BIODIVERSITAS
Volume 24, Number 9, September 2023
Pages: 5096-5104

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240954

Isolation and characterization of potential indigenous bacteria from the former bauxite mining area for heavy metal reduction

TRISNA AMELIA[1,2], LILIASARI[1,♥], KUSNADI[1], PINGKAN ADITIAWATI[3]

[1]Doctoral Program of Science Education, Faculty of Mathematics and Natural Sciences, Universitas Pendidikan Indonesia. Jl. Setiabudi 229, Bandung 40154, West Java, Indonesia. Tel./Fax.: +62-222-001108, ♥email: liliasari@upi.edu

[2]Department of Biology Education, Faculty of Teacher Training and Education, Universitas Maritim Raja Ali Haji. Jl. Raya Dompak, Tanjungpinang 29124, Riau Islands, Indonesia

[3]School of Life Sciences and Technology, Institut Teknologi Bandung. Jl. Ganesa 10, Bandung 40132, West Java, Indonesia

Manuscript received: 9 June 2023. Revision accepted: 29 September 2023.

Abstract. *Amelia T, Liliasari, Kusnadi, Aditiawati P. 2023. Isolation and characterization of potential indigenous bacteria from the former bauxite mining area for heavy metal reduction. Biodiversitas 24: 5096-5104.* This study aimed to isolate, identify, and characterize some indigenous bacterial strains from the former bauxite mining area of Tanjungpinang, Bintan Island, Indonesia. Furthermore, this study focused on evaluating the potential of these bacteria for reducing the heavy metals lead (Pb) and chromium (Cr). The measurements of heavy metal concentrations at four sampling points, collection of soil samples as a bacteria source, and laboratory assessments for bio-removal capabilities were all conducted. Screening experiments were performed to identify bacterial strains resistant to heavy metal, using basic growth media such as Nutrient Agar (NA) and Nutrient Broth (NB) enriched with 100 ppm Pb and Cr metals. The reduction of heavy metal was analyzed with atomic absorption spectrophotometry (AAS), while the bacteria species were determined using MALDI TOF-MS. A modified version of the Kirby Bauer method was employed for toxicity reduction testing. Two bacterial strains were identified as Pb and Cr reducers and demonstrated resistance to both metals. Based on a 99.9% similarity value, the isolates were identified as *Bacillus altitudinis* (Isolate A) and *Klebsiella pneumoniae* (Isolate B), reducing Pb by approximately 72.7% and 34.5%, and Cr by 87.4% and 86.2%, respectively. The results indicated reduced toxicity in the metal-enriched media, with bacterial growth following three hours of incubation but no toxicity after 21 hours.

Keywords: Bauxite residue, chromium, former mining area, indigenous bacteria, lead

INTRODUCTION

Mine waste is a significant global environmental concern due to metal pollution, impacting both humans and ecosystems (Priyadarshanee and Das 2021; Marzan et al. 2020; Newsome and Falagán 2021). The accumulation of heavy metal waste, a consequence of bauxite mining, is escalating worldwide in countries such as Australia (Gräfe et al. 2010), China (Qi et al. 2018), Malaysia (Lee et al. 2017), Brazil (Nogueira et al. 2017), and Indonesia (Damayanti and Khareunissa 2017).

The name "bauxite" originated from the French town of Les Baux, where this metal was first discovered. Serving as a direct precursor in aluminum production, bauxite possesses a red-brown tint and comprises various aluminum hydroxide minerals alongside elements including Arsenic (As), Beryllium (Be), Cadmium (Cd), Chromium (Cr), Lead (Pb), Manganese (Mn), Mercury (Hg), and Nickel (Ni). After aluminum extraction, these heavy metal components tend to persist in bauxite residues (Lee et al. 2017).

The term heavy metal pertains to a group of metals and metalloids with an atomic density exceeding 4 g/cm^3, which is five times denser than water (Priyadarshanee and Das 2021). While the human body requires trace levels of certain heavy metals, including Cu^{2+}, Zn^{2+}, and Fe^{2+}/Fe^{3+}, most heavy metals, such as As, Cd, Pb, Cu, and Cr, are harmful to organisms. Elevated levels of these beyond thresholds established by health organizations such as the WHO (World Health Organization), can disrupt various physiological processes within living systems (Khalil et al. 2022).

Hazardous heavy metal contaminants encompass lead and chromium, both associated with adverse effects (Xu et al. 2020; Yani et al. 2020). Lead toxicity uniquely induces oxidative stress and free radical imbalance, without playing any biological role in the human body. Furthermore, it is poisonous to organs, mutagenic, teratogenic, and carcinogenic, capable of causing damage to DNA, proteins, and cell membranes (Igiri et al. 2018; Marzan et al. 2020; Newsome and Falagán 2021). Chromium in the hexavalent state Cr^{6+} is toxic and has no biological utility for bacteria. Cr (III) serves as a micronutrient for mammals, Cr (VI) is highly hazardous and carcinogenic due to its potential to disrupt DNA and proteins (Igiri et al. 2018; Newsome and Falagán 2021; Priyadarshanee and Das 2021).

Mining areas are where there are lots of heavy metal residues that pollute the environment. Bintan Island has been reported as the area where a lot of bauxite mining is carried out. Heavy metal residues in the area make the ex-bauxite mining land unfit for use both as an agricultural area and as a residential area. The metal concentrations of Cd, Pb, Cu, and Fe in the ex-mining area have a higher value than in other areas (Raza'i et al. 2021).

Considering the environmental harm posed by lead and chromium, concerted efforts are required for their remediation. Microorganisms offer a potent means to

address heavy metal contamination through various cell mechanisms (Yani et al. 2020). Microbial activity can mitigate the impact of metal mining waste, thereby curbing its detrimental effects on environmental well-being. Several microorganisms, including bacteria, fungi, and algae, have been employed for the remediation of heavy metal-polluted settings, where bacteria play a pivotal role as they exhibit remarkable symbiotic interactions with other organisms (Igiri et al. 2018; Xu et al. 2020).

Bacteria show flourishing activity within metal-rich mine waste due to their adaptability. This organism employs diverse mechanisms such as active efflux, ion permeability changes, adsorption, and biotransformation to alleviate heavy metal impacts (Khalil et al. 2022). Bacterial biosorption emerges as a secure treatment strategy for toxic non-degradable heavy metal pollutants.

