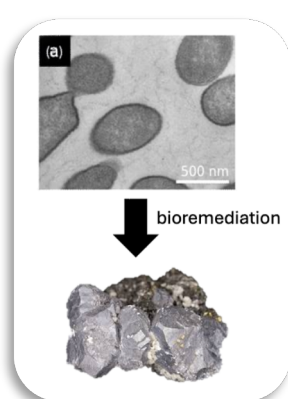


Application of *Staphylococcus aureus* for the Bioremediation of Lead (Pb) Pollution Contamination

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Abstract: Lampulo Marine Fishery Port in Banda Aceh serves as a major hub for fishing and trade activities, making it vulnerable to anthropogenic pollution, particularly heavy metal contamination. This study, conducted in December 2021, investigated lead (Pb) concentrations in sediment and water and evaluated the bioremediation potential of *Staphylococcus aureus*. Sampling sites were selected using a purposive method, and Pb concentrations were measured using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Initial analysis revealed high Pb concentrations: 210.62–247.93 mg/Kg in sediment and 161.91–168.23 mg/L in water, exceeding thresholds established by the Swedish Environmental Protection Agency (SEPA) and Indonesian environmental standards. Bioremediation was then applied using *S. aureus*, a gram-positive bacterium known for its biosorption capabilities. Post-treatment results showed a significant reduction in Pb concentrations to 0.72–2.72 mg/Kg in sediment and 0.18–1.55 mg/L in water. These findings confirm that *S. aureus* plays a vital role in reducing lead pollution and has strong potential as a bioremediator in marine environments affected by industrial and port-related activities.

Keywords: Ocean Fishing Port, Lead, Inductively Coupled Plasma-Mass Spectrometry, Bioremediation

1. Introduction

Heavy metals in aquatic environments pose a serious threat to the survival and health of aquatic organisms. One of the most hazardous heavy metals is lead (Pb), which can accumulate in sediments due to sources such as peeling paint from ship hulls. In addition to marine traffic, water pollution may be caused by various anthropogenic activities, including aquaculture, port operations, oil spills, domestic wastewater, and industrial discharges (Henny, 2018). Naturally, the concentration of heavy metals in aquatic systems is very low; however, anthropogenic activities significantly increase these levels, leading to environmental contamination (Kamarati et al., 2018).

The Lampulo Oceanic Fishing Port (Pelabuhan Perikanan Samudera, PPS Lampulo) in Banda Aceh is one such area experiencing intense human activity. It serves as a central hub for fish trading and is one of the largest fish-landing sites in the region (Zulfahmi et al., 2020). Rapid growth in coastal services such as aquaculture, settlements, and industry has heightened the risk of heavy metal contamination in the surrounding waters (Pratama et al., 2019). Heavy metals are persistent and tend to bioaccumulate in the environment. Continuous human consumption of marine organisms contaminated by heavy metals can pose significant health risks, including gastrointestinal irritation, reduced red and white blood cell production, skin disorders, and pulmonary irritation (Maddusa et al., 2017). Therefore, proper treatment strategies are urgently required to address lead pollution in marine environments.

Bioremediation offers a sustainable and environmentally friendly approach to pollution control. This technique utilizes biological processes, particularly microbial activity, to reduce contaminants. Effective bioremediation depends on several factors, including microbial species, availability of nutrients, pH, and environmental conditions (Hardiani et al., 2016). A previous study by Pratama et al. (2019) reported that Pb concentrations in Lampulo waters were below detectable limits (<0.0001 mg/L) and within regulatory thresholds. However, due to increasing anthropogenic pressure, a reassessment of Pb contamination is necessary to determine whether the levels have risen since 2019. This study aims to evaluate the current concentration of lead in Lampulo waters and investigate the potential of *Staphylococcus aureus* as a bioremediating agent to reduce Pb levels through experimental.

