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Identification of Endophytic Bacteria from Water Hyacinth (*Eichhornia crassipes*) in Lake Tondano as Potential Agents for Lead Bioremediation

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Abstract. Water hyacinth (*Eichhornia crassipes*) is a herbaceous plant known to accumulate heavy metals from polluted waters. In Lake Tondano, this plant grows abundantly and helps reduce lead (Pb) levels through bioremediation. This study aimed to identify the genera of endophytic bacteria from water hyacinth (*E. crassipes*) that have potential as bioremediation agents for lead. This study employed an exploratory, descriptive, quantitative laboratory approach. Samples were collected from *E. crassipes* plants found in Lake Tondano at three different locations. The isolation method used was direct plating. The research stages included bacterial isolation, lead resistance testing at 50 ppm, lead reduction testing at 10, 20, and 30 ppm, lead concentration analysis, and bacterial identification using morphological and biochemical tests. Three isolates (EG.A1.5, EG.A2.4, and EG.D3.4) were found to be resistant and capable of reducing lead levels. The reduction percentages reached 35.9%, 72.2%, and 55.5%, respectively. Based on morphological and biochemical characteristics, the bacterial genera of three isolates were identified as *Salmonella, Enterobacter,* and *Bacillus*.

Keywords: bioremediation; *Eichhornia crassipes*; endophytic bacteria; lead

I. INTRODUCTION

Lake Tondano is a natural lake in North Sulawesi that plays a vital role for the surrounding community, serving as a water source, a fishery, and a tourist attraction. However, increased activity can lead to declines in water quality and increases in nutrient concentrations, such as nitrogen, phosphate, and potassium. This condition can trigger the rapid growth of water hyacinth (*Eichhornia crassipes*) [1]. Although water hyacinth can absorb heavy metals, uncontrolled growth can disrupt aquatic ecosystems.

Lead (Pb) heavy metal pollution in the Tondano waters can become a serious problem, as lead levels in the Tondano River have been detected to exceed national quality standards, ranging from 0.09 to 0.14 mg/L [2]. The heavy metal lead (Pb) can enter waterways through various anthropogenic activities, including the use of leaded fuel and the discharge of industrial waste into rivers. Long-term exposure to heavy metals can have significant ecological and health impacts; therefore, effective control strategies are necessary.

A suitable approach is bioremediation, which uses living organisms, such as plants and microbes, to reduce the toxicity of environmental pollutants [3]. Previous studies have demonstrated that Eichhornia crassipes can accumulate heavy metals, including lead (Pb), from contaminated aquatic environments, making it a widely studied phytoremediation plant [4]. Several studies have also reported that this phytoremediation capacity is

associated with microbial communities residing within plant tissues [5]. Investigations into the endophytic microbiome of E. crassipes have identified bacterial genera such as Bacillus, Enterobacter, and Pseudomonas, many of which are known for their metal tolerance and biosorption capabilities [6].

Therefore, this study aims to identify the potential of endophytic bacteria in water hyacinth (*E. crassipes*) as bioremediation agents for lead (Pb). The results are expected to contribute to understanding the biological mechanisms of bioremediation and to serve as a basis for the application of environmentally friendly technologies in the management of polluted waters.

II. METHODS

Sampling Location

Endophytic bacterial isolates were collected from water hyacinth (*E. crassipes*) in Lake Tondano at three different locations: sampling point 1 was located near a residential area, representing domestic input, sampling point 2 was located near a restaurant, representing organic waste input, and sampling point 3 was located near a peripheral fish pond, representing aquaculture-related activities.

These locations were selected to capture potential differences in endophytic bacterial communities associated with varying environmental pressures.

Bacterial Isolation

Root and leaf tissues of water hyacinth (E. crassipes) were used as samples for endophytic bacterial isolation. The sample surface was sterilized by immersing it in 70% alcohol for 1 minute, followed by 1% NaOCl for 5 minutes, then 70% alcohol for 1 minute, and finally rinsed three times with sterile distilled water. The sample was cut into several pieces and then inoculated onto Nutrient Agar media using the direct plating method. Incubated at 37°C for 24-48 hours. Isolate purification was carried out by selecting a colony with a distinct shape and transferring it to new NA media. Isolates were propagated by making stocks using slant agar media. Pure isolates were then grown on new NA media enriched with 5 ppm $Pb(NO_3)_2$ [7].

