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Bacillus species for sustainable management of heavy metals in soil: Current research and future challenges

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ABSTRACT

A significant environmental problem that has recently gained global notoriety is heavy metal pollution. Therefore, removal techniques are required. The environmental dissemination of heavy metals (HMs) can result in serious ecological and health risks. A possible alternative to traditional physical and chemical approaches is bioremediation, which uses microorganisms to eliminate contaminants. The ability of *Bacillus* species to create biosurfactants, siderophores, and enzymes that can sequester, solubilize, and convert HMs sets them apart from other microbes employed in bioremediation, which has led to their discovery as one of the most efficient. This review paper highlights the application of *Bacillus* species for the remediation of HMs in the environment. The paper focuses on elucidating the diverse heavy metal remediation mechanisms employed by *Bacillus* bacteria and provides an overview of the factors that influence the efficacy of bioremediation utilizing *Bacillus* sp. The benefits and drawbacks of employing *Bacillus* sp. for bioremediation are also discussed in the paper, along with recent advancement and difficulties in this area. The findings of this review demonstrate that *Bacillus* sp. has a significant potential for bioremediation of HMs, as evidenced by several studies. A cost-effective, sustainable, and ecologically acceptable method for bioremediation, *Bacillus* sp. has the added benefit of being able to remove a variety of HMs. This review paper comes to the conclusion that *Bacillus* sp. may be an effective choice for use in the bioremediation of heavy metal contamination in the years to come.

1. INTRODUCTION

The health of human populations is anticipated to be greatly impacted by the widespread issue of environmental pollution. It is becoming evident how important environmental elements are to human population health and well-being. As global industrialization progresses, numerous pollutants are being released, endangering all life forms severely [1]. Global apprehension regarding the impact of environmental pollution on public health has intensified in the past three decades. The amount of pollution that individuals are exposed to today is allegedly at an all-time high [2]. Significant public health issues are caused by the interaction between anthropogenic activities that are not environmentally sustainable [3]. Dangers like climate change are prevalent in the 21st century. It is really concerning how the contamination of the air, water, and soil is escalating over time. The presence of elevated concentrations of heavy metals (HMs) and other toxic compounds often reaches levels that pose a significant risk to downstream ecosystems and life forms in proximity to contaminated sites, while industrial emissions combined with vehicle exhausts significantly worsen the air pollution. Industrial wastes are frequently dumped on unused or public areas, in rivers, or in sewers built to handle solely municipal garbage, which makes the problem worse due to the lack of hazardous waste facilities [4].

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Different air pollutants, such as sulfur dioxide, nitrogen oxides, carbon monoxide, ozone, HMs, and respirable particulate matter, volatile organic compounds have different chemical compositions, reaction characteristics, emission rates, times at which they degrade, and capacities for long- or short-distance diffusion [5]. Air pollution has detrimental effects on human health, manifesting in both immediate and prolonged impacts that affect various systems and organs. It can range from minor irritation of the upper respiratory tract to severe consequences such as lung cancer, chronic bronchitis among adults, respiratory infections in children, asthma exacerbations, and worsening of pre-existing cardiovascular and respiratory conditions [6]. Water pollution occurs when undesirable materials enter into water, alters the quality of water and adversely impacts the environment and human health [7]. The quality of water is influenced by various factors, including climatic conditions and the type of soil present, vegetation, precipitation geology, ground water, and human activities [8]. Urbanization and mining have an impact on water quality. The various chemicals, sediment and hazardous substances are additional sources of water contamination [9] [Figure 1]. The presence of low-quality water adversely disrupts the food chain, productivity of crops and contaminates our food, and endangering both human and aquatic life [10,11]. In addition, fishes are adversely affected by heavy metal; particularly iron (Fe), which poses harm to their respiratory system. Salem *et al.* [12] found that water contaminated with HMs leads to hair loss, liver cirrhosis, and kidney failure in human. The health of people and the environment may be at risk due to the rise in soil contamination over the past few decades. Biological processes related to plant growth and soil fertility are adversely affected by the continual presence of salts, pathogens, persistent hazardous substances, chemical compounds, or radioactive wastes [13]. The increased quantities of harmful substances, primarily HMs, pesticides, and petroleum derivatives, in the soil have adverse effects on the balance of ecosystems and human health [14].

Infections of the respiratory system, conditions of the heart, and several kinds of cancer are all become more common because of increasing pollution levels. Ecologically detrimental pollutants, such as oil hydrocarbons, HMs, and pesticides, have a detrimental impact

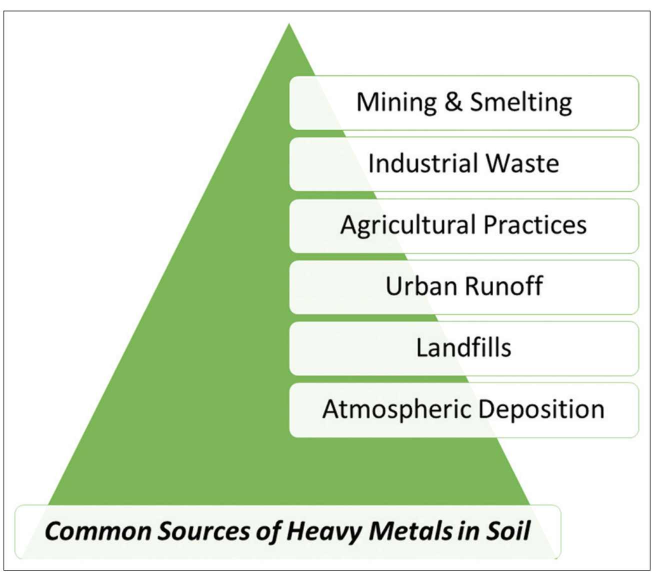

Figure 1: Sources that commonly contribute to heavy metal contamination in soil such as mining and smelting, industrial waste, agricultural practices, urban runoff, landfills, and atmospheric deposition.

on the sustainability of ecosystems. da Silva *et al.* [15] highlighted the prevalence of carcinogenesis, mutagenesis, and other harmful effects, particularly in humans. On entering the soil, pollutants can be absorbed, carried away by wind and runoff, or seep into the deeper layers and contaminate groundwater. Soil pollution primarily arises from agricultural residues, byproducts, air pollutants, irrigation practices, unintentional oil spills, inadequate municipal waste, sewage management, and deposition of hydrocarbons [13]. Due to the problems associated with global population, pressure on soil quality and requirement for sustainable soil fertility are both continuously growing. The confluence of all the aforementioned problems makes soil pollution a prominent concern for microbial community, plant, and agricultural as well as human health [16]. Consequently, the restoration of polluted sites becomes imperative to recover the functionality of the impaired environment, ensuring both environmental preservation and facilitating urban growth. At present, there are three major techniques for the remediation of contaminants from the soil sample including chemical, physical, and sustainable [17] [Figure 2]. Recent years have seen a significant increase in research into bioprocesses including phytoremediation and bioremediation because they are more cost effective than traditional methods, eco-friendly, and capable of removing a wide range of toxins efficiently [18].