Numerous studies investigated the potential of bacteria in nature as heavy metal bioremediation agents (Pambudiono et al. 2018; Anno et al. 2020; Wang et al. 2020; Oziegbe et al. 2021). Only limited data presently exist on indigenous bacteria from former bauxite mining areas that possess the ability to bio-remove Pb and Cr. Therefore, this study aims to identify indigenous bacteria from former bauxite mining areas and assess the capacity to bioremediate Pb and Cr.

MATERIALS AND METHODS

Study area

Indigenous bacteria strains were sourced from soil samples collected from Tanjungpinang, Bintan Island, Province of Kepulauan Riau, Indonesia. This area has witnessed bauxite mining activities since the 1920s, leading to several abandoned mining sites transforming into unused open spaces. Soil samples were obtained from four distinct points: (i) Lat 0.94533° Long 104.478592°, (ii) Lat 0.95073° Long 104.474869°, (iii) Lat 0.959904° Long 104.494706°, and (iv) Lat 0.873473° Long 104.526136°, as indicated in Figure 1. The sampling area experiences an average annual temperature of 27°C and an average yearly rainfall of 161.97 mm.

Data collection and analysis

Soil sampling

The soil samples (up to 150 g) were collected at depths of 15-20 cm using a soil sampler and stored in sterile plastic containers for subsequent laboratory analysis. The samples were homogenized, and large particle components were manually removed to facilitate immediate testing of metal levels and bacteria isolation (Anusha and Natarajan 2020).

Heavy metal analysis of soil samples

The content of heavy metal in the soil samples, specifically chromium (Cr) and lead (Pb) was quantified with atomic absorption spectrophotometry (AAS Shimadzu AA-7000). The obtained measurements were then compared against heavy metal thresholds established by the World Health Organization (WHO).

Bacterial isolation from former bauxite mining soil

Bacterial isolation was performed on NA media containing yeast extract, peptone, sodium chloride (NaCl), agar, and distilled water. To specifically cultivate bacteria resistant to Pb and Cr metals during inoculation and isolation, the NA media was enriched with $Pb(NO_3)_2$ and $K_2Cr_2O_7$ metals, each measuring 100 ppm and 200 ppm. The inoculation process commenced with the serial dilution of soil samples up to the fifth dilution level (10^{-5}). Sterile distilled water was employed for dilution to prevent bacterial contamination from outside the samples. Subsequently, the pour plate technique was used to inoculate the 10^{-4} and 10^{-5} dilution results. The isolated bacteria were incubated at 37°C for 24 hours (Marzan et al. 2020).

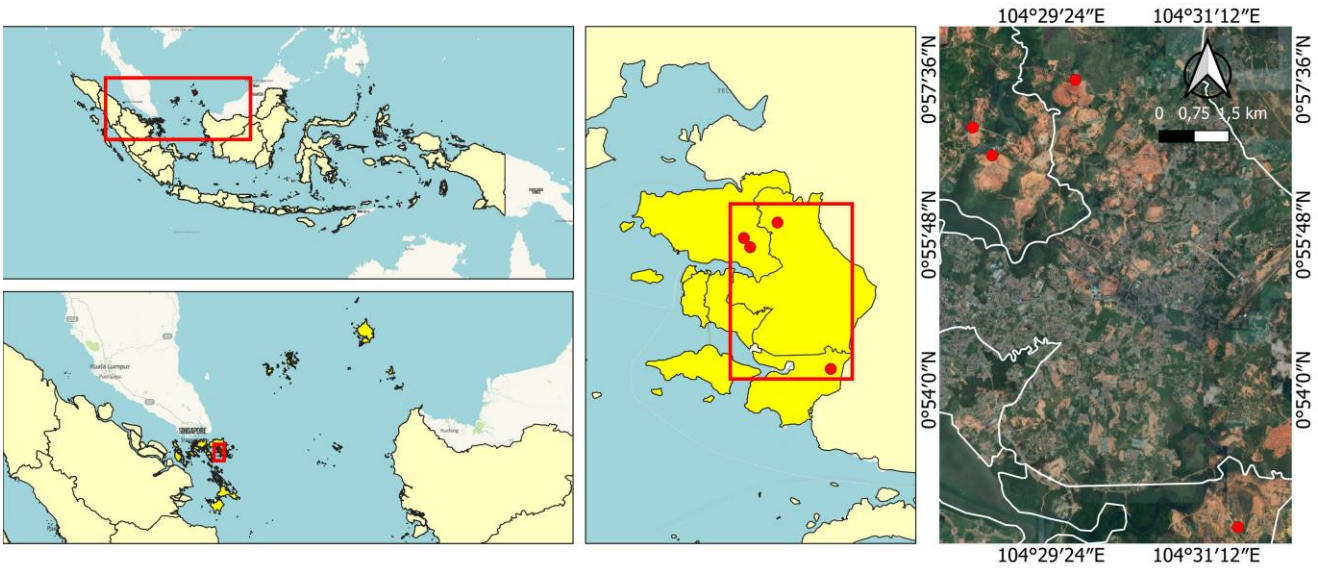

Figure 1. Sampling points of former bauxite area in Tanjungpinang, Bintan Island, Indonesia

Isolation of bacteria

Bacterial strains were isolated from several colonies that grew on the agar plates from the isolation process using the streak plate technique. A single ose of the colony to be isolated was scratched on the NA plate to generate three separate streak areas, then incubated at 37°C for 24 hours.

Preparation of bacterial inoculum

The cultivated bacterial isolates were suspended in 50 mL NB medium enriched with 100 ppm of $Pb(NO_3)_2$ and $K_2Cr_2O_7$ metals. This suspension was incubated in a shaker incubator at 37°C for 24 hours with a rotation speed of 120 rpm (Batta et al. 2013; Yani et al. 2020). Absorbance values were measured using an ultraviolet spectrophotometer, initially and after 24 hours of incubation, to track the increase in absorbance as an indicator of bacterial growth (Anusha and Natarajan 2020).

