A Seminar Paper

On

Potential of Endophytes for Environmental Heavy Metal Bioremediation

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Potential of Endophytes for Environmental Heavy Metal Bioremediation[1]

By

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ABSTRACT

Heavy metals create toxic effects on environment as they lead to the pollution of soil, ground water, sediments etc. Heavy metals are non-biodegradable in nature and possess a serious threat to human health and the ecosystem. That's why it has become a major concern nowadays. Bioremediation of heavy metals using endophytic microorganisms has emerged as a promising strategy due to their potential to enhance plant growth and alleviate heavy metal toxicity. The objective of this review is to assess the potential of endophytes in heavy metal bioremediation and involvement of endophytes in plant metal tolerance and hyperaccumulation. This paper reviewed some endophytic bacterial strains (*Enterobacter ludwigii* SAK5, *Exiguobacterium indicum* SA22, *Paenibacillus* sp. RM, *Bacillus* spp. L14 etc) and fungal strain (*Aspergillus* sp. A31, *Curvularia* geniculata P1, Lindgomycetaceae P87, and *Westerdykella* sp. P71) in heavy metal bioremediation. Endophytes have also been shown to enhance plant growth and reduce heavy metal toxicity by producing phytohormones, solubilizing nutrients, and inducing systemic resistance. The reviewed papers suggest that endophytic microorganisms have significant potential for the bioremediation of heavy metals in contaminated environments and that further research is needed to explore the efficacy of these microorganisms in real-world settings.

Keywords: Heavy metal, bioremediation, endophytic bacteria, endophytic fungi

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CHAPTER 1

INTRODUCTION

The term "heavy metal" refers to a group of metals with higher atomic numbers (above 20) and greater densities (5 g/cm^3) such as cadmium, lead, mercury, nickel, chromium, arsenic, copper, and zinc. These metals are linked to environmental pollution and biological toxicity issues due to their strong ability to hinder biodegradation processes (Mahendra *et al.,* 2014; Nagajyoti *et al.*, 2010). The introduction of heavy metals into the environment has toxic effects, as it leads to the pollution of various components such as soils, groundwater, sediments, natural water, and air. This contamination can cause harm to human health through various means of exposure (Bade *et al.*, 2013). The issue of soil and water being contaminated with heavy metals has become a significant concern on a global scale. The primary reasons for heavy metal pollution on a global level are rapid urbanization, industrialization, and intensive agriculture. Without intervention, heavy metals can remain in soil for centuries since they are non-degradable. The ecosystem can be negatively impacted by the long-term presence of heavy metals, which can harm not only agricultural products and water quality, but also soil microorganisms and human health (Kidd *et al.,* 2012). The bioconcentration and biomagnification of heavy metals, which can result in toxic levels within biological organisms, highlights the importance of removing heavy metal contamination from polluted soil and water (Govarthanan *et al.,* 2014).

Numerous techniques have been suggested to eliminate heavy metals from polluted soil and water, including methods based on physical, chemical, and biological processes (Shi et al., 2009). The majority of physiochemical techniques for remediation are expensive and can cause harm to the soil ecosystem (Hooda, 2007). Whereas, bioremediation was developed as a cost-efficient, environment-friendly, and sustainable option for hazardous waste treatment (Xiong *et al.*, 2022). Bioremediation is a method used to decontaminate a polluted environment by altering enzymes that are involved in the biosorption of various toxic metals (Okoduwa *et al.*, 2017). It employs microorganisms to detoxify heavy metal-contaminated substrates, which is both innovative and environmentally friendly. Microorganisms are particularly well-suited to this task as they have developed various detoxification mechanisms and can survive in harsh environmental conditions (Leitão, 2009; Narsi & Bishnoi, 2005; Ojuederie & Babalola, 2017; Sag & Kutsal, 2001). Using or inoculating endophytic microbial strains for the remediation of environmental substances has been done regularly for the management of various contaminants, such as heavy metal during the last few decades (Fe, Cd, Pb, Ni, Mn, Mg, As, and Cu) (Sharma & Kumar, 2021).

"Endophyte" is a term that describes an organism which inhabits the internal tissues of a plant host for a certain period of time. Endophytic microorganisms make up one of the largest and most diverse groups of living organisms, with an estimated one million species (Mishra *et al.*, 2019). After conducting research for over a decade, it has been discovered that huge number of plant species in natural ecosystems harbor various types of endophytic microorganisms. This finding has led to the realization that endophytic microbes represent one of the most important and yet untapped natural resources for bioprospecting of biosynthetic enzymes and secondary metabolites (Manganyi & Ateba, 2020). Endophytes possess the potential to be utilized for bioremediation purposes (Deng & Cao, 2017).Endophytes have the ability to promote the growth of plants even when they are exposed to toxic heavy metals. This is because endophytes can enhance the plant's capacity to take in, break down, and transport the harmful substances away from the plant, resulting in a positive effect on plant growth (Deng & Cao, 2017). The relationship between plant roots and microorganisms in the soil can enhance the uptake of metals in the plant's root system. This is because some types of soil microorganisms can form beneficial associations with the roots, which can increase their ability to absorb nutrients and metals from the soil. This interaction can be especially beneficial in soils where essential micronutrients are scarce, and some microorganisms can also help to prevent toxic levels of metals from accumulating in the plant tissues (Saravanan *et al.*, 2007). The in-depth study about the potential of endophytes in heavy metal bioremediation as well as involvement of endophytes in plant metal tolerance and hyperaccumulation has not been tapped yet.

Considering the facts present study was undertaken with the following objectives:

1. To evaluate the potential of endophytes in bioremediation of heavy metals.
2. To assess involvement of endophytes in plant metal tolerance and hyperaccumulation.