2. HEAVY METAL ACCUMULATION

One of the foremost environmental challenges that can have a negative impact on both environmental quality and human health at the moment is the widespread contamination of soil by HMs. According to Ali and Khan [19], a heavy metal is characterized as an element with an atomic number exceeding 20 and an atomic density surpassing 5 g/cm^3, exhibiting metal-like properties. Thakare *et al.* [20] further HMs into two broad categories: Necessary and nonessential HMs. Essential HMs are vital for the fundamental biological processes of growth, metabolism, and organ development in living organisms. Plant species, such as those studied by Gratão *et al.* [21], require various essential HMs including copper (Cu), Fe, manganese (Mn), cobalt (Co), zinc (Zn), and nickel (Ni). These metals play essential roles in the production of cofactors that maintain the structural and functional integrity of enzymes and proteins. On the other hand, non-essential HMs such as cadmium (Cd), lead (Pb), mercury (Hg), chromium (Cr), or aluminum (Al) are not required by plants or any of their metabolic processes, even in trace amounts [22]. Both anthropogenic and natural factors can cause the discharge of HMs into the soil [23]. Despite the fact that heavy metal environmental contamination dates back to prehistoric times, the issue only became a serious concern following the industrial revolution because of the huge rise in the usage of HMs in contemporary technology.

In soil, commonly encountered HMs include Ni, Pb, Cd, Cr, Cu, Co, Zn, Mn, Al, and Hg [24]. The accumulation of HMs in agricultural soils can Pb to increased uptake of these contaminants by food crops and vegetables, presenting significant health risks to human populations [25]. According to reports, HMs can harm the bones, kidneys, nervous system, brain, and skin as well as cause cardiovascular disease, cancer, cognitive decline, chronic anemia, and damage to the skin, bones, and skin and brain [26-28]. There is a global imperative to maintain heavy metal levels in agricultural soil and crops below regulatory thresholds to mitigate the potential risks associated with heavy metal exposure. Increasing awareness of the adverse effects on human and environmental health stemming from heavy metal-contaminated soils has fostered the development of solutions to remediate such areas [29].

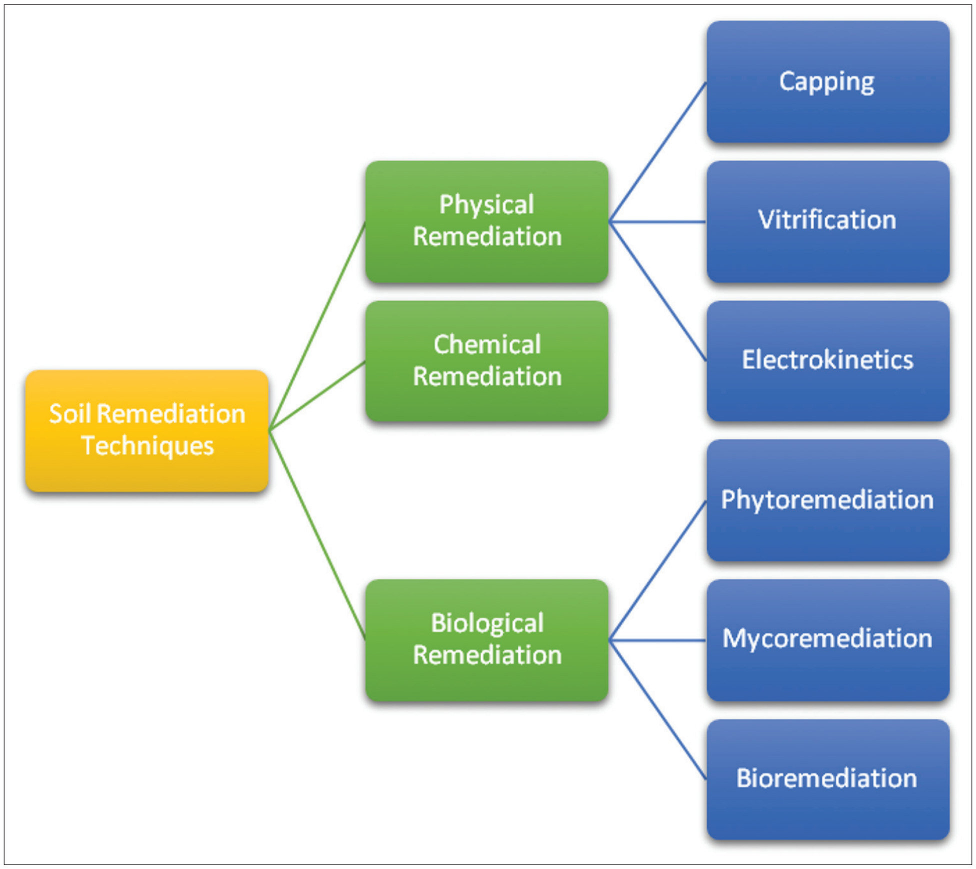

Figure 2: Different types of soil remediation techniques.

2.1. Chemical Fertilizers

In the past, agriculture was the main human activity that had a significant influence on the soil [30]. In addition to macronutrients nitrogen (N), phosphorus (P), potassium (K), sulfur (S), calcium, and magnesium, plants require essential micronutrients for their growth and life cycle. However, certain soils may exhibit deficiencies of necessary HMs, including Co, Cu, Fe, Mn, Zn, Ni, and Molybdenum, which are crucial for healthy plant development [31], thus crops can be given these nutrients by adding them to the soil or by spraying them on the leaves. On rare instances, Cu is applied to soil as a remedy for Cu-deficient soils to address the nutritional needs of cereal crops. According to Alloway [32], Mn supplementation is also beneficial for cereal and root crops. In intensive agricultural practices, substantial quantities of fertilizers are regularly applied to provide an adequate supply of N, P, and K for crop growth. However, these fertilizer compounds may contain trace amounts of HMs such as Cd and Pb as impurities. Prolonged use of fertilizers can significantly elevate the concentrations of these metals in the soil [33]. Cd and Pb are classified as metals that lack recognized physiological functions in biological systems. The application of certain phosphatic fertilizers can inadvertently introduce Cd and other potentially hazardous metals, including F, Hg, and Pb, into the soil, as highlighted by Moolenaar and Lexmond [34].

