JURNAL BIOLOGI PAPUA
Volume 18, Number 1, pages: 79–88
April 2026

ISSN 2086-3314
E-ISSN 2503-0450
DOI: 10.31957/jbp.5286
http://ejournal.uncen.ac.id/index.php/JBP

Isolation and characterization of nickel, lead, copper, and cadmium-multiresistant bacteria from the Citarum River, West Java

DEOKWARD F. RUGA, WAHYU IRAWATI*
Biology Education Study Program, Faculty of Education, Universitas Pelita Harapan, Tangerang, Indonesia

Received: 16 January 2026 – Accepted: 26 March 2026

ABSTRACT

Industrial activities along the Citarum River, West Java, have resulted in serious heavy metal contamination, causing negative impacts on aquatic ecosystems and human health. Bioremediation using indigenous bacteria with multi-metal resistance offers an efficient and environmentally friendly solution because these bacteria are directly obtained from contaminated environments and are resistant to several heavy metals simultaneously. This study aimed to isolate and characterize indigenous bacteria from the Citarum River that are multiresistant to a mixture of four heavy metals, namely nickel, lead, copper, and cadmium. The bacteria were isolated using the spread plate method on Luria–Bertani Agar supplemented with a mixture of those heavy metals at a concentration of 1 mM. The isolates were purified by the streak plate method and characterized based on colony morphology and cell morphology through Gram staining. The results showed that 24 bacterial isolates were successfully obtained, all of which were able to grow in a 1 mM mixture of nickel, lead, copper, and cadmium. Morphological characterization revealed diverse colonies dominated by yellow, white, and brown pigmentation, with circular shapes and smooth margins. Gram staining showed that all isolates were Gram-negative bacteria. A total of 16 isolates exhibited darker pigmentation, ranging from dark brown to blackish, when cultivated on heavy-metal-containing media. Dark-colored colonies indicate intensive interactions through biosorption and bioprecipitation mechanisms, resulting in the formation of insoluble compounds such as metal sulfides on the cell surface. The discovery of these 24 multiresistant bacterial isolates is expected to serve as preliminary research for exploring the potential of bacteria as heavy metal bioremediation agents to address pollution in the Citarum River.

Key words: cadmium; multi-resistant; nickel; copper; lead.

INTRODUCTION

The development of the industrial sector in Indonesia has become a driving force for national economic growth. However, behind this positive contribution lies a serious consequence in the form of increasing pressure on the environment. Solid, liquid, and gaseous wastes generated by industrial activities are often discharged without adequate treatment, resulting in persistent pollution (Bersih *et al*., 2024). One of the most hazardous forms of pollution is heavy metal contamination, as heavy metals cannot be naturally degraded and may accumulate in ecosystems. Toxic heavy metals such as copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd), nickel (Ni), and mercury (Hg) have long been identified as serious threats to environmental sustainability and human health (Das *et al*., 2016).

The Citarum River in West Java is a clear example of a water body experiencing quality degradation due to heavy metal pollution. As the longest river in the province, the Citarum plays a strategic role in the supply of raw water,

* *Corresponding author:*
Biology Department, Faculty of Education, Universitas Pelita Harapan. Jl. M.H. Thamrin Boulevard 1100, Lippo Karawaci, Tangerang 15811, Banten, Indonesia.
E-mail: w.irawati3@gmail.com.

agricultural irrigation, aquaculture, and hydropower generation through the Saguling, Cirata, and Jatiluhur reservoirs. However, the intensity of industrial and domestic activities along the river basin has caused high COD, TDS, turbidity, and concentrations of Ni, Pb, Cu, and Cd that exceed environmental quality standards (Fahimah *et al*., 2023). Recent data indicate that approximately 47.1%, or 127 km, of the Citarum River has been categorized as heavily polluted, with both organic and inorganic wastes as the main sources of water quality degradation (Kamila *et al*., 2024). These conditions highlight the urgent need for mitigation efforts because several of these heavy metals are toxic.

Heavy metals have a dual nature in biological systems. Some essential metals such as Cu, Ni, Fe, and Zn are required in small amounts for specific physiological functions, but at high concentrations they may become toxic and carcinogenic (Panjaitan *et al*., 2019). In contrast, non-essential heavy metals such as Cd, Pb, and Hg have no biological function and are highly dangerous even at low concentrations (Hamzah & Priyadarshini, 2019). The main characteristics that make heavy metals a serious threat are their persistence and inability to be naturally degraded, thereby allowing bioaccumulation and biomagnification to occur (Mardiana & Handayani, 2017). The presence of mixed heavy metals in aquatic environments may produce synergistic effects that aggravate ecological damage and complicate natural recovery processes.