CHAPTER 2

MATERIALS AND METHODS

The purpose of this seminar paper is to provide a review, and all information presented here was obtained from secondary sources. The sources used included e-journals, reports, internet searches, and books available online. I was fortunate to receive sufficient guidance from my major professor and course instructors, which proved helpful in completing my seminar report. To acquire knowledge, I conducted searches on similar websites on the internet. The collected information was then compiled to create this seminar paper, which drew from a variety of publications, journals, and websites.

CHAPTER 3

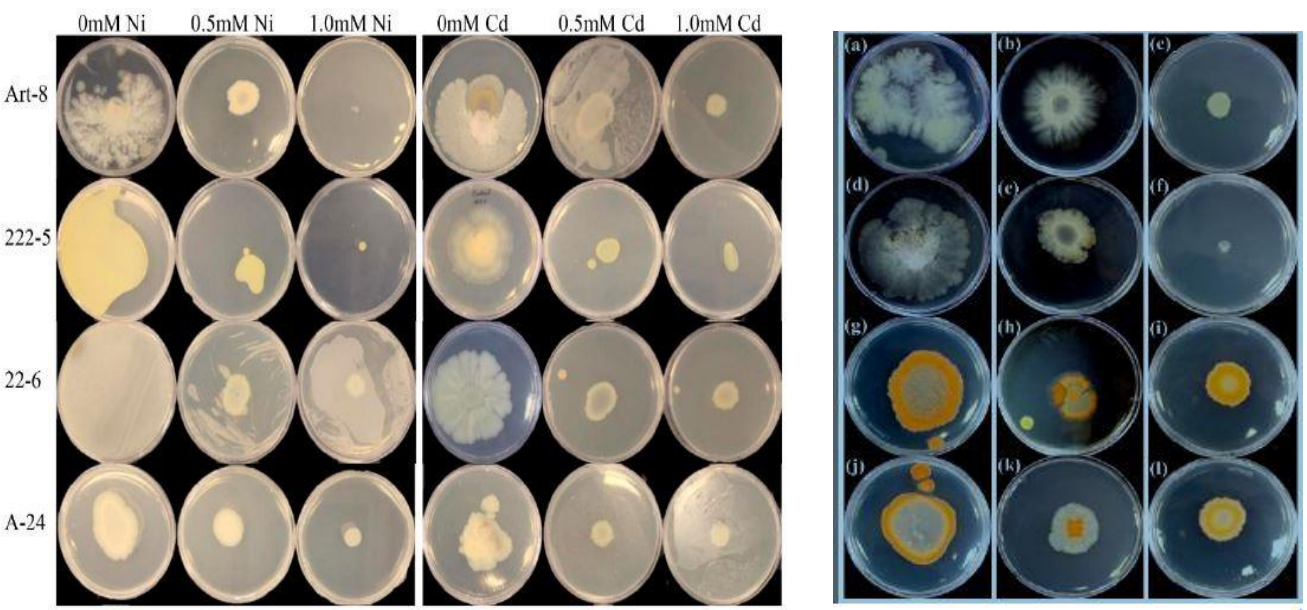
REVIEW OF FINDINGS

3.1.1 Endophytic bacteria

Endophytic bacteria are microorganisms that live within the tissues of plants, specifically below the outermost layer of cells. They are capable of colonizing internal plant tissues and developing various types of relationships with their hosts, including symbiosis, mutualism, commensalism, and trophobiosis (Schulz & Boyle, 2006). Usually, endophytic bacteria enter plant tissues mainly through the root system, but they can also use aerial parts of plants, such as flowers, stems, and cotyledons, as a means of entry (Kobayashi & Palumbo, 2000). It is possible that endophytic bacteria are more effectively shielded from both biotic and abiotic pressures than rhizospheric bacteria (Hallmann *et al.*, 1997). Plants and endophytic bacteria have co-evolved over a long period of time, resulting in a close-knit ecosystem that helps plants adapt and survive in challenging biotic and abiotic stress conditions such as pathogen infections, drought, salinity, and exposure to contaminants. This relationship also helps maintain a healthy ecological balance in natural systems (Ryan *et al.*, 2008). Endophytic bacteria have developed various mechanisms to counteract metal toxicity and overcome metal stress. These mechanisms involve expelling metal ions outside the cell, converting metal ions into less harmful forms, containing metals on the surface or inside the cell using polymers, and precipitation, adsorption, desorption, or biomethylation (Rajkumar *et al.*, 2013). Recent studies on hyperaccumulator plants have shown that introducing metal-resistant endophytic bacteria into the soil, seeds, or seedlings can boost plant growth and speed up the phytoremediation process in naturally or artificially metal-contaminated soil. This is achieved by improving nutrient uptake, promoting cell elongation, enhancing metal accumulation or stabilization, and reducing the negative effects of metal stress on plants (Babu *et al.*, 2013; Luo *et al.*, 2011; Zhu *et al.*, 2014). The use of endophytic bacteria in bioaugmentation can aid in phytoremediation by reducing the toxic effects of metals and altering the availability of heavy metals in contaminated soils. These bacteria possess various traits that promote plant growth, including resistance, detoxification, accumulation, transformation, and sequestration of metals. Therefore, they are an ideal choice for microbial-assisted phytoremediation studies (Ma, Prasad, *et al.*, 2011; Rajkumar *et al.*, 2009).