Determination of Cr and Pb uptake capacity of bacterial strains

Bacterial strains were introduced into sterilized NB media and kept in an autoclave for 15 minutes at 121°C. The media was subsequently placed in a shaker incubator set at 37°C and 120 rpm. After 24 hours of incubation, both growth and remediation potential were assessed. Bacterial remediation potential was gauged based on optimal growth, determined by measuring absorbance values at a wavelength of 600 nm with an ultraviolet spectrophotometer. The cultures were centrifuged for 20 minutes at 1700 rpm, and following the bacterial incubation, the supernatant was collected in a sterile vial to evaluate the remaining metal content. The following formulas were applied to investigate heavy metal reduction values using AAS (e.g. Kaczorek 2012; Kim et al. 2017; Oziegbe et al. 2021).

$$\%\ reduction = \frac{\text{Initial heavy metal} - \text{Residual heavy metal}}{\text{Initial heavy metal concentration}}\ x\ 100$$

The following formula was employed to calculate the quantity of heavy metal ingested by bacteria strains (Marzan et al. 2020).

$$\text{utilization of heavy metals (ppm)} = \text{initial heavy metals (ppm)} - \text{residual heavy metals (ppm)}$$

The growth pattern of heavy metal-immobilizing strains

To comprehend the growth pattern of bacterial strains contributing to heavy metal reduction, a growth curve evaluation was conducted. Initially, a standard curve of the selected bacteria must be established. A single ose of the culture was suspended in 50 mL NB media and incubated for 24 hours at 37°C (Ndeddy Aka and Babalola 2017) with a rotation speed of 120 rpm (Marzan et al. 2020). Furthermore, the absorbance value of the inoculated bacteria was measured and their concentrations were altered by varying the inoculum-to-NB media ratios between 3:7, 5:5, and 7:3.

Each inoculum was serially diluted up to 10^{-7} and the absorbance values of the final dilution were determined at a wavelength of 600 nm using a UV spectrophotometer (Marzan et al. 2020). Subsequently, the pour plate technique was employed to infect agar plates, followed by the use of the total plate count (TPC) method to calculate the number of proliferating bacterial cells. The equation below encompassed the absorbance value measurement results and the estimated TPC value:

$$y = ax + b$$

Where:
y : absorbance value
a and b : constants
x : total plate count (TPC)

The regression equation produced from the standard curve technique formed the foundation for constructing the bacterial growth curves. To determine the bacterial growth pattern in NB media enriched with 50 ppm of $Pb(NO_3)_2$ and $K_2Cr_2O_7$, their growth curves were required. A total of 5% v/v 24-hour culture was pipetted into 125 mL of NB media. The suspension was incubated at 120 rpm and 37°C for 24 hours. During incubation, an inoculum sample was collected every 3 hours to evaluate the absorbance value and metal content toxicity. The measured absorbance values at each sampling point were employed in the standard curve calculation to construct the growth curves.

Determination of metal toxicity reduction

A modified disk diffusion method was employed to ascertain whether bacteria contributed to heavy metal toxicity reduction. This was achieved by measuring the inhibition zone surrounding paper discs previously soaked in the inoculum suspension and the procedure required 6 mm paper discs. *Escherichia coli*, known to be susceptible to antimicrobial chemicals, was used as test microbes by swabbing the cultures onto Mueller Hinton Agar (MHA) plates. The paper discs were immersed in the inoculum suspension for 2 minutes before being placed on Mueller Hinton Agar plates containing the test bacteria, then incubated for 24 hours at 37°C (Igiri et al. 2018).

Characterization of potential bacteria

To assess bacteria with potential for heavy metal bioremediation, both Gram staining and protein profile analysis were conducted using the Matrix Assisted Laser Desorption/Ionization, Time-of-Flight Mass Spectrometry (MALDI TOF-MS) method. The identification of microorganisms through MALDI TOF-MS, a fast and reliable method, was based on comparing the mass spectra of the investigated microorganisms with reference strains (Starostin et al. 2015; Shu and Yang 2017).

Culture samples for MALDI-TOF analysis were prepared by mixing or coating with an energy-absorbing organic matrix solution. During the drying of the matrix solution, the sample within became crystallized. A laser beam was used to automatically ionize the sample in the matrix, generating individual protonated ions from the analyte through laser light desorption and ionization. These ions were accelerated and separated based on their mass-to-

charge ratio, with a time-of-flight (TOF) analyzer detecting and measuring the loaded analyte (Croxatto et al. 2012; Clark et al. 2018; Zhang et al. 2022).

RESULTS AND DISCUSSION

Heavy metal analysis of soil samples

Analysis of soil samples collected from a former bauxite mining area in Tanjungpinang City showed the presence of Pb and Cr. The measured metal concentrations were compared against the criteria set by WHO. According to WHO standards, none of the sampling points met the threshold limits for Pb and Cr levels in agricultural soils.

The four sample points designated as the former bauxite mining area exhibited distinct characteristics. Soil from sample point 3 displayed a different color compared to other samples and contained the highest concentrations of Cr and Pb (Table 1). The total four soil samples used as a source of indigenous bacterial isolates were investigated for their heavy metal bioremediation potential.

Isolation of heavy metal-resistant bacteria

The bacterial inoculation method applied to soil samples led to the successful isolation of 11 strains resistant to Pb and Cr. Differential bacterial growth was observed at varying concentrations of Pb and Cr, with higher growth occurring at 100 ppm compared to 200 ppm metal concentrations, as presented in Figure 2.

In this study, two isolates designated as A and B, with the greatest potential, were selected from the 11 obtained for further examination. Both isolates were transferred to agar plates and slanted agar media for subsequent analysis, and they were characterized as indicated in Table 2.

In this study, two isolates designated as A and B, with the greatest potential, were selected from the 11 obtained for further examination. Both isolates were transferred to agar plates and slanted agar media for subsequent analysis, and they were characterized as indicated in Table 2.