Bacterial Resistance Test

Pure isolates were inoculated with up to 0.5 mL into Nutrient Broth (NB) media enriched with Pb(NO3)2 at 50 ppm and incubated for 48 hours at 37°C. The screening process was carried out by observing the media's turbidity. Isolates exhibiting the highest turbidity and most pronounced color intensity in Pb-enriched Nutrient Broth at each sampling location were selected for further Pb reduction testing.

Lead (Pb) Reduction Test

Lead reduction tests were conducted on three selected isolates at initial Pb concentrations of 10, 20, and 30 ppm, representing increasing Pb stress levels commonly used to evaluate bacterial Pb reduction capacity [7]. This process utilized 80 mL of Nutrient Broth medium, 10 mL of bacterial suspension, and 10 mL of lead (Pb) working solution, and was incubated for 72 hours at 37°C. The solution was centrifuged for 5 minutes at 10000 rpm, and the supernatant was used for lead (Pb) analysis by Atomic Absorption Spectrophotometry. Optical Density (OD) measurements using a UV-Vis Spectrometer at 600 nm were performed before and after the Pb reduction test. The data obtained were analyzed descriptively using quantitative measurements of lead concentration and reduction percentage.

Bacterial Identification

Morphological and biochemical characterization were employed to identify bacterial isolates at the genus level, as these methods are widely used for preliminary bacterial identification in environmental and microbiological studies.

The bacterial identification process was carried out on bacterial isolates that were able to live in NA media that had been enriched with 5 ppm $Pb(NO_3)_2$ through macroscopic observation of colonies in the form of size, colony shape, colony edges, elevation, appearance, texture, and colony pigmentation, as well as through microscopic observation using the gram staining method and observation of bacterial shape using a microscope.

Biochemical tests were also conducted on the isolates using catalase, citrate, motility, and sugar fermentation tests (TSI) to identify the bacterial genus with potential as a lead (Pb) bioremediation agent.

III. RESULTS AND DISCUSSION

Bacterial Isolation

The process of isolating endophytic bacteria from water hyacinth (*E. crassipes*) yielded 14 bacterial colonies. The bacterial colonies were cultivated on NA medium enriched with 5 ppm Pb, yielding 9 pure isolates. Isolate codes were assigned based on plant part and sampling location, as summarized in Table 1. Representative macroscopic colony morphology of the selected Pb-resistant isolates is shown in Figure 1.

Table 1.
Description of endophytic bacterial isolates obtained from *Eichhornia crassipes*

Isolate Code	Plant Part	Sampling Location
EG.A1.5	Root	Residential area
EG.D1.5	Leaf	Residential area
EG.A2.1	Root	Restaurant area
EG.A2.3	Root	Restaurant area
EG.A2.4	Root	Restaurant area
EG.D2.4	Leaf	Restaurant area
EG.D3.1	Leaf	Peripheral fish pond area
EG.D3.3	Leaf	Peripheral fish pond area
EG.D3.4	Leaf	Peripheral fish pond area

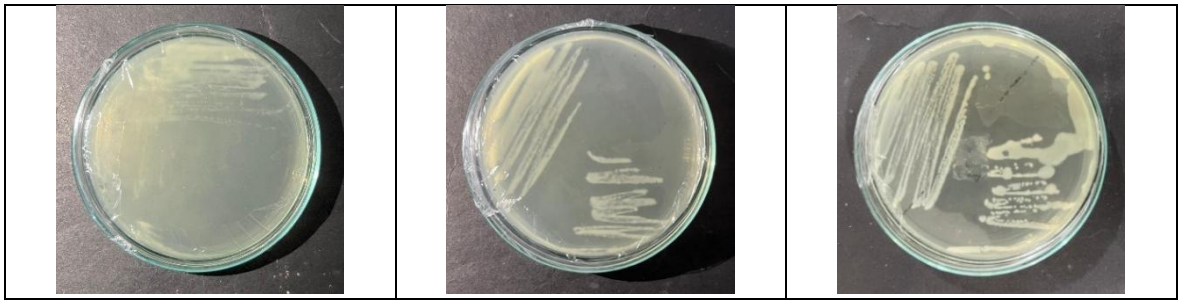

Figure 1. Macroscopic colony morphology of representative Pb-resistant endophytic bacterial isolates (EG.A1.5, EG.A2.4, and EG.D3.4) grown on Nutrient Agar supplemented with $Pb(NO_3)_2$

