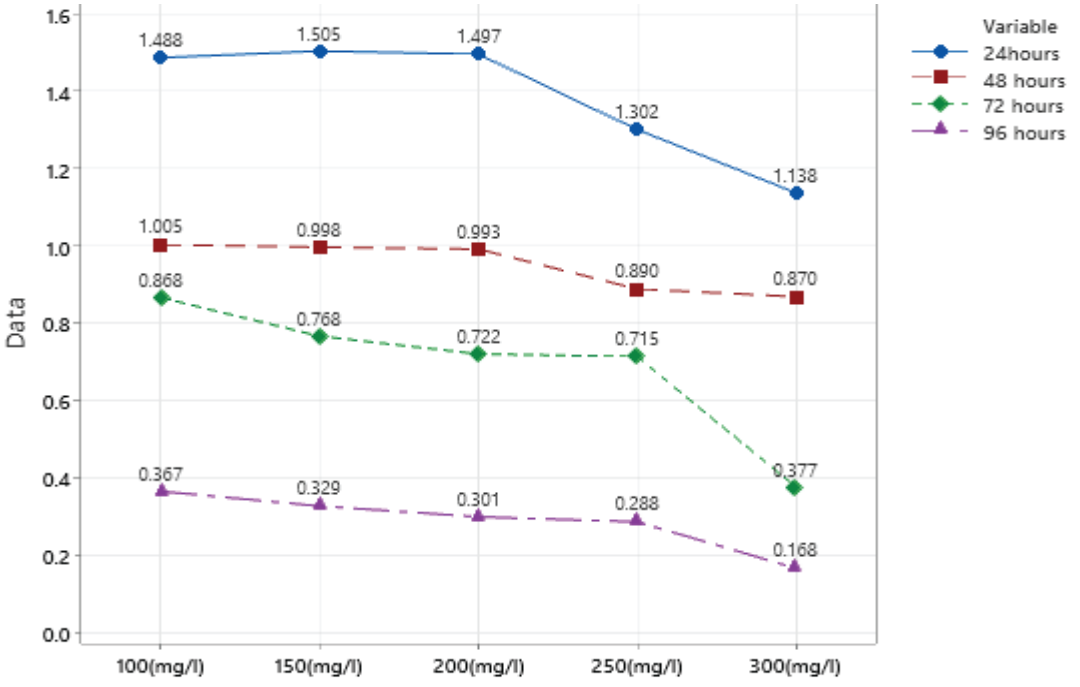

Fig. 4. Tolerance values of AM184307 Pantoea agglomerans, to different concentrations of lead at 24, 48 ,72 and 96 hours interval. Data are presented as mean ± standard deviation (n = 3)