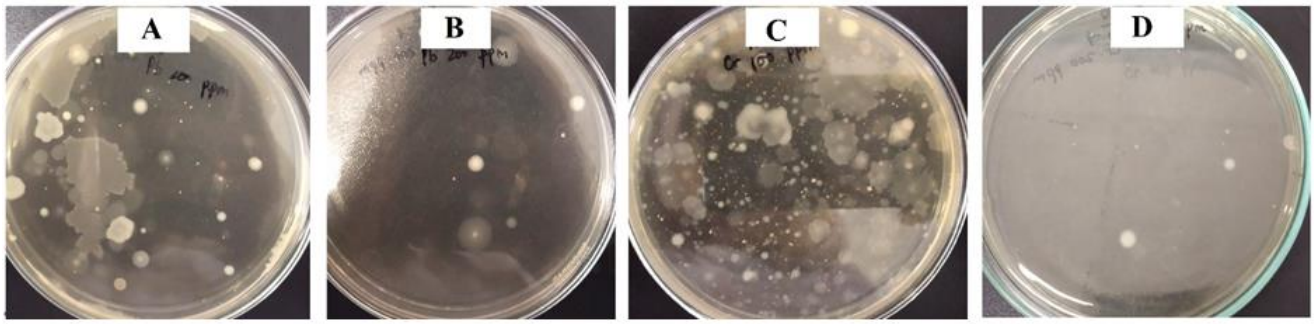

Figure 2. Bacterial growth on NA media with 100 ppm and 200 ppm metal concentrations; (A) bacterial growth at 100 ppm of Pb, (B) bacterial growth at 200 ppm of Pb, (C) bacterial growth at 100 ppm of Cr, (D) bacterial growth at 200 ppm of Cr

Table 1. Levels of heavy metals Pb and Cr at sampling points

Heavy metals	Unit	Sampling point				WHO standards
		1	2	3	4	
Pb	ppm	1.84	3.83	28.03	8.40	0.1
Cr	ppm	30.03	nd	48.02	nd	0.1
Color of soil		Reddish yellow	Reddish yellow	Brown	Reddish yellow	

Note: nd represented "not detected."
Samples 1, 2, 3, and 4 were from former bauxite mining area

Table 2. Morphological characteristics of bacterial isolates

Characteristic	Code of bacterial isolates	
	A	B
Colony size	7-8 mm	5-6 mm
Colony shape	Round	Irregular
Colony elevation	Flat	Convex
Edge	Entire	Undulate
Colony color	White	White
Gram reaction	Negative (-)	Positive (+)
Shape of cell	Rod-shape	Rod-shape
Result of MALDI TOF-MS	Similar to *Klebsiella pneumoniae* (Similarity value 99.9%)	Similar to *Bacillus altitudinis* (Similarity value 99.9%)

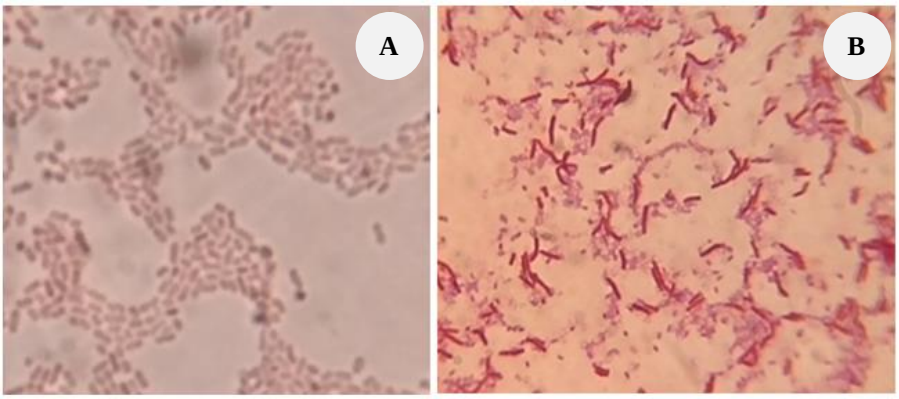

Figure 3. Isolated bacterial cell morphology: A. Isolate A, *Klebsiella pneumoniae*; B. Isolate B, *Bacillus altitudinis* (1000 × magnification)

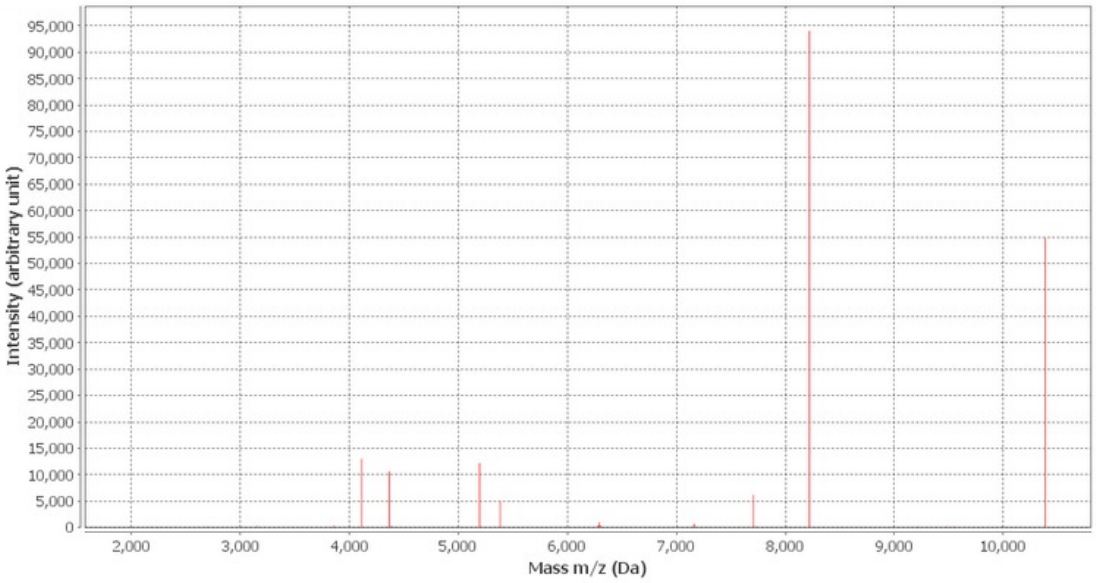

Figure 4. MALDI mass spectra of isolate A (*Klebsiella pneumoniae*)

