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Bioleaching Assisting Phytoextraction of Metals from Contaminated Soil: An Overview

¹Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, University Kebangsaan Malaysia, 43600, UKM Bangi, Selangor, Malaysia

²East Coast Environmental Research Institute, Gong Badak Campus, University Sultan Zainal Abidin, Kuala Terengganu 21300, Malaysia

ABSTRACT

Human activities such as mining, industrial operations and waste management can lead to soil pollution by heavy metals including chromium, cadmium, mercury, lead and arsenic. These contaminants cause harm both to humans and the ecosystems where they are found. Of all the previously used techniques, phytoremediation is the most promising one for cleaning up heavy metal-contaminated soils. Phytoremediation refers to a technique where plants use roots for absorbing, storing and immobilizing soil contaminants while also removing them. Bioleaching is a method which uses microorganisms to dissolve metals that have been shown to facilitate phytoextraction in increasing the availability of metals. It is anticipated that research advancements and technological innovations will make it more efficient and appropriate. Root absorption is increased by bioleaching through modification of rhizosphere thus making it more bioavailable for plant uptake. Plant-bacterial interactions are proven to speed up the remediation rates. Both processes can help clear off pollutants from the soil environment. However, further research is needed to find and improve the best strains of microorganisms, assess long-term soil impacts and control massive influxes of bacteria. The combination of bioleaching and phytoextraction offers a reliable and efficient system for removing metals from polluted soils.

Keywords: Bacteria, Bioleaching, Contaminated soil, Phytoremediation, Phytotechnology.

1. INTRODUCTION

High levels of heavy metals in the soil cause pollution and therefore are a major problem for both the environment and public health **(Purwanti et al., 2019; Yuliasni et al., 2023)**. These heavy metals include lead **(Imron et al., 2021b)**, mercury **(Imron et al., 2019a;**

*Corresponding author

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Arliyani et al., 2023), cadmium **(Shojaei et al., 2021)**, arsenic **(Titah et al., 2018)** and chromium **(Ramli et al., 2023a; 2023b)**. Heavy metals are naturally occurring elements which can be accumulated in the environment as a result of industrial activities like mining **(Ighalo et al., 2022; Wibowo et al., 2023)**, agricultural practices such as waste disposal through inappropriate management and industries operations like mining, farming, etc. The presence of heavy metals in the soil sometimes is catastrophic to ecosystems as well as human lives.

Several researchers have reported that these metals can persist in the environment for a long time **(Titah et al., 2019; Ismail et al., 2020; Wiradana et al., 2024)** and also that their toxic effects may interfere with biological processes **(Ahmad et al., 2022a; Kurniawan et al., 2023)**. The growth of plants can be hindered by heavy metal pollution **(Ahmed et al., 2021; Almansoory et al., 2021)**, causing declining crop yields as well as build-up of metals in edible parts **(Ramli et al., 2023b)**, which thus endanger both human and animal health. In this context, direct contact with polluted soil or by blood consumption of dishes and traffic can potentially cause a range of chronic health effects, showing adverse impact in our nervous system disorder, kidney failure problems, difficulty breathing and also cancers among other human beings that are currently explained **(Oginawati et al., 2021; 2022).**

Phytoremediation is an ecologically sustainable and perhaps the most effective approach for the environmental remediation of soils contaminated by heavy metals **(Al-Ajalin et al., 2020; Purwanti et al., 2020a; Kurniawan et al., 2022c)**. This is a way whereby pollutants that are present in the soil, will be taken by plants translocated and stored. The latter extracts and eliminates the toxins from above-ground parts of plants **(Al-Ajalin et al., 2022; AL Falahi et al., 2022; Imron et al., 2023)**. Plants take metals up from the substrate, move them within the plant and sequester most of these elements in their biomass through processes including root absorption (root-to-shoot transfer), movement inside plants (storage) or volatilization **(Aransiola et al., 2013; Imron et al., 2019b; Osama et al., 2022)**.