3.1.2 Potential of endophytic bacteria in heavy metal bioremediation

Numerous studies have found that certain endophytic bacteria, such as *Paenibacillus* sp., *Bacillus* sp., *Exiguobacterium* sp., *Alcaligenes* sp., *Pantoea* sp., *Brevibacillus* sp., and *Pseudomonas* sp., are very effective at accumulating heavy metals like Cd and Ni (Siripan *et al.*, 2018; Truyens *et al.*, 2014; Vullo *et al.*, 2008). In a study done by Jan *et al.* (2019) found that inoculating plants with *Enterobacter ludwigii* SAK5 and *Exiguobacterium indicum* SA22 could significantly improve growth parameters when exposed to Cd and Ni stress. Plant growth promoting endophytes (PGPE) not only help detoxify heavy metals but also boost the physiological and biochemical processes in plants, leading to increased production of stress-inhibiting hormones through the upregulation of certain genes. The same study showed that SAK5 and SA22 strains exhibited high levels of tolerance to both Cd and Ni and displayed typical growth patterns when grown on PDA media supplemented with these metals.



Source: (Jan *et al.*, 2019)

Figure 1. Growth pattern of selected endophytic isolates (*Enterobacter ludwigii* SAK5 and *Exiguobacterium indicum* SA22) in 0.5, 1.0, and 1.5 mM Cd-supplemented and Ni-supplemented media.

The A, B, and C plates represent the SAK5 strain grown with 0.5, 1.0, and 1.5 mM Cd, respectively, and the D, E, and F plates show the same strain grown with 0.5, 1.0, and 1.5 mM Ni, respectively. Similarly, the G, H, and I plates represent the SA22 strain grown with 0.5, 1.0, and 1.5 mM Cd, respectively, and the J, K, and L plates show the growth of the same strain with 0.5, 1.0, and 1.5 mM Ni, respectively (Figure 1). Both strains showed greater tolerance to Cd and Ni at a concentration of 0.5 mM compared to 1.0 mM. However, growth inhibition occurred at concentrations above 1.5 mM for both strains (Figure1).

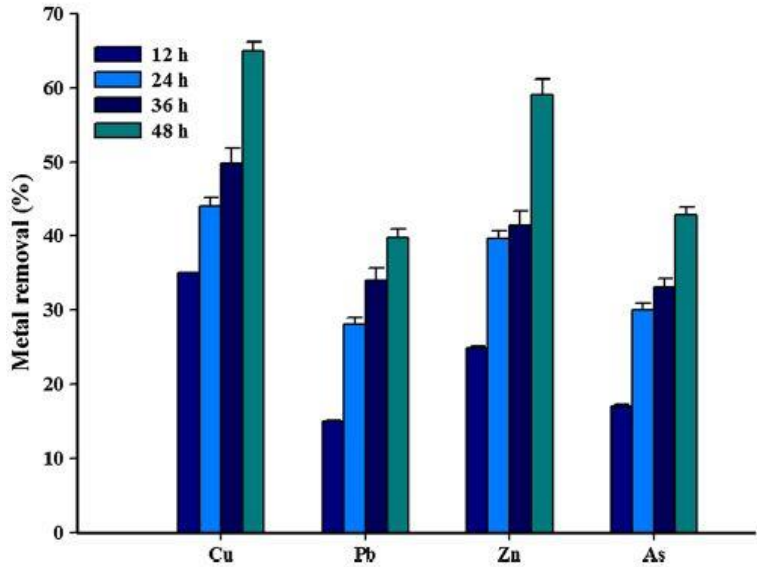
In another study, Govarthanan *et al.* (2016) found that, one of the bacterial strain *Paenibacillus* sp. RM were found inside the plant *Tridax procumbens* (Tridax daisy) and were resistant to several heavy metals including Cu, Zn, As, and Pb. Further experiments showed that *Paenibacillus* sp. RM had the ability to remove Cu and Zn from contaminated environments. Govarthanan *et al.* (2016) found that, on Luria–Bertani (LB) agar plates strain RM shows the highest minimal inhibitory concentration (MIC) against Cu which is about 750 mg/l compared to other metals like AS, Zn, Pb (Table 1).

Table 1. Multi-metal resistance pattern of the endophytic bacterial strain *Paenibacillus* sp. RM

Bacterial isolates	**As (mg/l)**	**Zn (mg/l)**	**Cu (mg/l)**	**Pb (mg/l)**
RM	400	500	750	450
RM1	200	350	150	100
RM2	200	250	200	100
RM3	150	200	350	300
RM4	200	150	200	250

Source: (Govarthanan *et al.*, 2016)

The metal removal potential of RM strain is dependent on pH and temperature. When they got optimum pH (7.0) and temperature (37^{0}c) this strain shows the highest metal removal rate (Govarthanan *et al.*, 2016). The highest percentage of Cu removal (65.0%) occurred after 48 hours of incubation, while the lowest percentage of Pb removal (15.1%) was observed after 12 hours. (Figure 2).



Source: (Govarthanan *et al.*, 2016)

Figure 2. Influence of optimum pH and temperature on Cu, Pb, Zn, and As removal by the isolate *Paenibacillus* sp. RM

In another study done by Guo *et al.* (2010) found that, a bacterial strain *Bacillus* spp. L14 have multi-metal resistant and according to subcellular fractionation studies, a large percentage of the Cd (II) and Pb (II) absorbed by the cells (around 80.8% and 76.5%, respectively) is located in the membrane fraction. The cytoplasmic area only contains 5.5% and 7.4% of Cd (II) and Pb (II), while the cell wall has 13.7% and 16.1%, respectively. These findings suggest that most of the removal and transformation of Cd (II) and Pb (II) ions occur in the membrane fraction. The results also suggest that bio-accumulation plays a significant role in the heavy metals removal process (Table 2).