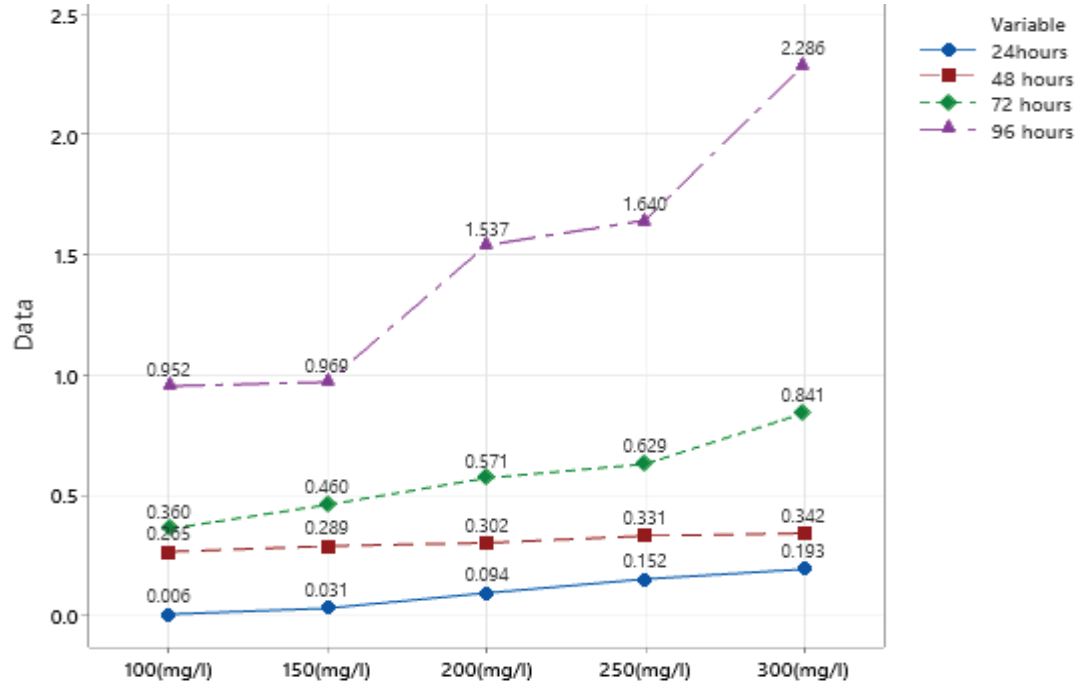

Fig. 5. Tolerance values of MT023693 Bacillus paraflexus strain rnr 13 to different concentrations of lead at 24, 48, 72 and 96 hours interval. Data are presented as mean ± standard deviation (n = 3)

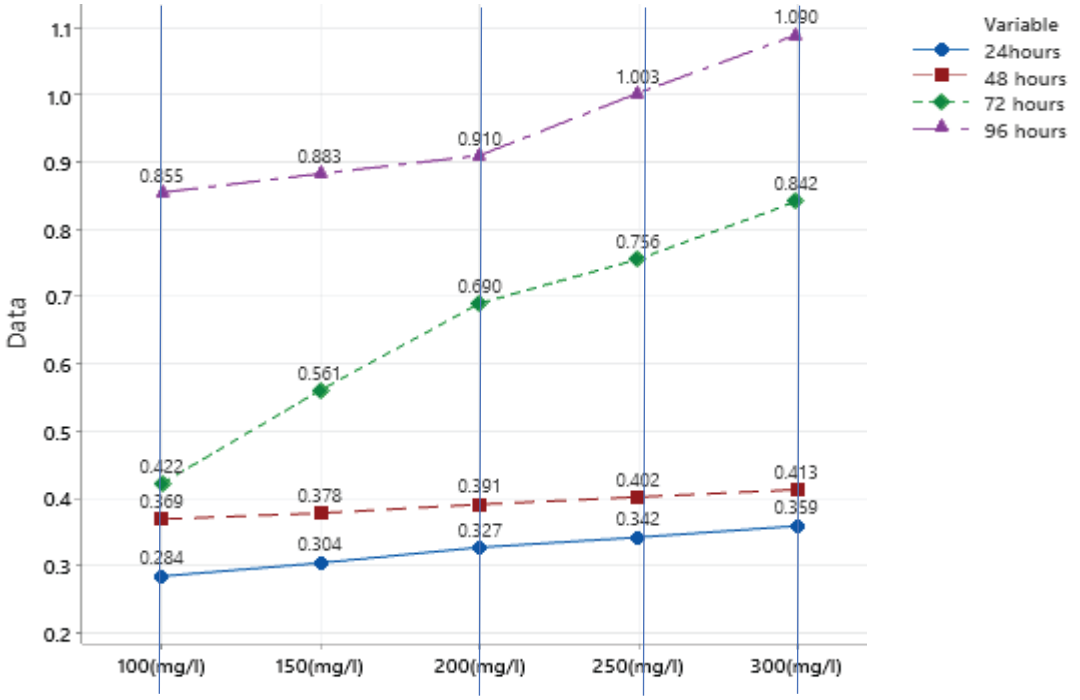

Fig. 6. Tolerance values of MT023702 Providentia struarti Strain MF1 to different concentrations of lead at 24, 48 ,72 and 96 hours interval. Data are presented as mean ± standard deviation (n = 3)

Figure 5 illustrates the tolerance of *Providencia stuartii* strain MF1 (MT023702) to varying lead concentrations at 24, 48, 72, and 96-hour intervals. Analytical results provided sufficient evidence to conclude that the growth of *P. stuartii* strain MF1 increased with increasing lead concentrations and over time.

