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Bacterial-based bioremediation: A sustainable strategy for mitigating copper and lead contamination in aquatic ecosystems

Eko Purnomo[a], Hermin Pancasakti Kusumaningrum[a*], Anto Budiharjo[a], Arina Tri Lunggani[a], Tri Retnaningsih Soeprobowati[a,b]

[a] *Biology Department, Faculty of Science and Mathematics, Diponegoro University, Semarang, Indonesia.*

[b] *Cluster for Paleolimnology (CPalim), Diponegoro University, Semarang, Indonesia.*

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ABSTRACT

The contamination of aquatic ecosystems by heavy metals, particularly copper (Cu) and lead (Pb), has emerged as a significant environmental concern driven by escalating anthropogenic activities. These metals are persistent, bioaccumulate across trophic levels, and exert toxic effects on aquatic organisms and human health. To address this issue, bacterial-based bioremediation has gained prominence as a sustainable and eco-friendly solution. This approach leverages the intrinsic capabilities of specific microorganisms to absorb, sequester, and neutralize heavy metals through mechanisms including bioadsorption, the expression of heavy metal resistance genes (HMRGs), and nanoparticle biosynthesis. Notably, species such as *Bacillus subtilis* and *Pseudomonas aeruginosa* have demonstrated remarkable efficiency, achieving up to 100% bioremoval of Pb and Cu, respectively. Advances in biotechnology, including omics technologies, genetic engineering, and nanobiotechnology, have significantly enhanced the capacity of bacteria for effective heavy metal remediation. Future strategies are likely to involve synergistic approaches, such as the coupling of microbial agents with functionalized nanoparticles, real-time monitoring systems powered by Geographic Information Systems (GIS), and the reinforcement of industrial waste regulations to optimize overall remediation efficacy. Although challenges persist, particularly concerning the complex interactions between microbes and their environments, the integration of multidisciplinary approaches offers a holistic and environmentally responsible framework for mitigating Cu and Pb pollution. Furthermore, this strategy fosters greater community involvement in sustainability initiatives. Consequently, bacterial-based bioremediation is not only a promising method for restoring aquatic ecosystems but also a critical pillar in the development of future-oriented environmental management strategies.

*Corresponding author Tel.: + +62 81325874805
E-mail: herminpk@live.undip.ac.id
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1. Introduction

Widespread heavy metal contamination has become a critical global environmental concern due to the increasing scale of anthropogenic activities, as illustrated in Figure 1. Copper (Cu) and lead (Pb) are among the most prevalent heavy metal pollutants found in various ecosystems. Although Cu and Pb occur naturally in the Earth's crust, their release and accumulation in aquatic environments have been significantly intensified by the extensive use of inorganic fertilizers, improper disposal of industrial waste, and irrigation with contaminated water [1, 2]. Furthermore, mining and ore extraction processes contribute to pollution, as residual materials are often dispersed through wind and flooding [3].

Both Cu and Pb are persistent pollutants in nature. When present in excessive concentrations, they pose a significant toxic threat to aquatic ecosystems and have the potential to bioaccumulate along food chains [4, 5]. Exposure to these metals in marine organisms including mollusks and plankton can disrupt metabolic processes, inhibit growth, and reduce biological productivity [4, 6, 7]. In humans, prolonged accumulation of heavy metals may lead to severe health issues including neurological disorders, reproductive dysfunctions, kidney damage, and liver injury [8].

Developing countries face significant challenges in managing Cu and Pb pollution due to limitations in economic capacity, scientific knowledge, and technological infrastructure. Consequently, there is an urgent need for eco-friendly and sustainable solutions. One promising approach is the application of bacteria-based bioremediation to restore contaminated aquatic ecosystems. This technique integrates various scientific disciplines, including genomics, transcriptomics, proteomics, and synthetic biology, to engineer more efficient bacterial systems for remediating Cu and Pb [9, 10]. Microbial-based bioremediation of heavy metals offers several advantages over conventional methods, as summarized in Table 1.

Certain bacterial strains possess unique metabolic capabilities that enable them to interact with toxic metals, either by producing organic compounds that stabilize metal ions or by synthesizing natural nanoparticles that mitigate their effects. As part of their adaptive survival mechanisms, these bacteria can adsorb and detoxify heavy metals by expressing specific heavy metal resistance genes (HMRGs) [14].

Moreover, bacterial cell wall components, such as peptidoglycan, phospholipids, and anionic lipopolysaccharides, play a critical role in binding positively charged metal ions [15, 16].

