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Research Article

Heavy Metal Adsorption (As, Zn, Pb, and Fe) using Indigenous Gram-Positive Bacteria from ASGM Wastewater in Lampung

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ABSTRACT

Small-scale gold mining (ASGM) produces toxic wastewater containing high concentration of heavy metals such as arsenic (As), lead (Pb), zinc (Zn), and iron (Fe) which threaten the environment and human health in the surrounding area. A biotechnological approach using metal-tolerant bacteria is an environmentally friendly alternative to physical and chemical methods. This study aims to test the ability of gram-positive bacteria, namely *Micrococcus*, isolated from ASGM tailings to absorb heavy metals As, Pb, Zn, and Fe. Isolation was carried out using Nutrient Agar (NA) and Nutrient Broth (NB) media, while tolerance tests were carried out at a concentration of 100 ppm for each metal. The results showed that *Micrococcus* bacteria could survive and resist exposure to contamination from the four heavy metals up to the highest concentration tested. These findings suggest that gram positive bacteria have significant potential for use as bioremediation agents in managing heavy metal pollution. In conclusion, *Micrococcus* can be used as an alternative in the remediation of As, Pb, Zn, and Fe metals, although further studies are still needed to evaluate their absorption capacity in more detail and their application in real environmental conditions.

INTRODUCTION

Small-scale gold mining (ASGM) activities are a significant contributor to heavy metal pollution in aquatic environments. Metals such as arsenic (As), zinc (Zn), lead (Pb), and iron (Fe) are toxic and persistent, and they tend to accumulate in aquatic organisms and food chains, causing serious impacts on human health and ecosystem balance. One of the dangerous pollutants is lead (Pb). It is a heavy metal that can be dangerous to the environment and toxic

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to human health (Ramadhaniar, 2019). The presence of these heavy metals not only reduces water quality but also disrupts the biological functions of aquatic organisms and lead to long-term risks to related terrestrial ecosystems. Heavy metal accumulation in humans can cause kidney, liver, and nervous system disorders, while in aquatic organisms, it can disrupt growth, reproduction, and survival.

The ASGM sector accounts for 17–20% of global gold production, with around 15 million people involved (KLHK, 2017). The main factors that driving the sustainability of these activities include limited employment opportunities, high global gold prices, and low compliance with existing regulations (Meutia, 2023). In addition, weak supervision and the limited ability of law enforcement officials to reach mining sites, most of which are located in remote areas, further exacerbate the problem (Agustina et al., 2025). The environmental impacts include water pollution, land degradation, and the accumulation of mercury and other heavy metals, which can endanger public health (Nugraha et al., 2024).

The use of indigenous bacteria from the mining environment is believed to be effective in reducing the impact of heavy metal pollution. Local bacteria are highly adaptable to local environmental conditions, including high concentrations of heavy metals. Some bacteria such as iron-reducing bacteria (IRB), can play a role in geochemical processes by anaerobically respiring Fe^{3+} oxide, thereby offering potential use in heavy-metal bioremediation strategies (Li et al., 2022).

Although many studies have reported the impact of ASGM-related heavy metal pollution, research on the ability of indigenous bacteria to absorb heavy metals (As, Zn, Sn, Fe) from gold and silver mining wastewater remains limited, especially in Lampung Province. This study aims to evaluate the potential of local bacteria to absorb these heavy metals, while hoping that the results will serve as a basis for developing microbe-based bioremediation strategies to reduce heavy metal pollution in water bodies.

MATERIALS AND METHOD

Materials

Soil and mine wastewater samples were collected at PT. Lampung Kencana Cikantor (LKC), Sejahtera, and terminology (ASGM) in Pesawaran Regency, Lampung Province, Indonesia. Sample testing and research were conducted at the Bio Metallurgy Laboratory (BRIN Lampung).