2.2. Insecticides

In the field of horticulture and agriculture, numerous conventional pesticides that were extensively employed in the past were found to contain elevated concentrations of metals [35]. Some pesticides, including Bordeaux mixture and Cu oxychloride, are fungicidal sprays that contain Cu [36]. Historically, Pb arsenate was extensively utilized in fruit orchards as a means to combat parasitic insects. Abandoned sites that were treated with Cu, Cr, and arsenic-based formulations chromated copper arsenate to preserve timbers exhibited significantly higher soil concentrations of these elements than background levels, as highlighted by McLaughlin *et al.* [37]. This form of contamination can pose challenges, particularly if these sites are later repurposed for agricultural or non-agricultural activities. The application of these materials has been more geographically restricted and primarily associated with specific regions or crops, in contrast to the widespread use of fertilizers [38].

2.3. Biosolids

Land application of various biosolids, including composts and municipal sewage sludge, inadvertently results in the contamination of soil with a wide range of HMs [39]. In agriculture, it is customary to utilize animal wastes, including poultry, livestock, and pig manures, by applying them as solids or sludges to crops and pastures [40]. Although manures are generally recognized as valuable fertilizers, the use of Cu and Zn in pig and poultry industries as growth promoters and poultry health products raises concerns regarding potential soil contamination with these metals [41]. Repeated application of manures produced by animals fed diets containing elevated levels of arsenic (As), Cu, and Zn to confined land areas can Pb to substantial accumulation of these metals in the soil [42].

There is growing interest in exploring the potential of composting biosolids together with organic materials such as wood shavings, straw, or yard waste. However, if this trend continues, there is a legitimate concern regarding the potential for soil contamination by HMs. The utilization of biosolids in agricultural practices has raised substantial concerns regarding their potential to introduce toxic substances into the soil [43]. The HMs that are most frequently detected in activated sludge are Pb, Ni, Cd, Cr, Cu, and Zn. The concentrations of metal are affected by the type of industrial activity, its intensity, the procedure used to treat the biosolids, and many other factors [44]. In specific situations, metals that are deposited in soils through the application of biosolids can potentially percolate through the surface soil and contaminate groundwater [45].

2.4. Metal Mining

Metal pollutants in soil have been widely distributed as a result of metal ore mining, ore milling, and related sectors in many different nations. During mining operations, the discharge of tailings, consisting of larger and denser particles that settle at the bottom of flotation cells, directly into natural depressions, including on-site wetlands, leads to elevated concentrations of contaminants [46]. A risk to the health of people and the environment has been created by the extensive extraction and processing of Pb and Zn ore through mining and smelting operations [47]. The soil reclamation techniques utilized at these sites are often time-consuming, costly, and may not effectively enhance soil productivity. The bioavailability of HMs in the soil plays a crucial role in determining the potential environmental risks to human health [48]. The food chain and the availability of contaminants are present in the soil for oral absorption and assimilation [47]. Other materials are produced by numerous industries, including fabric, bronzing, petrochemical products from unintentional oil spills or use of crude oil ingredients, herbicides, and pharmacological amenities, and they have a very varying composition [49]. Despite some being dumped on land, very few are useful for forestry or agriculture. Moreover, some substances are rarely, or never, utilized for land application due to their potential hazard, such as the presence of HMs (such as Cr, Pb, and Zn) or toxic organic compounds. In addition, certain substances exhibit inadequate soil conditioning properties or possess minimal nutritional value for plant growth [40].

2.5. Sewage Water

It has been a regular practice for 400 years in various regions of the world to apply municipal, industrial, and associated wastes and effluents to land [50]. An estimated 20 million acres of cultivable land globally are reported to utilize wastewater for irrigation purposes. Studies indicate that in numerous Asian and African towns, wastewater irrigation-based agriculture provides 50% of total of the city's supply of vegetables [51]. Farmers are typically more concerned with increasing their yields and earnings than they are with the advantages or risks to the environment. Although the metal concentrations in industrial wastewaters are typically low, prolonged irrigation of land with such waters can eventually result in the accumulation of HMs in the soil.

3. HAZARDOUS EFFECT OF HMS ON SOIL

In the industrialized world, heavy metal poisoning of soil is a major concern [52]. In addition to having negative effects on a number of plant quality and yield-related indices, heavy metal pollution also alters the size, make-up and productivity of the microbial community [53]. The detrimental impact of HMs on the biological and chemical properties of soil is widely recognized. The extent of these effects on biological and biochemical characteristics is heavily influenced by specific soil attributes, such as organic matter content, clay concentration, and pH levels [54]. HMs have an indirect effect on soil enzymatic activity by altering the microbial population that generates enzymes [55]. The presence of HMs in soil adversely affects soil biota by disrupting critical microbial functions and diminishing the abundance and activity of microorganisms. On the other hand, long-term effects of HMs can boost bacterial population tolerance as well as fungi like arbuscular mycorrhiza fungi's tolerance, which can be crucial for the regeneration of damaged ecosystems [56]. Heavy metal contamination resulted in reduction in biomass as well as diversification of the microbial populations in polluted soils, as well as a relative rise in soil actinomycetes. It is said that due to the various biochemical inclinations of the enzymes with in soil system, different metals have varying effects on the enzyme activity [57]. Pb is less harmful to enzymes than Cd because of its weaker affinity for soil colloids and greater mobility. More than cellulose activity, cu inhibits the activity of beta-glucosidase. Urease, catalase, invertase, and acid phosphatase all exhibit significant reductions in activity when exposed to Pb. As (V) inhibits the enzymes phosphatase and sulfatase; however, this did not influence the urease [58]. Protease, urease, alkaline phosphatase, and arylsulfatase are negatively impacted by cd contamination; however, invertase is not significantly affected.

It is crucial to study the behavior of soil microorganisms in ecosystems with prolonged exposure to heavy metal contamination [59]. In soils, Cr exists in two forms: Cr (III) and Cr (VI), each with distinct chemical properties and levels of toxicity. Cr (VI) is a powerful oxidizing agent and highly toxic, while Cr (III) is an essential nutrient and relatively non-hazardous, being 10–100 times less toxic [60]. At high concentrations, Cr (VI) is known to have negative impact on microbial cellular metabolism and has been observed to change the makeup of soil microbial populations [55]. It has also been noted that heavy metal pollution (Cr, Zn, and Cd) has an impact on the metabolism of beneficial microorganisms in all circumstances. This is because HMs have harmful effects on soil microorganisms, changing their variety, population size, and general activity. In general, an increase in metal concentration has a negative impact on the microbiological characteristics of the soil, including the rate of respiration and enzyme activity, both of which seem to be excellent markers of soil pollution [61]. A small alteration in the soil microbial spectrum was seen in lead-contaminated soil [56]. The potential health risks associated with the uptake of significant quantities of HMs by plants from soil warrant careful consideration in relation to their impact on the food chain. A significant food chains route for human exposure is the use of food crops that are polluted with HMs [58]. The ability to collect nutrients from soils is quite strong in food plants whose evaluation system relies on extensive and ongoing culture. Since, the vegetative tissues of such plants have the capacity to absorb HMs growing them in contaminated soil poses a possible concern [62]. When HMs accumulate in the soft tissues without being metabolized, they can become toxic [63]. Chronic exposure to hazardous metals in humans has negative effects that do not become apparent for years after the initial exposure [22].