Public and industrial understanding of waste treatment remains limited, so hazardous wastes are often discarded without proper treatment (Handayani *et al*., 2022). Efforts to control heavy metal pollution are still dominated by physical and chemical methods; however, these approaches have limitations, including high costs, complex technology, and the potential generation of secondary wastes (Rachman *et al*., 2024). Bioremediation has emerged as an efficient alternative by utilizing bacteria that exhibit resistance to several heavy metals simultaneously. Indigenous bacteria are a strategic option because they possess adaptive capabilities through heavy metal resistance mechanisms, including biosorption, bio-accumulation, precipitation, and cellular detoxification (Fahimah *et al*., 2023). Indigenous bacteria that have long adapted to contaminated environments have great potential to be developed as effective bioremediation agents (Trikurniadewi, 2025).

Several Gram-negative and Gram-positive bacteria are known to have multiresistance to heavy metals. *Streptococcus mutans*, *Flavobacterium*, and *Pseudomonas* sp. exhibit multiresistance to Cd, Cu, Ni, and Pb (Mahrus *et al*., 2024). *Acinetobacter* sp. IrC2, isolated from a waste treatment center in Surabaya, shows multiresistance to mercury, cadmium, and lead (Irawati *et al*., 2020). *Cupriavidus pauculus* IrC4 is multiresistant and can accumulate copper, lead, and cadmium at 371.42 mg, 254.4 mg, and 5.8 mg per gram of dry cell weight, respectively (Irawati *et al*., 2021). This study aimed to isolate and characterize indigenous bacteria from the Citarum River that are multiresistant to nickel, lead, copper, and cadmium and to characterize their colony and cellular morphology. This work is expected to serve as preliminary research exploring the potential of indigenous heavy-metal-multiresistant bacteria from the Citarum River as bioremediation agents.

MATERIALS AND METHODS

Time and place of research

Sampling was conducted in the Citarum River because it is the longest and largest river in Tatar Pasundan, West Java Province, Indonesia, and is known to have a high level of pollution (Septiansyah *et al*., 2023). The sampling location was determined in the Citarum River basin near several industrial areas, where production processes and waste outputs are associated with the use of heavy metals. Previous studies have reported the distribution of heavy metals in the Citarum

River basin; therefore, this site was selected as the sampling area.

Water samples were collected using sterile bottles by the grab sampling method. During sampling, the bottle opening was directed opposite to the river flow to minimize surface contamination. The collected samples were immediately transferred to sterile containers and stored under cold conditions (4°C) in the laboratory until further analysis. Figure 1 shows the map of the sampling location, while Figure 2 presents an example of the river water sample used in the isolation and characterization of indigenous bacteria.

Tools and materials

Various laboratory equipment and materials were used in this study to support the isolation process, resistance testing, and preliminary characterization of bacteria against heavy metals. The main equipment included a laminar air flow (LAF) cabinet as a sterile working area, an autoclave for sterilizing equipment and media, and an incubator for incubating bacterial cultures at the optimum temperature. Supporting equipment included sterile petri dishes, test tubes and racks, erlenmeyer flasks and 100 mL beakers, measuring cylinders, micropipettes and tips, inoculating loops, spreaders, spatulas, stirring rods, an analytical balance, a hot plate, a microscope and glass slides, and other supporting items such as labels, a stopwatch, wrapping paper, plastic, cotton, tissue, and waste containers. Personal protective equipment in the form of gloves and masks was also used. The materials included bacterial isolates obtained from Citarum River water, Luria Bertani Agar (LBA) as the growth medium, 98% alcohol for sterilization, and heavy metal solutions of nickel ($NiCl_2$), copper ($CuSO_4$), lead ($Pb(NO_3)_2$), and cadmium ($CdCl_2$) as resistance test agents. For preliminary bacterial identification, Gram staining reagents consisting of crystal violet, Lugol's solution (iodine), alcohol as the decolorizer, and safranin as the counterstain were used.

Research stages

Preparation of LBA medium

In this study, Luria Bertani Agar (LBA) medium was used, with a main composition of peptone, yeast extract, NaCl, and agar. The procedure began with sterilization of Petri dishes using an autoclave at 121°C and 1.1 atm (15 psi) for 20 minutes. Four grams of LBA powder were dissolved in 100 mL of distilled water in an Erlenmeyer flask, homogenized on a hot plate, and then sterilized again under the same autoclave conditions.