Isolation of Bacteria from Gold Mining Wastewater (ASGM)

The heavy metals used in this study included arsenic (As) under the Smart Lab brand, produced in Tangerang, Indonesia; lead (Pb) under the Merck brand, produced in Darmstadt, India; zinc (Zn) under the Merck brand, produced in Darmstadt, Germany; and iron (Fe) under the Merck brand, produced in Darmstadt, Germany. These elements were selected due to their prevalence as major contaminants in polluted environments, particularly those associated with industrial effluent. Each metal was administered in solid form at concentrations appropriate for laboratory analysis. Wastewater from small-scale gold mining activities was used as a source of bacteria for isolation. Samples were collected from liquid waste disposal sites around small-scale mining areas using sterile 500 mL bottles. Sampling was performed aseptically, and samples were immediately stored in a cool box. Isolation and characterization of bacteria followed Aguilar et al. (2020) with modifications. A total of 0.5 mL of gold mine wastewater sample was added to a 50 mL Erlenmeyer flask containing Luria Broth (LB) medium, and the flask was incubated for 3 days at 37 °C. After incubation, 0.1 mL of the turbid enrichment culture was transferred to a Petri dish containing nutrient agar. Bacterial colonies exhibiting morphological variation such as differences in shape, colour, and margin were further sub cultured on solid nutrient agar for purification. The purified isolates were characterized based on colony morphology, including dimensions, form, elevation, and edge characteristics.

Gram Staining in the Characterization of Bacterial Isolates

Cell morphology was observed using Gram staining: a drop of distilled water was placed on a slide, then the slide was inoculated with bacteria and heated by passing it over a flame. Next, the primary stain, Crystal Violet, was applied to the slide and left to react for one minute before being gently rinsed with distilled water. After that, Gram's iodine stain was applied and left for one minute before being rinsed with 95% ethanol. Finally, the secondary stain, Safranin, was applied for 1 minute, followed by rinsing with water. The prepared slide preparation was then observed under a microscope (Borthakur, 2025).

Analysis of Bacterial Motility Using Semi-Solid Medium

Motility assays were performed in Luria Broth (LB) medium solidified with 0.3% or 0.8% agar by inoculating 1 µL or 5 µL of bacterial suspension into the center of the medium, then incubating at 27 °C for 48 hours. Three parallel replicates were prepared per group, and all experiments were conducted in triplicate (Liu et al., 2023).

Biochemical Testing of Bacterial Isolates

Biochemical testing can be performed by measuring gas production from carbohydrate fermentation, as described in the working method (Lambui & Jannah, 2017). The isolates obtained were incubated for 24 hours in LB medium containing inverted Durham tubes. One tube without a carbohydrate source is incubated with 1 mL of culture that has been incubated for 24 hours. Incubation is carried out at 30 °C for 1 to 5 days, depending on the culture's growth. Gas formation occurs when gas bubbles appear in the Durham tube.

Potential Reduction of As, Zn, Pb, And Fe By Indigenous Bacteria

This method was adapted from Schalk et al. (2021) with modifications. Bacterial cultures were inoculated into Lactose Broth (LB) medium and incubated for 3 days at 37 °C, with the addition of arsenic (As), zinc (Zn), lead (Pb), and iron (Fe) at 100 ppm each. The mixture was then homogenized using an orbital shaker and incubated in an incubator for 3 days. Next, TPC was performed to observe the growing colonies.

RESULTS AND DISCUSSION

This section describes and discusses the results of bacterial isolation from gold mining waste and its ability to survive in media containing heavy metals. The test was conducted by preparing Nutrient Broth media inoculated with Micrococcus bacteria, then adding heavy metals arsenic (As), lead (Pb), zinc (Zn), and iron (Fe) at a concentration of

100 ppm each. A concentration of 100 ppm was used as a testing method to provide controlled metal pressure on the media, so that the ability of bacteria to tolerate heavy metals could be clearly observed. At this concentration, each metal was equivalent to 100 mg/L in the culture media, so that the bacterial growth response could be compared under the same exposure conditions.

Isolation of Bacteria from Gold Mine Wastewater (ASGM)

Bacterial isolation from gold mine wastewater in Lampung Province in this study was performed using the spread plate method. This method was chosen because it has a relatively lower risk of contamination, considering that only the surface of the agar medium is exposed during the inoculation process. In addition, bacterial colonies grow on the agar surface in accordance with the spread area using a hockey stick tool, so that the colonies formed are more separated and easier to observe and count (Sanders et al., 2012).