The discharge of mine effluents poses a significant environmental challenge in Nigeria and globally, leading to soil and water pollution due to increased industrial activities (Hou *et al.* 2017). Various methods have been proposed for the chemical removal of lead, but microorganisms have shown promising efficiency (Kumar *et al.* 2022). In this study, effluent samples from three sources were analyzed: sample A from a personal mine, sample B from a company mine, and sample C, a common discharge point for both. The aim was to identify indigenous microorganisms capable of treating lead mine effluents before discharge into water bodies.

The analysis revealed the presence of heavy metals, with lead concentrations reaching up to 0.111 mg/L, particularly high in the company mine effluent. This level significantly exceeds the USEPA standard for lead in the environment. Other heavy metals detected in high concentrations in the effluent samples included chromium (Cr), cadmium (Cd), manganese (Mn), zinc (Zn), mercury (Hg), copper (Cu), and nickel (Ni). These findings indicate heavy metal pollution in the effluent samples, likely due to extensive mining activities over time as reported by Hu *et al*. (2014).

Effluents from heavy metal mines contain microorganisms resistant to heavy metals, which can be utilized for bioremediation (Verma and Kuila, 2019). This approach offers greener and more sustainable technological solutions to address contaminated environments (Hou *et al*. 2017; Hou *et al*. 2020).

The metal uptake capacity of tolerant microorganisms is influenced by environmental conditions such as pH and temperature (Fan *et al*. 2014). Graphical representations obtained from the data showed a significant level of tolerance by three isolated microorganisms, while two others showed no appreciable tolerance. Tolerance levels for *Pseudomonas aeruginosa* strain A3 and *Bacillus paraflexus* strain rnr 13 increased with time and concentration, while *Providentia stuartii* Strain MF1, *Pantoea agglomerans*, and *Commamonas thioxydans* strain MA1 showed varying levels of tolerance over time and concentration. *Providentia stuartii* Strain MF1 had the lowest tolerance average (2.77), while *Pantoea agglomerans* had the highest. Percentage tolerance

Table I. Effect of pH on Lead (Pb) reduction at 24, 48, 72 and 96 hours

Organism		24 hours	48 hours	72 hours	96 hours
	1	0.210	0.200	0.193	0.190
	2	0.110	0.106	0.100	0.970
	3	0.200	0.183	0.180	0.169
	4	0.111	0.105	0.103	0.991

PH = 2.5; Temperature. = 30ºC; Concentration = 100mg/L

Data are presented as mean ± standard deviation (n = 3)

Table II. Effect of pH on Lead (Pb) reduction at 24, 48, 72 and 96 hours

Organism		24 hours	48 hours	72 hours	96 hours
	1	0.210	0.200	0.193	0.190
	2	0.110	0.106	0.100	0.970
	3	0.200	0.183	0.180	0.169
	4	0.111	0.105	0.103	0.991

PH = 2.5; Temperature. = 30ºC; Concentration = 100mg/L

Data are presented as mean ± standard deviation (n = 3)

Table III. Effect of temperature on Lead (Pb) reduction at 24, 48, 72 and 96 hours

Organism		24 hours	48 hours	72 hours	96 hours
	1	0.210	0.200	0.193	0.190
	2	0.110	0.106	0.100	0.970
	3	0.200	0.183	0.180	0.169
	4	0.111	0.105	0.103	0.991

PH = 2.5; Temperature. = 30ºC; Concentration = 100mg/L

Data are presented as mean ± standard deviation (n = 3)

Table IV. Effect of temperature on Lead (Pb) reduction at 24, 48, 72 and 96 hours