For preparation of selective media containing heavy metals (Ni, Pb, Cu, and Cd), all procedures were carried out aseptically in a Laminar Air Flow (LAF) cabinet while maintaining sterility of the working zone with a

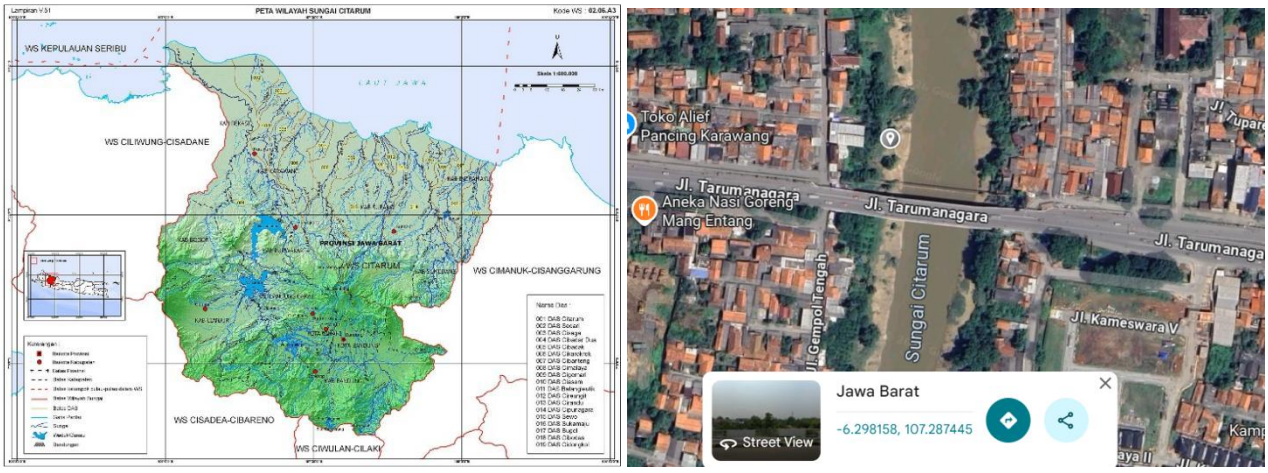

Figure 1. Research sampling location

Bunsen flame. The LBA solution was enriched by adding 25 µL of heavy metal stock solution to achieve a final concentration of 1 mM and then poured into sterile petri dishes. After solidification, the media were wrapped to minimize contamination risk and stored in an inverted position to prevent condensation droplets from falling onto the medium surface. All equipment and materials were disinfected with 96% alcohol before use to ensure sterile working conditions (Irawati *et al*., 2021).

1. Isolation of heavy-metal-multiresistant bacteria

The initial isolation step was carried out by taking 100 µL of waste sample with a micropipette and inoculating it onto solid Luria Bertani Agar (LBA) medium. The sample was evenly spread over the medium surface using a spreader with the spread plate technique, a method of inoculating microorganisms on solid media by homogeneously distributing the sample suspension across the agar surface (Irawati *et al*., 2022). After inoculation, the petri dishes were tightly wrapped and incubated for 24 hours at 37°C. All procedures were performed aseptically in a laminar air flow cabinet and near a Bunsen flame to minimize contamination risk. This step was intended to ensure that the growing colonies originated solely from the tested waste sample.

2. Characterization of bacterial isolates

The isolates obtained were then characterized morphologically and physiologically to provide a preliminary overview of their identity and resistance potential to heavy metals. Colonies growing during the isolation stage were purified by the streaking method (Marzan *et al*., 2017). Colony selection was based on morphological characteristics, including shape, color, surface appearance, margin, and colony size. Selected colonies were then cultivated on LBA medium supplemented with 1 mM of each heavy metal (Ni, Pb, Cu, and Cd) and incubated for three days at 37°C. Each emerging colony was labeled according to its morphological characteristics, and the number of colonies was recorded for each medium. This stage was important for assessing isolate dominance and diversity. Morphological characterization involved observation of six main aspects, namely color, shape (round, irregular, concentric), margin (smooth, undulate, serrated), surface appearance (transparent, opaque, shiny), elevation (convex, umbonate, plateau, flat, raised), and colony number. These observations formed the basis for selecting unique isolates with potential for further analysis.

3. Purification of heavy-metal-multiresistant bacterial isolates at 1 mM Ni, Pb, Cu, and Cd

Purification was carried out to obtain pure colonies originating from a single isolate (Pamaya *et al*., 2018). All stages were performed aseptically, with tools and materials sterilized using 96% alcohol before use in the laminar cabinet. The inoculating loop was heated until red-hot and then used to pick the selected isolate. Streaking was performed in three quadrants with different patterns: the first quadrant was streaked densely, the second more sparsely with the initial line taken from the first quadrant, and the third even more loosely with the initial line taken from the second quadrant. After each streak, the loop was reheated to reduce the number of bacteria

Figure 2. Citarum River water sample used in this study.

carried over. The final step involved sterilizing the loop, wrapping the petri dish, and incubating at 37 °C.

4. Gram staining

Gram staining was initiated by sterilizing the microscope slide with alcohol, followed by brief heat fixation. Bacterial isolates were smeared onto the slide using a flamed inoculating loop. The staining process was carried out sequentially with crystal violet (1 minute), Lugol's solution (1 minute), alcohol washing, and finally safranin (1 minute), with each step followed by rinsing with distilled water. The preparations were then observed under a microscope to identify membrane structure and cell shape. Gram staining separates bacteria into two major groups, namely Gram-positive bacteria with a single membrane layer protected by a thick cell wall, and Gram-negative bacteria with a more complex double-membrane structure (Irawati *et al.*, 2022). This technique facilitates isolate differentiation based on cell wall characteristics while also providing preliminary information on physiological properties.