Bioleaching, a method for using microbes to release metals from the stable components that bind them, may hold the potential to assist phytoextraction treat metal-polluted soils **(Nayak et al., 2020; Tran et al., 2020)**. Although research on the combination of bioleaching together with phytoextraction is relatively new and scant, coupling these two techniques might now be a more absorb idea to maximize soil decontamination. This review is conducted to summarize the current knowledge about how bioleaching works together with phytoextraction for soil contaminated by metal pollution in terms of processes and problems that are investigated as well as possible directions into future research. This review is likely to provide new perspectives in the domain of metal-contaminated soil phytoremediation.

2. PHYTOREMEDIATION OF METALS FROM CONTAMINATED SOIL

Previous literature mentioned that phytoremediation is a modern and eco-friendly technology to restore soils contaminated with high levels of metals **(Purwanti et al., 2019; Imron et al., 2021a; Al-Baldawi et al., 2021)**. Phytoremediation involves the mechanisms of accumulation of metals, in which specific species of plants may pose metal hyperaccumulation capabilities (hyperaccumulator species). Hyperaccumulator species absorb metals from the soil through their roots, transport them, and store them into their tissues (both below and above ground). The use of plants in treating metal-contaminated soil has attracted attention as an alternative approach to traditional physicochemical treatments

due to its cost and environmental benefits **(Tangahu et al., 2019; Rahim et al., 2022)**. Physical treatment of metal contaminated soil often involve soil digging which considered expensive in cost. In another view, chemical treatment of metal contaminated soil require the addition of chemicals, which residue may left and cause further contamination.

Several processes are involved in the phytoremediation of contaminated soils, including phytoextraction (the process where plants absorb and remove metals from soil) **(Li et al., 2020)**, phytostabilization (the process where plants prevent the spread of contaminants in soil by immobilizing them in their roots) **(Ismail et al., 2019)**, rhizodegradation (the process where plant roots release substances that help soil microbes break down contaminants) **(Arliyani et al., 2023)**, rhizofiltration (the process where plant roots absorb, filter, and remove contaminants, especially heavy metals) **(Kadir et al., 2020)**, phytovolatilization (the process where plants take up contaminants from soil or water, transform them into a gas, and release them into the atmosphere through their leaves) **(Bhandari, 2018)**, phytodegradation (the process where plants break down or degrade contaminants in soil or water through metabolic activities within their tissues) **(Al-Baldawi, 2018)**, and phytofiltration (the process where plants filter and remove contaminants from water or soil by absorbing them through their roots or shoots) **(Sandhi et al., 2018)**. Among the aforementioned processes, phytostabilization (immobilization of metals in soil and plant root matrix) and phytoextraction (mobilization, absorption, and conversion of metals to aboveground plant parts) play the most important roles in the remediation process of metalcontaminated soils **(Fig. 1).**

3. MECHANISMS OF PHYTOREMEDIATION OF METALS CONTAMINATED SOIL

To date, there are many studies analyzing the way plants absorb contaminants, including the involved basic processes. (**Du et al., 2020)** noted that plants can both store and push away materials. Plants that store contaminants in their above-ground parts keep living. They change pollutants into more stable forms within their tissues to break them down or transform them **(Zhang et al., 2022)**. Plants that exclude contaminants limit how much they take into their living matter.

Plants have come up with smart and useful ways to get important micronutrients from their surroundings even when there's not much of them around. The roots of plants can break down and take in micronutrients from very small amounts in the soil even from stuff that's hard to dissolve. They do this by making chelating agents **(Diarra et al., 2021)** and changing the pH and redox reactions. Plants use special systems to move and store these micronutrients **(Möller and Müller, 2012)**. These same methods also help plants take in, move, and build up harmful substances that have chemical features like important nutrients. Because of this how plants get micronutrients plays a big role in cleaning up polluted areas using plants.

