

I. INTRODUCTION

1.1 Background

Soil is a thin layer on the Earth's surface consisting of a mixture of minerals and organic matter that plays a crucial role in the survival of other living organisms. However, soil currently faces a serious threat in the form of pollution. One of them is pollution by heavy metals. Heavy metal contamination in soil is an important global environmental issue, primarily driven by rapid industrialization, urbanization, mining, and agricultural activities. Plants, soil organisms, and food crops can be negatively affected by heavy metals in the soil. Among the most prevalent and enduring pollutants in soils across the globe are heavy metals, including lead (Pb), cadmium (Cd), chromium (Cr), arsenic (As), mercury (Hg), nickel (Ni), copper (Cu), and zinc (Zn). These metals accumulate in ecosystems, causing severe health risks such as neurotoxicity, kidney damage, and carcinogenicity (Priya et al. 2023).

Industries such as metal plating, battery recycling, and manufacturing of lead-containing products are major lead pollutants. Improper procedures and methods in handling used batteries in recycling lead-acid batteries contribute significantly to lead pollution. Exposure to lead can significantly hinder the intellectual development of infants and may result in peripheral neuritis, damage to cells in the cerebellum and cerebral cortex, and, in severe cases, dementia (Ding et al. 2019). Cadmium, a toxic non-essential metal for humans, can impair the sense of smell by irritating the respiratory tract, potentially causing conditions such as pneumonia and pulmonary edema. Prolonged consumption of food contaminated with cadmium and other heavy

metals can impair renal tubular function and damage cartilage. Chronic cadmium poisoning primarily affects the kidneys and may also lead to anemia. Consequently, the detection and prediction of heavy metal contamination in soil are critically important (Koleleni and Mbike, 2018). Therefore, it is important to know about heavy metal binding and its relationship with soil and the environment in order to develop efficient soil remediation methods.

Caves are generally defined as areas of underground space that occur naturally in the environment and are accessible to living things. Limestone caves are formed by acid dissolution when groundwater flows through the floor and rock layers, interacting with carbon dioxide gas (CO_2) and producing carbon dioxide (H_2CO_3), which slowly dissolves carbonate rocks. When sulfate resolution occurs, hydrogen sulfide (H_2S) gases from rock folding (the narrow openings in rocks, often narrow openings in structural regions) react with atmospheric oxygen to form sulfuric acid (H_2SO_4) that dissolves carbonate rocks. Furthermore, the decomposition of organic matter by microorganisms can be a source of sulfate reduction (Beolchini et al. 2017). Microbial-induced carbonic acid bias (MICP) is a form of biogenicity and is a natural process of carbon transformation that occurs worldwide and is transmitted primarily by bacteria (Seifan and Berenjian, 2019).

Microbiologically induced calcium carbonate precipitation (MICP) allows microorganisms to react with chemical components to produce minerals as organic and inorganic compounds that act as authoritative agents, these substances act as adhesives and are commonly known as biocement. Calcium carbonate (CaCO_3) is one of the most abundant materials in nature, and the most abundant cement raw material. CaCO_3

precipitation carried out by heterotrophic microscopic organisms occurs through the nitrogen and sulfuric cycles (Iqbal et al. 2021).

Urea degrading bacteria or so-called ureolytic bacteria are bacteria that can produce the urease enzyme which hydrolysis urea. The urease enzyme is a key enzyme in the calcium carbonate precipitation process (Novanti and Zulaika, 2018). Ambarsari and Ridlo (2018) and Omoregie et al. (2021) stated that ureolytic bacteria play an important role in the calcium carbonate precipitation process and remove heavy metals. Ureolytic bacteria hydrolyse urea causing the formation of ammonia and carbonate, thereby increasing the pH of the environment and forming calcium carbonate precipitation and the process occurs in an environment rich in calcium, it will cause calcium carbonate (calcite) to precipitate, forming solid crystals. This process occurs in the formation of cave structures (Tomczyk-Żak and Zielenkiewicz, 2015).

Several previous studies have succeeded in isolating bacteria from various caves. In addition, many studies have also been conducted to utilize bacteria in remediating environments contaminated by heavy metals. Based on the results of research conducted by Maulas et al. (2024) found 7 isolates from the coastal area of Iligan City in Northern Mindanao Island, Philippines. After conducting a bio-cementation assay on the seven isolates, the isolates indicated to have the ability to produce urease and have the potential in MICP were *P. stutzeri*, *P. pseudoalcaligenes*, *S. stutzeri*, and *B. aryabhatai*. Based on the results of Nath et al. (2018) conducted studies observed an occlusal tolerance for PBs between 80 and 180 µg/ml, and determined that most of them were resistant to PB concentrations up to 100 µg/mL. In

the presence of CDs, up to 1000 µg/ml CD concentrations are tolerant. *P. aeruginosa* strain SN4 and *P. aeruginosa* strain SN5 can withstand the presence of toxic heavy metals in agricultural environments and promote seedling growth in pot research.

In Indonesia, industrial and agricultural activities such as mining, metallurgy, textiles, chemicals, and the use of fertilizers and pesticides are increasingly developing, and if accompanied by improper waste management, it can lead to an increase in heavy metal contamination levels in the environment, especially in the soil. Based on these issues, research was conducted on "Exploration and Identification of Bacteria from Padayo Bat Cave, Padang for Pb and Cd Heavy Metal Immobilization Using MICP" which utilizes bacteria in the process, making it more environmentally friendly and cost-effective. This study aims to identify potential isolates from Padayo Bat Cave, Padang that are able to induce biomineralization processes in immobilizing lead and cadmium contamination and determine optimal environmental conditions in maximizing the immobilization process.

1.2 Research Problem

Based on the background above, the problem formulation for the research conducted is as follows:

1. What are the characteristics of bacteria isolates from cave water, bat guano, and limestone rock samples from Padayo Bat Cave, Padang?
2. How is the urease enzyme activity of each bacteria isolate and the ability of these isolates to induce biomineralization in immobilizing heavy metals?
3. Which bacterial isolate is best in immobilizing both heavy metals (Pb and Cd)?

1.3 Research Obectives

The aims of this research are:

1. To isolate, identify, and characterize bacteria from cave water, bat guano, and limestone rock samples from Padayo Bat Cave, Padang.
2. To determine the ability of bacteria isolated from cave water, bat guano, and limestone rock samples from Padayo Bat Cave, Padang to form heavy metal precipitates.
3. To identify the most effective bacterial species for heavy metal immobilization.

1.4 Benefits of Research

From this research, it is expected to provide information related to what bacteria are present and what types of bacteria dominate each isolate taken from Padayo Bat Cave, Padang. In addition, it can also provide information on the ability of each identified bacteria to immobilize heavy metal contamination by inducing calcium carbonate precipitation. So that from this information, further research can be carried out on the application of potential isolates in the MICP process. Thus, this research is expected to provide a significant contribution to the development of science and technology.