Bacterial Identification

Most of the isolates obtained were gram-negative bacteria, except for EG.D1.5, EG.D3.1, EG.D3.3, and EG.D3.4, which were gram-positive. The dominant bacterial colony shape is circular, except for isolate EG.D1.5, which has an irregular colony shape. The colony edges are generally entire and undulate. The elevation of the bacterial isolates is raised, convex, and flat. Colony colors observed macroscopically include milky white, cream, and grayish white (Table 2). Biochemical test results showed that most isolates were motile, except for isolate EG.D1.5, which was non-motile. All bacterial isolates produced catalase and citrate enzymes, as summarized in Table 3.

The analysis results found five suspected bacterial genera in Lake Tondano. The presence of *Salmonella* at the residential and restaurant sampling sites indicates fecal contamination and suboptimal sanitation management [8]. The genus Microbacterium itself is an endophytic bacterium commonly found in plant tissue [6]. Bacteria of the *Enterobacter* genus are commonly found in plant tissue; their growth can also be supported by the organic material content in the restaurant and fish pond sampling sites, such as food waste and feed waste in fish pond areas [9]. Bacteria of the genus *Aeromonas* are commonly found in aquatic ecosystems with high levels of organic matter [10]. Bacteria of the genus *Bacillus* are commonly found living in plant tissue [6].

Table 2.
Bacterial Morphological Characterization

Isolate Code	Shape	Edges	Elevation	Color
EG.A1.5	Circular	Entire	Raised	Milky white
EG.A2.1	Circular	Entire	Convex	Cream
EG.A2.3	Circular	Entire	Raised	Milky white
EG.A2.4	Circular	Entire	Convex	Cream
EG.D1.5	Irregular	Undulate	Flat	Cream
EG.D2.4	Circular	Entire	Convex	Grayish-white
EG.D3.1	Circular	Entire	Convex	Cream
EG.D3.3	Circular	Undulate	Flat	Milky white
EG.D3.4	Circular	Undulate	Flat	Milky white

Table 3.
Bacterial Biochemical Characterization

Isolate Code	Gram	Motility	Catalase	Citrate	TSI	Suspected Genus
EG.A1.5	(-)	+	+	+	K/A, H_2S	*Salmonella* sp.
EG.A2.1	(-)	+	+	+	A/A	*Enterobacter* sp.
EG.A2.3	(-)	+	+	+	K/A, H_2S	*Salmonella* sp.
EG.A2.4	(-)	+	+	+	A/A	*Enterobacter* sp.
EG.D1.5	(+)	-	+	+	K/A	*Microbacterium* sp.
EG.D2.4	(-)	+	+	+	K/A	*Aeromonas* sp.
EG.D3.1	(-)	+	+	+	A/A, Gas	*Enterobacter* sp.
EG.D3.3	(+)	+	+	+	K/A	*Bacillus* sp.
EG.D3.4	(+)	+	+	+	K/A	*Bacillus* sp.

Bacterial Resistance Test

After 24 hours of incubation in Pb-enriched NB medium, differences in bacterial growth were observed among isolates. All bacterial isolates grew in NB medium containing 50 ppm Pb, indicating Pb tolerance. Based on the resistance screening, three isolates (EG.A1.5, EG.A2.4, and EG.D3.4), each representing a different sampling location and showing the strongest phenotypic responses, were selected for subsequent Pb reduction analysis.

During the resistance test, a visible change in medium color from clear to turbid yellowish-grey was observed, indicating bacterial growth under Pb stress. Changes in the color of bacterial colonies and media can occur in response to heavy-metal stress [11]. OD measurements at 600 nm, an indirect indicator of bacterial cell density, showed a noticeable decrease after Pb exposure, suggesting Pb's inhibitory effects on bacterial growth (Figure 2). This decrease may occur because Pb can disrupt bacterial cell metabolism, thereby reducing cell growth rates [12].