The plant cell plasma membrane contains many specialized proteins and transport systems that have an impact on ion uptake and movement **(Were et al., 2017)**. These include proton pumps that create electrochemical gradients and use energy, co- and anti transporters that take in ions by using the electrochemical gradients proton pumps generate, and channels that help ions enter the cell. All transport systems can absorb a wide range of ions. The way different ions interact when plants take in various heavy metal pollutants poses a key challenge. Moving nutrients from roots to shoots makes sense, as pulling out root biomass isn't practical **(Reboredo et al., 2021)**.

Figure 1. An overview of phytoremediation of contaminated soil **(Tangahu et al., 2011)**.

Plants have tight control over how they take in and move substances around **(Kabra et al., 2012; Li et al., 2021)**. They don't store more trace elements than they need for their bodily functions. Most trace elements are needed at 10 to 15 ppm, which is enough for most uses **(Tangahu et al., 2011)**. Some plants called "hyperaccumulators" can soak up harmful metal ions at levels as high as thousands of ppm **(Purwanti et al., 2020b; Imron et al., 2024a)**. Another key question is how these hyperaccumulators avoid metal poisoning and how they keep the dangerous metal ions inside **(Purwanti et al., 2018b; Ahmed et al., 2021; Ahmad et al., 2024)**. Several systems are at work, with the vacuole playing a big role in storage . The process of transpiration helps plants absorb nutrients and other elements from the soil into their roots **(Bolan et al., 2011)**. This involves water evaporating from plant leaves. This mechanism of evapotranspiration also has an impact on moving contaminants into plant branches. Contamination moves from the roots to the shoots, which are then harvested. This process allows to remove of contamination while keeping the original soil intact. Plants used in phytoextraction methods are called accumulators **(Masinire et al., 2021).** These plants have shoot-root metal concentration ratios higher than one. Non-accumulator plants can be categorized based on their low shoot-root ratios (which are mostly lower than 1). There are also some plants resistant to certain metals which show growth in metal-contaminated soil but not performing any metal uptake. Hyperaccumulator species show a high shoot-root

ratio, able to grow in a toxic environment, and produce high biomass **(Salido et al., 2003).** Currently, only a few species are considered metal hyperaccumulator plants and research to identify new species is emerging.

4. PHYTOEXTRACTION OF METALS FROM CONTAMINATED SOIL

Phytoextraction is a unique and environmentally favorable method for remediating soil that has been contaminated with high levels of heavy metals. This mechanism enables plants to mobilize absorbed metals into their branches and leaves **(Chellaiah, 2018)**. The process of metal absorption by plants is also somehow called bioconcentrating mechanisms. This process enables plants to accumulate metal concentrations that are significantly higher than those in the soil environment to which they are exposed. By performing this mechanism, the concentration of metals in the soil will be reduced and concentrated into the plants' biomass. Metals can be eliminated further from the soil system through the periodic harvesting of aboveground plant parts, provided that appropriate operation and maintenance are implemented. The appropriate disposal and handling of harvested plant biomass are essential to prevent further environmental contamination, as it contains high concentrations of metals **(Kurniawan et al., 2021)**.

Phytoextraction has demonstrated numerous advantages in the context of soil remediation. In comparison to conventional soil excavation and chemical treatments, it is comparatively simple to operate, involves fewer chemicals, and is cost-effective **(Alaboudi et al., 2018)**. It maintains ecosystem health and mimics natural processes, thereby reducing reliance on chemicals and benefiting the environment. Phytoextraction is appropriate for long-term remediation initiatives and can address extensive surface areas **(Keller et al., 2005)**. In addition, the utilization of plants in the remediation of contaminated soil offers the additional advantage of creating new habitats for a variety of organisms, thereby enhancing biodiversity **(Agarwal et al., 2018)**.

The success of phytoextraction is contingent upon the identification of specific hyperaccumulator species that can adapt to specific living conditions, despite the substantial advantages of this method **(Benizri et al., 2021).** In addition, this approach is also perceived as time-consuming when contrasted with conventional pharmacological and physical treatments **(Ali et al., 2020).** However, it should be emphasized that not all plants can take up all metals. Also how well they work may vary depending on the metal type or quantity present in soil. Throwing away contaminated plant material carefully is necessary to overcome pollution spread and contamination of the environment **(AL Falahi et al., 2022)**. Also, the soil properties and weather conditions may affect how well plants take up metals **(Herath and Vithanage, 2015)**.