4. SOIL REMEDIATION TECHNIQUES

4.1. Physical Techniques

4.1.1. Capping

Capping entails covering hazardous material, such as rubbish from landfills or contaminated soil. These coverings are known as "caps". Caps neither eliminate nor destroy pollutants. To stop the spread of infection, they segregate them and maintain them *in situ*. Caps shield both humans and animals from harmful substances. Surface capping, sanitary landfills, and encapsulation are a few of the methods used in soil replacement, which entails utilizing a significant amount of uncontaminated soil to mix with or cover the surface of polluted soils [64]. Due to its high cost and labor intensity, this technique is best suited for severely contaminated soil in areas with limited space. It can also effectively reduce the pollutant concentration. To stop additional dispersion from the site, barrier walls might be installed to isolate and contain pollutants. For capping, horizontal and vertical containment, impermeable physical barriers constructed of steel, clay, bentonite, and grout are used. It is clear that the practice of soil isolation or confinement was utilized to minimize the flow of HMs into the groundwater rather than as a direct remedial process [65].

4.1.2. Vitrification

The process of vitrification uses a strong energy source to melt soil at very high temperatures, immobilizing the majority of inorganic impurities and oxidizing or pyrolyzing the majority of organic contaminants. It is possible to use this technology including both *situ* and *ex situ*. *Ex situ* soil heating can be accomplished using a variety of technologies, but the most popular method is the use of electricity. The soil is put in a furnace and heated with electricity to a range of 1100–1400°C. *In situ,* treatment requires a higher temperature around 1600–2000°C [66]. Graphite electrodes are typically put into the soil to be treated to apply electrical energy [67].

4.1.3. Electrokinetics

The removal of organic, inorganic, and heavy metal particulates from the soil using direct electric current is known as electrokinetics (EK) [68]. When treating subsurface contaminants, this methodology provides an approach that disturbs the surface as minimally as possible [69]. It is a recently created technique that effectively cleans up heavy metal-polluted soils. This technique uses direct electric current to remove HMs from the soil matrix by a variety of methods, comprising electromigration, electroosmosis, electrophoresis, and electrolysis [70]. Chelates are additionally employed to increase the effectiveness of the EK in contaminated soils. By evaluating the impact of several chelators such as Ethylenediamine tetraacetic acid (EDTA), nitrilotriacetic acid, and citric acid in improving EK efficiency, HMs (As, Cd, Cr, Cu, Ni, Pb, and Zn) mobility was examined [71]. In addition, to overcome the drawback of flushing method in fine soil, several researchers have coupled flushing and EK in two different ways: Sequential and integration. The addition of a pump to the EK flushing remediation improved the effectiveness of removing Co^{2+} and Cs^{+} (caesium) from the polluted soils.

4.2. Chemical Remediation

It describes a particular technique for removing pollutants that makes use of chemical reagents, processes, and principles. Common methods for remediating contaminated soil include solidification/stabilization, soil washing, and soil flushing [70]. Solidification technology is often employed to reduce the mobility of heavy-metal pollutants by mixing contaminated soils with chemicals or materials. Stabilization involves implementing chemical reactions to reduce the mobility of pollutants, while solidification entails physically encapsulating the impurities within a solid matrix consisting of cement, asphalt, or thermoplastic binders [72]. Chemical bondings as well as encapsulation are the two basic interactions that can immobilize HMs in the glass matrix. Nitrification is a process of solidification and stabilization that requires thermal energy (1400–2000°C). It can be accomplished by combining polluted soil with glass-forming intermediates, heating the compounds until they become liquid, and then cooling the liquid to produce an amorphous relatively homogenous glass [73]. Chemical bondings as well as encapsulation are the two basic interactions that can immobilize HMs in the glass matrix [74]. Soil washing and soil flushing are highly effective techniques for remediation purposes aimed at eliminating pollutants from the soil. Various agents, such as water [75], organic acids [76], chelating agents [77], surfactants [78], saponin [79], and low-molecular-weight organic acids [80], have been identified as successful washing agents, ensuring optimal removal of HMs. Among these, EDTA has proven to be the most efficient chelating agent for extracting HMs from contaminated soils [81]. EDTA offers advantages such as minimal impact on soil microorganisms and enzyme activity, low biodegradability, enhanced metal extraction efficiency, accessibility to appropriate recycling methods, and availability for use in the remediation process [82].

4.3. Biological Techniques

4.3.1. Phytoremediation

Phytoremediation involves the utilization of plants, naturally occurring bacteria, and fungi to biodegrade or detoxify harmful pollutants that pose a threat to the environment or human health. These microorganisms can either originate from the contaminated site itself or be introduced from external sources. Through their metabolic activities, living organisms facilitate reactions that transform contaminant substances, aiding in the remediation process. The utilization of natural processes in bioremediation, which can successfully biodegrade a range of pollutants, even persistent ones, could be useful and effective method for reducing soil contaminations. This technique can make use of a variety of microorganisms, including ligninolytic fungi, methylotrophs, aerobic bacteria, and anaerobic bacteria. In an environmentally responsible manner, revegetating heavy metal-polluted soil with plants is possible through phytoremediation. A deeper comprehension of the mechanisms driving heavy metal concentration and tolerance in plants is essential to increasing the effectiveness of phytoremediation [83]. In this method, hazardous metals are removed from the environment, decomposed, or detoxified using green plants. Five different phytoremediation techniques, including phytovolatilization, phytostabilization, phytodegradation, rhizofiltration, and phytoextraction have been employed for soil decontamination [84]. Phytoremediation involves utilizing plants to eliminate hazardous substances from the surroundings or to make them less accessible in the soil. Ionic compounds with in soil can be taken up by plants via their rhizosphere even in small amounts. Plants strategically extend their root systems into the soil matrix, establishing rhizosphere ecosystems to uptake and control the bioavailability of HMs. This process aids in the restoration of polluted soil and regulation of soil fertility [85].