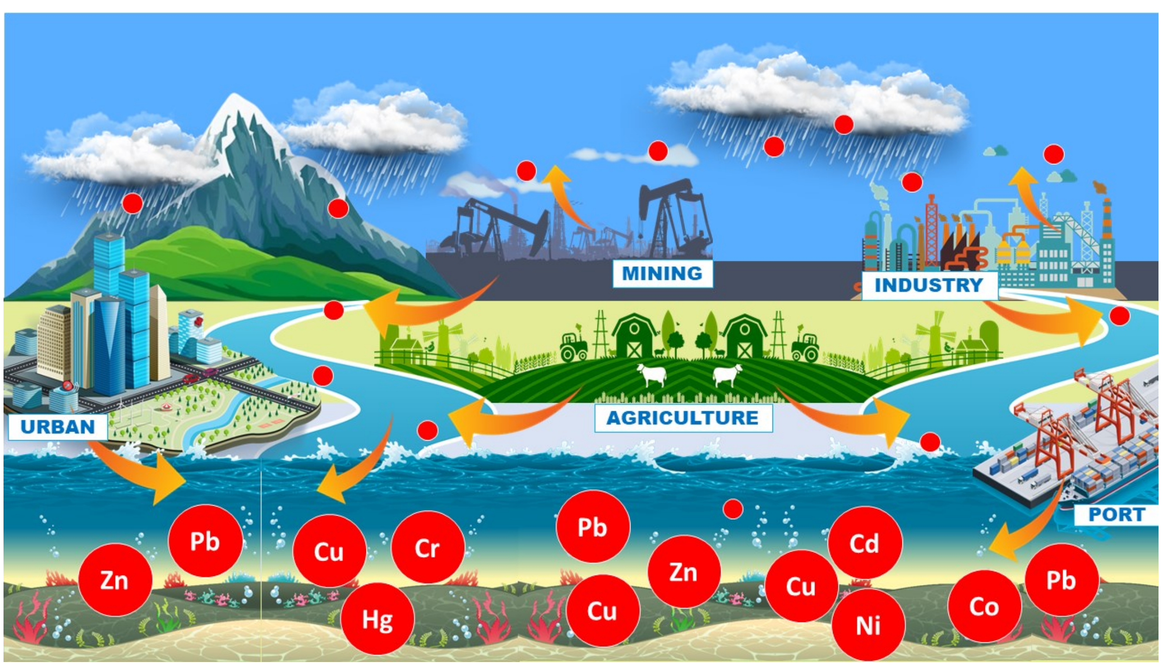

Figure 1. Impact of anthropogenic activities on Cu and Pb contamination in aquatic environments.

Table 1. Comparison of Bacterial Bioremediation and Conventional Remediation Methods

Parameter	Bacterial Bioremediation	Conventional Methods (Physical/Chemical)	Key References
Laboratory Efficiency	Exhibits generally high removal rates (60–99%, depending on strain and operational conditions), with pronounced efficacy for Pb and Cu when employing specialized bacterial strains.	Achieves high removal rates (70–99%) under elevated concentrations; however, performance declines markedly at trace or low metal concentrations.	[11, 12]
Field Efficiency	Performance tends to decrease (typically 40–60%) under real environmental conditions due to variability in physicochemical parameters, interspecific microbial competition, and fluctuating site conditions.	Remains generally robust, though susceptible to reductions caused by membrane fouling, extreme pH, or interference from co-occurring chemical species.	[11]
Cost	Involves lower operational expenditure, particularly at large scale or when leveraging locally sourced biomass or agricultural residues, with limited requirement for complex infrastructure.	Associated with significantly higher operational and capital costs due to reliance on chemical reagents, specialized equipment, substantial energy input, and the management of hazardous waste streams.	[12, 13]
Environmental Impact	Environmentally benign, producing minimal secondary waste, yielding predominantly biodegradable byproducts, and avoiding the generation of toxic sludge.	Imposes a substantial environmental footprint, generating hazardous chemical sludge and secondary pollutants, with processes typically requiring high energy input.	[11, 13]
Selectivity	Capable of achieving high selectivity, with potential customization for target metals such as Pb and Cu through strain engineering or biomass functionalization.	Exhibits generally low selectivity, as most systems remove a broad spectrum of ions; selective refinement is technically feasible but incurs elevated costs.	[12, 13]
Scalability	Currently constrained in scalability; successful transition to industrial scale necessitates precise optimization of environmental parameters, including pH, nutrient availability, temperature, and microbial community dynamics.	Well-established for large-scale industrial operations, supported by standardized protocols and readily available equipment.	[11, 12]
Residual Pollutant	Leaves minimal residual contamination, with most heavy metals sequestered or transformed; however, changes in environmental conditions may induce remobilization.	Residual contamination may persist if treatment is incomplete, and chemical sludge produced is challenging to manage and dispose of safely.	[11]
Operational Complexity	Operationally straightforward and applicable in situ or ex situ, requiring only moderate technical capacity but necessitating regular monitoring and environmental control.	Operationally more complex, requiring precise regulation of process parameters (e.g., pH, flow rate, pressure), advanced monitoring systems, and often multi-stage treatment sequences.	[12]
Sustainability	Demonstrates high sustainability by utilizing natural processes and renewable biomass, with potential for both metal recovery and water reuse.	Relies heavily on non-renewable chemicals and energy sources, producing persistent waste and limiting overall sustainability.	[11, 13]
Advantages	Characterized by economic efficiency, environmental benignity, effectiveness at trace metal concentrations, potential for resource recovery, applicability in situ, and low infrastructural demand.	Defined by high efficiency under elevated metal concentrations, rapid processing capacity, extensive industrial adoption, and the availability of standardized implementation protocols.	[11, 12]
Disadvantages	Potential decline in efficiency under field conditions, inherently slower remediation kinetics, and the need for site-specific environmental optimization.	Incur high capital and operational expenditure, generate secondary waste and sludge, and demand substantial energy and chemical inputs.	[11, 13]

Notable examples include *Staphylococcus epidermidis* AS-1 and *Bacillus pumilus* OQ931870, both isolated from contaminated environments, which exhibit significant biosorption potential for Pb and Cu and are thus considered promising bioremediation agents [17, 18]. However, the optimization of bacterial bioremediation strategies for Cu and Pb remains a work in progress. Emerging approaches, including genetic engineering, metagenomic analyses, and integrated omics platforms, are continuously being explored to enhance the efficiency and specificity of microbial-based detoxification processes.