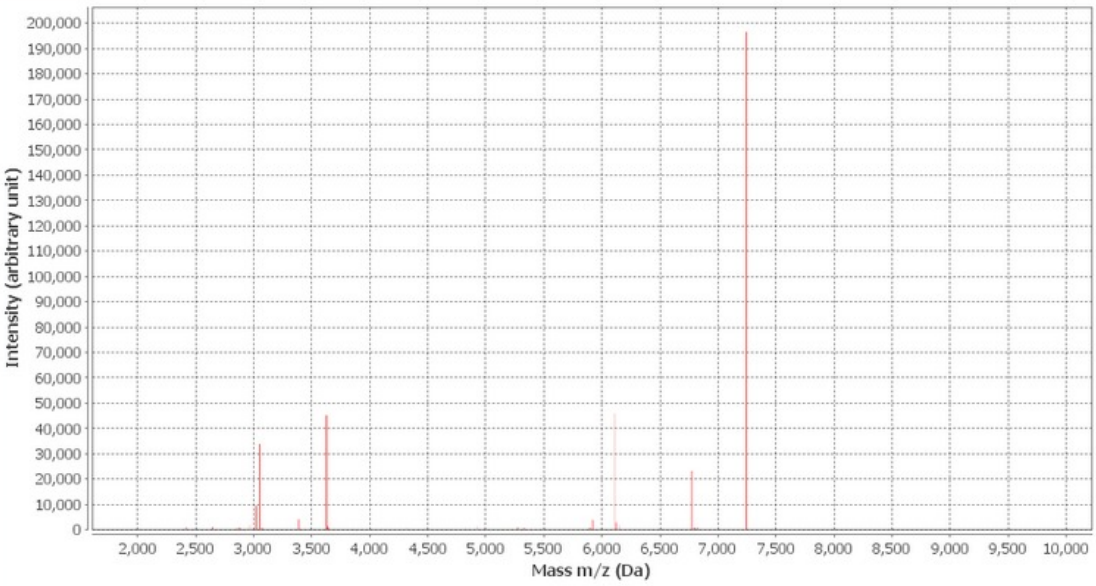

Figure 5. MALDI mass spectra of isolate B (*Bacillus altitudinis*)

Gram staining proved that the two isolates A and B were clearly different species. Figure 3 shows isolate A is a rod-shaped Gram-negative bacteria and isolate B with a longer rod-shape is a Gram-positive bacteria. The species of the two isolates were determined with protein analysis using MALDI TOF-MS. The protein mass spectra of the two isolates are depicted in Figures 4 and 5. Based on the protein profile analysis, isolate A exhibited similarities to *K. pneumoniae*, while B corresponded to *B. altitudinis*. The species identification of both isolates yielded a confidence value of 99.9%.

Determination of Cr and Pb uptake capacity of bacterial strains

Metal reduction percentages after 24 hours of incubation revealed the capacity of the isolated bacteria for Pb and Cr bioremediation, as indicated in Table 3. Isolates A (*K. pneumoniae*) and B (*B. altitudinis*) both achieved reductions of over 50% for Cr and isolate B caused a > 50% decline in Pb. Specifically, *K. pneumoniae* decreased Cr concentration from an initial 44.27 ppm to 6.11 ppm, while *B. altitudinis* lowered it to 5.57 after 24 hours. *K. pneumoniae* decreased Pb to 19 ppm from 29 ppm after 24 hours, and *B. altitudinis* reduced it to 7.9 ppm. *Bacillus altitudinis* demonstrated a superior ability to reduce both types of metals compared to *K. pneumoniae*.

Bacterial strain growth pattern in the media

A growth curve analysis was performed to assess the growth patterns of the isolates in heavy metal-enriched media, as presented in Figure 6. Isolates A and B displayed swift adaptation to NB media containing heavy metals by entering the exponential growth phase within 3 hours. Isolate A reached the peak of the exponential phase at the 12 hours, then the stationary phase at the following hour, and finally the death phase at the 15 hours. However, isolate B remained in the exponential phase until the 15 hours, transitioning to a stationary phase for the subsequent 6 hours before entering the death phase at the 24 hours.

Reducing metal content toxicity in the inoculum

During the incubation period, both isolates A and B effectively reduced the hazardous levels of heavy metal. The reduction in toxicity of the inoculum within the incubation period in NB media enriched with Pb and Cr is presented in Figure 7. The reduction in toxicity is indicated by the reduction of the inhibition zone in the test bacteria against a mixture of heavy metals in the growth media for bacteria A and B.

Table 3. Heavy metal (Pb and Cr) reduction capacity of isolates A and B after 24 hours of incubation

Heavy metal	Bacteria isolate	Heavy metal concentration in inoculum (ppm)		Reduction capacity (%)
		Initial	After 24 hours	
Cr	A (*K. pneumoniae)*	44.27	6.11	86.20
	B (*B. altitudinis)*	44.27	5.57	87.42
Pb	A (*K. pneumoniae)*	29.00	19.00	34.48
	B (*B. altitudinis)*	29.00	7.90	72.76

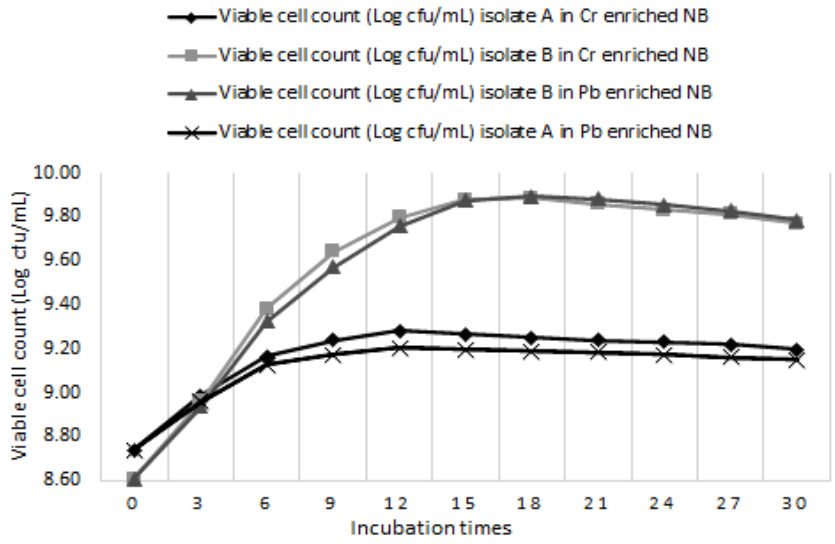

Figure 6. Growth curve of isolates A and B in Cr and Pb enriched nutrient broth

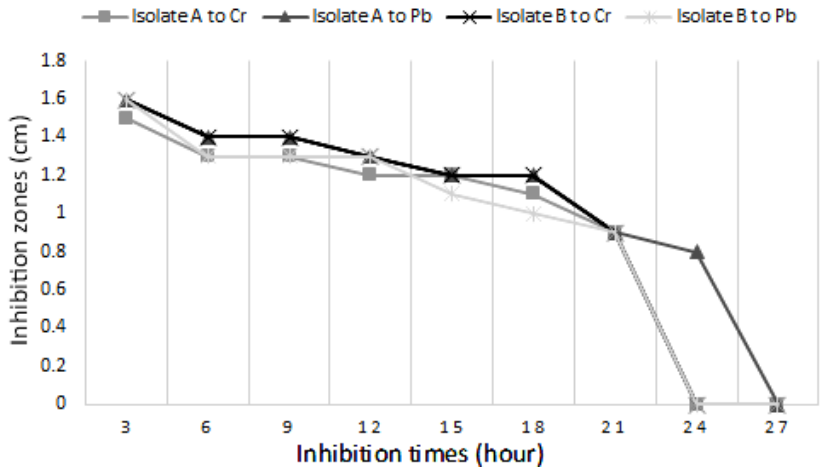

Figure 7. The reduction of inhibition zones indicates the reduction in metal toxicity per incubation time