Data analysis

Data obtained from the isolation of heavy-metal-multiresistant bacteria were analyzed using a qualitative approach focusing on the observation and description of the characteristics of bacteria isolated from Citarum River water samples, West Java. The analysis was conducted by observing colony morphology on media supplemented with the heavy metal mixture, including colony shape, color, size, and texture. In addition, Gram staining and microscopic observation were performed to identify bacterial type based on

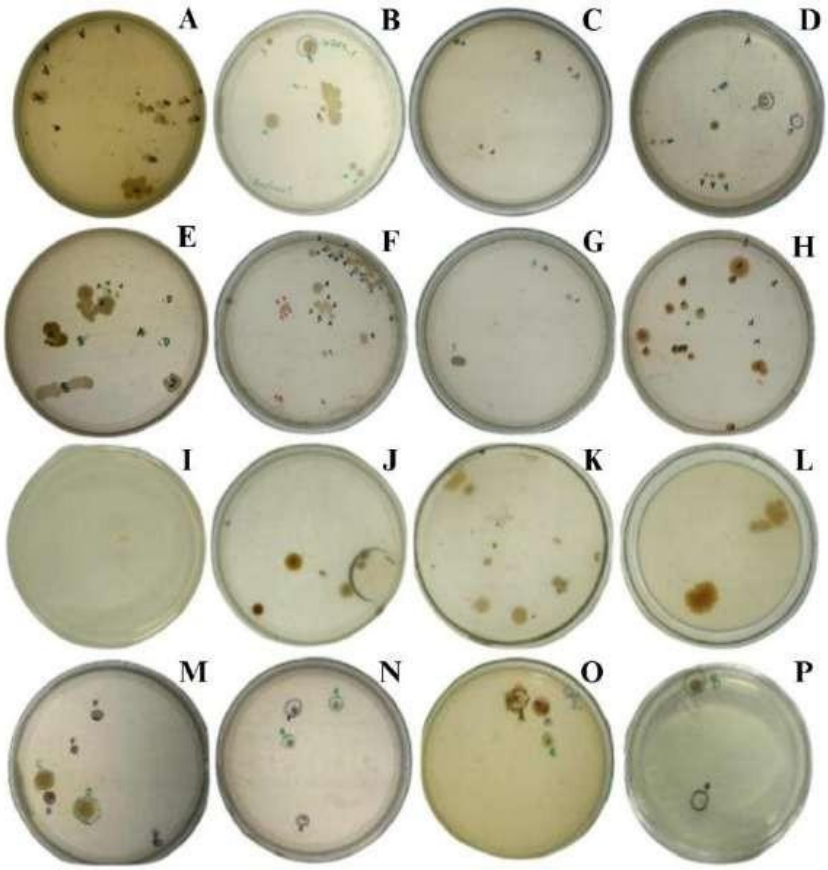

Figure 3. Bacterial communities growing on medium containing a 1 mM mixture of Ni, Pb, Cu, and Cd.

cell membrane characteristics.

RESULTS AND DISCUSSION

The isolation and characterization results in Figure 3 show the growth of several bacterial colony types resistant to a mixture of Ni, Pb, Cu, and Cd heavy metals. The Citarum River has been contaminated with heavy metals for a long period, thereby driving natural selection of resistant bacteria capable of surviving under such toxic pressure (Irawati *et al*., 2020). Bacteria lacking resistance mechanisms will die or be eliminated from the population because they are unable to withstand toxic stress (Mukhamatdyarov *et al*., 2023), whereas bacteria possessing resistance mechanisms are selected to grow as resistant bacteria (Baharudin *et al*., 2022). In heavy-metal-contaminated environments, bacteria have evolved and developed a series of complex adaptive strategies to survive and maintain cellular homeostasis (Mondal *et al*., 2025).

The mixture of Ni, Pb, Cu, and Cd heavy metals suppressed the number of bacterial colonies that grew (Figure 3). The lowest bacterial growth was observed in Figure 3-I, with only one colony, whereas the highest growth was observed in Figure 3-H, with 15 colonies. This highly limited bacterial colony growth indicates that a 1 mM mixture of Ni, Pb, Cu, and Cd is toxic to bacteria.

