CHAPTER I. INTRODUCTION

1.1 Problem Background

Heavy metal (HM) contamination in the environment has become a significant concern due to its detrimental effects on ecological value. Contamination of heavy metals nickel (Ni) and Copper (Cu) is a serious problem in countries with rapidly growing mining, industrial and urbanization activities, including Indonesia (Armid., et al 2021). The impacts of nickel (Ni) and copper (Cu) affect a variety of biological processes and ecological interactions. Both metals are persistent environmental pollutants that can accumulate in the food chain, causing significant health risks to living organisms. High concentrations of Ni and Cu can lead to severe health issues, including cancer and neurological disorders. Ni contamination affects seed germination, nutrient uptake, and photosynthesis, leading to oxidative stress and decreased biomass production (Rizwan et al., 2024).

Copper contamination poses a significant threat to various ecosystems. It disrupts essential biological processes, from impairing temperature regulation in soil organisms to inhibiting plant growth and altering nutrient cycles in aquatic environments. Its persistence allowed it to accumulate in the environment, intensifying ecological impact. Additionally, copper can alter soil properties, potentially compromising soil structure (Ge et al., 2023). Heavy metal pollution is particularly hazardous to human health, with metals like mercury, lead, chromium, cadmium, and arsenic being the most common contaminants causing health risks through bioaccumulation (Balali-Mood et al., 2021).

Over the past few decades, remediation technologies such as membrane filtration, reverse osmosis, electrochemical treatment, chemical precipitation, and adsorption have been employed to address this issue (Cho et al., 2016; Mugwar and Harbottle, 2016). However, some of these technologies face some obstacles such as easy fouling and less effective in immobilizing small metal ions. Traditional polymer membranes are often easy to foul, which occurs when the pores of the filtration membrane are clogged by particles. This reduces the performance of the membrane in separating contaminants. As a result, the efficiency of water treatment technology decreases, which reduces its efficiency and service life (Liu et al., 2024). The importance of alternative bioremediation technology alongside conventional methods for heavy metal immobilization lies in its enhanced efficiency, sustainability, and versatility.

Studies have demonstrated that MICP can achieve an immobilization rate exceeding 96% for heavy metals, making it a superior alternative compared to many conventional remediation techniques (Chen and Achal., 2019). Microbial Induced Calcite Precipitation (MICP) has emerged an innovative biotechnological approach (Basri et al., 2023). MICP is a biogeological process that improve soil strength through facilitating the microbial to induced formation of calcium carbonate ($CaCO_3$) crystals (Wang et al., 2023). Moreever, biocementation is soil improvement method by adopts natural biological process to utilize the role of microorganisms to precipitate $CaCO_3$ through microbiologically induced calcium carbonate precipitation (MICP). This approaches utilizes microorganisms to effectively enhance soil properties and mitigate soil degradation across various soil and environmental conditions (Gowthaman et al., 2019; Jiang et al., 2019; Liu et al., 2020; Liu et al., 2021).

Biocementation by microbial-induced carbonate precipitation (MICP) employed the ability of bacteria occurs several metabolic pathways such as ureolysis, denitrification, sulfate reduction, and ion reduction. Ureolysis is the most effective method for precipitating $CaCO_3$ due to its ability to produce up to 90% chemical conversion efficiency (CCE) in the form of $CaCO_3$ precipitate (Al-Thawadi, 2011). Among the various biocemantaation technique, MICP widely used due to its efficiency, minimal energy consumption, and low environmental impacts (Dejong at al., 2013). In addition, the minerals that produced through MICP can significant increasing the soil's physical, chemical, and mechanical properties (Jiang et al., 2021). This highlights the potential of MICP as a sustainable and effective method for soil improvement and environmental engineering applications, as it not only enhances soil stability and strength but also offers a low-carbon alternative to conventional soil treatment methods, thereby contributing to environmentally friendly and cost-effective engineering solutions.

Advances in microbiology have highlighted the potential of microorganisms as biocementation agents as a green solution for geotechnical applications (Dejong et al., 2013). To date, biocementation has been successfully applied in several applications, including soil strengthening and stabilization (Cheng and Shahin, 2019; Shu et al., 2022; Kim and Roh, 2024), erosion control (Imran et al., 2019; Fattahi et al., 2020; Dubey et al., 2021; Moqsud et al., 2024), and bioremediation (Basri et al., 2023; Huynh et al., 2023; Hu et al., 2024). Bioremediation through biocementation offers a promising approaches that can effectively stabilize and immobilize heavy metals contaminants in soil and water. This approach also enhances soil integrity and sustainability. Soil contaminated with heavy metals poses significant health and environmental risks (Huynh et al., 2023).