However, there are times when these difficulties have been bypassed by researchers. To mention, such plants as Alpine pennycress also known as *Thlaspi caerulescens* possess high capabilities for accumulating zinc and cadmium **(Cosio et al., 2004)**. Again, Indian mustard or *Brassica juncea* is effective in lead accumulation **(Sut-Lohmann et al., 2023)**. Removal of arsenic from soils can be done using ferns such as Chinese brake fern whose scientific name is *Pteris vittata* **(Zhao et al., 2023)**. Genetic engineering advancement could possibly allow higher metal extraction capacity by plants **(Mallikarjuna and Yellamma, 2019)** thus making them more tolerant to heavy metals or able to assimilate larger quantities of them. In addition, research on plant-microbe interactions might result in bioleaching microorganisms including microbial inoculants that promote metal uptake **(Kumar and Gopal, 2015; Nayak et al., 2020)**. Knowing the challenges and the future research

directions, phytoextraction is a viable option for metal contaminated soil remediation with continuous research developments as well as technological advancements to improve its effectiveness and applicability **(Ahmad et al., 2022a; Kurniawan et al., 2022a; 2022b)**.

5. BIOLEACHING MICROORGANISMS ASSISTING PHYTOEXTRACTION

Researchers have stated that bioleaching can assist phytoextraction in cleaning up metalcontaminated soil **(Gomes et al., 2018; Nayak et al., 2020).** Bioleaching can reduce the severity of metal contamination in soil by dissolving stable metals in a solid matrix, allowing further uptake by plants **(Purwanti et al., 2020b; Kurniawan et al., 2022c; Imron et al., 2024b).** *Acidithiobacillus ferrooxidans* and *Aspergillus niger* are said to be superior in carrying out this action because of their ability to produce organic acids, as well as other metabolic by-products, which dissolve metal compounds in soil **(Cui et al., 2021; Giese, 2021).** Organic acids, such as citric acid, malic acid, and oxalic acid, released by microorganisms can assist metal uptake by plants by increasing the bioavailability of metal compounds, which then increases the efficiency of phytoextraction **(Fig. 2)**.

Figure 2. An overview of how bioleaching microorganisms assist the phytoremediation of contaminated soil **(Kurniawan et al., 2022c)**.

One important benefit of bioleaching that supports phytoextraction is its facilitation of easier entry to the metals **(McCutcheon and Jørgensen, 2018)**. Microbes convert metal compounds into dissolvable forms hence increasing the amount of plant-available metals in the soil with respect to A. ferrooxidans which can oxidize iron and sulfur compounds **(Valdés et al., 2008)**, thereby releasing copper, zinc, nickel among others into the soil solution. Other organisms such as *A. niger*, produce citric and oxalic acids. These acids can stick to heavy metals **(Odoni et al., 2017)** making them more soluble and easier for plants to absorb.

Acidophilic aerobic, and chemolithotrophic microbes play a key role in autotrophic bioleaching. These bacteria and archaea have the ability to turn inorganic sulfur compounds and/or ferrous ions into sulfuric acid and ferric ions **(Jones and Santini, 2023)**. These substances kick off weathering processes. In autotrophic leaching, Acidithiobacillus stands out as the most common and well-studied bacterium. Meanwhile, *Sulfolobus* spp. take the spotlight as the best-known autotrophic archaea in metal leaching. You'll often find these microbes in acidic, sulfide-rich spots. They have a knack for converting carbon through the Benson-Calvin cycle **(Ayangbenro et al., 2018)**.