Heavy metals at low concentrations (micromolar range) inhibit enzyme activity, damage cell membranes, and trigger oxidative stress in microorganisms. At a concentration of 1 mM, these toxic effects increase significantly so that only bacteria with specific resistance mechanisms are able to survive (Gillieat & Coleman, 2024). In general, bacterial resistance mechanisms include efflux, whereby heavy metals

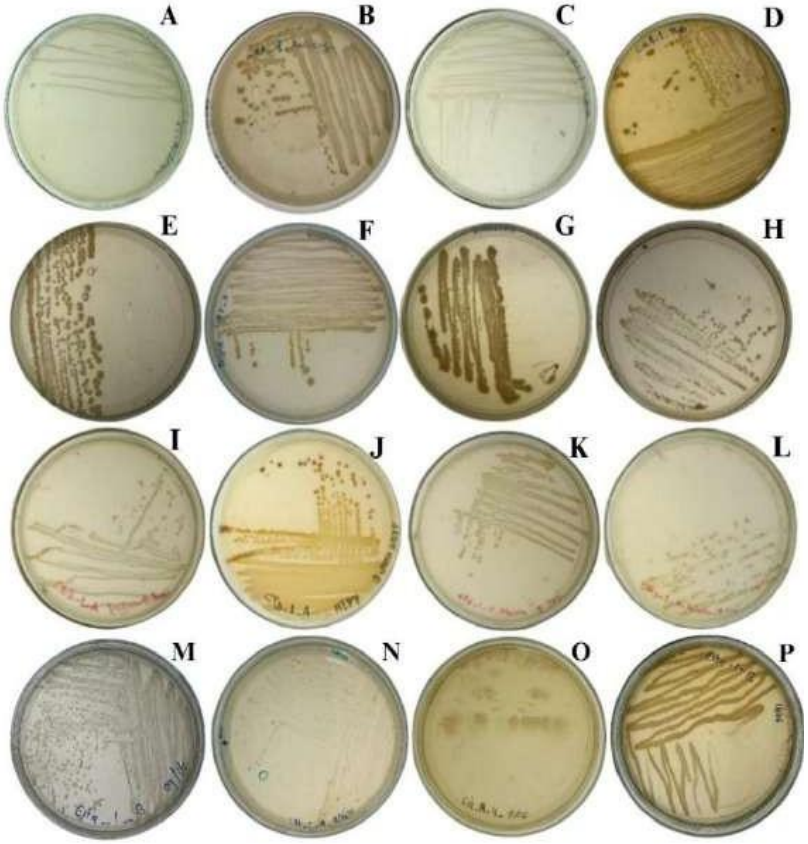

Figure 4. Heavy-metal-multiresistant bacterial isolates at 1 mM.

are pumped out through activation of specific transport systems in the cell membrane (Corona *et al*., 2025). This system functions as an active pump that selectively recognizes heavy metal ions entering the cytoplasm and exports them back into the environment, allowing bacteria to maintain internal heavy metal concentrations below the toxic threshold and prevent damage to vital cellular components (Patil *et al*., 2025).

Based on differences in colony color, shape, margin, optical appearance, and elevation, 24 bacterial isolates were obtained (Table 1). Most colonies were yellow, with variations including pale yellow, dark yellow, dull yellow, and bright yellow, while the remainder were white, milky white, or a combination of white and yellow. In general, the bacteria had round colonies, smooth margins, an opaque appearance, and convex elevation. Variations in colony margin shape and elevation generally reflect growth rate, cell-surface interaction with the medium, and cell wall composition (Wahyuni, 2017).

Gram staining results showed that all isolates were Gram-negative bacteria. Physiologically, Gram-negative bacteria possess a double-membrane structure containing lipopolysaccharides (LPS). According to Paracini *et al*. (2022), this structure serves as the first physical barrier that binds metal ions in the periplasmic space before they reach the cytoplasm and is supported by more complex efflux pump systems that expel toxic ions from the cell.

Differences in colony color, ranging from cream and white to yellowish brown, also indicate differences in secondary metabolites or pigments produced by each isolate. These pigments function as protection against oxidative stress or heavy metals and as adaptive components in extreme environments (Ren *et al*., 2025). Baharudin *et al*. (2022) reported that darker colonies are indicated to have a higher heavy metal absorption capacity than white or transparent colonies; therefore, dark-pigmented bacterial isolates were selected and purified as candidates for further bioremediation studies (Figure 4). The selection of representative colonies with darker pigmentation was based on the biological consideration that dark pigments, such as melanin- or melanoidin-type compounds, have strong potential in heavy metal binding and detoxification mechanisms. These colonies are suspected to undergo more intensive metal interactions on their cell surfaces than light-colored or transparent colonies (Destriana *et al*., 2025).

Figure 4 shows the purification results of 16 dark-colored bacterial isolates. Colony color is directly correlated with the bacterial ability to respond to heavy metals. Greenish isolates are theoretically associated with a specific ability to absorb copper (Cu), whereas dark brown to blackish colonies correlate with lead (Pb) and cadmium (Cd) absorption activity (WeiXie *et al*., 2022). In line with Irawati *et al.* (2021), Acinetobacter sp. IrC2, which is resistant to Cu, was reported to accumulate up to 508 mg Cu/g biomass; colonies on medium containing $CuSO_4$ changed to green, indicating Cu uptake and the expression of CopA/CopB proteins associated with Cu resistance.