4.3.2. Mycoremediation

The widespread presence of fungi in the natural environment and their extensive utilization in industrial settings are widely recognized. Given their ability to adapt to their surroundings, fungus is essential for natural processes such as nitrogen cycling and decomposition [86]. Through mycoremediation, pollutants from diverse environmental niches are removed using fungus, either alive or dead [87]. Mycoremediation is a cost-effective approach that avoids the production of harmful waste products, making it a comprehensive solution due to the complete mineralization of pollutants in the environment [88]. The effectiveness of mycoremediation heavily relies on the identification and utilization of a specific fungal species tailored to the targeted HMs or other pollutants. Fungi exhibit efficient accumulation of HMs in their fruit bodies, which renders them inaccessible or lowers their abundance in the environment [89]. Future availability of HMs and other pollutants in the environment is significantly influenced by the longevity of fungus, the chemical characteristics of elements, and the availability of fungi following sequestration [90]. Studies have indicated that *Saccharomyces cerevisiae* has the potential to sequester a significant amount of Pb and Cd from contaminated soil, ranging from 65% to 79% [91]. Fungal cell walls with functional groups such as carboxyl, amino, hydroxyl, phosphate or sulfate, as well as proteins, glucans, lipids, pigments, and polysaccharides are involved in the biosorption process, which is mediated by interactions including ion-exchange, complexation, and adsorption [92].

4.3.3. Bioremediation

Bioremediation is the process of converting environmental pollutants into less harmful forms by using living organisms, primarily microbes. Methodology used in bioremediation is mostly based on biodegradation. It refers to the complete conversion of harmful or naturally occurring organic contaminants into substances that are safe for human, animal, plant, and marine life [93]. The biodegradation of a wide range of organic compounds has been explained by a variety of mechanisms and pathways; for instance, it can occur both with and without oxygen. Bioremediation is an effective method that aims to mitigate the presence of toxic compounds, including HMs, by converting them into less harmful substances. This technique contributes to the removal of potentially hazardous materials from contaminated environments, as well as the degradation of organic substances, leading to the complete mineralization of organic matter into components such as carbon dioxide, water, and nitrogen gas [94]. The application of bioremediation can be carried out using *in situ* or *ex situ* approaches, both of which are suitable for treating soil contamination [Figure 3]. There are several *in -situ* methods, including bioattenuation, biostimulation, biosparging, and bioventing. To encourage the growth of native or introduced microorganisms, bioventing entails adding oxygen to the soil. Biostimulation entails the provision of particular nutrients and ideal environmental conditions at the site to promote the growth of local microorganisms [95]. A contaminated site can be passively remedied through bioattenuation, which is applicable for both disintegrating and recalcitrant chemicals and necessitates ongoing monitoring. Biosparging is the process of supplying air under compression underneath the water table to raise the oxygen concentration and speed up microbial activity [96]. Over the past two decades, significant advancements have been achieved in bioremediation techniques with the primary goal of restoring polluted areas in an environmentally friendly and cost-effective manner [97].

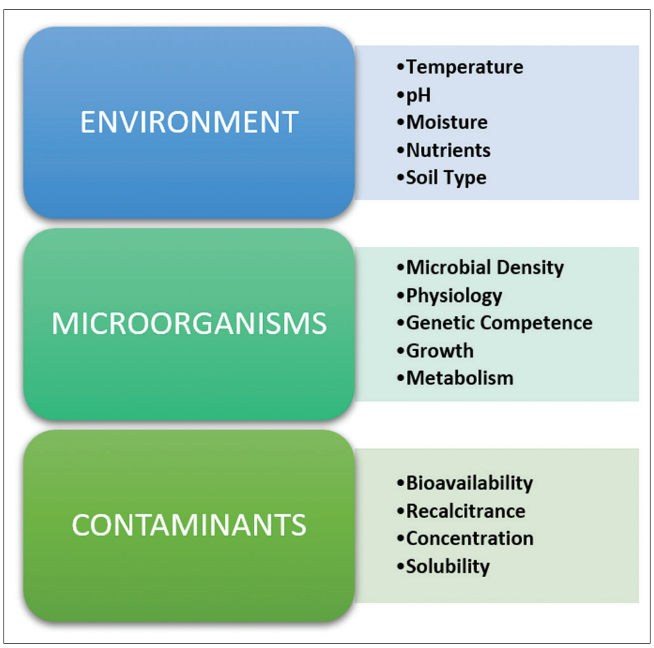

Figure 3: Factors affecting bioremediation.

5. MICROBIAL MANAGEMENT OF HMS IN SOIL

Microorganisms play a crucial role in the essential biogeochemical cycles of metals, facilitating the transformation of metals between insoluble and soluble forms. They are abundant in nature and contribute significantly to these cycles [98]. The interactions between metals and microbes can be advantageous or detrimental. These transformations depend on environmental variables such as pH, humidity, temperature, the occurrence of many other charged particles, in addition to the type of bacteria, and the chemical composition of metal, both of which are essential for microbial colonization and biofilm formation [87]. There are several *in situ* methods, including bioventing, biostimulation, bioattenuation, and biosparging. To encourage the growth of native or introduced microorganisms, bioventing entails adding oxygen to the soil. Biostimulation entails the provision of particular nutrients and ideal environmental conditions at the site to promote the growth of local microorganisms. A contaminated site can be passively remedied through bio-attenuation, which is applicable for both disintegrating calcitrant chemicals and necessitates ongoing monitoring. Biosparging is the process of supplying air under compression underneath the water table to raise the oxygen concentration and speed up microbial activity [99]. Everywhere you look in the environment, you can find bacteria. A simple and effective method for removing contaminants from wastewater, involving non-biodegradable substances like HMs, is biosorption by bacteria. Cells that make up bacterial biomass might be alive or dead. For the survival, bacterial species have developed mechanisms for resistance to metal ions and remediation. Bacterial species have evolved mechanisms to ensure their survival in the presence of metal ions and to contribute to remediation efforts. Metals including Cu, Zn, Pb, Cd, and Cr can be quickly removed using bacterial biomass [87]. The main physical interface connecting metal ions and bacterial biomass is the bacterial cell wall. Gram-positive bacteria possess cell wall components such as peptidoglycan, teichoic acids, and teichuronic acids, while Gram-negative bacteria have peptidoglycan, lipopolysaccharides, and phospholipids. These components contain anionic functional groups, including amine, hydroxyl, carboxyl, sulfate, and phosphate, which contribute to the metal-binding capability of the cell wall [92]. Extracellular processes are used by decaying biomass cells to remove HMs. These interactions are caused by functional groups on the cell wall, such as carboxyl, phosphonate, amine, and hydroxyl groups [100]. Microorganisms may induce redox reactions that Pb to metal mobilization and immobilization, affecting the bioremediation process. HMs go through cycles of oxidation and reduction, including Fe, As, Cr, and Hg [101]. An element's transformation from its immobile, insoluble form in sediments to its mobile, soluble state facilitates bioremediation. When hazardous metal ions are reallocated and liberated from their solid phase in sediments into the solution phase, mobilization can potentially have negative effects. As a result, HMs are more bioavailable and can enter microbial metabolic processes. By breaking down Hg (II), the bacteria can produce Hg (0), which is more volatile. By reducing certain ions, such as Fe (III) and As (V), to Fe (II), and As (III), respectively, microbial reduction can also increase the solubility of certain ions and promote leaching from soil. In soil and water, the process of heavy metal biomethylation is crucial and can change the toxicity, instability, and mobility of the metals. Due to the ability to eliminate volatile methylated species from cells, it also functions as a significant detoxification method