This review focuses on recent advancements in biotechnology aimed at protecting aquatic ecosystems through detoxification and mitigation of Cu and Pb contamination. It highlights the ecological and human health impacts of Cu and Pb contamination, examines omics-based approaches for exploring indigenous microbial communities to enhance bacterial bioremediation, and identifies future priorities that include real-time monitoring, policy alignment and public engagement for sustainable remediation. Specifically, it aims to (i) assess the environmental impacts of Cu and Pb pollution, (ii) map current research trends in bacterial-based bioremediation, and (iii) explore future research directions. It also provides in-depth insights into the mechanisms by which Cu and Pb exert toxicity and how bacteria mediate their remediation in aquatic environments.

2. Copper and lead pollution in aquatic ecosystems

2.1. Human health effects

Copper is an essential micronutrient required by the human body in trace amounts, approximately 1–2 mg per day, to support various metabolic processes [19]. In contrast, lead has no known biological function in the human body [20]. However, at elevated concentrations, both copper and lead become toxic [5, 21]. Human exposure to these metals primarily occurs through air, food, and drinking water, particularly via the consumption of fish and shellfish that have bioaccumulated these contaminants. Although copper is considered less toxic than other heavy metals such as mercury, cadmium, lead, and chromium, chronic overexposure may cause severe health disorders. When copper levels in the body exceed physiological needs, the liver and kidneys respond by producing metallothionein, a metal-binding protein that facilitates copper excretion [22]. Nevertheless, excessive exposure can still cause irritation of the nose, mouth, and eyes, which may lead to symptoms such as headaches, dizziness, vomiting, diarrhea, and damage to hepatic and renal tissues [23, 24]. In extreme cases, chronic exposure to copper and lead has been linked to neurodegenerative diseases, cancer, Wilson's disease, reproductive system impairment, cardiovascular dysfunction, and kidney failure [20, 21].

2.2. Effects on microbial function in aquatic environments

Heavy metal toxicity in aquatic bacteria occurs via multiple pathways, including the generation of reactive oxygen species (ROS), which induces oxidative stress and damages critical cellular components such as DNA, membrane lipids, and cytoplasmic proteins. Additionally, heavy metals can inactivate vital enzymes such as superoxide dismutase (SOD), thereby exacerbating intracellular oxidative stress. Disruption of ion regulation and membrane permeability also occurs when metals enter cells through ion channels or transport proteins, leading to ionic imbalances that may culminate in cell lysis or death. Furthermore, these metals can cause DNA damage, inhibit replication, and interfere with RNA and protein synthesis, ultimately impairing metabolic function and reducing bacterial viability [25].

Copper and lead contamination in aquatic ecosystems significantly alters microbial function and diversity. These pollutants have been shown to increase the prevalence of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) [26]. From a public health perspective, the emergence of such bacteria poses serious risks, including difficult-to-treat infections and potential contributions to global health crises if they infiltrate drinking water systems. Moreover, copper contamination induces shifts in bacterial community structure, characterized by a decline in autotrophic bacteria and the proliferation of heterotrophic taxa [27]. These community shifts suggest the dominance of a few opportunistic species exploiting organic resources derived from phytoplankton decomposition [28]. Heavy metal pollution accounts for over 80% of structural changes in bacterial communities at the phylum, class, and order levels [14]. Specific bacterial taxa such as *Hirschia* (Hyphomonadaceae), *Formosa*, and *Tenacibaculum* have been reported to increase in copper-exposed environments [28].

2.3. Effects on aquatic biota

Copper and lead contamination exerts both direct and indirect toxic effects on aquatic organisms. Fish and shellfish are exposed to these metals through gills, water, sediment, and trophic transfer within the food web [29]. Copper exposure leads to multifaceted toxicity via oxidative stress, DNA damage, and cellular dysfunction. One of the primary effects is gill damage, which disrupts osmoregulatory balance in fish [29]. On the other hand, lead impairs immune function and neurotransmission, resulting in neurotoxicity in aquatic species such as fish [30]. Copper also interferes with olfactory perception in fish, which is essential for foraging, predator avoidance, and migration. Such disruptions have been shown to impair olfactory responses and navigation abilities in salmon [31]. Long-term exposure to copper and lead leads to reproductive disorders and population declines in various aquatic organisms including fish, shellfish, echinoderms, annelids, cnidarians, and crustaceans [29, 32]. Additionally, these metals inhibit the growth of algae species such as *Selenastrum gracile* and *Chlorella vulgaris*, which form the base of aquatic food chains, thereby destabilizing the entire ecosystem [33, 34].

3. Bacterial resistance to copper and lead

3.1. Mechanisms of Bacterial Resistance to Copper

Bacteria have evolved a variety of mechanisms to withstand copper toxicity, including efflux systems, sequestration, enzymatic oxidation, and the reduction of intracellular copper transport. Efflux pumps, such as copper-exporting ATPases, actively remove copper ions from the cytoplasm to prevent excessive accumulation that could damage essential cellular components. These ATPase pumps utilize energy derived from ATP hydrolysis to expel Cu+ ions from the cell, thereby mitigating their toxic effects [35]. In addition, bacteria can sequester copper by binding it to specific proteins such as metallothioneins or storing it within intracellular compartments. This process decreases the bioavailability of copper ions and reduces their cytotoxic impact [36]. Another critical resistance strategy is enzymatic oxidation. Gram-negative bacteria, for example, can synthesize multicopper oxidases, enzymes that convert the more reactive and toxic Cu+ ions into Cu^{2+}, a more stable and less harmful oxidation state. This transformation is vital since Cu+ ions have a higher propensity to generate reactive oxygen species (ROS), which can damage DNA, proteins, and bacterial cell membranes [35]. Furthermore, some bacteria downregulate the expression of copper transporters in their membranes, thereby limiting copper uptake from the surrounding environment [37].