Experts group autotrophic bioleaching processes into two types: contact and non-contact bioleaching. These labels have replaced "direct" and "indirect" leaching, as scientists haven't proven that metal sulfides and attached cells transfer electrons **(Gavrilescu, 2022)**. In noncontact bioleaching, microbes produce substances that dissolve metals. Contact bioleaching requires microbes to stick to sulfide mineral surfaces. This means they need to stay close and connected. Bacterial cells attach to metal particles often within minutes or hours. A. ferrooxidans make sticky substances that cover metal particles. These substances contain complex iron ions that help break down sulfide minerals. This works like the free-floating iron ions in non-contact bioleaching **(Sarkodie et al., 2022)**. Some experts suggest a third method cooperative bioleaching, to explain situations where both contact and non-contact bioleaching happen together. Autotrophic bioleaching involves microbes interacting with metal compounds through biological and chemical oxidation **(Purwanti et al., 2023; Al-Ajalin et al., 2024)**. So, in real situations, people should speed up whichever method they're using (contact or non-contact bioleaching) based on what materials they need to leach.

Heterotrophic leaching has an impact on the extraction of metals from metal-containing materials. This process uses heterotrophic microorganisms to produce organic acids or complexing agents. Many bacteria, fungi, and yeast play a role in heterotrophic bioleaching. The main difference between autotrophic and heterotrophic bioleaching lies in the need for organic resources to grow heterotrophic organisms. Researchers have studied *Penicillium* spp. and *Aspergillus* spp. to explore their use in bioleaching **(Sarkodie et al., 2022)**. The most common heterotrophic bacteria used in this process are *Bacillus* spp. and *Pseudomonas* spp. Heterotrophic bioleaching is considered to be less competitive as compared to autotrophic due to the generation of common chelating (leaching) agents by heterotrophic leaching microorganisms e.g., organic ligands, including succinate, citrate, malonate, and oxalate. In addition to that, heterotrophic leaching is also considered superior in extracting metals from non-sulfide compounds **(Sarkodie et al., 2022)**.

The rhizosphere environment can be altered by bioleaching organisms, which in turn influences root assimilation **(Niu et al., 2021)**. The release of organic acids and other byproducts by bioleaching organisms and plants results in a synergistic action that alter the redox conditions in the rhizosphere, thereby increasing the bioavailability of metals in the soil matrix and enabling plant roots to assimilate them more efficiently. A slightly more acidic condition is preferred by microorganisms to boost their metabolic activities, while also benefiting the plants due to the increased metal bioavailability. In an alternative perspective, plants also emit chemicals known as exudates, which aid in the proliferation of rhizosphere microorganisms. The secretion of exudates, which contain natural bioactive compounds, by plants also called as phytostimulation mechanism. This collaboration has the potential to lead to a more efficient and effective remediation of metal-contaminated soils **(Zahoor et al., 2017; Hawrot-Paw et al., 2019)**. The presence of bioleaching organisms can increase the time required for phytoextraction, thereby facilitating a more efficient soil remediation process **(McCutcheon and Jørgensen, 2018)**.

Although the integration of phytoextraction and bioleaching appears to be promising, it continues to encounter numerous obstacles. The current research should concentrate on the identification and enhancement of specific microbial strains that are most effective in solubilizing specific metals while coexisting with hyperaccumulator plants **(Briffa et al., 2020)**. Optimizing the amount of microorganisms to be introduced to the soil ecosystem for bioleaching needs to be carefully conducted and monitored. The long-term effects of bioleaching microorganisms and their processes on soil health and microbe ecosystems need to be investigated to avoid unwanted effects on the soil ecosystem **(Pande et al., 2022)**. Most improbably, several laboratory studies already demonstrated successful application, while field or even real-scale application is currently still limited and not without its challenges **(Kurniawan et al., 2020; Ahmad et al., 2022b; Sohaimi et al., 2024)**.

In conclusion, the efficacy of metal removal from contaminated soils is influenced by the combination of bioleaching and phytoextraction. Some species that may be suitable for bioleaching are listed in **Table 1**. Based on **Table 1**, it is worth to mention that some species e.g., *Acidithiobacillus thiooxidans, Aspergillus* sp. and *Aspergillus niger* achieved >99% bioleaching efficiencies. Among several mentioned metals, Zn showed to be the most targeted metal for bioleaching, followed by Cu, Pb, and Cd.