Bacterial isolates from the Citarum River exhibited high multiresistance compared with previously reported multiresistant bacteria in terms of both heavy metal concentration and number of metals tolerated. Based on Table 2, the Citarum isolates showed multiresistance comparable to *Pseudomonas aeruginosa*, namely resistance to a 1 mM mixture of four heavy metals, although with a different metal combination. The Citarum isolates were multiresistant to Ni, Cu, Pb, and Cd, whereas *Pseudomonas aeruginosa* was resistant to Cu, Zn, Ni, and Cr. The Citarum isolates exhibited higher multiresistance than *Bacillus subtilis, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae*, and *Staphylococcus aureus*. The Citarum isolates tolerated up to 1 mM, whereas the other bacteria tolerated only about 0.05 mM to 0.5 mM. The Citarum isolates were resistant to four heavy metals, whereas *Bacillus subtilis*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* were resistant to only three heavy metals.

Table 1. Morphological characterization of bacterial colonies multiresistant to 1 mM Ni, Cu, Pb, and Cd.

No	Isolate Code	Color	Shape	Margin	Optical appearance	Elevation	Bacterial Type
1.	Cit_1_A	Brownish white	*Round*	*Smooth entire*	*Translucent*	*Convex*	Gram-negative
2.	Cit_1_B	Yellowish brown	*Round*	*Smooth entire*	*Translucent*	*Convex*	Gram-negative
3.	Cit_1_C	Yellowish white	*Round*	*Smooth entire*	*Translucent*	*Convex*	Gram-negative
4.	Cit_1_D	Yellowish white	*Round*	*Smooth entire*	*Translucent*	*Convex*	Gram-negative
5.	Cit_1_E	Pale yellow	*Round*	*Smooth*	*Translucent*	*Convex*	Gram-negative
6.	Cit_1_F	Milky white	*Round*	*Smooth*	*Translucent*	*Convex*	Gram-negative
7.	Cit_1_G	Dark yellow	*Round*	*Smooth*	*Opaque*	*Convex*	Gram-negative
8.	Cit_1_H	Milky white	*Round*	*Smooth*	*Opaque*	*Convex*	Gram-negative
9.	Cit_1_I	Yellowish brown	*Round*	*Smooth*	*Opaque*	*Convex*	Gram-negative
10.	Cit_1_J	Yellowish white	*Round*	*Smooth*	*Opaque*	*Convex*	Gram-negative
11.	Cit_1_K	Yellowish brown	*Round*	*Smooth*	*Translucent*	*Convex*	Gram-negative
12.	Cit_1_L	White	*Round*	*Smooth*	*Opaque*	*Convex*	Gram-negative
13.	Cit_1_M	Yellow	*Round*	*Smooth*	*Translucent*	*Convex*	Gram-negative
14.	Cit_1_N	White	*Round*	*Smooth*	*Opaque*	*Convex*	Gram-negative
15.	Cit_1_O	Yellow	*Round*	*Smooth*	*Opaque*	*Convex*	Gram-negative
16.	Cit_1_P	Yellow	*Round*	*Smooth*	*Opaque*	*Convex*	Gram-negative
17.	Cit_1_Q	Yellowish brown	*Irregular*	*Lobate*	*Opaque*	*Umbonate*	Gram-negative
18.	Cit_1_R	Brown	*Round*	*Smooth*	*Opaque*	*Umbonate*	Gram-negative
19.	Cit_1_S	Brownish white	*Round*	*Lobate*	*Opaque*	*Plateau*	Gram-negative
20.	Cit_1_T	Yellowish brown	*Irregular*	*Lobate*	*Opaque*	*Convex*	Gram-negative
21.	Cit_1_U	Brown	*Irregular*	*Entire*	*Opaque*	*Convex*	Gram-negative
22.	Cit_1_V	White	*Round*	*Entire*	*Translucent*	*Convex*	Gram-negative
23.	Cit_1_W	Yellowish brown	*Irregular*	*Umbonate*	*Opaque*	*Umbonate*	Gram-negative
24.	Cit_1_X	Brown	*Round*	*Entire*	*Opaque*	*Convex*	Gram-negative

Table 2. Superiority of Citarum isolates in multiresistance compared with other multiresistant bacteria

No.	Bakteri	Type of heavy metals	Number of heavy metals	Heavy metal resistance
1.	**Citarum isolate**	**Ni, Cu, Pb, Cd**	**4**	**1 mM**
2.	*Pseudomonas aeruginosa*	Cu, Zn, Ni, Cr	4	0,5 – 1 mM
3.	*Bacillus subtilis*	Pb, Cd, Hg	3	0,1 – 0,5 mM
4.	*Enterobacter cloacae*	Cr, Ni, Cd	3	0,2 – 1,5 mM
5.	*Escherichia coli*	Cu, Zn, Pb	3	0,1 – 0,3 mM
6.	*Klebsiella pneumoniae*	Ni, Cd, Pb	3	0,2 – 0,8 mM
7.	*Staphylococcus aureus*	Cd, Hg, Pb	3	0,05 – 0,2 mM