Organism		24 hours	48 hours	72 hours	96 hours
	1	0.210	0.200	0.193	0.190
	2	0.110	0.106	0.100	0.970
	3	0.200	0.183	0.180	0.169
	4	0.111	0.105	0.103	0.991

PH = 2.5; Temperature. = 30ºC; Concentration = 100mg/L

Data are presented as mean ± standard deviation (n = 3)

Table V. Effect of temperature on Lead (Pb) reduction at 24, 48, 72 and 96 hours

Organism		24 hours	48 hours	72 hours	96 hours
	1	0.210	0.200	0.193	0.190
	2	0.110	0.106	0.100	0.970
	3	0.200	0.183	0.180	0.169
	4	0.111	0.105	0.103	0.991

PH = 2.5; Temperature. = 30ºC; Concentration = 100mg/L

Data are presented as mean ± standard deviation (n = 3)

for *Commamonas thioxydans* strain MA1 and *Pantoea agglomerans* decreased, while there was an increase for *Pseudomonas aeruginosa* strain A3, *Bacillus paraflexus* strain rnr 13, and *Providentia stuartii* Strain MF1 at a concentration of 300 mg/l. Supporting the findings of Kumar and Bharadvaja (2020) on microbial remediation of heavy metals.

Findings of this research are not far from results recorded by Hafeez *et al*. (2018) who isolated a *Pseudomonas aeruginosa strain* HF5 with good potential to grow in presence of considerable concentration of lead (Pb) and as well decolonize RR120 and other dyes in media amended with lead. Another strain of *Pseudomonas aeruginosa* ZGKD2 had been reported by Zhang *et al*. (2012) to also exhibit potential roles in Bioremediation of different heavy metals with high removal percentage when compared with other bacterial strains. A specie of *Providentia* sp isolated from municipal solid waste dumps site has been observed to have multiple tolerance to heavy metals (Anjanapriya *et al*. 2002). Sivan *et al*. 2015 and Oyetibo *et al.* 2017 isolated *Bacillus* sp from agricultural soil and industrial water in Mauritius and sediments polluted with petroleum cells and industrial wastes waters from Ikeja Industrial estate. Moreso *Bacillus species* with multiple tolerance to metal ions species have been reported by several researchers. Fashola *et al. (*2016); Ndeddy and Babalola (2016) have reported the isolation of Bacillus species from a gold mine tailing in South Africa. *Commamonas acquatica* was reported to record biosorption by Qurbani and Hamzah (2021). Some of these indigenous organisms are reported to show multiple tolerance which is common among heavy metal tolerant bacteria. The metal tolerance ability of the bacteria is said to depend on the metallic activities of their cells both intrinsic, biochemical and structural properties which includes physicochemical and genetic adaptations, environmental modifications and metal speciation availability and toxicity (Cooksey, 1993). Bacteria could bind to metals via metalothiones to form complexes or they could pump

absorbed metals out through an efflux P type ATPases (O'Neil *et al*. 1999). The ability of *Bacillus spp* to form spores in nutrient limiting environment among other properties also makes this genus a self-sustaining bioremediation agent. Intracellular and extracellular sequestration can be achieved by *Pseudomonas* spp as observed in *Pseudomonas syringe* strains that were responsible for binding of microbial colonies and copper ion as recorded by Pande *et al.* (2022). Biofilm agglomerate also plays an important function in metal uptake by extracellular polymeric molecules from water resources. Tolerance against lead (Pb) Zinc (Zn) and Copper (Cu) has been displayed by the biofilm of *Pseudomonas aeruginosa* (Tietzel and Parsek 2003). In addition to biofilms and cell walls, other Exopolysaccharides (EPS) have also proven to be a barrier against metals for instance adsorption of lead (Pb) ions were reported in *Pseudomonas aeruginosa, Acinetobacter jenuni* LPb *1* and *Azotobacter chroococcum* XU1 (Rasulov *et al* 2013; Kushwaha *et al.* 2017). Moreso Copper inducible protein Co PA, CoPB (periplasmic protein) and CoPC outer membrane proteins are produced by copper resistant organisms. Adding trace amount of heavy metals to environment of microbial cells could stimulate microbial growth but an increased concentration results in severe reduction of microbial activity (Gikas, 2007). This may have been the case with *Pantoea agglomerans* as it recorded a drop in tolerance with increased concentration and time.