The combined action of these mechanisms enables bacteria to survive in copper-rich environments, such as those found in mining operations and metal-contaminated ecosystems.

A key cytoplasmic membrane system involved in copper resistance is encoded by the *copA* gene, which produces a Cu(I)-transporting ATPase that pumps copper ions from the cytoplasm into the periplasm to avoid toxic intracellular accumulation [38]. Once in the periplasmic space, the multi-component CusCFBA efflux system further regulates copper levels by exporting ions into the external environment. In parallel, the multicopper oxidase enzyme CueO modulates copper redox states by oxidizing Cu(I) to Cu(II), thereby decreasing its reactivity and potential toxicity [37]. There is a strong correlation between the presence of the *cusA* gene, encoding a critical component of the CusCFBA system, and the minimum inhibitory concentration (MIC) of copper. Bacterial isolates harboring this gene exhibit resistance to copper concentrations ranging from 3 mM to 6 mM, indicating that *cusA* expression significantly enhances bacterial survivability in copper-rich environments [39]. These mechanisms highlight bacterial evolutionary adaptation under selective pressure from heavy metal exposure and provide valuable insights into microbial resistance and its potential applications in bioremediation.

3.2. Mechanisms of bacterial resistance to lead

To survive lead toxicity, bacteria utilize several resistance mechanisms, including extracellular immobilization, surface adsorption, intracellular precipitation, and efflux systems [40]. In extracellular immobilization, certain bacteria bind lead ions to their cell surface, preventing their entry into the cytoplasm and thereby reducing toxic effects on cellular function. Similarly, surface adsorption involves the attachment of lead ions to the cell wall, providing a physical barrier that protects the cell and aids in the reduction of environmental lead levels. Intracellular precipitation is another important mechanism, in which absorbed lead ions are transformed into insoluble compounds within the cell, thereby reducing their reactivity and toxicity in metabolic processes [37]. As with copper resistance, bacteria also deploy efflux systems to expel lead ions from the cell, avoiding intracellular accumulation that could disrupt enzymatic activity and cellular structure. The combination of these mechanisms allows bacteria to thrive in lead-contaminated environments, including natural habitats and industrially polluted sites.

Lead resistance in bacteria is also mediated by specific genes encoding proteins that effectively capture and isolate Pb(II) ions from sensitive cellular regions. A primary mechanism involves the expression of metallothioneins (MTs), cysteine-rich proteins that bind heavy metals such as Pb(II) via their sulfhydryl groups [41]. MTs contain two functional domains, α and β, which form stable metal–sulfhydryl complexes [40]. Genetic engineering efforts have demonstrated that enhancing MT expression, such as *smtAB* from *Salmonella choleraesuis* or metallothioneins from *Proteus penneri*, significantly increases lead bioaccumulation [42]. Additionally, protein-based resistance systems such as PbrR, PbrR691, and PbrD from *Cupriavidus metallidurans* CH34 exhibit high selectivity for Pb(II) [43].

These proteins function by recognizing the unique hemidirected coordination geometry of lead ions, which limits interactions with other metals and enhances resistance specificity.

Remarkably, these resistance mechanisms have been identified across diverse bacterial taxa, suggesting that adaptation to lead is not restricted to a single lineage. Several bacteria have demonstrated a high capacity for lead uptake, including *Acidithiobacillus ferrooxidans* [44], *Bacillus pumilus* sp. [45], and strains such as *Serratia* sp. L2, *Raoultella* sp. L30, and *Klebsiella* sp. [46]. Recent studies indicate that these bacteria not only tolerate high Pb concentrations but also hold significant promise for bioremediation—utilizing microorganisms to remove or neutralize heavy metal pollutants from the environment [47]. The widespread occurrence of these resistance traits across bacteria from different ecosystems points to convergent evolutionary strategies in response to heavy metal pollution, offering valuable insights for the development of microbe-based environmental restoration technologies [48].

4. Bacterial mechanisms involved in copper and lead bioremediation

4.1. Biosorption

Biosorption is a passive mechanism by which microbial biomass interacts physicochemically with heavy metal ions such as copper (Cu) and lead (Pb), as illustrated in Figure 2. This process involves various pathways including surface adsorption, physisorption, chemisorption, ion exchange, and complexation [16]. It primarily relies on the presence of negatively charged functional groups on the bacterial cell wall—such as hydroxyl, carboxyl, sulfate, phosphate, and amine groups—which interact electrostatically and through coordination bonds with positively charged metal ions. In physisorption, metal ions transfer from the aqueous phase to the solid phase through Van der Waals forces and Coulombic interactions, as observed in the biosorption of copper and lead by *Klebsiella* sp. R19 and *Raoultella* sp. L30 [46, 49]. Ion exchange mechanisms involve the displacement of pre-existing cations on the bacterial surface by metal ions, a process evident in the biosorption of Pb and Cu by *Pseudomonas pseudoalcaligenes* and *Micrococcus luteus* [16, 50]. Meanwhile, complexation involves the formation of coordination compounds between metal ions and active groups like carboxyl, phosphate, and amine groups on the microbial cell surface, as documented in *Ochrobactrum cicero*, *Stenotrophomonas maltophilia*, and *Pseudomonas* *putida* [51]. Compared to bioaccumulation, biosorption offers several advantages: it is metabolism-independent, occurs rapidly, utilizes dead biomass (which is more resistant to metal toxicity), and allows for easier recovery of adsorbed metals [52]. Several studies have shown that certain microorganisms, such as *Azotobacter nigricans* NEWG-1, demonstrate higher biosorption capacity in non-viable (dead) states than in viable ones, making them a highly efficient alternative for remediating heavy metal-contaminated environments [53].