Table 2. Distribution and uptake of cadmium and lead in endophytic bacterial strain *Bacillus* spp. L14 after 24 h of incubation

Fraction	**Cadmium conc. (μg)**	**Lead conc. (μg)**	**Percentage (Cd^{2+}) (%)**	**Percentage (Pb^{2+}) (%)**
Culture broth				
Whole cells	780 ± 24	825 ± 25	75.7 ± 2.3	80 ± 2.4
Culture media	250 ± 7	200 ± 8	24.3 ± 0.7	19.5 ± 0.8
Subcellular fraction				
Cell wall	107 ± 4	133 ± 4	13.7 ± 0.5	16.1 ± 0.5
Cytoplasmic	43 ± 1.3	61 ± 2	5.5 ± 0.2	7.4 ± 0.2
Membrane fraction	630 ± 25	631 ± 30	80.8 ± 3.2	76.5 ± 3.6

Source: (Guo *et al.*, 2010)

EB L14 was found to be effective in removing and transforming a majority of Cd (II) and Pb (II) ions *in vivo*, without causing significant secondary pollution during bioremediation (Guo *et al.*, 2010).

3.2 Endophytic Fungi

Fungus is one of an absolute candidates for tolerating and detoxifying metals through various mechanisms, such as transforming the valence of metals, precipitating them both inside and outside the cell, and actively taking them up (Ashida, 1965). Microorganisms have a great potential to serve as an alternative to synthetic resins for the remediation of dilute solutions of metals and solid wastes, due to their high surface to volume ratio and ability to detoxify metals. This is because microorganisms possess a larger surface area in comparison to their volume, which enhances their ability to interact with metals. Additionally, they have the capacity to break down and render harmless toxic substances such as heavy metals, making them a promising solution for environmental remediation (Kapoor *et al.,* 1999; Magyarosy *et al.,* 2002). Fungi have the biochemical and ecological capability to reduce the risks associated with metals, metalloids, and radionuclides by modifying their chemical properties or by influencing their bioavailability.

Moreover, their ability to form extended mycelial networks makes them well-suited for bioremediation processes.

The majority of fungi utilized for bioremediation purposes belong to either the rhizosphere or mycorrhizal categories. This includes both endophytic mycorrhizal fungi and ectomycorrhizal fungi (Dixit *et al.,* 2021; Hao *et al.,* 2021). In numerous cases, it has been demonstrated that mycorrhizal fungi can aid plants in dealing with the stress caused by heavy metals (Cornejo *et al.*, 2017). Endophytic fungi have effective mechanisms for capturing or binding heavy metals, which allows them to withstand high levels of these toxic substances. In addition, their ability to grow in large quantities makes them well-suited for use in bioremediation, the process of removing pollutants from the environment (Aly *et al.*, 2011). There have been limited reports on the influence of fungi, aside from arbuscular mycorrhizal fungi, on the ability of plants to extract metals from the soil (Pawlowska *et al.,* 2000). Mycorrhizal fungi take up nutrients that are immobile in the soil and transport them to the plants they are associated with. They also have the ability to capture and store heavy metal ions that could be harmful to the plants. In addition, these fungi can help facilitate the transfer of nutrients between different plants, and they can positively impact the water relations of the plants they are associated with (Fomina *et al.*, 2005).

3.2.1 Potential of endophytic fungi in heavy metal bioremediation

A study done by Pietro-Souza *et al.* (2020) revealed that, four types of endophytic fungi, namely *Aspergillus* sp. A31, *Curvularia geniculata* P1, Lindgomycetaceae P87, and *Westerdykella* sp. P71, have shown potential in bioremediating mercury in both laboratory and plant-based systems. These fungi were found to enhance the growth of their host plants, *Aeschynomene fluminensis* (jointvetches) and *Zea mays* (maize) regardless of whether mercury was present or not. Additionally, they helped in the bioremediation process by aiding in the accumulation and/or volatilization of the metal. Four endophytic fungal strains removed 86 to 100% of Hg^{2+} added to the culture media, indicating that they had high mercury bioremediation capacity *in vitro* (Table 3). At both growing periods, (i) *Aspergillus* sp. A31 and *C. geniculata* P1 bioremediated all the mercury from culture media supplemented with 30 mg/mL Hg^{2+}; and (ii) the increase in Hg^{2+} concentration diminished the bioremediation efficiency of *Aspergillus* sp. A31, *C. geniculata* P1, and Lindgomycetaceae P87 by nearly 5.15, 8.95, and 10.55%, respectively, but enhanced the bioremediation efficiency of *Westerdykella* sp. P71 by 5% (Table 3). Mycelial bioaccumulation of

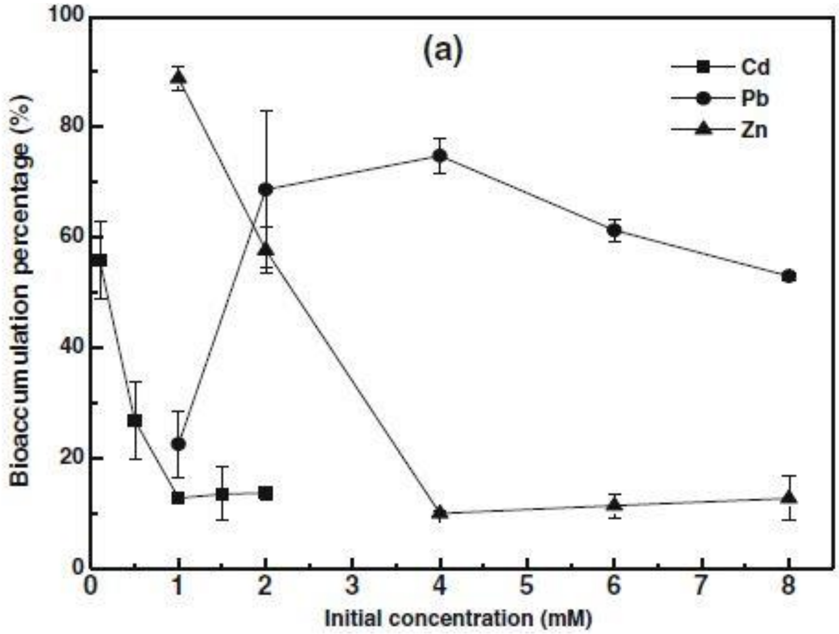
Hg^{2+} in all the fungal strains was proportional to the Hg^{2+} concentration added to culture media (Table 4). The growing period did not influence mycelial bioaccumulation of Hg^{2+} in *Aspergillus* sp. A31 and Lindgomycetaceae P87. In contrast, mycelial bioaccumulation of Hg^{2+}in *Westerdykella* sp. P71 treated with 30 and 90 mg/mL Hg^{2+} was respectively 56% and 80% greater at the seventh day of growth (Table 3).

