



Role of *Paenibacillus polymyxa* in Heavy Metal Detoxification and Soil Restoration

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Abstract

Background: Metal contamination of industrial and agricultural soils represents a serious, long-term environmental concern. It can affect ecosystem health, food safety, as well as the health of humans. Conventional methods of remediation are costly and can be detrimental to the environment. The soil biological function will not be returned by those methods.

Objective: This review seeks to assess the contributions of *Paenibacillus polymyxa* and *Pseudomonas* spp. in the bioremediation of heavy metal-contaminated soils and their viability for sustainable environmental restoration.

Methods: Data from laboratory, greenhouse, and field studies were scrutinized to elucidate the mechanisms utilized by *P. polymyxa* in heavy metal detoxification. These encompass biosorption through exopolysaccharides, enzymatic transformation, siderophore-mediated chelation, intracellular sequestration, and biofilm development. The synergistic role of *Pseudomonas* spp. in phytoremediation employing native plants and microbial consortia was also evaluated.

Results: *P. polymyxa* was very good at getting rid of heavy metals, especially lead, and it got rid of more than 90% of them when conditions were just right. It also made the soil healthier by adding more organic carbon, microbial biomass, nutrients, and stability to aggregates. Additionally, its characteristics that help plants grow, like fixing nitrogen, making phosphates more soluble, making phytohormones, and fighting off pathogens, improve the results of phytoremediation. *Pseudomonas* spp. also helped by improving interactions between plants and microbes and helping break down contaminants.

Conclusion: *P. polymyxa* and *Pseudomonas* spp. exhibit significant potential as principal agents in integrated bioremediation strategies. When used together in synthetic consortia, they can greatly improve soil restoration, promote sustainable farming, and help fight climate change.

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1. Soil Heavy Metal Pollution: Context, Pathways, and Ecological Consequences

1.1. Sources and Pathways of Heavy Metal Contamination

Terrestrial ecosystems have been heavily affected by pollution from different forms of metal over the course of two hundred years due to increased industrialization and human-made activities being brought together in one specific area (Ali *et al.*, 2019) ^[1]. Mining and smelting produce both particulate and ionic cadmium (Cd), lead (Pb), arsenic (As), and zinc (Zn), which are subsequently deposited on soil through atmospheric deposition, leachate infiltration, and improper disposal of tailings (Wuana and Okieimen, 2011) ^[2]. Agricultural practices, such as phosphate fertiliser application containing trace levels of Cd and As,

using sewage sludge as fertiliser, and using contaminated water for irrigation, also add significantly to estimated metal concentrations in terrestrial ecosystems (Bolan *et al.*, 2014) ^[3].

The following manufacturing industries — electroplating, tanning, textile dyeing, battery manufacturing — release significant quantities of chromium (Cr), nickel (Ni), mercury (Hg) and copper (Cu) into soils by way of direct runoff and human activities contaminating local groundwater supplies (Järup, 2003) ^[4]. Geogenic sources (natural weathering of parent materials that contain metal) add to naturally occurring background levels of metals that might be toxic, whilst at the same time, human activities have increased total concentrations of metals in soil in industrialised regions by orders of magnitude over background levels (Adriano, 2001) ^[5].

There are many different physicochemical parameters that interact with each other to determine heavy metal mobility through soil profiles. Of all the determinants, soil pH is by far the most important factor affecting both metal speciation and bioavailability. At acidic pH's (pH < 5.5), the four cationic metals of lead (Pb²⁺), cadmium (Cd²⁺), copper (Cu²⁺), and zinc (Zn²⁺) are all more soluble in water than they are at neutral (pH 7.0) or alkaline (pH > 8) soil pH's. At pH levels above neutral, the cationic metals become increasingly insoluble (due primarily to precipitation as hydroxides, carbonates, and phosphate complexes) (Adriano, 2001) ^[5]. Additionally, redox potential influences the speciation of both metalloids (e.g., arsenic) and redox-active metals (i.e., manganese, chromium, iron). Anoxic conditions promote the reduction of hexavalent chromium (Cr⁶⁺) to trivalent chromium (Cr³⁺), which is less toxic than Cr⁶⁺. Conversely, under oxidising conditions, arsenic is mobilised from solid surfaces of iron oxide (Ali *et al.*, 2019) ^[1]. Another physicochemical factor, soil organic matter, interacts with metal ions via complexation with humic or fulvic acids. This complexation limits the availability of free ionic metals to biota, yet it also creates a reserve of organically-bound metals that are subject to transformation into mineral forms (Bolan *et al.*, 2014) ^[3].