4.2. Bioaccumulation

Bioaccumulation of copper (Cu) and lead (Pb) in bacterial cells occurs through both passive and active transport mechanisms, allowing metal ions to enter and accumulate intracellularly [54]. This process begins with the adsorption of metal ions onto the bacterial cell wall through interactions with functional groups such as teichoic acids and lipopolysaccharides, as illustrated in Figure 2. The ions may then enter the cell either passively through porins and membrane channels, or actively via specific transporters, such as ATP-binding cassette (ABC) transporters [16]. Active transport requires energy derived from ATP hydrolysis to move metal ions against their concentration gradient into the cytoplasm [55]. Once internalized, metal ions may undergo various detoxification pathways, including sequestration by metal-binding proteins such as metallothioneins, precipitation within polyphosphate granules, or methylation into volatile compounds that are more readily expelled [56, 57]. Copper, being essential for cellular metabolism, is accumulated via both general and specific transport systems, including siderophores or Cu-specific binding proteins [58]. In contrast, lead is typically more cytotoxic and is often found bound to polyphosphates or complexed with detoxifying enzymes [59]. Unlike biosorption, which is limited to surface interactions, bioaccumulation involves active internal transport and retention, making it more effective for long-term metal sequestration [52]. Consequently, bioaccumulation is frequently employed in the bioremediation of industrial effluents contaminated with heavy metals.

4.3. Bioprecipitation

Bioprecipitation of copper and lead by bacteria involves three main mechanisms: metal reduction, sulfide precipitation, and phosphate precipitation, as illustrated in Figure 2. In the reduction mechanism, bacteria such as *Shewanella oneidensis* and *Geobacter* species reduce toxic metal ions to more stable and less soluble forms, such as elemental selenium or uranyl carbonate [60]. This reduction is driven by the availability of electron acceptors in the bacterial environment. Sulfide precipitation is primarily facilitated by sulfate-reducing bacteria (SRB), which are obligate anaerobes. These organisms oxidize organic compounds or hydrogen while using sulfate as a terminal electron acceptor, producing sulfide ions. The sulfide then reacts with heavy metal ions like Cu and Pb to form insoluble metal sulfide precipitates, thereby reducing metal toxicity in both bacterial cells and their surrounding environment. Examples include *Desulfomicrobium norvegicum* and *Alteromonas putrefaciens* [61]. Additionally, certain bacteria such as *Salmonella typhimurium* and *Klebsiella planticola* produce enzymes like thiosulfate reductase, which facilitate sulfide production and enhance heavy metal precipitation [62, 63]. The third mechanism involves phosphate precipitation. Here, enzymes such as phosphatases, produced by bacteria like *Citrobacter* sp., release inorganic phosphate from organic substrates. These phosphates then react with heavy metal ions such as Cu and Pb to form insoluble metal phosphate precipitates [16].

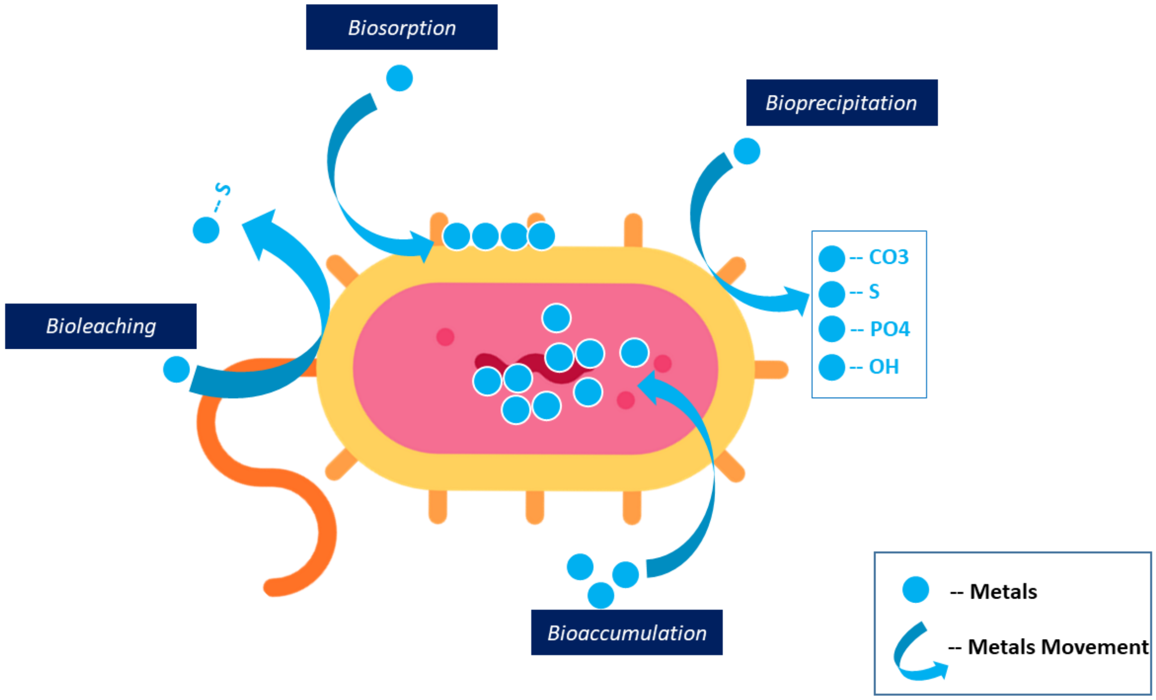

Figure 2. Bioremediation mechanisms of Cu and Pb by bacteria.