Based on the isolation and purification results, one bacterial isolate was obtained. The selection of isolates was carried out by considering the differences in colony morphology observed on the medium, including colony shape, elevation, edges, surface, and color. These morphological characteristics were used as a basis for distinguishing and determining the bacterial isolates shown in **Figure 1**.

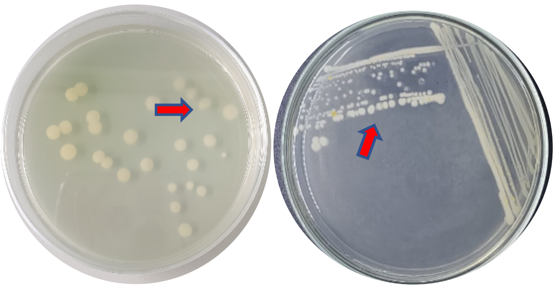

Figure 1 Red arrows indicate bacterial colonies

The morphological characteristics of the bacterial isolate colonies showed relatively uniform colony growth on the culture medium. The morphological observation data of the colonies are presented in **Table 1**. The observation results show that isolate C1 has a round colony shape, convex elevation, smooth colony edges, and white color. These morphological characteristics provide an initial description of the growth properties of the bacterial colonies obtained from the isolation process.

Table 1. Bacterial colony morphology

Isolate	Shape	Elevation	Margin	Color
C1	*Circular*	Convex	Smooth	White

Gram Staining in the Characterization of Bacterial Isolates

The characteristics of bacterial isolates were analyzed using the Gram staining method as an initial identification step to determine the type of bacterial cell wall. Gram staining is a technique commonly used in microbiology to distinguish bacteria based on the ability of the cell wall to retain the main dye during the staining process. Gram-negative bacteria are characterized by a pink color, while Gram-positive bacteria show a purple color because they are able to retain crystal violet after the decolorization process. Based on microscopic observations, the bacterial cells in this study appeared blue-purple and coccus-shaped, indicating that the isolates were Gram-positive bacteria. The results of the Gram staining observation are shown in **Figure 2**. These findings are in line with the statement by Putri and Kusdiyantini (2018), which states that bacteria that retain crystal violet dye even after undergoing a decolorization process with alcohol or acetone are classified as Gram-positive bacteria.

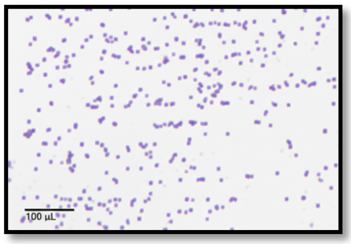

Figure 2 Gram positive staining of bacterial isolates from gold mine wastewater medium

Analysis of Bacterial Isolate Motility Test Results Using Semi-Solid Medium

The motility test is a test that aims to confirm whether the microorganisms found belong to the group of bacteria that have flagella as their means of movement. The motility test shown in **Figure 3** shows a positive result, as evidenced by the spread of colony growth and the media becoming cloudy like fog after one bacterial isolate was planted perpendicularly on the media (Rahmawati and Isnaeni, 2016). According to research conducted by Casado et al. (2021), bacteria can move using their flagella by rotating them, allowing them to move within media with lower agar content. Passive bacterial movement occurs due to Brownian motion. Factors that can influence the observation of bacterial motility include fresh media, acid production, and toxin production (Pattuju et al., 2014).

Figure 3 Motility of bacterial isolates using control semi-solid medium

Biochemical Test

Based on research conducted on gas production tests through carbohydrate fermentation, the main objective is to determine the ability of bacterial isolates to metabolize substrates into fermentation products, particularly gas. Based on observations, isolate C1 did not produce gas or bubbles yet was cloudy and formed a biofilm. This may indicate that bacterial growth was rapid but uneven in the liquid medium, or that gas was produced mainly through initial enzyme activity.