Table 3. Endophytic fungal strains treated with different mercury concentrations for 7 and 14 days and the mercury bioremediation, bioaccumulation

Fungal strain	Parameter	Hg^{2+} (µg/mL)	Cultivation period	
			7	14
Aspergillus sp. A31	Bioremediation	30	100 ± 0.00	100 ± 0.00
		90	95.13 ± 0.78	94.64 ± 1.66
	Bioaccumulation	30	2026.79 ± 226.48	2367.30 ± 204.57
		90	14142.05 ± 751.50	16050.09 ± 1301.24
Curvularia geniculata P1	Bioremediation	30	100 ± 0.00	100 ± 0.00
		90	87.38 ± 0.22	92.48 ± 1.60
	Bioaccumulation	30	2653.91 ± 237.99	2213.20 ± 133.56
		90	10583.49 ± 1914.79	8607.69 ± 1169.16
Lindgomycetaceae P87	Bioremediation	30	93.71 ± 0.57	98.60 ± 1.46
		90	86.74 ± 0.37	88.29 ± 3.27
	Bioaccumulation	30	2522.41 ± 322.94	2779.84 ± 100.93
		90	8576.17 ± 567.48	11977.09 ± 2448.33
Westerdykella sp. P71	Bioremediation	30	85.70 ± 0.84	86.10 ± 2.32
		90	90.06 ± 0.70	90.29 ± 0.74
	Bioaccumulation	30	3460.98 ± 297.88	2209.14 ± 59.44
		90	13467.93 ± 1113.72	7472.69 ± 791.00

Source: (Pietro-Souza *et al.*, 2020)

The endophytic fungus, *Lasiodiplodia* sp. MXSF31 isolated from *Portulaca oleracea* (Common purslane) plant exhibited significant abilities in adsorbing and accumulating Cd, Pb, and Zn from solutions contaminated with metals. Additionally, it improved the efficacy of metal extraction from soil that contained multiple metals, thereby benefiting the growth of rape in such contaminated soils (Deng *et al.*, 2014). In the same study researchers found that, MXSF31 strain have the potential to of bioaccumulation of heavy metals.



Source: (Deng *et al.*, 2014)

Figure 3. Effects of initial concentrations of Cd, Pb, and Zn on the bioaccumulation percentages of *Lasiodiplodia* sp. MXSF31 isolate.

The percentage of Cd bioaccumulation was 56% when the initial concentration was 0.1 mM, whereas the percentage of Zn bioaccumulation was 89% when the initial concentration was 1 mM. As the initial concentrations of Cd (0.1 to 2 mM) and Zn (1 to 8 mM) increased, the bioaccumulation percentages of Cd and Zn by MXSF31 decreased. On the other hand, the bioaccumulation percentage of Pb increased from 1 to 4 mM, reaching a maximum of 75%, but decreased as the initial Pb concentrations increased up to 8 mM (Figure 3).

3.3 Association of endophytes with hyperaccumulator plants

The pollution caused by heavy metals can have negative impacts not only on the quality and yield of plants, but also on the size, composition, and activity of the microbial community associated with the plants (Giller *et al.*, 1998). But in the mysterious nature, some plants have evolved through by mitigating the stress effects inside out with the assist of microorganisms living inside them.

These are known as hyperaccumulator. Plants that grow in areas contaminated with heavy metals possessing a unique ability to accumulate high amounts of these metals without any adverse effects on their growth and development (Baker & Brooks, 1989; Freitas *et al.,* 2004) are known as hyperaccumulating plants such as *Thlaspi*, *Urtica*, *Chenopodium*, *Polygonum sachalase*, and *Alyssum.*

For plants especially for hyperaccumulators, microbes show interesting phenomenon found on roots and in the rhizosphere by taking advantage of the nutrients released by the roots, by becoming endophytes that don't harm the plant forming a mutually beneficial relationship with it and even by showing external metal-resistance (Compant *et al.,* 2005; Hallmann *et al.,* 1997).