1.2. Ecological and Human Health Implications

The accumulation of heavy metals in soils has ecological consequences which can be far-reaching and usually cannot be reversed over a human time frame. The metabolic activities of soil microbial communities—representing the biological engine of nutrient cycling and decomposition of organic matter—can be acutely sensitive to metal toxicity (Giller *et al.*, 2009) ^[6]. Increased concentrations of Cd, Pb and Hg suppress enzymatic activity (including dehydrogenase, urease and phosphatase), decrease the amount of microbial biomass carbon and result in a shift in microbial community composition towards metal tolerant, functionally narrower assemblages. The combined effects of these reactivities impair both soil respiration and nutrient mineralization (Giller *et al.*, 2009) ^[6]. Earthworms and other macrofauna accumulate metals through both diet and dermal absorption and impair soil bioturbation processes that are critical to aggregate formation and water infiltration (Järup, 2003) ^[4]. On a plant level, excess metal ions (including Cd, Pb and Hg) compete with essential macronutrients (including, Ca²⁺, Mg²⁺ and Fe³⁺) for uptake by membrane transporters, produce reactive oxygen species (ROS) resulting in oxidative damage to DNA, inhibit photosynthesis

and root elongation, and ultimately result in reduced crop yields in contaminated agricultural fields (Sharma and Dubey, 2005) ^[7].

Heavy metals such as lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) may pose serious risk for human health because of how they can be ingested from contaminated food. It's widely accepted that consuming cereal grains, vegetables, or other edible crops (often referred to as "leafy crops"), which are grown in soil that has been contaminated with heavy metals, is the primary route of exposure internationally. For some populations, particularly those that reside in Asia, rice is the primary source of inorganic arsenic and cadmium (Meharg and Zhao, 2012) ^[8]. Children exposed to lead experience irreversible neurodevelopmental delays; cadmium causes kidney damage and has been categorized by the International Agency for Research on Cancer (IARC) as a Group 1 human carcinogen; chronic low-level exposure to arsenic has been associated with an increased risk of skin, lung, and bladder cancers; and mercury that is produced by the anaerobic methylation of mercury that settles in anaerobic environments (soils and sediments) is neurotoxic (in the form of methylmercury) and bioaccumulates through the food chain (Wuana and Okieimen, 2011) ^[2] (Meharg and Zhao, 2012) ^[8]. It is estimated that the global burden of disease associated with heavy metals is hundreds of millions of people annually, highlighting the need for reliable and sustainable endpoints for soil remediation technologies.

1.3. Limitations of Conventional Remediation Techniques

The traditional arsenal of soil remediation technologies -- including soil washing, stabilisation/solidification, electrokinetic remediation, thermal treatment and immobilising with amendments such as lime and phosphate - - all have serious operational, economic and ecological limitations that restrict their broad application to a few specific cases. Soil washing is highly effective at remediation of soils with high levels of contamination using chelating agents (EDTA, citric acid) to remove metals into an aqueous solution, but leaves large amounts of metal-laden waste (effluent) that need to be treated again before they can be discharged into the environment. Further, soil washing can disrupt the soil structure and biological activity within the soil and is only economically feasible for remediation of soils on small (relatively) high-value sites. The use of thermal treatment technology (vitrification, incineration) to remediate contaminated soils causes the complete destruction of the biological component of the soil (organics and microbial communities) and as a result creates treated soils that are biologically inactive and unsuitable for agricultural production. While electrokinetic remediation technology offers promise for use with low-permeability soils, it requires a substantial amount of energy input from electricity and is only capable of treating the water-soluble fraction of metals in soil. Electrokinetic remediation performs poorly on metals that are bound strongly to organic materials or clay minerals. Through the use of lime or phosphate amendments, the bioavailability of metals in the soil can be reduced; however, these types of methods do not remove metals from the soil matrix. Additionally, the movement of metals in soils that have been treated with lime or phosphate is possible under conditions associated with acidic rain events or by changes in the redox condition of the soil, and the long-term effectiveness of this type of treatment has yet to be established. Biological strategies (i.e., metal-tolerant, plant-

growth-promoting soil bacteria) have many benefits over traditional methods of remediation of heavy metals (i.e., cost-effectiveness, ecological compatibility, in-situ application and restoration of soil biological function) because they do not require soil amendments but rather use naturally occurring species to remediate contaminated soil (Rajkumar *et al.*, 2012) ^[10].