Bioprecipitation may occur through metabolism-dependent pathways, which rely on active bacterial processes, or metabolism-independent pathways, which are purely chemical interactions between metal ions and reactive cell surface groups [54]. Living cells are generally more effective in this process due to their active biological machinery. Bioprecipitation is particularly effective in remediating industrial wastes such as mining effluents and electroplating wastewater, offering the added benefit of facilitating metal recovery [64].

4.4. Bioleaching

Bioleaching is the microbially mediated extraction of metals from ores and waste materials, occurring through either direct or indirect mechanisms. In direct bioleaching, bacteria such as *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* oxidize sulfur and iron compounds in metal sulfide ores to produce soluble sulfates, which then release metals like Cu and Pb into solution [16]. In contrast, indirect bioleaching involves the microbial oxidation of sulfur compounds into sulfuric acid, lowering the environmental pH and enhancing metal solubility, as illustrated in Figure 2. Bioleaching can also be facilitated by heterotrophic bacteria such as *Pseudomonas* sp., which secrete organic acids, such as citric, oxalic, and lactic acids, that help solubilize metals from non-sulfide mineral matrices [65, 66]. Certain microbes like *Citrobacter* sp. contribute to bioleaching by releasing inorganic phosphate, which precipitates with heavy metals to stabilize their toxic forms [67]. For Cu and Pb recovery, *Acidithiobacillus ferrooxidans* and *Leptospirillum ferriphilum* have proven effective in solubilizing these metals from mine tailings and industrial effluents via iron and sulfur oxidation pathways [68, 69]. Additionally, *Bacillus* sp. and *Thiobacillus ferrooxidans* have been shown to facilitate lead bioleaching by converting sulfide forms into soluble species suitable for downstream recovery [16]. Although bioleaching presents a more environmentally sustainable alternative to conventional hydrometallurgical techniques, its progress is typically slow and highly dependent on optimal pH and temperature conditions to achieve high efficiency [54]. Therefore, continued research is essential to optimize microbial strains and operational parameters, aiming to enhance the industrial applicability of bioleaching in metal recovery and environmental remediation.

5. Influence of environmental change

Environmental factors play a critical role in determining the efficiency of copper (Cu) and lead (Pb) bioremediation by bacteria. Among the most influential parameters is pH, which affects bacterial enzyme activity, cell surface charge, and the mobility and hydration state of heavy metal ions [70]. Optimal pH levels, typically ranging between 5.5 and 6.5, enhance metal adsorption and removal rates. However, when pH exceeds this optimal range, the formation of insoluble hydroxide precipitates can occur, reducing microbial uptake efficiency [71, 72]. For instance, *Bacillus jeotgali* has been shown to perform more effectively at a neutral pH of around 7 for bioremediation purposes [73]. Temperature is another crucial factor, as it directly influences microbial growth, metabolic rates, and enzymatic activity [74]. Each bacterial species exhibits a specific temperature range for optimal performance. For example, *Bacillus jeotgali* demonstrates peak biodegradation efficiency for Cd^{2+} at 35°C and for Zn^{2+} at 30°C [75]. While elevated temperatures can increase metal ion diffusion rates and bioavailability, excessive heat may also inhibit microbial viability and enzymatic functions if the thermal threshold is surpassed [76].

Furthermore, the type and concentration of substrates significantly affect the outcome of bioremediation processes. The adsorption characteristics of metal ions vary depending on soil properties, the specific metal ions present, and the use of soil amendments. Soils with high adsorption capacities, such as coastal soils with a Freundlich adsorption constant (K) of 93.79, can immobilize heavy metals, thereby reducing their bioavailability to microbes and lowering adsorption efficiency [52]. Conversely, the addition of soil amendments like $FeSO_4 \cdot 7H_2O$ at concentrations up to 20 g/L has been shown to double the release rates of Cu and Zn, although excessive supplementation can conversely reduce efficacy [77]. The concentration of metal ions in the surrounding medium also influences microbial adsorption performance. Moderate increases in Pb^{2+}, Cd^{2+}, and Zn^{2+} concentrations can enhance adsorption rates up to a saturation point, beyond which no further increase in uptake occurs [78]. The Langmuir isotherm model is commonly used to describe monolayer adsorption behavior, while the Freundlich model is better suited to represent heterogeneous surface adsorption and equilibrium dynamics [79]. Considering these environmental factors, optimizing pH, temperature, substrate type, and metal ion concentration is essential for maximizing the bioremediation efficiency of Cu and Pb by bacterial systems.

6. Limitations and challenges in the implementation of bacteria-based bioremediation

Although bacteria-based bioremediation offers an environmentally friendly solution for addressing copper (Cu) and lead (Pb) contamination in aquatic environments, its direct application in natural ecosystems faces several significant challenges. The high rates of metal absorption or neutralization achieved by bacteria under laboratory conditions often do not translate to comparable success in the field. Environmental fluctuations, such as changes in pH, temperature, salinity, and nutrient availability, can drastically compromise bacterial performance. As a result, laboratory bioremediation efficiencies that may reach up to 90% can decrease by half under real-world conditions [80-82]. Additionally, the presence of diverse indigenous microbial communities, the bioavailability of heavy metals influenced by sediment matrices, and interactions with co-occurring pollutants further complicate the remediation process. Consequently, approaches relying solely on single bacterial strains are often insufficient [83, 84]. Community-based strategies or metagenomic analyses are therefore recommended to facilitate better adaptation of the introduced bacteria to native environmental conditions [85].