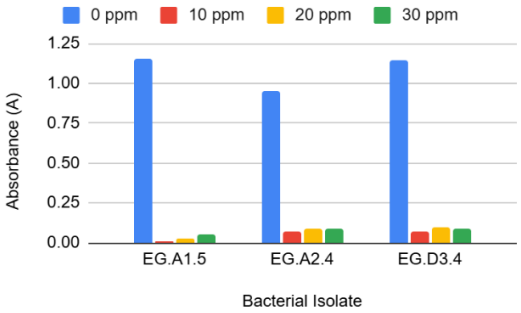

Figure 2. Optical density (OD_{600}) values are an indirect indicator of bacterial cell density after 7 hours of incubation under Pb exposure

Table 4.
Bacterial Ability of Reducing Lead Content In 3×24-Hour

Isolate Code	Initial Lead Content (ppm)	Final Lead Content (ppm)	Reduction Percentage (%)
EG.A1.5	10	6,41	35,9
	20	13,95	30,25
	30	22,01	26,63
EG.A2.4	10	2,78	72,2
	20	13,06	34,7
	30	22,27	25,76
EG.D3.4	10	5,6	44
	20	8,9	55,5
	30	20,11	32,96

Lead (Pb) Reduction Test

The results of the lead reduction test revealed differences in metabolic capacity among isolates in their response to toxic compounds (Table 4). The effectiveness of bacteria in reducing lead levels is thought to be influenced by the bacteria's initial habitat conditions. EG.A1.5, which originates from residential areas, showed the lowest effectiveness. This may indicate that bacterial colonization is more likely due to fecal contamination rather than environmental selection, which requires adaptation to heavy metals [8]. Isolate EG.A2.4 demonstrated the highest effectiveness, a result supported by a habitat rich in organic matter. The organic waste can support bacterial metabolic activity, making it resistant to heavy metals [13]. Isolate EG.D3.4, which exhibited

moderate Pb reduction effectiveness, originated from a fish pond environment. Aquatic systems with fluctuating physicochemical conditions may impose selective pressures that favor bacteria capable of interacting with heavy metals through mechanisms such as biosorption or immobilization. These adaptive traits may contribute to the observed Pb reduction performance rather than solely to metal tolerance [14].

The difference in the effectiveness of endophytic bacteria and their host plants can occur because the plant's accumulation capacity is on a much larger scale than that of endophytic bacteria [15]. Endophytic bacteria in plants play a supporting role by modifying the chemical form of Pb and stimulating the growth or resistance of host plants [5].

Bacteria of the genus *Salmonella* have the potential to serve as bioremediation agents via biosorption, in which Pb^{2+} ions bind to functional groups on the cell wall [16]. Bacteria of the genus *Enterobacter* are able to biosorb on cell walls with the help of siderophores and precipitate Pb into an insoluble form [17-20]. Bacteria of the genus *Bacillus* are capable of carrying out biosorption mechanisms on cell walls and mineralizing Pb into insoluble lead phosphate compounds [19-22].

Other bacterial genera, such as *Aeromonas* sp. and *Microbacterium* sp., have also been reported in the literature as having potential roles in heavy metal tolerance [23-25]. However, in this study, Pb reduction testing was limited to selected isolates that exhibited the strongest phenotypic responses during resistance screening. Therefore, the presence of *Aeromonas* and *Microbacterium* in this study does not indicate a lack of bioremediation potential, but rather reflects the selection criteria applied for Pb reduction evaluation.

IV. CONCLUSION

Based on the research results, it can be concluded that endophytic bacteria isolated from water hyacinth (E. crassipes) have potential as bioremediation agents for lead (Pb). Among the tested isolates, EG.A2.4 exhibited the highest Pb reduction efficiency (72.2%), followed by EG.D3.4 (55.5%) and EG.A1.5 (35.9%). These results indicate variability in Pb reduction capacity among endophytic bacterial isolates associated with Eichhornia crassipes from Lake Tondano.

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