It is a commonly recognized fact that bacteria that are found in polluted environments have a greater tolerance for high concentrations of metals compared to those found in unpolluted areas. Moreover, when metals are introduced, the tolerance of bacterial communities can increase due to the death of susceptible species, leading to competition and adaptation among the surviving bacteria (Diaz-Ravina & Baath, 1996). In nature, many examples are available of such plant's microbes, heavy metal interactions. The following table reflects some of those:

Table 4. Overview of hyperaccumulator plants and their associated endophytic bacteria

Hyperaccumulator	Plant portion	Endophytes	References
Thlaspi goesingense	Stem	*Alphaproteobacteria, Holophaga Acidobacterium, Betaproteobacteria, Gammaproteobacteria, Bacillus sp.*	(Idris *et al.,* 2004)
Alyssum bertolonii	Leaves	*Staphylococcus, Microbacterium,* and *Pseudomonas*	(Barzanti *et al.,* 2007)
	Stem	*Staphylococcus, Curtobacterium, Microbacterium,* and *Curtobacterium*	
	Root	*Staphylococcus, Bacillus, Arthrobacter, Pseudomonas, Curtobacterium, Microbacterium, Paenibacillus*	

Hyperaccumulator	Plant Portion	Endophytes	References
Brassica napus	Root	*Pseudomonas fluorescens,* and *Microbacterium sp*.	(Sheng *et al.*, 2008)
Thlaspi caerulescens	Stem	*Sphingomonas sp., Methylobacterium sp.,* and *Sphingobacterium multivorum*	(Lodewyckx *et al.*, 2002)
	Root	*Phyllobacterium sp., Devosia sp., Afibia sp., Sphingomonas sp.,* and *Rhodococcus sp.*	
Nicotiana tabacum	Seed	*Enterobacter sp., Xanthomonadaceae, Pseudomonas sp., Pseudomonas fulva, Clostridium*	(Mastretta *et al.*, 2009)

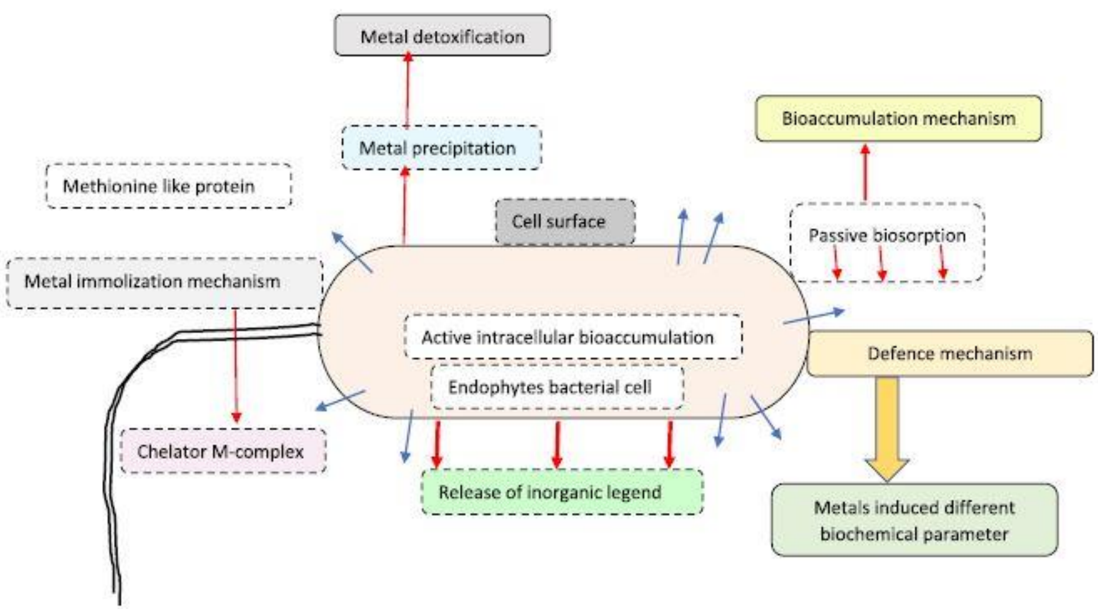
Hyperaccumulating plants have the ability to amass significant quantities of heavy metals. Consequently, they create a distinct environment that can support bacterial endophytes capable of thriving in conditions with elevated metal concentrations (Idris *et al.*, 2004). The relationship between endophytes and hyperaccumulator plants has recently become a subject of interest among many researchers, as it has potential biotechnological applications in bioremediation and for studying the bacterial communities present in naturally contaminated environments (Idris *et al.*, 2004; Lodewyckx *et al.*, 2002). As hyperaccumulating plants accumulate huge amount of heavy metals therefore they create a specific environment for the endophytic bacteria to adapt with that heavy metals concentration's environment (Idris *et al.*, 2004).