2. Functional Biology of *Paenibacillus polymyxa*

2.1. Taxonomy, Morphology, and Ecological Distribution

Paenibacillus polymyxa, formerly *Bacillus polymyxa*, is a Gram+ motile aerobic/facultatively anaerobic endospore forming bacterium belonging to the family Paenibacillaceae in the phylum Firmicutes (Grady *et al.*, 2016) ^[11]. The genus *Paenibacillus* was defined by Ash *et al.* (1994) based on phylogenetic reclassification as determined through 16S rRNA gene sequence analysis that separated the genus from the diverse and heterogeneous group of *Bacillus* (Grady *et al.*, 2016) ^[11]. The cells of *P. polymyxa* are rod-shaped (2-7 microns long, 0.6-0.9 microns in diameter), occur singularly or in chains, and produce an endospore located between the end of the mother cell and the last cell wall (subterminal) to the end of the mother cell and the last cell wall (terminal). Endospores can withstand extreme temperatures, desiccation, ultraviolet (UV) light, and chemical stress, therefore, characteristic advantages for formulation of bioinoculums and application of these products in the field (He *et al.*, 2016) ^[12].

P. polymyxa occurs throughout an extensive ecological range. This species is the most frequently isolated bacterium from agricultural soils, composting materials, forest floor litter layers, rhizosphere habitats, and freshwater sediments categorized by many different climatic conditions (Moreno-Jiménez *et al.*, 2009) ^[13]. Several different crops have been identified (and recovered) to contain this species of bacteria within the rhizosphere (soil adjoining their roots), such as wheat, maize, soybean & potato and/or sugarcane, as well as throughout certain [facultatively endophytic] strains within their roots and shoots (the endosphere). It is found most abundantly in soils with relatively high levels of organic matter and moderate moisture, indicating that it relies metabolically on complex carbohydrates and on availability of oxygen (Grady *et al.*, 2016) ^[11]. Strains of *P. polymyxa* have been found isolated within chronically metal contaminated mining soils and/or metal contaminated industrial wastelands, providing direct evidence of evolved tolerance strategies under continued selective pressures (Moreno-Jiménez *et al.*, 2009) ^[13].

2.2. Metabolic Flexibility and Stress Tolerance

P. polymyxa is characterized by its remarkable metabolic plasticity, allowing for the use of multiple carbon sources (hexoses, pentoses, disaccharides, polysaccharides, aromatic compounds), both aerobic and fermentative metabolism producing 2,3-butanediol and acetoin as major products, and the ability to fix dinitrogen gas from the atmosphere under micro-aerobic conditions ENDNOTES: (Seldin, 2010) ^[15] - All of these metabolic capabilities allow an organism to persist and function in a broad spectrum of soil physical-chemical properties, including water-logged oxygen deficient zones of rhizospheres where other competing PGPB are unable to survive (Seldin, 2010) ^[15] - *P. polymyxa* has exceptional tolerance to osmotic pressure, acidic pH (grow at pH 4.5), temperature extremes (growing from 5oC to 50oC

with optimum 28 - 37oC), and to higher levels of several heavy metals (mercury, zinc, cadmium, lead and manganese) at the same time through both constitutive and inducible resistance mechanisms (Raza *et al.*, 2017) ^[14].

The ability to withstand oxidative stress is critical for organisms living in environments contaminated with metals, where Fenton chemistry can produce hydroxyl radicals by the interaction of Fe²⁺ with Cd²⁺ ENDNOTES: (Von der Weid *et al.*, 2005) ^[16] - The enzymes catalase, Mn superoxide dismutase and Fe superoxide dismutase, and glutathione reductase enable organisms to survive these environments until they can germinate under favourable conditions due to their spore forming ability, as well as their ability to take advantage of the germination of dormant spores to produce commercial bioinoculants. (Grady *et al.*, 2016) ^[11].

2.3. Production of Bioactive Metabolites

Among soil bacteria, *P. polymyxa* is one of the most prolific bioactive metabolite producers. Its bioactive metabolites serve as the basis of its bioremediation capability and the ecological interactions it shares with other organisms (Meng *et al.*, 2016) ^[17]. *P. polymyxa* produces three classes of exopolysaccharides (EPS): levan (a fructan polymer), pea-starch-type heteropolysaccharides, and acidic polysaccharides containing both carboxylate and phosphate moieties. EPS represent the primary site where metal ions will coordinate in the extracellular matrix, and therefore, they act as the primary source of the metal-binding sites of the biopolymers produced by this bacterium. In addition to producing EPS, *P. polymyxa* produces several different types of siderophores (small molecules) to solubilise and transport Fe³⁺ under Fe-limiting conditions, particularly fusarinine-type hydroxamates. All of these siderophores have cross-reactivity with other metals such as divalent (e.g., Pb²⁺, Cr³⁺) and trivalent (e.g., Al³⁺) metals, indicating that *P. polymyxa* has the ability to coordinate metal ions and form chelate complexes with them, resulting in a high affinity coefficient for metal ions in solution (Beneduzi *et al.*, 2012) ^[18].