CONCLUSION

Isolation of indigenous bacteria from the Citarum River successfully yielded 24 isolates capable of growing on media containing a 1 mM mixture of nickel, lead, copper, and cadmium and demonstrating multiresistant properties. Colony morphology characterization revealed diversity in color and shape, whereas Gram staining showed that all isolates were Gram-negative. Dark pigmentation changes in some isolates indicate metal interactions through

biosorption and bioprecipitation mechanisms. These results show that indigenous bacteria have potential to be developed as heavy metal bioremediation agents in future studies.

ACKNOWLEDGMENTS

The authors thank the 2023 students of the Biology Education Study Program, as well as Andreas Rambe, Eddowardho Otniel Legesang, and Andriana Purba, Faculty of Education, Universitas Pelita Harapan, for their participation in conducting this research. The authors also thank the students of the Biology Education Study Program for their dedication in assisting the preparation of this publication manuscript.

REFERENCES

Baharudin, A., N. Widyorini, & D. Ayuningrum. 2022. Isolasi dan identifikasi bakteri toleran logam berat Pb (timbal) dan Cu (tembaga) dari sedimen mangrove di Mangrove Tapak, Semarang. *Juvenil: Jurnal Ilmiah Kelautan dan Perikanan*. 3(3): 61–65.

Bersih, Y., B. Afra, H. Haifa, A. Yogi, & O.U. Kamal. 2024. Tantangan dan solusi pengelolaan limbah industri: Upaya menuju lingkungan. *Jurnal Ilmiah Wahana Pendidikan*. (23): 1133–1139.

Corona, S.C., L.G. Avila, C.H. Cortez, J.R. Vargas, G.C. Escarpulli, & H.C. Sanchez. 2025. Impact of heavy metal and resistance genes on antimicrobial resistance: Ecological and public health implications. *Ecological and Public Health Implications*. 16(6): 625.

Das, S., H.R. Dash, & J. Chakraborty. 2016. Genetic basis and importance of metal resistant genes in bacteria for bioremediation of contaminated environments with toxic metal pollutants. *Applied Microbiology and Biotechnology*. 100: 2967–2984.

Destriana, S., R.N. Rahma, M.I. Sena, F.H. Khotimah, & A. Primasari. 2025. Kopi sebagai pangan fungsional: Aktivitas biologis, manfaat kesehatan dan risiko toksisitas. *Jurnal Al- Azhar Indonesia Seri Sains dan Teknologi*. 10(3): 279.

Fahimah, N., I.R. Salami, K. Oginawati, S.H. Susetyo, A. Tambun, A.N. Ardiwinata, & S. Sukarjo, S. 2023. The assessment of water quality and human health risk from pollution of chosen heavy metals in the upstream Citarum River, Indonesia. *Journal of Water and Land Development*. 58: 153–163.

Gillieat, B.F., & N.V. Coleman. 2024. Unravelling the mechanisms of antibiotic and heavy metal resistance co-selection in environmental bacteria. *FEMS Microbiology Reviews*. 48(4): 1-20.

Hamzah, A., & R. Priyadarshini. 2019. *Remediasi tanah tercemar logam berat*. Unitri Press.

Handayani, C.O., S. Sukario, & T. Dewi. 2022. Penilaian tingkat cemaran logam berat pada lahan pertanian di hulu Sungai Citarum, Jawa Barat. *Jurnal Ilmu Lingkungan*. 20(3): 508–516.

Irawati, W., A. Hasthosaputro, & L. Kusumawati. 2020. Multiresistensi dan akumulasi *Acinetobacter* sp. IrC2 terhadap logam berat. *Jurnal Biologi Papua*. 12(2): 114–122.

Irawati, W., P.P. Ambarita, D.L. Sihombing, F.E.S.R. Advenita, & E.B. Marvella. 2022. Isolation and characterization of indigenous copper resistant bacteria from Yogyakarta tannery factory waste. *Jurnal Biologi Tropis*. 22(3): 795–802.

Irawati, W., E.S. Djojo, L. Kusumawati, T. Yuwono, & R. Pinontoan. 2021. Optimizing bioremediation: Elucidating copper accumulation mechanisms of *Acinetobacter* sp. IrC2 isolated from an industrial waste treatment center. *Frontiers in Microbiology*. 12: 713812.

Irawati, W., S.E. Winoto, L. Kusumawati, & R. Pinontoan. 2021. Indigenous multiresistant bacteria of *Cupriavidus pauculus* IrC2 isolated from Indonesia as a heavy metal bioremediation agent. *Biodiversitas*. 22(6): 3349–3355.