Scalability and practical field management also pose substantial obstacles. While in situ bioremediation offers advantages in cost efficiency and minimal physical disruption, its effectiveness depends heavily on the uniform distribution of bacterial inoculum and nutrients throughout water bodies and sediments. In practice, periodic bioaugmentation is required to maintain active bacterial populations, demanding additional time and resources. Furthermore, the monitoring of remediation success increasingly relies on advanced molecular technologies, which are not yet fully standardized and remain limited in terms of accessibility and data interpretation [86, 87]. The potential risks associated with secondary contamination or the release of genetically engineered bacteria whose safety has not been fully established also warrant careful consideration [88]. Therefore, the integration of diverse technological platforms, the development of real-time monitoring systems, and the establishment of adaptive regulatory policies are essential to bridge the gap between laboratory and field applications. These measures are critical to ensure that bacteria-based bioremediation remains a vital strategy for restoring aquatic ecosystems contaminated with Cu and Pb.

7. Current trends in bioremediation research on copper and lead

7.1. Co-Occurrence Analysis of Scientific Publications

This section utilizes a co-occurrence analysis of journal articles sourced from the SCOPUS database to explore the stratification and evolving trends in bacterial bioremediation of copper (Cu) and lead (Pb). The parameters of the analysis span from 2016 to 2025, using the keywords "bacteria bioremediation heavy metal copper and lead," yielding a total of 263 publications. The data was derived from titles, abstracts, and author keywords. The resulting visualization, presented in Figure 3., highlights the most frequently occurring terms in scientific literature over the past decade. Each node in the network represents a referenced article, while the connecting lines indicate inter-article relationships.

The analysis underscores that research on the bioremediation of Cu and Pb remains highly relevant, driven by an increasing intensity of anthropogenic activities. These include urban expansion [89], land-use change and agricultural intensification [90], transportation [91], and mining operations [3]. Such activities have significantly escalated the contamination burden of Cu and Pb in aquatic ecosystems, emphasizing the urgent need for effective remediation strategies.

As illustrated in Figure 3, recent studies emphasize metagenomic approaches, including microbial community profiling, gene sequencing, and 16S rRNA analysis, as emerging frontiers in Cu and Pb bioremediation. Metagenomics has advanced our understanding of bacterial potential in detoxifying Cu and Pb through the identification of key microbial taxa and the underlying genetic mechanisms of heavy metal resistance. Analyses of 16S rRNA and metagenome-assembled genomes (MAGs) have revealed a predominance of microbial groups such as *Actinobacteria*, *Rhizobiaceae*, and *Burkholderiaceae* in contaminated environments.

These microbes harbor resistance genes (e.g., *czc*, *cop*, *ars*C) that facilitate metal detoxification via ion efflux and reduction of toxicity [92]. Moreover, horizontal gene transfer (HGT) plays a pivotal role in disseminating resistance traits across microbial populations, enhancing community adaptability under extreme environmental stress [93].

Functional metagenomics has also identified critical metabolic pathways such as sulfur oxidation (*sox*) and carbon fixation, which support microbe–plant interactions in phytoremediation systems [94]. In aquatic ecosystems, species such as *Bacillus cereus* have demonstrated the ability to immobilize Pb and Cd through biochemical transformation processes—an effect further enhanced by adjuncts like magnetic biochar [95, 96].

These findings affirm the dual role of metagenomics in characterizing microbial diversity and informing the design of targeted, bacteria-based bioremediation strategies. The integration of metagenomics with other omics technologies, such as metatranscriptomics and in silico modeling, has expanded the applicability of these approaches in Cu and Pb bioremediation. Amplicon sequencing of 16S rRNA and comparative genomic analyses have successfully identified *Pseudomonas* and *Geobacter* species possessing genes that encode metal transporters and detoxifying enzymes, enabling them to reduce and immobilize heavy metals effectively [97].