Discussion

The elevated concentrations of Pb and Cr detected at the four sampling points in Tanjungpinang, Bintan Island, Indonesia, exceeded the soil quality criteria established by WHO. This heavy metal pollution could be attributed to the historical bauxite production in the area. A previous study reported that the process of alumina extraction from bauxite involved the use of concentrated sodium hydroxide, leading to the formation of a byproduct known as bauxite residue or red mud (Nogueira et al. 2017). A comparison of Pb and Cr levels between former bauxite mining areas and adjacent residential zones with no mining history confirmed residual bauxite mining activities as the primary source of Pb and Cr. The observations were consistent with prior studies indicating the presence of heavy metal components, including Pb and Cr, in bauxite residue after alumina extraction (Lee et al. 2017).

Soil quality and biological activity significantly depend on the presence of microorganisms. The bioremediation of contaminated soil is largely driven by microorganisms, and some beneficial microbes help to reduce soil pollution. Metal contamination disrupts soil processes and critical element cycles, as bacteria require energy for cell sustenance and repair. However, microbial communities can adapt and tolerate metal pollutants, particularly when these metals are essential elements regulated by homeostasis or known to possess metabolic equivalents (Newsome and Falagán 2021).

Metal ions play diverse roles in microbial cellular processes, both directly and indirectly. Several metals and metalloids found in mine waste can function as electron donors or acceptors during energy production (Newsome and Falagán 2021; Igiri et al. 2018). Moreover, active Cr absorption occurs through the sulfate transport system in prokaryotes and eukaryotes. Cr-resistant bacteria are capable of mitigating the contamination of heavy metals in soil by lowering their redox value (Ahemad 2019). For instance, Cr (VI) is reduced within the cell to less toxic forms such as Cr (V), Cr (IV), and Cr (I-II) (Newsome and Falagán 2021).

Many bacterial species possess physiological abilities and genetic mechanisms for alleviating heavy metal toxicity. Some of these organisms convert Cr (VI) to less toxic Cr (III) through enzymatic reduction (Ndeddy Aka and Babalola 2017). Additionally, bacteria employ various other mechanisms, including bioaccumulation within cell biomass, efflux systems, complexation, precipitation, and oxidation reactions, to mitigate heavy metal toxicity. These processes involve phosphate, carboxyl, carbonyl, sulfhydryl, and hydroxyl functional groups which create a negatively charged surface on bacterial cell walls (Alotaibi et al. 2021).

In this study, the isolation of indigenous bacteria from the bauxite mining area led to the growth of two isolates on NA media enriched with 100 ppm Pb and Cr. The isolated *B. altitudinis* exhibited superior reduction capabilities compared to *K. pneumoniae*. *B. altitudinis* achieved reduction efficiencies of 87.42% for Cr and 72.76% for Pb, while *K. pneumoniae* reduced Cr by up to 86.2% and Pb by 34.48%.

Previous studies highlighted the Pb resistance of *Bacillus* sp. which used active transport and the pbr operon as defense mechanisms against Pb toxicity. Organic macromolecules within the cell wall, including polypeptides, polysaccharides, and proteins can adsorb Pb through electrostatic forces. Functional groups present in *Bacillus* sp. cell walls facilitate the binding of Pb, forming insoluble compounds (Alotaibi et al. 2021).

Firmicutes, a prominent bacterial phylum, is frequently found in old bauxite soils, with *Bacillus* being the most abundant genus (Nogueira et al. 2017). *Bacillus* species, such as *B. cohnii, B. pseudofirmus,* and *B. clarkia,* also exhibit resistance to environmental conditions in former bauxite sites (Nogueira et al. 2017). The results of this study aligned with previous reports stating that *Bacillus* sp. exhibited a significant capacity for Cr (VI) bio-removal (up to 72%) from Cr concentrations of 1000 g/mL.

Bacillus sp. is a rod-shaped gram-positive bacterium known for spore production. Furthermore, *B. altitudinis* has demonstrated potential as an effective biological fertilizer

for plant cultivation under abiotic stress in metal-contaminated soils (Pranaw et al. 2020). This versatile bacterial genus is abundant in soil and extensively used in bioremediation (Kowalczyk and Kramkowski 2023). This present study emphasized the continually increasing efficacy of *Bacillus* sp. in bioremediation, a quality enhanced by its ability to thrive in challenging environments.

The results obtained concerning the potential of *B. altitudinis* correlated with previous studies on the efficacy of this species in the biosorption of Ni (Babar et al. 2021), Cu (Khan et al. 2022), and Zn (Khan et al. 2022). Although *B. altitunidis* has the ability to reduce heavy metal levels through biosorption, the presence of these metals in the environment can inhibit bacterial cell growth.

Microorganisms mitigate Cr toxicity by converting Cr (VI) ions to Cr (III), distinct from the process used to reduce Pb toxicity. Cr (III) is comparatively less toxic than Cr (VI) and tends to precipitate, rendering it more easily removable. Microbes exposed to Cr in nature develop resistance by avoiding metal stress through metal depletion, absorption, or detoxification involving decreased metal ion immobilization. Microbial biodegradation employs enzyme-regulated biotransformation to convert hazardous Cr (VI) into a non-toxic state. The enzyme ChrR (chromate reductase) mediates this Cr reduction in both Gram-positive and Gram-negative bacteria. Additionally, it is present in *Bacillus* sp., *K. pneumoniae*, and other Cr-resistant bacteria. The reduction of Cr ions in *Bacillus* sp. and *K. Pneumoniae* follows an aerobic mechanism involving electron transfer from Cr (VI) to Cr (III), facilitated by the formation of an unstable Cr (V) intermediate and regulated by NADH/NADPH (Alotaibi et al. 2021).