Table 1. Bioleaching microorganisms with the potential to assist phytoremediation of metals contaminated soil.

6. CONCLUSIONS

Phytoremediation, specifically phytoextraction, is a promising and eco-friendly method for removing heavy metals from soils. *Thlaspi caerulescens* showed a high phytoextraction capability of cadmium and zinc, *Brassica juncea* showed a good lead accumulation, while *Pteris vittata* capable of extracting arsenic from soil. Bioleaching, a method using microorganisms to dissolve metals, can enhance phytoextraction by increasing metal accessibility. Advancements in research and technology are expected to improve its efficiency and effectiveness. Bioleaching improves root absorption by modifying the rhizosphere due to the release of organic acids and some functional by-products, enhancing metal availability to plants. Several species, such as *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans*, and *Aspergillus niger*, were known to have good bioleaching

capabilities. While bioleaching and phytoextraction can improve soil pollution remediation, further research is needed to identify potential microbial strains, monitor the soil health impacts, regulate microorganism introduction, and scale up from a successful laboratory story to real-field application.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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استخالص المعادن بمساعدة التحلل الحيوي من التربة الملوثة: مراجعة عامة

1,2 ستيو بودي كيرنياوان

¹قسم الهندسة الكيميائية وعملية الهندسة، كلية الهندسة والبيئة المبنية، جامعة كيبانجسان ماليزيا، UKM ،43600، باليزيا 2 معهد بحوث البيئة الساحلية، حرم غونغ باداك، جامعة سلطان زين العابدين، كواال ترينجانو ،21300 ماليزيا

الخالصة

تؤدي الأنشــــطة البشـــــرية مثل التعدين، العمليات الصـــــناعية، وإدارة النفايات إلى تلوث التربة بالمعادن الثقيلة مثل الكروم، والكادميوم، والزئبق، والرصـاص، والزرنيخ. تتسبب هذه الملوثات في أضـرار للإنسـان والأنظمة البيئية التي توجد فيها. من بين جميع التقنيات المســـتخدمة ســــابقًا، تعتبر تقنية معالجة النباتات (الفيتوري ميدياشـــن) هي الأكثر وعدًا لتتظيف التربة الملوثة بالمعادن الثقيلة. تشـير الفيتوري ميدياشــن إلى تقنية تســتخدم فيها النباتات جذورها لامتصـــاص وتخزين وتثبيت الملوثات في التربة، مع إزالتها أيضًا. التحلل الحيوي هو طريقة تستخدم الكائنات الدقيقة لذوبان المعادن، وقد ثبت أنها تسهل عملية استخراج المعادن من خلال زيادة توفر المعادن. من المتوقع أن تســــهم التقدمات البحثية والابتكارات التكنولوجية في جعل هذه العملية أكثر كفاءة وملاءمة. يتم زيادة امتصـــاص الجذور من خلال التحلل الحيوي عن طريق تعديل منطقة الجذور ، مما يجعلها أكثر توافراً للنباتات. أثبتت التفاعلات بين النباتات والبكتيريا أنها تســـرع من معدلات المعالجة. يمكن أن تســـاعد كلا العمليتين في إزالة الملوثات من بيئة التربة. ومع ذلك، هناك حاجة إلى مزيد من البحث للعثور على أفضـــل ســـلالات من الكائنات الدقيقة، وتقييم التأثيرات طويلة الأمد على التربة، والتحكم في تدفقات البكتيريا الكبيرة. يوفر الجمع بين التحلل الحيوي واستخراج المعادن نظامًا موثوقًا وفعالًا لإزالة المعادن من التربة الملوثة.
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<mark>الكلمات المفتاحية :</mark> بكتيريا، تحلل حيوي، تربة ملوثة، معالجة النباتات، تقنية النبات.