The gas production test in this study aimed to determine the ability of bacterial isolates to metabolize substrates through fermentation. The test was qualitative and did not involve quantitative measurements, so the interpretation of the results was based on visual observation of several indicators that reflected the metabolic activity of the bacteria. The test results are presented in **Figure 4**. The parameters observed included gas formation, media turbidity, and the appearance of bubbles during the incubation process. These three indicators were used as a basis for evaluating the biochemical response of the bacterial isolates tested.

Based on the results of observations in the carbohydrate fermentation test, isolate C1 did not show gas or bubble formation, but the culture medium appeared cloudy, and a biofilm formed on the surface. This condition indicates that bacterial growth continued in the liquid medium even though it was not followed by gas production as a fermentation product. The cloudiness of the medium indicates an increase in the number of bacterial cells, while the formation of biofilm may indicate localized growth activity on the surface of the medium. These results indicate that isolate C1 may have a metabolic ability that does not predominantly produce gas during the fermentation process.

Figure 4 Biochemical test results of bacterial isolate from gold mine waste, (A) Control and (B) Isolate C1

This may indicate that bacterial growth is rapid but unevenly distributed in the liquid medium, or that gas is predominantly produced by initial enzymatic activity. According to research by Yanti and Dali (2013), isolates containing gas bubbles are heterofermentative, whereby fermentation produces lactic acid, carbon dioxide (CO_2), and ethanol. Heterofermentative fermentation converts about half of the total sugar into lactic acid, while the rest is converted into gas or alcohol. According to research (Koo et al., 2017), bacterial cells in biofilms are more resistant to stress conditions or the host's immune system. Biofilms are considered a form of biofouling on man-made industrial surfaces, but they have also been implicated in chronic bacterial infections.

Potential Reduction of As, Zn, Pb, And Fe By Indigenous Bacteria

Based on the test results, the native bacteria showed significant potential in reducing As, Zn, Pb, and Fe. The difference in the reduction levels between these metals indicates differences in the bacteria's adaptability and metabolic activity towards different types of metals. This is evidenced by the color change in the bacterial culture before and after treatment, which shows the reduction of metals by bacterial activity as shown in **Figure 5**. Visual observation of bacterial cultures after the addition of heavy metals Pb, As, Zn, and Fe showed changes in color and turbidity levels of the medium, reflecting the growth response of bacteria to each metal. In the lead (Pb) treatment, the culture medium appeared cloudy yellow with biomass accumulation at the bottom of the medium, indicating that bacteria were still able to grow even in conditions containing heavy metals. Similar conditions were also observed in the arsenic (As) and zinc (Zn) treatments, where the medium showed turbidity and bacterial cell precipitation at the bottom of the medium as an indication of bacterial growth activity during the incubation period.

Meanwhile, in the iron (Fe) treatment, there was a change in the color of the medium, which tended to be darker or brownish compared to the other treatments. This difference in color intensity indicates an interaction between Fe metal and the culture medium as well as bacterial metabolic activity. In general, the color change, media turbidity, and biomass accumulation in each treatment indicate that the bacterial isolates are still able to survive and grow in media containing heavy metals, even though each metal has a different effect on the characteristics of the bacterial culture.

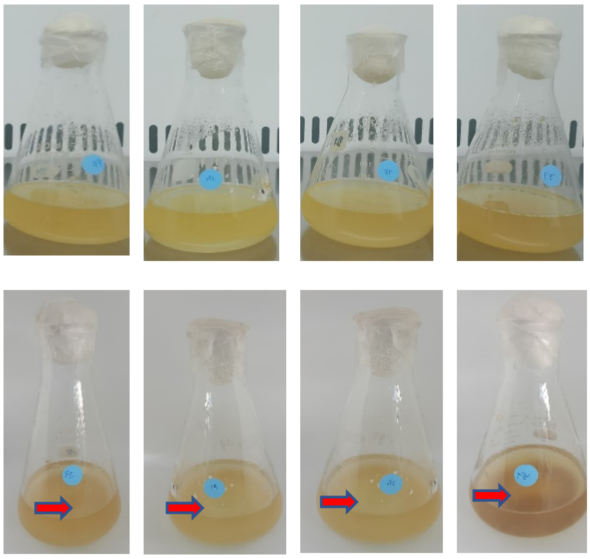

Figure 5 Culture of isolate *Micrococus* bacteria before and after inoculation with heavy metals. Red arrows indicate changes in bacterial cultures that have been inoculated with heavy metals