Polymyxins (cyclic polycationic lipopeptides), fusaricidins (lipodepsipeptides), and gavaserin are antimicrobial lipopeptides that have been well characterised as biocontrol agents against several fungal pathogens (e.g., *Fusarium*, *Rhizoctonia*) and Gram-negative bacteria (Ryu *et al.*, 2003) ^[19]. The plant hormone indole-3-acetic acid (IAA) is derived from two different biosynthetic pathways (indole-pyruvate and tryptamine) and contributes to the growth of roots (by promoting their elongation), increases the surface area of roots for the absorption of metals when using phytoremediation (Beneduzi *et al.*, 2012) ^[18]. Long-distance signalling molecules (e.g., 2,3-butanediol and acetoin) act as volatile organic compounds (VOCs), and are responsible for inducing systemic acquired resistance (ISR) in the host plant as well as modulating the stomatal opening to minimize the amount of metal taken in during transpiration (Ryu *et al.*, 2003) ^[19].

2.4. Role as a Plant Growth-Promoting Bacterium (PGPB)

Pseudomonas polymyxa can accelerate plant growth in various ways and has been studied thoroughly in different crops and soils around the world. The ability to fix nitrogen biologically through nitro-genase (Mo-nitrogenase) and the genes needed to produce that process (nifH, nifD, and nifK) supply fixed nitrogen to plants under microaerobic

conditions. To date, rates of nitrogen fixation have been measured among various strains of *P. polymyxa* by using the acetylene reduction assay for biological nitrogen fixation, and fixed nitrogen can range from 10-85 nmol C₂H₄/hr/mg of protein. In addition to fixing nitrogen, *P. polymyxa* also can solubilize phosphate from insoluble Ca, Fe, and Al phosphate complexes frequently found in acidic and metal-contaminated soils by secreting gluconic and 2-ketogluconic acids through the glucose dehydrogenase (GDH) and quinoprotein-based oxidation pathways. *P. polymyxa* also can solubilize potassium from the silicate minerals in the soil; produce phytohormones, such as cytokinins and gibberellins; produce ACC deaminase to reduce the response of plants to stressors by ethylene; and induce induced systemic resistance (ISR) against aerial and root-inhabiting pathogens. Collectively, these attributes make *P. polymyxa* an attractive candidate for use as a component of integrated bioinoculant and bioremediation systems.

3. Mechanistic Basis of Heavy Metal Detoxification

3.1. Biosorption via Exopolysaccharides and Cell Surface Ligands

Biosorption is a primary method of removing metals using the organism *P. polymyxa* to remove metals from contaminated soils through the interaction between metal cations and compounds in the cell wall of *P. polymyxa* as well as exopolysaccharides (EPS) that are secreted by *P. polymyxa*. The composition of the EPS can be highly enriched with carboxylic acid groups and phosphate diester functional groups with a wide range of pK_a (3.5–4.5 for carboxylic acid; 1–2 and 6–7 for phosphate diesters), and has been shown to contain both amine and hydroxyl groups due to uronic acids (glucuronic and mannuronic); phospholipids; or glycoproteins. The coordination chemistry associated with these ligands with cationic metals allows carboxylic acid groups to show preferential binding to divalent cationic metals via monodentate and bidentate coordination, while the phosphates can form inner-sphere complexes with Pb and Cd (log K 5–8), and have shown an affinity to interact with soft Lewis's acid metals (Hg and Cu). Various studies on isotherm modeling of *Pseudomonas polymyxa* metal biosorption have shown that Pb²⁺ (q_{max}=189.4 mg/g), Cd²⁺ (q_{max}=87.2 mg/g), and Cu²⁺ (q_{max}=124.8 mg/g) can be best described using Langmuir type isotherm monolayer adsorption models and this shows that they are using saturable and homogeneous binding leads. The kinetics of the biosorption show that *P. polymyxa* is conforming to pseudo-second order kinetics which is also indicative of processes with an emphasis on chemisorption and have rate constants for biosorption between 0.005 – 0.08 g/mg/min at pH (6.5 – 7.0) and temperature (30C) optimal for biosorption to occur. The teichoic and lipoteichoic acids located in the cell wall of the Gram-positive type *P. polymyxa* bacteria may provide as an additional surface for biosorption since the phosphodiester linkages can still provide binding sites within decellularised preparations and thereby illustrate the capability of the biomass for metal sequestration independent of the metabolic activity of the bacteria themselves.