Microbes employ various methods to alleviate heavy metal toxicity, such as the ion exchange mechanism. For instance, some investigations showed ion exchange occurrence in the absorption of Pb (II) by extracellular polymeric molecules from *Klebsiella* sp., involving counter ions K^+ and Mg^{2+} (Wei et al. 2016; Priyadarshanee and Das 2021). Gram-negative bacteria, including *K. pneumoniae*, possess a high affinity for heavy metals due to their lipopolysaccharide, oleic acid, and polychromic acid components (Yani et al. 2020; Ndeddy Aka and Babalola 2017). Previous studies also reported the resistance of *K. pneumoniae* to Pb, Hg, Cd, Cu, Ni, and Zn (Mohan et al. 2019; Atikpo and Ihimekpen 2020; Orji et al. 2021).

In conclusion, this study shows that in former bauxite mining areas, there are indigenous bacteria that have the potential to reduce heavy metal contamination. *B. altitudinis* and *K. pneumonia* have a firm ability to reduce Pb and Cr metals. These two bacterial species can be used as bioremediation agents for heavy metal-contaminated environments. This study can become the basis for future research to examine the optimization of metal-reducing bacteria groups. It is also recommended that future research carry out an experimental design with appropriate repetition and statistical analysis of data.

ACKNOWLEDGEMENTS

The authors are grateful to *Lembaga Pengelola Dana Pendidikan* (LPDP) and *Pusat Layanan Pembiayaan Pendidikan* (PUSLAPDIK) for supporting this study through the Scholarships for Indonesian Education (BPI) program. The authors are also grateful to the Indonesian Ministry of Education and Culture (Kemendikbud) for providing grants to facilitate the study execution.

REFERENCES

Ahemad M. 2019. Remediation of metalliferous soils through the heavy metal resistant plant growth promoting bacteria: Paradigms and prospects. Arabian J Chem 12: 1365-1377. DOI: 10.1016/j.arabjc.2014.11.020.

Alotaibi BS, Khan M, Shamim S. 2021. Unraveling the underlying heavy metal detoxification mechanisms of *Bacillus* species. Microorganisms 9: 1-31. DOI: 10.3390/microorganisms9081628.

Anno FD, Brunet C, Zyl LJ. Van, Trindade M, Golyshin PN, Anno AD, Ianora A, Sansone C. 2020. Degradation of hydrocarbons and heavy metal reduction by marine bacteria in highly contaminated sediments. Microorganisms 8: 1-18. DOI: 10.3390/microorganisms8091402.

Anusha P, Natarajan D. 2020. Bioremediation potency of multi-metal tolerant native bacteria *Bacillus cereus* isolated from bauxite mines, Kolli Hills, Tamilnadu- A lab to land approach. Biocatalysis Agric Biotechnol 25: 1-9. DOI: 10.1016/j.bcab.2020.101581.

Atikpo E, Ihimekpen NI. 2020. Bioremediation of lead-contaminated agricultural soil using *Klebsiella pneumoniae*. J Appl Sci Environ Manag 24: 805-808. DOI: 10.4314/jasem.v24i5.11.

Babar Z, Khan M, Chotana GA, Murtaza G, Shamim S. 2021. Evaluation of the potential role of *Bacillus altitudinis* MT422188 in nickel bioremediation from contaminated industrial effluents. Sustainability 13: 1-22. DOI: 10.3390/su13137353.

Batta N, Subudhi S, Lal, Devi A. 2013. Isolation of a lead-tolerant novel bacterial species, *Achromobacter* sp. TL-3: Assessment of bioflocculant activity. Indian J Exp Biol 51: 1004-1011.

Clark CM, Costa MS, Sanchez LM, Murphy BT. 2018. Coupling MALDI-TOF mass spectrometry protein and specialized metabolite analyses to rapidly discriminate bacterial function. PNAS 115: 4981-4986. DOI: 10.1073/pnas.1801247115.

Croxatto A, Prod G, Greub G. 2012. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. FEMS Microbiol Rev 36: 380-407. DOI: 10.1111/j.1574-6976.2011.00298x.

Damayanti R, Khareunissa H. 2017. Composition and characteristics of red mud: A case study on Tayan bauxite residue from alumina processing plant at West Kalimantan. Indones Mining J 19: 79-190. DOI: 10.30556/imj.vol19.no3.2016.660.

Gräfe M, Landers M, Tappero R, Klauber C, Hutomo G, Gan B, Grabsch A, Austin P, Davies I. 2010. Chemistry of trace and heavy metals in bauxite residues (red mud) from Western Australia. Proceeding of 19th World Congress of Soil Science, Soil Solutions for a Changing World. Brisbane, Qld, 1-6 August 2010. [Australia]

Igiri BE, Okoduwa SIR, Idoko GO, Akabuogu EP, Adeyi AO, Ejiogu IK. 2018. Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater. J Toxicol 27: 1-17. DOI: 10.1155/2018/2568038.

Kaczorek E. 2012. Effect of external addition of rhamnolipids biosurfactant on the modification of gram-positive and gram-negative bacteria cell surfaces during biodegradation of hydrocarbon fuel contamination. Pol J Environ Stud 21: 901-909.

Khalil A, Shaikh S, Tawabini B. 2022. Removal of heavy metals from contaminated water by thermophilic bacteria isolated from hot springs in Saudi Arabia. J Bacteriol Mycol 10: 60-64. DOI: 10.15406/jbmoa2022.10.00328.

Khan M, Ijaz M, Chotana GA, Murtaza G, Malik A, Shamim S. 2022. *Bacillus altitudinis* MT422188: a potential agent for zinc bioremediation. Bioremediat J 26: 228-248. DOI: 10.1080/10889868.2021.1927973.

Khan M, Kamran M, Kadi RH, Hassan MM, Elhakem A, Alhaithloul HA, Soliman MH, Mumtaz MZ, Ashraf M, Shamim S. 2022. Harnessing

the potential of *Bacillus altitudinis* MT422188 for copper bioremediation. Front Microbiol 13: 1-16. DOI: 10.3389/fmicb.2022.878000.

Kim SW, Chae Y, Moon J, Kim D, Cui R, An G, Jeong SW, An YJ. 2017. In situ evaluation of crop productivity and bioaccumulation of heavy metals in paddy soils after remediation of metal-contaminated soils. J Agric Food Chem 65: 1239-1246. DOI: 10.1021/acs.jafc.6b04339.