Analysis of pH Changes in Bacterial Cultures in Media Containing Heavy Metals

Measurements taken during the incubation period revealed a change in the pH value of the culture medium from 7 to 8, indicating a shift in environmental conditions due to bacterial metabolic activity. This change in pH reflects the growth and biological activity of microorganisms in the culture medium. Bacterial metabolic activity is known to produce various compounds that alter the medium's pH, making it more acidic or more alkaline. Therefore, the subsequent pH change was analyzed in relation to bacterial growth as indicated by the Total Plate Count (TPC) value. Data on pH changes during the incubation period are presented in **Table 2**.

Based on the data in **Table 2**, an increase in pH from 7 to 8 occurred in treatments containing Pb, As, and Zn metals after incubation of isolate C1. This indicates that the bacteria were still able to remain active and affect the media conditions even in an environment containing heavy metals.

Meanwhile, in the treatment with Fe metal, there was no change in pH, indicating that the metabolic activity of the bacteria or the interactions occurring in the medium were relatively stable during the incubation period. The differences in pH changes in each treatment indicate that the type of metal in the medium can affect the growth response and activity of the bacteria.

Table 2 Changes in medium pH values during the Absorption process of Pb, As, Zn, and Fe metals by local bacterial isolates from Gold Mine Wastewater (ASGM)

Isolate	Metal Type	Initial pH	Final pH
	Pb	7	8
C1	As	7	8
	Zn	7	8
	Fe	7	7

Bacterial Growth Based on Total Plate Count (TPC)

Bacterial growth observed through Total Plate Count (TPC) measurements showed that the number of colonies in all heavy metal treatments was higher than in the control, where the control TPC value was recorded at 2.34 ± 0.21 Log CFU/mL, while the Pb, Fe, As, and Zn treatments showed values of 3.27 ± 0.29; 3.18 ± 0.38; 3.13 ± 0.47; and 2.99 ± 0.52 Log CFU/mL, respectively. The highest number of colonies was obtained in the Pb treatment, while the lowest value in the heavy metal treatment was found in Zn, although it was still higher than the control. These conditions indicate that the bacterial isolates have a tolerance to environments containing heavy metals, so that the presence of metals at the concentrations used did not completely inhibit bacterial growth, and the microorganisms were still able to maintain their growth activity during the incubation period. This is in line with research conducted by Nadya et al. (2024), which states that *Micrococcus* Pb bacteria have the ability to reduce accumulated Pb levels of 100 ppm. Data regarding TPC is presented in **Table 4**.

Table 4 Variations in bacterial colony growth resulting from Total Plate Count (TPC) tests on medium containing Pb, As, Zn, and Fe metals

Heavy metal	Average Total Bacterial Colonies (Log CFU/mL)
Control	2.34 ± 0.21
Pb	3.27 ± 0.29
Fe	3.18 ± 0.38
As	3.13 ± 0.47
Zn	2.99 ± 0.52

CONCLUSION

This study shows that local bacterial isolates (*Micrococcus*) obtained from small-scale gold mining (ASGM) waste in Lampung Province are able to survive and adapt to exposure to heavy metals As, Pb, Zn, and Fe at concentrations of up to 100 ppm. Based on the characterization results, isolate C1 is classified as a Gram-positive bacterium that is motile and metabolically active. The Total Plate Count (TPC) results showed the highest number of colonies in the Pb treatment and the lowest in Zn, indicating differences in tolerance levels to each metal. Changes in medium color and pH values indicate the occurrence of biosorption and biotransformation of metals by bacterial activity. Thus, *Micrococcus* isolates have great potential as environmentally friendly bioremediation agents for managing heavy metal-contaminated waste from ASGM activities.

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