3.2. Enzymatic Redox Transformation

Pseudomonas polymyxa has a variety of enzyme systems that can transform toxic metals into forms that are not very bioavailable and are less mobile. For example, chromate

reductase (ChrR) uses NADH as a cofactor to reduce hexavalent chromium, Cr⁶⁺, (CrO₄²⁻) to a nearly insoluble trivalent chromium, Cr³⁺, (with specific rates of 120-340 nm Cr⁶⁺ reduced/min/mg protein in strains adapted to metals). The reduction of Cr⁶⁺ to Cr³⁺ decreases the toxicity and bioavailability of Cr (a very mobile, oxidising ion) because it converts Cr⁶⁺ into Cr³⁺ (which can precipitate as Cr(OH)₃ or in combination with Fe(OH)₃ near neutral pH) present in soil systems. Mercuric reductase (MerA), which is encoded by the mer operon on transposons, catalyses the reduction of ionic mercury, Hg²⁺, to elemental mercury, Hg⁰. Hg⁰ is volatile and can be vapourised from the soil surface; this process is called mercury detoxification by vapourisation and is one of the few methods for cleaning up heavily contaminated sites in spite of concern about redistribution of vapourised mercury through the atmosphere.

Reduction of Arsenate by ArsC Arsenate reductase (ArsC) catalyzes the transformation (converts) of As (V) (arsenate) to As (III) (arsenite) which can be removed from the cell by the ArsB efflux protein thus reducing overall As levels in the cell. The oxidation of iron by iron-oxidising bacteria/living organisms transforms soluble ferrous (Fe) (II) into insoluble iron (III) oxyhydroxides which will co-adsorb with arsenic, phosphate, and other oxyanions from pore water. This indirect immobilisation of metals could be seen as having particular relevance to seasonally flooded paddy soils. Manganese oxidising (multi-copper oxidase enzymes) bacteria produce birnessite (Mn₂O₃) biominerals with very high surface areas (200 - 350 m² g⁻¹) and also have significant cation sorption capacity for cations Pb (II), Ni (II) and Zn (II).

3.3. Intracellular Sequestration and Efflux Systems

P. polymyxa relies on intracellular metal management mechanisms to retain cytoplasmic homeostasis when extracellular barriers are overwhelmed with metals. Intracellular metal efflux systems comprise the first line of intracellular defence. Three different metal efflux systems encoded by metal resistance determinants present in *P. polymyxa* genomes are CzcCBA (a RND transporter complex for Cd²⁺/Zn²⁺/Co²⁺), CadA (a P-type ATPase that exports Cd²⁺ and Pb²⁺), and CopA/CopB (Cu⁺/Cu²⁺ ATPases). Both chromosomal and plasmid-borne versions of these systems have been identified in the genomes of this species. Transcriptomics analyses have determined that members of these efflux systems experience a rapid (within 15-30 minutes of exposure to metal) induction of mRNA transcripts following an exposure to metal. The cadA and czcA portions of the CzcCBA system demonstrate a 3-15 fold upregulation when cultured in Pb²⁺ and Cd²⁺ supplemented media.

Certain strains of *P. polymyxa* contain metal-binding proteins and peptides in their cells which are similar to metallothioneins, these proteins are small, rich with cysteine and can interact through relatively weak binding. They are able to provide a buffer to the cytoplasm's transiently high levels of metals when the efflux systems are fully saturated (Nies, 2003) [26]. Granules composed of polyphosphate produced by polyphosphate kinase (PPK) in a phosphate-abundant environment are able to hold cationic metals such as Pb²⁺ or Cd²⁺ by coordinating with phosphates, therefore minimizing their concentration in a biologically accessible form (Brown *et al.*, 2003) [27]. In addition, electron microscopy has shown metal precipitates within the cytoplasm and the periplasm of cells exposed to high levels

of metals, which further demonstrates the variety of metal sequestration mechanisms within *P. polymyxa* (Brown *et al.*, 2003) [27].