Microorganisms are effective biosorbents, and their biosorption efficiency varies based on several aspects, including ambient factors, sorbing materials, and the metals to be removed. pH is one such environmental factor that significantly affects the capacity of microorganisms to remove heavy metals, as noted by Igiri *et al*. (2018). Heavy metals tend to form free ionic species at acidic pH levels, where more protons are available to saturate metal binding sites (Young, 2013). This increased hydrogen ion concentration leads to a more positively charged absorbent surface, reducing the attraction between the absorbent and metal cations and increasing their toxicity.

In this study, the reduction of lead by lead-tolerant microorganisms was affected by pH, with different organisms exhibiting varying levels of lead reduction at different pH values. MN515055 *Pseudomonas aeruginosa* strain A3 showed the highest reduction of lead at pH 5.2, while MN294683 *Commamonas thioxydans* strain MA1 exhibited the lowest reduction at the same pH. These variations occurred because the optimum pH for each organism differs, and unsuitable pH levels present adverse effects on microbial growth for several reasons.

The results obtained in this study align with findings by Rodriguez tirado *et al*. (2012), who reported that the removal rates of heavy metals by microorganisms increased with an increase in pH over a limited range. However, the removal rate may begin to decrease after the pH rises above a certain limit. For lead (Pb2+) and zinc (Zn2+), the removal rate continued to rise at pH values from 2.0 to 5.5, but decreased at pH values higher than 5.5, returning to the same level as pH 2.0. This phenomenon is due to the formation of hydroxide precipitates of metal ions, which are less suitable for microbial absorption at higher pH levels.

Temperature is another parameter that significantly affects heavy metal accumulation, as it is directly linked to microbial growth and metabolism. Results from this study showed that as temperature increased, there was an increase in the reduction of lead, with MT023693 *Bacillus paraflexus* strain rnr 13 showing the highest reduction and MN294683 *Commamonas thioxydans* strain MA1 showing the lowest at 30°C. Similarly, at 37°C and 45°C, AM184307 *Pantoea agglomerans* showed the highest reduction, while MN515055 *Pseudomonas aeruginosa* strain A3 showed the lowest.

These findings are in agreement with previous studies, such as those by Igiri *et al*. (2018), which suggest that the actions of microorganisms increase with the rise in temperature within a suitable range, enhancing microbial metabolism and enzyme activity, thereby accelerating bioremediation. Optimum temperatures for each organism play a significant role in metal ion uptake, with the most favorable temperature range for the reduction of lead falling between 37-45°C, which is conducive for the growth of *Bacillus* and *Pseudomonas* species.

Conclusion

The study highlights the tolerance and reduction capabilities of bacterial isolates to lead, indicating the potential for using indigenous bacteria to mitigate the environmental impact of mine effluent discharge. In conclusion, pH and temperature are critical factors that affect the efficiency of microorganisms in removing heavy metals from contaminated environments. Understanding the optimum conditions for metal reduction by microorganisms can help in developing more effective bioremediation strategies for heavy metal-contaminated sites.

Recommendation

Based on the findings, it is recommended that government regulations be enforced to ensure proper treatment of mine effluents before discharge to reduce harmful metal contami-

nation. Additionally, the study suggests the use of a consortium of indigenous lead-tolerant bacteria to enhance the lead reduction process, which could be a cost-effective and environmentally friendly approach to remediate lead-contaminated effluents from mines. Further research could focus on optimizing the conditions for bacterial lead reduction and assessing the long-term effectiveness of using bacterial consortia in mine effluent treatment.

Conflict of Interest: The authors declare no conflict of interest

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Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on request.

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