[102]. Microbial breakdown of organic matter contributes to the indirect process of metal mobilization by accelerating the emission of these ions [Table 1].

6. UTILIZATION OF *BACILLUS* SP. FOR HEAVY METAL REMEDIATION

Bacillus spp., belonging to the phylum *Firmicutes*, are Gram-positive bacteria characterized by their rod-shaped morphology and ability to form spores. Categorized primarily as soil microorganisms, *Bacillus* spp. have the ability to thrive in both aerobic and anaerobic conditions. Furthermore, they are widely distributed across diverse environments such as air, water, food sources, and even the human gut [103]. The *Bacillus* group is among the most diverse in terms of genetic or economic applications since certain species have gained recognition for their opportunistic nature as infectious agents and their ability to produce toxins, while others have found extensive applications in industrial and pharmaceutical settings [104]. The capacity of *Bacillus* spp. to produce spores in harsh settings, which is typically driven by a nutrient shortage, is one of their distinctive traits [105]. Spores can withstand extreme environmental stress, including desiccation, high temperatures, humidity, and radiation, thanks to their strong structural makeup. Due to this characteristic, they demonstrate a diverse range of commercial implications and are more ideal for use than vegetative cells [106]. The beneficial applications of *Bacillus* spp. in numerous domains have drawn attention to their distinctive properties, and research has made it possible to employ these traits to the benefit of humankind. Biocontrol products made from *Bacillus* are used [107]. The utilization of Gram-positive bacteria, specifically *Bacillus* spp., presents considerable advantages across medical, industrial, and environmental domains. One notable advantage is their reduced tendency to engage in the transmission of genetic material compared to Gram-negative bacteria. This characteristic enhances their suitability for various applications, making them a valuable resource in these fields.

Numerous *Bacillus* species, including *Bacillus subtilis*, *Bacillus coagulans*, *Bacillus pumilus, Bacillus licheniformis,* and *Bacillus cereus*, are used across the globe for a variety of uses [106]. Due to the evidence of its antibacterial and anti-cancerous properties, numerous

Table 1: List of microorganisms associated with heavy metal remediation.

Microorganism	Genus	Heavy metal removed	References
Bacteria	*Bacillus* sp.	Pb, Cd, Cr, Cu, As, Zn, Ni, Hg	Alotaibi *et al.* [161]
	Vibrio sp.	Cd, Pb, Hg	Parmar *et al.* [162]
	Pseudomonas sp.	Cd, Ni, Pb, Hg, Zn, Cu	Chellaiah [163]
	Geobacter sp.	Cr	He *et al.* [164]
	Rhodococcus sp.	Cu	Maslanová *et al.* [165]
	Alkaligenes sp.	Cd, Pb, Hg	Sodhi *et al.* [166]
	Sphingomonas sp.	Ni, Cu, Pb, Cd,	Heidari *et al.* [167]
	Enterobacter sp.	Ni, Cu, Pb,	Heidari *et al*. [167]
	Kocuria sp.	Cu	Kumari *et al.* [168]
	Sporosarcina sp.	Cr	Li *et al.* [169]
	Acinetobacter sp.	Ni	Wu *et al.* [170]
	Staphylococcus sp.	Cr, Cu, Pb	Kumar *et al.* [171]
	Streptomyces sp.	Cd, Pb	Mosbah and Sahmoune [172]
Fungi	*Aspergillus* sp.	Ni, Pb, Cr, Cd	Mishra and Malik [173]
	Rhizopus sp.	Cu	Gomes *et al.* [174]
	Trichoderma sp.	Cd	Herliana *et al.* [175]
	Saccharomyces sp.	Pb, Hg, Ni	Infante J *et al.* [176]
	Ganoderma sp.	Cd, Pb	Chang *et al.* [177]
	Fusarium sp.	Pb, Cd, Cu	Al-Hagar *et al.* [178]
	Candida sp.	Cr	Bahafid *et al.* [179]
	Penicillium sp.	Cd, Pb, Hg, As	Sánchez-Castellón *et al.* [180]
	Agaricus sp.	Cd, Pb	Long *et al.* [181]
	Ulva sp.	Pb, Cu, Cd, Zn, Ni	Areco *et al.* [182]
Algae	*Cladophora* sp.	Pb, Cu	Deng *et al.* [183]
	Spirullina sp.	Pb, Cu, Cd, Zn, Ni	Mane and Bhosle [184]
	Sargassam sp.	Pb, Cr, Cd	Tamilselvan *et al.* [185]
	Spirogyra sp.	Pb, Cu, Cd, Zn, Ni	Mane and Bhosle [184]
	Codium sp.	Pb, Cu, Cd, Zn, Ni	Laib and Leghouchi [186]
	Caulerpa sp.	Pb, Cr, Cd	Tamilselvan *et al*. [185]
	Chaetomorpha sp.	Cd	Kumar *et al.* [187]

Pb: Lead, Cd: Cadmium, Cr: Chromium, Cu: Copper, As: Arsenic, Zn: Zinc, Ni: Nickel, Hg: Mercury.

study findings have suggested that *B. subtilis* is safe for usage as a probiotic. In the food sector, *Bacillus* spp. are also employed to produce a number of different enzymes such as amylase, protease, cellulase, and pectinase, as well as a number of additional nutrients such as vitamins and carotenoids [108]. In addition to these applications, extensive research has been conducted on *Bacillus* spp. due to their active involvement in mitigating heavy metal pollution through diverse mechanisms, including bioaccumulation and biosorption [Table 2]. Their ability to alleviate HMs from contaminated environments has been a subject of intensive study and offers promising prospects for environmental remediation. This is because it has been suggested that Gram-positive bacteria frequently predominate in contaminated locations due to their flexible metabolic capabilities and superior biosorption abilities [109].