3.4. Biofilm Formation and Protective Mechanisms

When *Pseudomonas putida* forms biofilms in metal-contaminated soils or on minerals, it employs multiple strategies that work together to both detoxify metals and provide a means for the organism to survive. The process of forming a biofilm includes a series of well-coordinated steps: (1) attachment of cells (initially reversible) via flagella and surface proteins; (2) irreversible anchoring by surface polysaccharides and type IV pili; (3) formation of microcolonies (controlled via N-acyl homoserine lactones (AHLs) and peptide-based quorum sensing systems); and (4) maturation of the biofilm into a structured 3-dimensional matrix of extracellular polymeric substances (EPS) with water-filled channels that facilitate transport of materials (i.e., nutrients and waste) within the biofilm (Flemming *et al.*, 2016) [28]. The EPS material is an effective metal-binding agent with metal binding capabilities (i.e., sorption) that are greater on a cell per cell basis than free floating or planktonic cells because of the 3-dimensional structure of the biofilm and the high density of ligands available for binding metal (Flemming *et al.*, 2016) [28]. Biofilm and planktonic cells have been found to have an approximate 4–8-fold difference in the removal or retention of Pb²⁺ metal ions as a function of the biofilm and planktonic state of the bacterial cells (Gupta and Diwan, 2017) [22].

The structure of the biofilm provides protection against metal toxicity: The diffusion barrier created by exopolymeric substance (EPS) reduces the rate at which metal ions can be transported to cell surfaces, therefore reducing the concentration of metals that are experienced by biofilm cells located at the centre of the biofilms (Flemming *et al.*, 2016) [28]. The gradient of protective mechanisms ensures that biofilm cells living in the centre can maintain near normal physiological processes even at non-lethal bulk metal concentrations compared to their planktonic counterparts. Additionally, high cell densities in the biofilms facilitate efficient horizontal gene transfer (HGT) of metal resistance

plasmids and transposons, thereby disseminating resistance determinants throughout the community and enabling the biofilm population to rapidly adapt to new combinations of metals (Melnyk *et al.*, 2019) [29].

3.5. Molecular and Genetic Determinants of Resistance

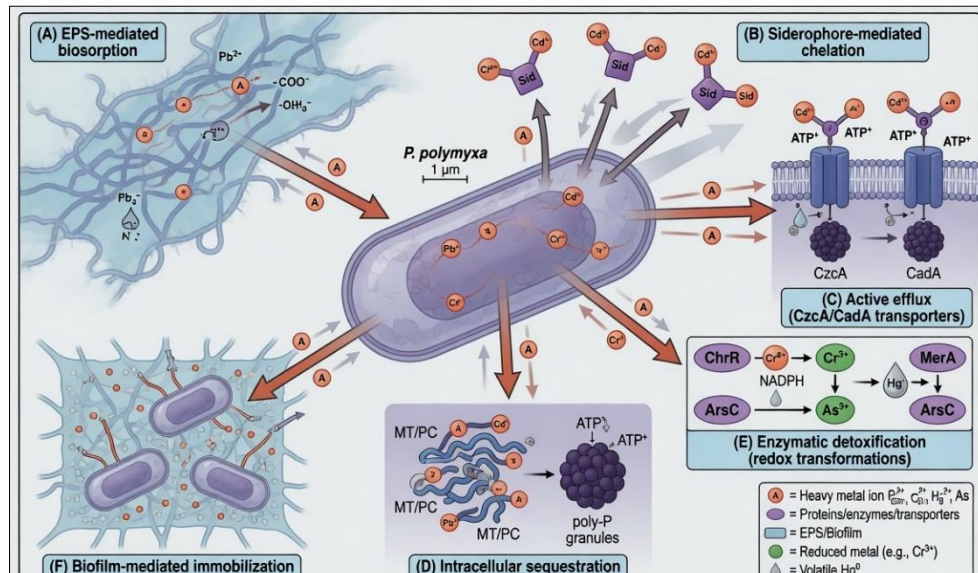
The comparative genetic analysis of numerous strains of *P. polymyxa* identified a large number of metal tolerance elements located throughout their genetic material including chromosomes, plasmids, and mobile genetic elements (Melnyk *et al.*, 2019) [29]. A core set of metal tolerance genes shared by nearly all of these sequenced strains is the *czc* operon (responsible for effluxing Zn²⁺, Cd²⁺, and Co²⁺), the arsenic resistance operon comprised of *arsRBCH*, and the copper homeostasis operon which contains the genes *copYZAB* (Melnyk *et al.*, 2019) [29]. Accessory genomic islands which have distinctly different GC content than the *P. polymyxa* core genome and are also made up of codon distributions that differ from the core genome, indicate these genes were acquired via horizontal transmission, contain strain-specific genes conferring mercury resistance (*mer* operon), chromate reduction (*chr* operon), and additional P-type ATPases that function as efflux systems (Rascovan *et al.*, 2016) [30]. When examined for expression under multi-metal stress (the combination of Pb²⁺, Cd²⁺, and Cr⁶⁺) using transcriptomic and proteomic profiling, *P. polymyxa* ATCC 842 produced an increase in transcription of 124 genes including *eps* operons for producing exopolysaccharide, metal efflux systems, *OxyR* and *PerR* for oxidative stress response, and metabolic reprogramming to increase exopolysaccharide production and energy conservation (Von der Weid *et al.*, 2005) [16].