2. Results

2.1. Analysis of Lead (Pb) Concentration in Sediment and Water Samples

The analysis of lead (Pb) concentrations in sediment and water samples collected from the Lampulo Oceanic Fishing Port (PPS Lampulo) was conducted using Inductively Coupled Plasma–Mass Spectrometry (ICP-MS). Results indicated that the Pb concentrations in the sediment samples exceeded the threshold values established by the Swedish Environmental Protection Agency (SEPA), which categorizes Pb levels between 100–400 mg/Kg as high contamination. The detected concentrations confirm that sediment in the study area is heavily polluted with lead. Similarly, Pb concentrations in water samples also exceeded environmental quality standards as regulated by Indonesian Government Regulation (PPRI) No. 22 of 2021 on Environmental Protection and Management. The regulation stipulates maximum allowable Pb concentrations of 0.05 mg/L for port waters, 0.005 mg/L for marine tourism areas, and 0.008 mg/L for waters supporting marine biota. The observed values at PPS Lampulo were significantly above these thresholds, indicating serious contamination.

2.2. Bioremediation of Lead (Pb) in Sediment and Water Using *Staphylococcus aureus*

The bioremediation process was carried out using *Staphylococcus aureus*, a gram-positive bacterium known for its ability to reduce lead ion concentrations and degrade Pb in aquatic environments (Syari et al., 2018). The application of *S. aureus* to sediment samples resulted in a marked decrease in Pb levels, showing effective stabilization and reduction of lead contamination. The bioremediation test on water samples demonstrated similarly significant reductions in Pb concentration. The bacterium effectively reduced lead levels, bringing post-treatment concentrations closer to or below regulatory standards. These findings highlight the strong bioremediation potential of *S. aureus* in mitigating lead pollution in heavily impacted coastal environments such as PPS Lampulo.

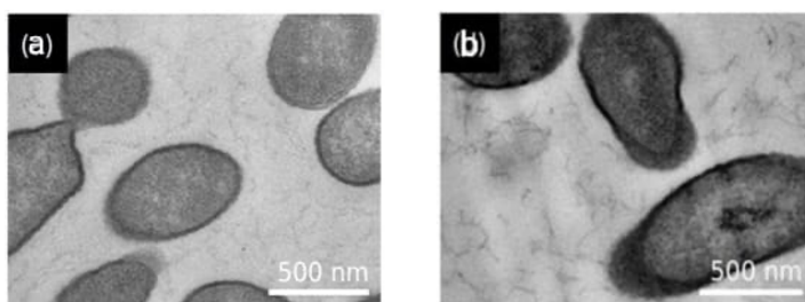


Figure 1. Morphological comparison of *Staphylococcus aureus* colonies: (a) Control of *S. aureus* cultured on LB medium without lead (Pb), showing typical colony appearance; (b) *S. aureus* cultured on LB medium supplemented with 1 mmol/L Pb, exhibiting darker pigmentation and altered morphology, indicating potential lead accumulation or stress response.

3. Discussion

The high concentration of lead (Pb) detected in sediment samples from the Lampulo Oceanic Fishing Port (PPS Lampulo) confirms significant anthropogenic pressure on the local marine environment. Lead is a toxic heavy metal with strong affinity for particulate matter, allowing it to persist in sediments where it binds to organic and inorganic compounds. In this study, Pb concentrations ranged from 210.62 to 247.93 mg/Kg, categorizing the sediment as heavily polluted based on the Swedish Environmental Protection Agency (SEPA) guidelines. These findings reflect not only natural accumulation

processes but also the ongoing contribution from port-related activities such as vessel maintenance, fuel handling, and fish unloading, which are known to release Pb into the environment through paint residues, oil spills, and waste discharge.

Bioremediation using *Staphylococcus aureus* showed promising results, with Pb levels in sediment decreasing to 0.72–2.72 mg/Kg following treatment. This reduction is significant and supports the role of *S. aureus* in the biosorption and bioaccumulation of heavy metals. As a facultative anaerobic bacterium, *S. aureus* can survive under varying oxygen conditions and uses its cell wall functional groups to bind and sequester Pb ions. These groups include carboxyl, phosphate, and hydroxyl moieties that facilitate ion exchange and metal immobilization. Enzymatic processes may also contribute to reducing Pb toxicity by transforming it into less bioavailable forms. This microbial interaction, coupled with favorable incubation conditions, enhances the efficiency of the bioremediation process.