6.1. As Remediation

Arsenic is difficult to remove from the environment since it is soluble. Arsenic is remedied physically and chemically by an oxidation process that changes As^{3+} into As^{5+}. It can happen with air oxygen, which normally happens very slowly, or with chemical oxidants like ozone, chlorine, or hydrogen peroxide. This approach costs a lot of money and results in undesirable externalities [110]. The enzyme As oxidase, primarily located within the protoplasm of bacteria capable of oxidizing As, plays a significant role in As metabolism, converts the poisonous As^{3+} into its less toxic version, As^{5+}, as a result of microorganisms using Arsenic as a source of energy in their metabolic processes [111]. Numerous researches undertaken over the years have revealed that *Bacillus* spp. can absorb Arsenic, making it one of the most significant bacteria for minimizing As contamination [Liao *et al*., 2011]. Arsenic removal conducted by *Bacillus* spp. such as *B. cereus*, *Bacillus aryabhattai* [112], and *Bacillus megaterium* [113]. Anaerobic respiration of As^{5+} is also done by *B. cereus* [114]. Oxidative phosphorylation is essential for all living things, and this is unavoidable. Since As^{5+} and phosphate are structurally identical, it may act as an inhibitor of oxidative phosphorylation [115]. The pathways known as Pit and Pst, commonly utilized for phosphate uptake, serve as the means through which As^{5+} enters the biological system of organisms [116]. Adenosine triphosphate production is inhibited and phosphorylation metabolic pathways are hampered [117]. When it internalizes, it attaches to the respiratory enzymes right away using their sulfur residue [118]. In *Bacillus* species, the Ars operon uses genes such as *arsA, arsB, arsC, arsD,* and *arsR* to bioremediate As. *ArsA* and *ArsB* are ATPases, ArsC converts As^{3+} to As^{5+}, ArsD is a metallochaperone, and ArsR is a repressive inhibitor [119,120]. Normally, As^{3+} is carried out of the cell by ArsB after being converted from As^{5+} that enters the cell by ArsC [121].

Table 2: List of *Bacillus* sp. associated with heavy metal remediation.

S. No.	*Bacillus* species	Heavy metal removed
1.	*Bacillus cereus*, *Bacillus aryabhattai*, *Bacillus megaterium*	Arsenic
2.	*Bacillus altitudinis*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus jeotgali*, *Bacillus licheniformis*	Zinc
3.	*Bacillus thuringiensis*, *Bacillus megaterium*, *Bacillus subtilis*	Nickel
4.	*Bacillus licheniformis*, *Bacillus safensis*, *Bacillus catenulatus*	Cadmium
5.	*Bacillus cereus*	Lead
6.	*Bacillus thuringiensis*, *Bacillus sphaericus*, *Bacillus licheniformis*, *Bacillus subtilis*	Copper
7.	*Bacillus licheniformis*, *Bacillus cereus*	Chromium
8.	*Bacillus cereus*, *Bacillus thuringiensis*	Mercury

6.2. Zn Remediation

The capacity of *Bacillus* spp. effectively eliminate Zn from polluted environments has been emphasized in various bioremediation trials. *Bacillus* species can either evolve resistance mechanisms or acquire resistance using plasmids to control this ability [Christofi and Ivshina, 2002]. The Zur family controls Zn absorption in *B. subtilis*, allowing Zn ions to be transported using two transporter proteins. When there is oxidative stress, the Zn ion absorption pathway in *B. subtilis* termed ZosA (P-type ATPase) is expressed [122]. In the bacterium *B. subtilis*, a CPx-type ATPase extrusion pump called CadA plays a crucial role in facilitating the excretion of Zn from highly concentrated solutions. Furthermore, it has also been revealed in latest studies that certain *Bacillus* species, including *Bacillus altitudinis*, *B. subtilis*, *B. cereus, Bacillus jeotgali,* and *B. licheniformis* remove Zn from the environment [123-125].

6.3. Ni and Cd Remediation

Although there are various ways to remove Ni from solid matrices, the best ones are those that can do it before the Ni enters the environment. Throughout the years, various physicochemical techniques have been employed to eliminate Ni from aqueous media [126]. Regardless of the techniques previously employed, recent advancements have introduced more cost-effective and efficient methods for Ni removal. Among these methods, adsorption techniques, particularly utilizing biomass such as sugarcane, corn cobs, citrus peels, and barks, have gained prominence [127]. The more effective way to remove Ni ions from media that has been contaminated with Ni is through bioremediation employing gram-positive bacteria like *Bacillus* spp. Extensive studies [128,129] have shown the consumption and removal of nano particles from polluted sites like soils, sewage, and streams. It has also often been reported that *Bacillus thuringiensis* may absorb and eliminate Ni from polluted surroundings [130]. In a latest study, *B. megaterium* was obtained from soils that had been contaminated with Ni, and it was able to absorb more than 500 mg of Ni there, despite the presence of more than 3000 mg/L of Ni salt there earlier [131]. Bacterial resistance mechanisms, which ultimately make it easier for metal ions to enter and exit the cell, are necessary for the elimination of Ni by bacteria. It has long been known that members of the CDF family, which includes Ni, facilitate the efflux of several metal ions [132]. The cation diffusing transporter CzcD in *B. subtilis* is considered to prevent the cell from excessive $Ni^{2+,}$ Cu+, Zn2+, and Co2+ concentrations. Under favorable conditions, metal ions in association with citrate can be absorbed by *B. subtilis* through the metaldicitrate absorption system known as CitM, thereby potentially increasing their toxicity [133]. Since *B. licheniformis* eliminated more than 98% of Cd^{2+}, it is a highly efficient adsorbent for the effective removal of Cd^{2+} ions [134]. There are other reports that *Bacillus safensis* and *Bacillus catenulatus* are capable of eradicating Cd^{2+} [135,136].

6.4. Pb Remediation

There have been reports of Pb- resistant capability of *Bacillus* spp. in the past. *Bacillus* speciesexploit the pbr operon and active transport as possible defenses against the harmful effects of Pb [137,138]. It is immobilized by microorganisms through biosorption, adsorption, chelation, extrinsic sedimentation, complexation, and adsorption.

Several methods incorporate phosphate, carboxyl, carbonyl, sulfhydryl, and hydroxyl groups in bacterial cell walls, which give the cell wall a negative charge. When Pb binds to any of these, an insoluble material is produced. The capacity of organic macromolecules such as proteins, polypeptides, and polysaccharides to sequester Pb using electrostatic forces including covalent bonds, ionic bonds, and Van der Waal's forces make adsorption through the cell wall a viable alternative route [139]. By altering the DNA, proteins, and lipids and even substituting the necessary ions inside the enzymes, Pb interacts with bacterial metabolism, structure, and biological activities [140]. To protect themselves from Pb toxicity, microbes exhibit interfacial deposition, ostracization, volatilization, biomethylation, cellular binding, intrinsic sequestration, and increased siderophore synthesis. *Bacillus* spp. especially *B. cereus* use one or more of these techniques, similar to other Gram-positive bacteria, to remove Pb from polluted sites [141].