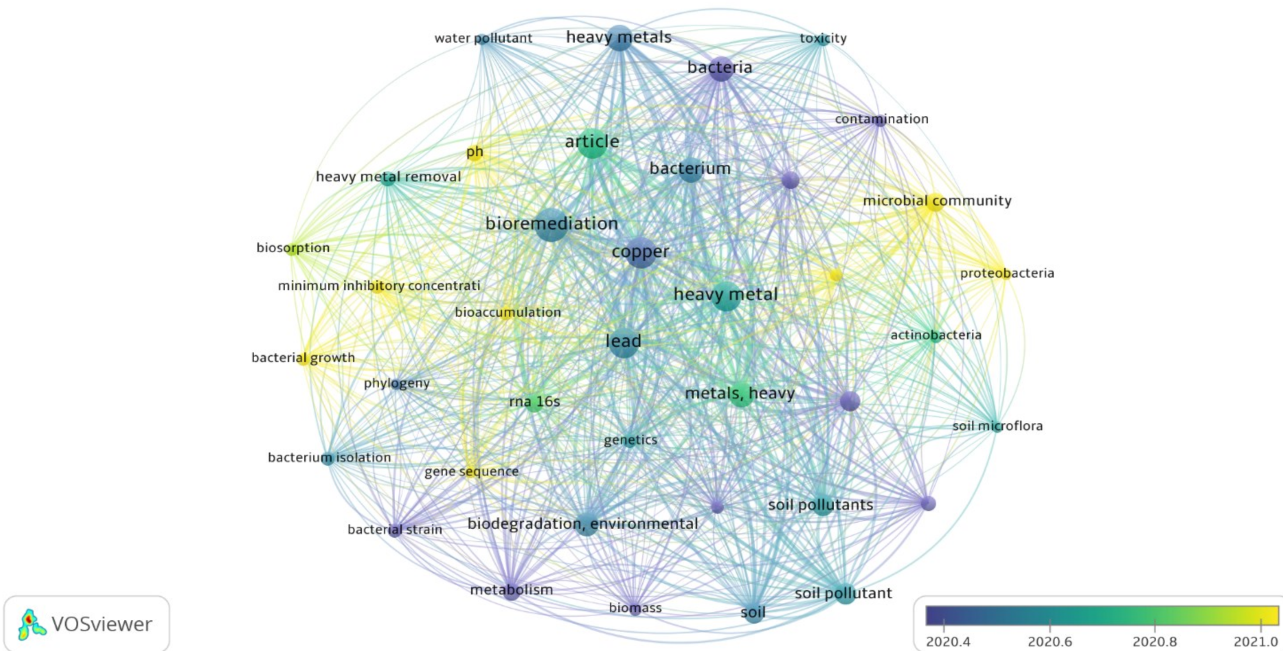

Figure 3. Co-occurrence network analysis of research trends in bacterial bioremediation of Cu and Pb.

Computational (in silico) approaches further enable the prediction of metabolic pathways for engineering genetically enhanced microbes with superior metal accumulation capabilities, such as modified *Bacillus* strains designed to overproduce organic acids [98, 99]. Nevertheless, challenges persist, particularly the complexity of microbe–environment interactions and the incomplete characterization of microbial communities, which continue to limit the full optimization of these technologies [97]. Innovative solutions, including the combination of microbial consortia with nanoparticles or biochar [100], and the implementation of GIS-based environmental monitoring systems, offer promising pathways to increase remediation efficiency. Furthermore, metagenomic exploration holds potential for discovering novel hyperaccumulating microbes, such as *Salinimicrobium*, identified through 16S rRNA analyses [101]. In conclusion, the future of Cu and Pb bioremediation lies in the synergistic integration of metagenomics, advanced biotechnological tools, and supportive environmental policies. Such an interdisciplinary approach will be essential in developing sustainable and high-performance remediation strategies to address heavy metal pollution in diverse ecosystems.

7.2. Future Strategies for Bioremediation

Bioremediation of heavy metals such as copper (Cu) and lead (Pb) in aquatic ecosystems represents a pressing environmental challenge, particularly due to increasing industrial and agricultural pollution [90]. Bacterial-based bioremediation offers a cost-effective and environmentally sustainable alternative to conventional methods such as chemical filtration and physical treatments [15]. The integration of omics-based technologies to enhance bacterial resilience in contaminated water bodies has emerged as a promising strategy [102]. Moreover, advancements in nanobiotechnology can be leveraged to improve bacterial performance in heavy metal removal through mechanisms such as bioadsorption and bioprecipitation [103]. A comprehensive understanding of the physical, chemical, and biological characteristics of bioremediating bacteria is crucial for developing more efficient strategies to mitigate Cu and Pb pollution in aquatic systems. Future research should prioritize the optimization of bacterial applications in Cu and Pb bioremediation through omics approaches, genetic engineering, and the enhancement of microbial tolerance to extreme environmental conditions. Genetic modification of microorganisms, such as *Escherichia coli* BL21 engineered with the *PbrR* gene, which achieved Cu removal efficiencies up to 85.6%, and the insertion of *SynHMB*, which enhanced Pb removal efficiency to 90%, demonstrates the potential of synthetic biology in improving bioremediation outcomes [104, 105]. The combination of microbial systems with nanoparticles also holds promise for increasing heavy metal removal efficiency while mitigating toxicity effects on the microbial consortia [105, 106]. Additionally, the application of Geographic Information System (GIS)-based monitoring systems, combined with active community participation, can accelerate pollution detection and enhance the management of aquatic ecosystems [107-109]. For the long-term sustainability of this approach, stricter regulations governing industrial and agricultural waste discharge are essential, alongside policies that promote microbial bioremediation as a primary solution to Cu and Pb contamination in aquatic environments.

8. Conclusion

Copper and lead contamination in aquatic ecosystems poses significant risks to environmental integrity, human health, aquatic biodiversity, and the structural composition of microbial communities. Bioremediation using bacterial approaches offers a sustainable and ecologically sound solution to mitigate the toxic effects of Cu and Pb in the environment. Contemporary bioremediation strategies have evolved to incorporate a wide range of innovations, including omics technologies, nanobiotechnology, nanoparticle applications, and genetic engineering. However, the success and scalability of these methods require further in-depth investigation and broader public engagement. A holistic, interdisciplinary approach—supported by scientific innovation, regulatory enforcement, and community involvement—will be vital to advancing bioremediation as a core environmental management strategy for heavy metal pollution.

Authors contribution

Eko Purnomo: Data collection and manuscript writing, Hermin Pancasakti Kusumaningrum and Anto Budiharjo: Conceptualization, supervision and review, Arina Tri Lunggani and Retnaningsih Soeprobowati: Supervision and review.

Conflict of interest

No potential conflict of interest was reported by the
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Data availability

Not Applicable.

Declaration of using generative AI

This article was originally prepared in the Indonesian language. Generative AI tools were used solely to assist in refining the English sentence structure and improving linguistic clarity. The authors confirm that all scientific content, analysis, interpretations, and conclusions are entirely their own, and they take full responsibility for the integrity and originality of the work.

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