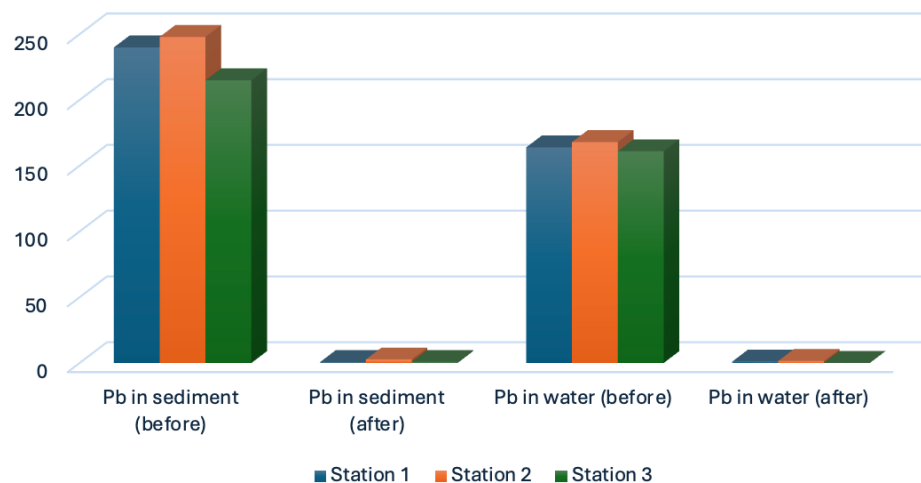


Figure 2. Concentration of lead (Pb) in sediment and water before and after bioremediation at three sampling stations in PPS Lampulo.

In water samples, Pb concentrations were also notably high, ranging from 161.91 to 168.23 mg/L, far exceeding national environmental standards. For instance, Indonesian Government Regulation No. 22 of 2021 sets maximum Pb limits for port waters at 0.05 mg/L. The elevated levels indicate acute pollution, likely stemming from localized point sources such as fuel spills, ship cleaning activities, and waste discharge. Following treatment with *S. aureus*, Pb concentrations decreased significantly, reaching 0.18–1.55 mg/L. While some values still exceeded environmental limits, the overall reduction—over 99% in several samples—demonstrates that *S. aureus* is an effective bioremediator under controlled experimental conditions. These results are consistent with earlier reports that facultative anaerobic bacteria can play a central role in heavy metal reduction. The mechanism involves cellular uptake of Pb through passive adsorption on the cell surface and active intracellular accumulation. Studies by Syari et al. (2018) and Rahadi et al. (2019) confirmed the potential of this species to bind and transform lead. The observable color change in bacterial colonies exposed to Pb further supports bioaccumulation activity. According to Wulandari et al. (2001), Pb reduction by *S. aureus* involves ion exchange, metal uptake during cell metabolism, and passive biosorption at the cell membrane, all of which contribute to decreasing the metal concentration in the surrounding environment.

On the other hand, compared to previous assessments conducted in PPS Lampulo, such as that by Pratama et al. (2019), which reported Pb concentrations in water to be below detection limits, the current study suggests a worrying upward trend in contamination. The increase may be attributed to intensified maritime activities and insufficient waste management. Furthermore, the persistence and bioaccumulative nature of Pb pose serious ecological risks, as heavy metals are non-biodegradable and tend to concentrate in marine organisms. Long-term exposure to even low levels of Pb can lead to toxicity in aquatic life, disrupting physiological functions and leading to bioaccumulation in the food chain. Several sampling locations around the port support these findings. Station 1, located near abandoned vessels, showed high contamination likely due to deteriorating ship paint. Station 2, used for docking and fish unloading, may contribute Pb through effluent from onboard operations. Station 3, adjacent to a fuel depot, is exposed to hydrocarbon spills that often co-occur with metal contamination.