6.5. Cu Remediation

Similar to several other HMs, copper is considered to play a key role in the physiology of *B. subtilis*, although quantities over typical ranges can be hazardous to the cells. *B. cereus, B. thuringiensis, Bacillus sphaericus,* and *B. licheniformis* species are known to participate in the removal of Cu from environments when its concentrations exceed the permissible levels [142-145]. The transport of Cu in the cytosol of *B. licheniformis* is facilitated by CueR, a protein that is similar to CueR found in other bacterial species [146]. *B. subtilis* CueR controls the copZA operon, which encodes a P-type ATPase for Cu extrusion, a constituent of the vital category that exports metal from the cell, along with a Cu facilitator. CopB is in charge of Cu extrusion and decontamination in the latter, whereas CopZ is essential as a Cu chaperone in the former, transporting Cu to CopA, which helps to Cu absorption [147]. *B. subtilis* has shown that YcnJ is associated with the Cu absorption function of the copZA operon. According to their model, this protein works in collaboration with specific Cop proteins to facilitate the transport of Cu both within and outside of the cell [148].

6.6. Cr Remediation

The innate resistance of microbes in Cr-contaminated habitats allows them to avoid metal stress by metal absorption, efflux, or detoxifying by lowering metal ion immobilization [149]. The enzyme-mediated biotransformation of Cr^{6+} by bacteria out of its hazardous to non-hazardous state (Cr^{3+}) is thought to be one of the most effective and affordable ways to remove Cr from polluted site and wastewater. The enzymatic reduction of chromate in both Gram-positive and Gram-negative bacteria is regulated by an enzyme known as ChrR, also known as chromate reductase. This enzyme is predominantly found in Cr-resistant bacteria and is not commonly associated with plasmids [150]. The membrane-bound component and the cytosolic component, respectively, can mediate this reduction both aerobically and anaerobically. Furthermore, the reduction of chromate ions in *Bacillus* spp. is mediated by an aerobic mechanism, in which electrons are moved from the hexavalent to the trivalent state of Cr. An unstable intermediate is produced during this transition (Cr^{5+}), which is controlled by NADH/NADPH [151]. Numerous studies Nguema *et al.* [152] and Joshi *et al.* [153] indicated that *B. licheniformis* removed 95% and 69.4% of Cr. Over 81% of the Cr was eliminated by *B. cereus*. The effectiveness of other *Bacillus* species in reducing Cr contamination has also been reported [154].

6.7. Hg Remediation

According to several studies conducted over the years, the several species of *Bacillus* contribute to the bioremediation of Hg in a positive way. Hg has a hazardous impact on biological systems due to its high toxicity. Bacteria are armed with a variety of methods to protect their survival when exposed to high levels of harmful Hg. Hg resistance genes and operons, such as the Mer operon, have been identified in both gram-positive and gram-negative bacteria [155]. A limited range Mer operon and a broad range operon are the two different forms of Hg resistance genes found in bacteria. Inside the cell, Hg is forced to undergo reversible detoxification from its hazardous to non-hazardous form, yet diffusion aids in Hg's exit from the body. Once the metal ions have left the cell, different bacterial species may once more subject them to oxidation [156]. Numerous bacteria have been used to study the phenomena, where the activation of this phenomenon by the activator protein MerR results in the target identification of Hg. The mercuric reductase and lyase enzymes, which are inherited by the MerA and MerB genes, assist the elimination of inorganic Hg^{2+} and organic Hg [157]. In addition, the various other forms of Hg might regulate its transit and bioaccumulation, which might lead to Hg toxicity in environments and ecosystems close to Hg mines or emission sites [158]. Metallothioneins play a significant role in the bioremediation of Hg by accumulating Hg ions in an inactive state and facilitating their transformation through enzymatic processes. Gram-positive and Gram-negative bacteria both share the Mer operon. Although the pathway of Hg resistance in Gram-negative bacteria is better understood compared to Gram-positive bacteria, significant progress has been made in unraveling the mechanisms of Hg resistance in both bacterial groups [159]. Moreover, it was reported that soil-isolated *Bacillus* spp. possessed Hg-resistant genes, which aided in the removal and subsequent bioremediation of Hg [160]. To enhance the biosorption and exudation of Hg from contaminated sites, genetic modification was performed on *B. cereus* by introducing a mer operon derived from another *Bacillus* species, *B. thuringiensis*. This modification aimed to confer Hg resistance to *B. cereus* and improve its capacity for Hg removal [161].

7. CONCLUSIONS

The promising approach of bioremediation, which involves the use of living organisms to detoxify polluted areas, has shown great potential in remedying heavy metal contamination in the environment. Among different types of bacteria, the potential of *Bacillus* spp. in the bioremediation of HMs has been well-established and validated. *Bacillus* spp. has been shown to have the capability to remove various HMs, including Pb, Cu, and Cd, from contaminated soils, groundwater, and industrial effluents. *Bacillus* spp. has been studied extensively for its molecular mechanisms in heavy metal bioremediation, including metal resistance, efflux, haulage, and detoxification. These processes enable *Bacillus* spp. to maintain metal homeostasis even in environments with elevated concentrations of HMs. The *Bacillus* spp. can be used to bioremediate settings that have been contaminated with HMs, although there are still unexplained molecular resistance mechanisms that need to be investigated and characterized. The bioremediation process driven by bacteria is not only efficient but also practical from an economic and environmental standpoint. Sewage sludge, effluents, polluted groundwater, and soil can all be treated using this method. *Bacillus* spp. can considerably lower the levels of HMs and other environmental pollutants when used in bioremediation. In conclusion, using bacteria-driven bioremediation, in particular using *Bacillus* spp., is a promising and long-lasting alternative for the treatment of environmental heavy metal contamination. To create more efficient methods for bioremediation, additional study is required to completely comprehend the molecular mechanisms underlying heavy metal resistance and detoxification by *Bacillus* spp. *Bacillus* spp. play a significant role in bioremediation, which will become clearer with the identification and characterization of hitherto unknown molecular resistance mechanisms. This will also open the door to their prospective application in the effective removal of heavy metal pollutants.

8. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors requirements/guidelines.

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This study does not involve experiments on animals or human subjects.

12. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

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