Kamila, A., I. Sadidan, & A.K. Fauzie. 2024. Penilaian status mutu air Sungai Citarum menggunakan metode indeks pencemaran. *Ruwa Jurai: Jurnal Kesehatan Lingkungan*. 18(2): 84–91.

Mahrus, M.H., R. Kartika, M. Fahmi, F. Rahman, R. Aswat, & M.A. Rizkirullah. 2024. Penyerapan logam berat secara biokimiawi oleh bakteri Gram positif dan Gram negatif: a mini review. *Prosiding Seminar Nasional Kimia*. 2(2): 69–73.

Mardiana, R.N., & N. Handayani. 2017. Antibacterial activity of the sambiloto leaf extracts (*Andrographis paniculata*) to *Bacillus cereus* and *Pseudomonas aeruginosa*. *Biofarmasi Journal of Natural Product Biochemistry*. 14(1): 19–24.

Marzan, L.W., M. Hossain, S.A. Mina, Y. Akter, A.M.A. Chowdhury. 2017. Isolation and biochemical characterization of heavy-metal resistant bacteria from tannery effluent in Chittagong city. *Egyptian Journal of Aquatic Research*. 43(1): 65–74.

Mondal, S., A. Melzi, A. Zecchin, & L. Cayalca. 2025. Quorum sensing in biofilm-mediated heavy metal resistance and transformation: environmental perspectives and bioremediation. *Frontiers in Microbiology*. 16: 1607370.

Mukhamatdyarov, S.R., E.V. Kuzina, M.G. Iskhuzina, & T.Y. Korshunova. 2023. Adaptation of microorganisms to heavy metals. *Izvestia Ufimskogo Nauchnogo Tsentra*. 4: 31–43.

Pamaya, D., S.I. Muchlissin, E.T.W. Maharani, S. Darmawati, & S.N. Ethica. 2018. Isolasi bakteri penghasil enzim

protease *Bacillus amyloliquefaciens* IROD2 pada oncom merah pasca fermentasi 48 jam. *Seminar Nasional Edusaintek*. 2018.

Panjaitan, M.O., A.M.S. Hertika & R.B.D.S. Putra. 2019. *Ekotoksikologi untuk lingkungan perairan*. Brawijaya Press.

Paracini, N., E. Schneck, A. Imberty, & S. Micciulla. 2022. Lipopolysaccharides at solid and liquid interfaces: models for biophysical studies of the Gram-negative bacterial outer membrane. *Advances in Colloid and Interface Science*. 301: 102603.

Patil, A., S. Chakraborty, Y. Yadav, B. Sharma, S. Singh, & M. Arya. 2025. Bioremediation strategies and mechanisms of bacteria for resistance against heavy metals. *Bioremediation Journal*. 29(4): 448–480.

Rachman, R.M., F.R. Rustan, D.E. Rahayu, B.A. Ampanganallo, S. Syaiful, A. Aryadi, Mansyur, A. Safar, A.A. Iskandar, B. Badrun, & S. Gusty. 2024. *Danau Jempang di masa El Niño 2023*. Penerbit Deepublish. Yogyakarta.

Ratnaningsih, D., N. Widayati, A. Prajanti, H. Puspita, & Y. Sofyan. *Keberadaan Pencemar Organik Persisten (Pops) di Lingkungan*. Ecolab, 1(1), 17-26.

Ren, X., L. Zhao, J. Shen, P. Zhou, K. Zhao, C. Yuan, R.R. Xing, & X. Yan. 2025. Engineered microbial platform confers resistance against heavy metals via phosphomelanin biosynthesis. *Nature Communications*. 16(1): 4836.

Septiansyah, B., T. Kushartono, & A.C. Nabila. 2023. Peran pemerintah desa dalam pencegahan dan penanggulangan pencemaran daerah aliran Sungai Citarum. *Jurnal Academia Praja*. 7(2): 217–226.

Trikurniadewi, N., A.L. Nasyanka, F.Nur'aini, R.A. Novitasari, A.A. Illahi 2025. Isolasi dan karakterisasi mikroba indigenus penghasil biosurfaktan dari ecoenzyme. *Jurnal Kesehatan Vokasional*. 10(1): 27-37.

Wahyuni, D. 2017. Karakterisasi morfologi dan uji aktivitas bakteri termofilik yang terdapat di kawasan wisata Ie Suum Kecamatan Mesjid Raya sebagai penunjang praktikum mikrobiologi. *Jurnal Pendidikan Biologi*.

WeiXie, L., R. Yang, B. Liu, N. Lei, S. Peng, J. Li, J. Tong, R. Deng, & J. Li. 2022. Effects of Pb-, Cd-resistant bacterium Pantoea sp. on growth, heavy metal uptake and bacterial communities in oligotrophic growth substrates of Lolium multiflorum Lam. *Environmental science and pollution research international*. 29(33): 50742–50754.