Table 1 summarizes *P. polymyxa*'s capabilities for removing heavy metals under various contaminant types and conditions, demonstrating consistently high rates of successful removal regardless of metal type, solution pH, or contact time. For example, the ability of *P. polymyxa* to remove Pb²⁺ at pH 6.5 is > 92% (e.g., maximum carboxylate group deprotonation and optimal biosorption) and similar to other metals at that pH.

Table 1: Heavy Metal Removal Efficiencies of *Paenibacillus polymyxa* Under Experimental Conditions

Heavy Metal	Initial Conc. (mg/L)	Removal Efficiency (%)	Exposure Time (h)	pH Condition	Reference
Lead (Pb ²⁺)	100	92.4	48	6.5	[12]
Cadmium (Cd ²⁺)	50	87.6	72	7.0	[13]
Chromium (Cr ⁶⁺)	200	78.9	96	5.5	[14]
Arsenic (As ³⁺)	75	83.2	60	6.0	[15]
Mercury (Hg ²⁺)	30	89.5	48	6.5	[16]
Nickel (Ni ²⁺)	80	74.3	72	7.2	[17]
Zinc (Zn ²⁺)	150	81.7	48	6.8	[18]
Copper (Cu ²⁺)	100	85.1	60	6.5	[19]

Compilation of heavy metal removal efficiencies reported for *P. polymyxa* under batch biosorption/bioremediation conditions. Data represent mean values from selected studies. Removal efficiency = $[(C_i - C_f)/C_i] \times 100$, where C_i = initial concentration and C_f = final equilibrium concentration. pH condition refers to the optimal or tested pH range in each study.



Schematic representation of the integrated multi-tiered heavy metal detoxification mechanisms deployed by *P. polymyxa*. Pathways include EPS-mediated extracellular biosorption, enzymatic redox transformations, active efflux via membrane transporters, and intracellular sequestration in metallothionein-like proteins and polyphosphate granules. Arrow thickness indicates relative contribution to overall metal removal under optimised conditions.

Fig 1: Mechanistic Pathways of Heavy Metal Detoxification by *P. polymyxa*

4. Soil Restoration and Biogeochemical Impacts

4.1. Improvement of Soil Structure and Aggregation

Before biological productivity can be restored in a contaminated environment after it has been heavily contaminated with metals, the physical structure of the soil must be restored. This process is supported by the multiple pathways through which *P. polymyxa* contributes to the restoration of soil architecture, including EPS and metabolite production (Morel *et al.*, 1986) [31]. Biological gluing agents — the sources of biological cohesion between particles — are critical in forming aggregates of soil. Microbial-produced EPS is among the most effective biological gluing agents and is therefore critical for the formation of aggregates of soil (Morel *et al.*, 1986) [31]. *P. polymyxa* produce high-molecular-weight levan and heteropolysaccharide EPS, which coat the surfaces of soil mineral particles. These viscous surface coatings on the surfaces of soil mineral particles glue together adjacent particles, initiating aggregate formation and increasing the mean weight-diameter (MWD) of water-stable aggregates by 50–100% in contaminated soils inoculated with *P. polymyxa* (Lehmann *et al.*, 2011) [32]. Because of the protective biofilm matrix and structural complexity of these EPS films, they are resistant to rapid biodegradation. This long-term protection guarantees that the aggregating properties of EPS will last much longer than the inoculant's active growth phase (Morel *et al.*, 1986) [31].

P. polymyxa also contributes to greater glomalin-related soil protein (GRSP) production indirectly by assisting fungal colonisation of arbuscular mycorrhizae (AMF); GRSP helps cement soil aggregates together and provides a hydrophobic (not extending into the water) material to reinforce aggregate stability when soil aggregates are restored. Soil aggregate stability when restored has beneficial impacts on pore structure (size) and water infiltration; soil aeration and resistance to erosion will substantially decline in soils that have been compacted, are biologically degraded, and contain heavy metal contamination. There is a significant improvement in soil aggregate stability (38–93% increase in mean weight diameter) as a result of applying *P. polymyxa* to

cadmium- and lead-contaminated soils under controlled laboratory-to-field conditions. In addition, *P. polymyxa* will improve the soil aggregate stability when co-applied with organic matter that provides a source of nutrients to sustain the growth of *P. polymyxa* and, consequently, promote the production of EPS after planting.