Based on explorations, several types of bacteria have been used due to their ability to induced precipitate of calcium carbonate and heavy metal immobilization, such as *Sporosarcina pasteurii* (Stefaniak et al., 2023), and *Bacillus megaterium* (Soda et al., 2024). Previous studies (Nugroho, et al.,2019; Rohmah, et al.,2021) have identified ureolytic bacteria in various cave formation, such as cave ornaments, karst cave environments, and limestone caves. While the focus on cave microbiology is growing, challenges remain in balancing the exploration of other unique ecosystems with their conservation. The potential for discovering new strains of ureolytic bacteria underscores the importance of continued research in this area. The discoveries could contribute not only to scientific advancement but also to the more sustainable and tailored solutions for environmental engineering problems. These may possess unique metabolic capabilities and environmental adaptations that could enhance the efficiency and applicability of MICP in diverse biotechnological and geotechnical contexts.

Karst is widely distributed in Indonesia, covering an area of approximately 15.4 million hectares (Cahyadi, 2017). Karst landscapes develop through the chemical weathering and dissolution of carbonate and other soluble lithologies by infiltrating meteoric water, which is often slightly acidic due to dissolved CO_2 (White, 2020). This process generates extensive subterranean drainage networks and cave systems characterized by oligotrophic conditions, with total organic carbon concentrations typically below 2 mg/L. Despite the low nutrient availability, these environments support diverse and specialized microbial consortia adapted to low-energy niches (Jones & Northup, 2021). Consequently, karst caves represent unique ecological niches that integrate geological, hydrological, and biological processes.

One of the karst regions in Indonesia is Ngalau Basurek, located within Silokek Geopark, Muaro Silokek, Sijunjung Regency, West Sumatra. This cave features a reactive limestone environment that provides carbonate substrates ($CaCO_3$) essential for the MICP process. Furthermore, the presence of an underground river inside the cave plays a crucial role in the dissolution of karst rocks as well as the transport of nutrients and distribution of important ions that support the activity of ureolytic microbes within the cave. Moreover, secondary carbonate formations such as cave ornament including stalactites, stalagmites, and flowstones inside the cave serve as indicators of carbonate precipitation processes naturally involving microbial activity. This is due to the presence of ureolytic bacteria that, through the hydrolysis of urea, increase the pH and facilitate the precipitation of calcium carbonate, thereby biologically strengthening and stabilizing the speleothem structure. Previous studies by Rohmah (2021) have shown that ureolytic bacterial isolates from cave speleothems are capable of producing urease and mediating calcium carbonate precipitation with high efficiency, making these microbes important contributors to carbonate mineral formation in cave environments.

Besides these abiotic factors, biotic factors also play a significant role, particularly the presence of swiftlets (*Aerodramus* spp.). Their nesting activities lead to the accumulation of guano deposits rich in nitrogenous and phosphorous compounds, which contribute substantially to the nutrient dynamics within the cave ecosystem. These guano deposits serve as an important nutrient source that enhances the growth and metabolic activity of ureolytic bacteria. The interaction between organic inputs derived from swiftlets and microbial communities indicates a complex biogeochemical coupling that sustains the oligotrophic karst ecosystem and potentially regulates the MICP processes.

1.2 Problem Statement

The formulating of the research problem to be answered as follows:

1. Do the bacteria isolated from Ngalau Basurek Cave, Sijunjung, West Sumatra exhibit urease activity and heavy metal tolerance?
2. Do the bacteria isolated from Ngalau Basurek Cave, Sijunjung, West Sumatra, have the ability to form precipitates?
3. What are the partial characteristics of positive ureolytic bacteria isolates, and which isolate exhibits the most effective for the immobilization of heavy metal?

1.3 Research Objectives

The objectives of the research are:

1. To determine the urease activity and specific urease activity of bacteria isolated from Ngalau Basurek Cave, Sijunjung, West Sumatra in heavy metal-stressed environments.
2. To determine the ability of bacteria isolated from Ngalau Basurek Cave, Sijunjung, West Sumatra to form precipitates in heavy metal-stressed environments.
3. To characterize isolates with heavy metal immobilization capability and to identify the most effective ureolytic bacterial species for heavy metal immobilization.

1.4 Research Benefit

This study aims to isolate and characterize urease-producing bacteria from natural sources, specifically from Ngalau Basurek Cave, in Geopark Silokek, Sijunjung, West Sumatra. By assessing the urease activity and heavy metal precipitation ability of these isolates, this study will contribute to the development of bioremediation strategies for heavy metal contamination.