3.4 Endophytes mediated heavy metal accumulation by plants

Hyperaccumulating plants may store heavy metals in their shoots at considerably higher concentrations than the soil around them or other non-accumulating plant species can. Moreover, these plants offer a special habitat for bacterial endophytes, which may adapt to thrive in environments with high metal concentrations. Less studies have been done on the involvement of endophytes in plant metal tolerance and hyperaccumulation, despite the fact that there has been a lot of study on the physiological mechanisms behind metal transport, storage, and tolerance in hyperaccumulators. It has been discovered that metal-resistant endophytes contain a number of characteristics that can change the toxicity and availability of heavy metals to the plant, such as acidification, the production of iron chelators, siderophores, organic acids, and the mobilization of metal phosphates. Additionally, these endophytes have the potential to promote plant growth (Saravanan *et al.*, 2007; Sheng *et al.*, 2008). In a study conducted by Saravanan *et al.* (2007), they investigated the ability of *Gluconacetobacter diazotrophicus* to solubilize zinc under laboratory conditions using various zinc compounds. The researchers found that *Gluconacetobacter diazotrophicus* was able to produce a significant amount of 5-ketogluconic acid, which is a derivative of gluconic acid that aids in the solubilization of zinc compounds. A study by Sheng *et al.* (2008) found that inoculating *Brassica napus* (canola/rapeseed) with Pb-resistant endophytic bacteria resulted in increased uptake of lead into the plant's shoots. The researchers observed an increase in Pb uptake from 76% to 131% with *Pseudomonas fluorescens* and from 59% to 80% with *Microbacterium* sp. compared to a control group that was inoculated with dead bacteria. This suggests that the bacteria facilitated the release of Pb from non-soluble phases in the soil, making it more available to *B. napus*. One possible explanation for this is that the bacteria produced siderophores or solubilized Pb. Mastretta *et al.* (2009) reported similar results to those of Sheng *et al.* (2008). They found that inoculating *Nicotiana tabacum* (Common tobacco) with a Cd-resistant endophyte called *Sanguibacter* sp. increased the concentration of Cd in shoot tissues by approximately 3 folds compared to a control group that was not inoculated. These studies shows that endophytes improve the potential of hyperaccumulator plants in heavy metal accumulation. Although other things can be occurred in the plants in the presence of endophytes. Endophytes can decrease the metal accumulation and increase the biomass of the plants in metal stress condition. Madhaiyan *et al.* (2007) reported that inoculating tomato plants with *Magnaporthe oryzae* and *Burkholderia sp.* isolated from rice tissues led to a reduction in the uptake of Ni and Cd in both the roots and shoots of the plants, as well as a decrease in their availability in the soil. This reduced accumulation of metals may be due to the immobilization of metals by bacteria in the rhizosphere. Lodewyckx *et al.* (2001) aimed to enhance the phytoremediation of heavy metals by introducing genes for nickel tolerance (nickel–cadmium–cobalt resistance) from *Ralstonia metallidurans* 31A into two endophytic strains, *Burkholderia cepacia* and *Herbaspirillum seropedicae.*

3.5 Bioremediation by endophytes

Microbes have various ways to survive and interact with inorganic metals. These include biotransformation, extrusion, enzyme utilization, the production of exopolysaccharides (EPS), and the synthesis of metallothionein, which are mechanisms used by microorganisms to withstand metal toxicity (Sharma, Tripathi, Chaturvedi, *et al.,* 2021; Sharma, Tripathi, Vadakedath, *et al.*, 2021). In response to various compounds, microbes have developed innate resistance to metals and systems that help detoxify them (Dixit *et al.*, 2015; Yang *et al.*, 2015). Microbes can eliminate accumulated toxins through chemical precipitation and volatilization in their surroundings. Their cell surfaces have anionic structures that facilitate the attachment of microbes to metal cations, resulting in a negative charge on their surface (Sharma & Kumar, 2021). Endophytes have some mechanism to remediate heavy metals. Metal immobilization, metal detoxification, bioaccumulation, and defense mechanism these are some mechanisms that an endophyte use to remediate heavy metals (Figure 4).



Source:(Sharma & Kumar, 2021)

Figure 4. Schematic illustration of interaction between endophytic bacteria and heavy metals.

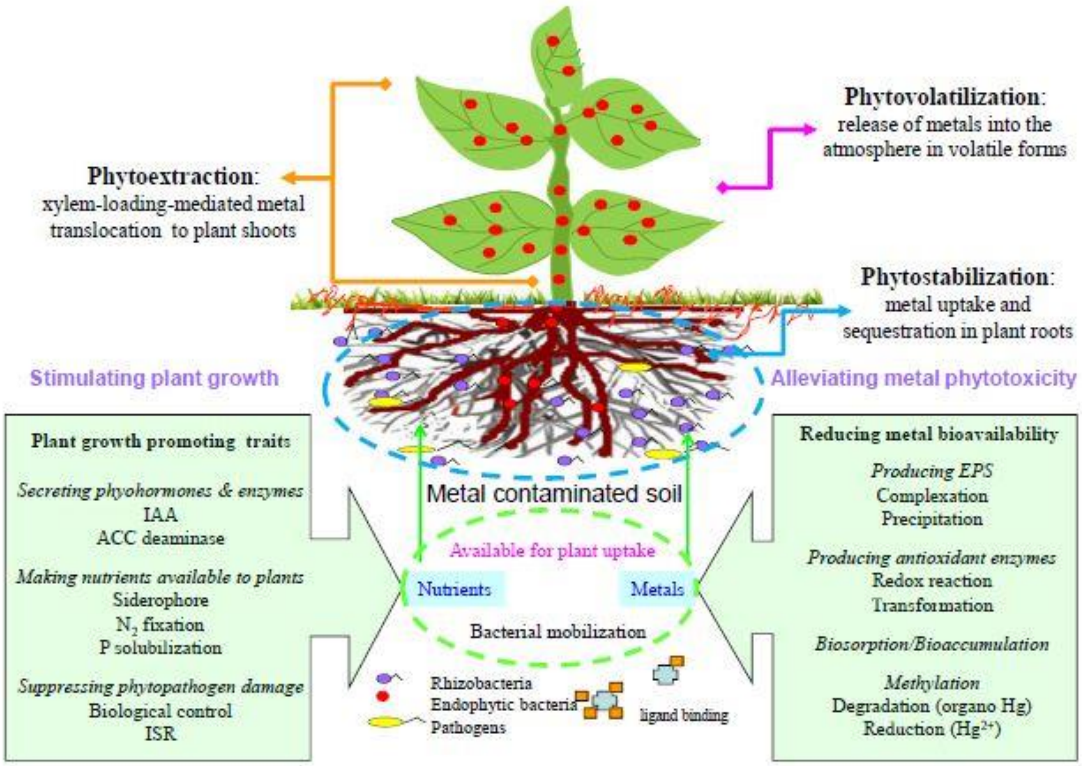
In case of metal immobilization endophyte create chelator metal complex. For metal detoxification mechanism endophytes precipitate the metals. In bioaccumulation mechanism there are 2 ways, one is passive biosorption (ion bounds on the surface) and other one is intracellular bioaccumulation (intracellular accumulation of ions) (Figure 4).