Taken together, these data demonstrate that *S. aureus* has strong potential as a biological agent for remediating lead pollution in both water and sediment in heavily impacted port environments. However, future research is needed to validate

these results under natural conditions, assess long-term stability, and explore the feasibility of scaling up for in situ applications.

4. Materials and Methods

This research was conducted from December 2021 to February 2022 at the Lampulo Oceanic Fishing Port (PPS Lampulo), located in Kuta Alam District, Banda Aceh City, Indonesia. All laboratory analyses, including lead (Pb) concentration testing and bioremediation experiments, were carried out at the Laboratory of Natural Sciences, National Institute of Education, Nanyang Technological University, Singapore. A range of materials and equipment were used in this study, including 4 mL glass vials, Styrofoam boxes, digital cameras, GPS devices, an Inductively Coupled Plasma–Mass Spectrometer (ICP-MS), plastic sampling bags, Erlenmeyer flasks, volumetric pipettes, beakers, volumetric flasks, watch glasses, glass funnels, vacuum filtration apparatus, filter paper, distilled water, and PVC pipes.

Sampling locations were determined using purposive sampling, taking into account anthropogenic factors such as ship maintenance, fuel spills, and flaking paint from hulls, which may contribute to heavy metal contamination. Three sampling stations were selected, each approximately 500 meters apart. Sediment and seawater samples were collected at each station, stored in labeled 4 mL glass vials, and kept in Styrofoam boxes with ice packs during transportation to the laboratory.

Lead analysis began with sample preparation by placing both sediment and water samples in vessels pre-cleaned with nitric acid (HNO_3) and drying them at 105 °C. Each sample was then treated with 10 mL of HNO_3 and digested using microwave-assisted digestion. The digested solution was diluted with distilled water, homogenized, and filtered using a 0.20 μm RC/GHP syringe filter. The filtrate was analyzed for Pb content using ICP-MS following standard procedures. The Pb concentration was calculated based on sample concentration, total volume, dilution factor, and sample weight, with results obtained from sample replicates. Bioremediation experiments were initiated by inoculating water and sediment samples into Lactose Broth (LB) medium that had been supplemented with 1 mmol/L of Pb. The spread plate method was used, and incubation took place at 37 °C for 48 hours. Colony development was observed to assess bacterial activity. The bacterium *Staphylococcus aureus* used in this study was tested for its ability to degrade Pb in vitro and was cultured under controlled conditions at the Laboratory of Natural Sciences, NTU Singapore. Data analysis focused on quantifying Pb concentrations before and after bioremediation using the ICP-MS instrument. All measurements were taken in triplicate, and the results were statistically evaluated to assess the effectiveness of the bioremediation process using *S. aureus*.

5. Conclusions

The present study demonstrated that the concentration of lead (Pb) in sediment and water samples from the Lampulo Oceanic Fishing Port (PPS Lampulo) exceeded both national and international environmental quality standards. Initial Pb concentrations ranged from 210.62 to 247.93 mg/Kg in sediment and from 161.91 to 168.23 mg/L in water, indicating severe contamination likely caused by anthropogenic activities around the port area. Following bioremediation using the bacterium *Staphylococcus aureus*, a significant reduction in Pb concentration was observed. Post-treatment levels ranged from 0.72 to 2.72 mg/Kg in sediment and from 0.18 to 1.55 mg/L in water. These findings confirm the potential of *S. aureus* as an effective bioremediator capable of reducing heavy metal contamination in both sediment and aquatic environments. Overall, *S. aureus* demonstrates promising application for environmentally friendly remediation strategies to address Pb pollution in marine port ecosystems. Further in situ investigations are recommended to evaluate its performance under natural environmental conditions and to explore its feasibility for large-scale implementation.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.scientifia.com/article/doi>.

Author Contributions: A.K.S. and S.K. were responsible for the conceptual design of the study. The methodology development, formal analysis, investigation, data management, visualization, and initial manuscript drafting were conducted by A.K.S. Validation of the findings was performed collaboratively by A.K.S and S.K. All authors have reviewed and approved the final version of the manuscript for publication.

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Conflicts of Interest: The authors declare no conflicts of interest.

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