Kowalczyk P, Kramkowski K. 2023. Bioremediation of heavy metals by the genus *Bacillus*. Intl J Environ Res Public Health 20: 1-17. DOI: 10.3390/ijerph20064964.

Lee KY, Ho LY, Tan KH, Tham YY, Ling SP, Qureshi AM, Ponnudurai T, Nordin R. 2017. Environmental and occupational health impact of bauxite mining in malaysia. Intl Med J Malays 16: 137-150. DOI: 10.31436/imjm.v16i2.346.

Marzan LW, Hossain M, Mina SA, Akter Y, Chowdhury AM. 2020. Isolation and biochemical characterization of heavy-metal resistant bacteria from tannery effluent in Chittagong city, Bangladesh: Bioremediation viewpoint. Egypt J Aquat Res 43: 65-74. DOI: 10.1016/j.ejar.2016.11.002.

Mohan AK, Chiplunkar S, Kamath S, Martis S, Goveas L, Rao V. 2019. Heavy metal tolerance of *Klebsiella pneumoniae* kpn555 isolated from coffee pulp waste. Borneo J Resour Sci Technol 9: 101-106. DOI: 10.33736/bjrst.1538.2019.

Ndeddy Aka RJ, Babalola OO. 2017. Identification and characterization of Cr-, Cd-, and Ni-tolerant bacteria isolated from mines tailings. Bioremediat J 21: 1-19. DOI: 10.1080/10889868.2017.1282933.

Newsome L, Falagán C. 2021. The microbiology of metal mine waste: Bioremediation applications and implications for planetary health. Geo-Health 5: 1-53. DOI: 10.1029/2020GH000380.

Nogueira EW, Hayash EA, Alves E, Lima A, Adorno MT, Brucha G. 2017. Characterization of alkaliphilic bacteria isolated from bauxite residue in the southern region of Minas Gerais, Brazil. Biol Appl Sci 60: 1-7. DOI: 10.1590/1678-4324-2017160215.

Orji OU, Awoke JN, Aja PM, Aloke C, Obasi OD, Alum EU, Udu-Ibiam OE, Oka GO. 2021. Halotolerant and metalotolerant bacteria strains with heavy metals biorestoration possibilities isolated from Uburu Salt Lake, Southeastern, Nigeria. Heliyon 7: 1-8. DOI: 10.1016/j.heliyon.2021.e07512.

Oziegbe O, Oluduro AO, Oziegbe EJ, Ahuekwe EF, Olorunsola SJ. 2021. Assessment of heavy metal bioremediation potential of bacterial isolates from landfill soils. Saudi J Biol Sci 28: 3948-3956. DOI: 10.1016/j.sjbs.2021.03.072.

Pambudiono A, Suarsini E, Amin M. 2018. The potential of indigenous bacteria for removing cadmium from industrial wastewater in Lawang, East Java. J Trop Life Sci 8: 62-67. DOI: 10.11594/jtls.08.01.11.

Pranaw K, Pidlisnyuk V, Trögl J, Malinská H. 2020. Bioprospecting of a novel plant growth-promoting bacterium *Bacillus altitudinis* KP-14 for enhancing miscanthus × giganteus growth in metals contaminated soil. Biology 9: 1-25. DOI: 10.3390/biology9090305.

Priyadarshanee M, Das S. 2021. Biosorption and removal of toxic heavy metals by metal tolerating bacteria for bioremediation of metal contamination: A comprehensive review. J Environ Chem Eng 9: 1-83. DOI: 10.1016/j.jece.2020.104686.

Qi X, Wang H, Huang C, Zhang L, Zhang J. 2018. Analysis of bauxite residue components responsible for copper removal and related reaction products. Chemosphere 207: 209-217. DOI: 10.1016/j.chemosphere.2018.05.041.

Raza'i TS, Thamrin, Nofrizal, Amrifo V, Pardi, H, Pangestiansyah PI, Febrianto T, Fadhli IA. 2021. Accumulation of essential (copper, iron, zinc) and non-essential (lead, cadmium) heavy metals in Caulerpa racemosa, sea water, and marine sediments of Bintan Island, Indonesia. F1000Res 699: 1-24. DOI: 10.12688/f1000research.54445.1.

Shu LJ, Yang YL. 2017. *Bacillus* classification based on matrix-assisted laser desorption ionization time-of-flight mass spectrometry - effects of culture conditions. Sci Rep 7: 6-15. DOI: 10.1038/s41598-017-15808-5.

Starostin KV, Demidov EA, Bryanskaya AV, Efimov VM, Rozanov AS, Peltek SE. 2015. Identification of *Bacillus* strains by MALDI TOF MS using geometric approach. Sci Rep 5: 1-9. DOI: 10.1038/srep16989.

Wang T, Wang X, Tian W, Yao L, Li Y, Chen Z. 2020. Screening of heavy metal-immobilizing bacteria and its effect on reducing Cd 2+ and Pb 2+ concentrations in water spinach (*Ipomoea aquatic* Forsk.). Intl J Environ Res Public Health 7: 1-16. DOI: 10.3390/ijerph17093122.

Wei W, Wang Q, Li A, Yang J, Ma F, Pi S, Wu D. 2016. Biosorption of Pb (II) from aqueous solution by extracellular polymeric substances extracted from *Klebsiella* sp. J1: Adsorption behavior and mechanism assessment. Sci Rep 6 (1): 31575. DOI: 10.1038/srep31575.

Xu X, Hao R, Xu H, Lu A. 2020. Removal mechanism of Pb (II) by *Penicillium polonicum*: Immobilization, adsorption, and bioaccumulation. Sci Rep 10: 1-12. DOI: 10.1038/s41598-020-66025-6.

Yani A, Amin M, Rohman F, Suarsini E, Putra WE. 2020. Profiling indigenous lead-reducing bacteria from Tempe Lake, South Sulawesi, Indonesia as bioremediation agents. Biodiversitas 21: 4778-4786. DOI: 10.13057/biodiv/d211043.

Zhang R, Zhang Y, Zhang T, Xu M, Wang H, Zhang S, Zhang T, Zhou W, Shi G. 2022. Establishing a MALDI-TOF-TOF-MS method for rapid identification of three common Gram-positive bacteria (*Bacillus cereus*, *Listeria monocytogenes*, and *Micrococcus luteus*) associated with foodborne diseases. Food Sci Technol 42: 1-9. DOI: 10.1590/fst.117021.