3.6 Increase of plant growth and phytoremediation process on heavy metals contaminated soil by endophytes

Numerous studies have provided evidence that endophytic bacteria possess the natural capability to aid host plants in adapting to adverse soil conditions and to improve the effectiveness of phytoremediation through various means such as promoting plant growth, reducing metal phytotoxicity, alleviating metal stress, modifying metal bioavailability in soil, and regulating metal translocation in plant (Ma, Rajkumar, *et al.,* 2011). In general, bacterial endophytes contribute to the phytoremediation process in soils contaminated with heavy metals in two different ways: firstly, by boosting plant tolerance to metal toxicity and promoting their growth; and secondly, by modifying the way in which metals are accumulated in plants.

Similar to rhizobacteria, Plant Growth Promoting Endophytes (PGPE) employ multiple mechanisms to directly support the growth and proliferation of their host plants. These mechanisms include nitrogen fixation, mineral solubilization, production of phytohormones, specific enzymes, and siderophores. Some PGPE are capable of reducing metal toxicity and improving plant development by utilizing one or more of these mechanisms (Pereira & Castro, 2014; Rajkumar *et al.,* 2009).

Endophytic bacteria possessing robust associative nitrogen-fixing capabilities aid plants in thriving in nitrogen-deficient soil environments and have a significant impact on enhancing plant health and growth when compared to microorganisms in the rhizosphere (Hurek & Reinhold-Hurek, 2003)



Source: (Ma *et al.*, 2016)

Figure 5. Plant growth promoting mechanisms of endophytic bacteria in metal contaminated soils.

In situations where plants are subjected to heavy metal stress, certain endophytic bacteria that are resistant to these metals are capable of either solubilizing phosphates that have precipitated in the soil through processes such as acidification, chelation, ion exchange, and organic acid release; or breaking down organic phosphorus in the soil through secretion of extracellular acid phosphatase, thereby increasing the availability of phosphorus to the plants (Nautiyal *et al.*, 2000; Van Der Heijden *et al.*, 2008).

Higher plants acquire iron through two primary strategies. The first strategy involves the use of microbial siderophores, wherein plants are able to uptake iron from Fe-siderophore complexes through root-mediated chelate degradation as demonstrated by Rajkumar *et al.* (2009). The second strategy involves solubilization of iron that is otherwise unavailable to the plant through the release of Phyto siderophores. In a study conducted by Barzanti *et al.* (2007), it was found that 83% of total endophytic bacteria were capable of producing siderophores, and the production of these siderophores was stimulated by the presence of heavy metals such as cobalt, chromium, copper, nickel, and zinc. These siderophores were also able to reduce metal toxicity in the host plant *Alyssum bertolonii* by increasing iron acquisition. Recent research examining the impact of phytohormones in shielding plants from metal stress has shown that the colonization of endophytes often leads to an increase in nutrient uptake and plant biomass (Gravel *et al.*, 2007; Phetcharat & Duangpaeng, 2012; Y. Shi *et al.*, 2009). These findings suggest that the ability of endophytic bacteria to alleviate heavy metal stress is likely due to a combination of nutritional and biochemical advantages. The following table (Table 5) shows that endophytes stimulate the plant growth and therefore alleviate the metal stress.

Table 5. Effects of endophytic bacteria on the plant growth and the uptake of metals by plants

Endophytic bacteria	Source of bacteria	Beneficial features of endophytes	Metal	Role of endophytes	Reference
Sanguibacter sp., *Pseudomonas* sp. and the consortia of various Cd resistant endophytes	Seeds of *Nicotiana tabacum*	No data found	Cd	Stimulated plant growth and facilitated plant Cd, Fe and Zn uptake	(Mastretta *et al.*, 2009)
Pseudomonas fluorescens, and *Microbacterium* sp.	Root of *Brassica napus*	ACCD activity, siderophore, IAA production, P solubilization and Pb mobilization	Pb	Stimulated plant growth and facilitated plant Pb accumulation	(Sheng *et al.*, 2008)
Methylobacterium oryzae strain CBMB20 and *Burkholderia* sp.	Tissues of *Oryza sativa*	ACCD activity, phytohormone production and bioaccumulation of Ni and Cd	Ni,Cd	Stimulated plant growth and decreased plant Ni and Cd accumulation	(Madhaiyan *et al.*, 2007)
Bulkholderia capacia with nickel resistance system	Tissues of *Lupinus luteus*	Bioaccumulation of Ni	Ni	Ni accumulation in root	(Lodewyckx *et al.*, 2001)

CHAPTER 4

CONCLUSION

Endophytes not only have the capability to remediate heavy metals from the environment but also, they have the ability to stimulate the growth of plants in metal stress condition when make association with the plants.

The first objective of evaluating the potential of endophytes in heavy metal bioremediation is supported by numerous studies that have demonstrated the ability of endophytes to remove heavy metals from contaminated environments. Endophytes can sequester heavy metals and convert them into less toxic forms, making them a promising tool for bioremediation.

The second objective of assessing the involvement of endophytes in plant metal tolerance and hyperaccumulation is also well-supported by research. Endophytes can enhance plant growth and survival in heavy metal-contaminated soils by promoting nutrient uptake and detoxification. Furthermore, some endophytes have been shown to contribute to the hyperaccumulation of metals in plants, potentially allowing for the extraction of metals from contaminated soils.

Overall, the review paper concludes that endophytes have significant potential for heavy metal bioremediation and can play a vital role in improving the environmental and human health impacts of heavy metal pollution. Further research is needed to fully understand the mechanisms of endophyte-mediated bioremediation and their potential for use in large-scale applications.

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