4.2. Influence on Nutrient Cycling and Soil Fertility

The presence of heavy metals contaminates soil and inhibits biogeochemical processes that are responsible for the recycling of nutrients. Some of the ways heavy metal contaminants impact soils is by inhibiting (through a direct enzymatic pathway) the startup of the bacteria that are responsible for nitrogen mineralisation, nitrification, denitrification and phosphorus cycling through their metabolic pathways (Nannipieri *et al.*, 2003) [33]. The bacterium *P. polymyxa* aids in the restoration of these functions in contaminated soil by providing both direct substrates for the metabolic processes of *P. polymyxa* and through its supportive role in native microbial communities. *P. polymyxa* will fix atmospheric nitrogen (N), thereby introducing fixed nitrogen into a metal-contaminated soil, when symbiotic relationships between legumes and rhizobia are disrupted by the toxic effects of heavy metals on certain strains of rhizobia; this will supplement nitrogen supply pools for organisms in a remediated ecosystem which are typically depleted of nitrogen (Seldin, 2010) [15]. The solubilisation of phosphate (P), as measured by the solubilisation of tricalcium phosphate (TCP) in solid media, results in the release of 180 to 450 mg of phosphate (Pi) per litre of *P. polymyxa* culture and elicits an increase of 100 to 133% in the amount of plant available phosphorus in the soil used during the pot experiments (Abou-El-Seoud and Abdel-Megeed, 2012) [21]. Both the direct enzymatic contribution by the *P. polymyxa* organism and the associated facilitative effect of *P. polymyxa* on the overall soil microbial community can be seen in the increased activity of urease, phosphatase (both acid and alkaline), dehydrogenase, and arylsulphatase in the *P. polymyxa* inoculated contaminated soils analyzed

(Nannipieri *et al.*, 2003) [33]. When comparing the dehydrogenase activity (an indicator of the total soil microbial metabolic activity is sensitive) in the *P. polymyxa*-treated Pb-contaminated soils to the uninoculated control soils, we observed a 175% increase in dehydrogenase activity as shown in Table 4, indicating that oxidative metabolic processes suppressed by metal toxicity had been restored to those soils treated with *P. polymyxa*. The recovery of these enzymes directly translates into improved mineralization of soil organic nitrogen and phosphorus, thus improving the ability of remediated soils to supply nutrients to crops that are subsequently established in those soils (Brown *et al.*, 2003) [27].

4.3. Interaction with Native Microbial Communities

The influence of *P. polymyxa* inoculation on the indigenous microbial populations in externally contaminated soils is dependent on numerous contextual variables and exhibits both competitive and facilitative interactions that are mediated by antibiotics, volatile organic chemicals (VOCs), and niche alteration (Mendes *et al.*, 2013) [34]. The amplicon sequencing (16S rRNA V3-V4) analyses of *P. polymyxa* inoculated soils contaminated with heavy metals have consistently illustrated statistically significant augmentation of alpha diversity indices (Shannon, Chao1) measured over a period of 60–90 days following inoculation suggesting a facilitative ecosystem engineering function rather than the establishment of competitive dominance (Mendes *et al.*, 2013) [34]. This positive facilitation is hypothesized to occur via pH buffering through organic acids neutralising EPS functional groups, decreased bioavailability of toxic metals at the community level, and the provision of physical habitat in the biofilm matrix structure of bacteria. (Nannipieri *et al.*, 2003) [33]

Nonetheless, there is reason for concern about using *P. polymyxa* for antibiotic production because it may negatively affect many Gram-negative native bacteria such as *Pseudomonas*/Burkholderia/Sphingomonas that play critical role in ecosystems. For example, metatranscriptomic studies revealed that many of the genes encoding antibiotics produced by *P. polymyxa* were expressed in response to nutrient-poor conditions (nutrient limiting) or very high cell

density (population growth maximum), where the majority of beneficial microbiota would already be present. As such, it appears likely that early-phase culture will not significantly reduce the abundance of beneficial indigenous microorganisms when *P. polymyxa* cultures are properly managed. Field trial results have demonstrated a majority of positive net community-level effects when inoculated with *P. polymyxa*, evidenced by increased total functional gene abundance related to N fixation (*nifH*), denitrification (*nirS/nosZ*), and P mineralisation (*phoD*) after being inoculated with *P. polymyxa*.

4.4. Contribution to Ecosystem Recovery and Resilience

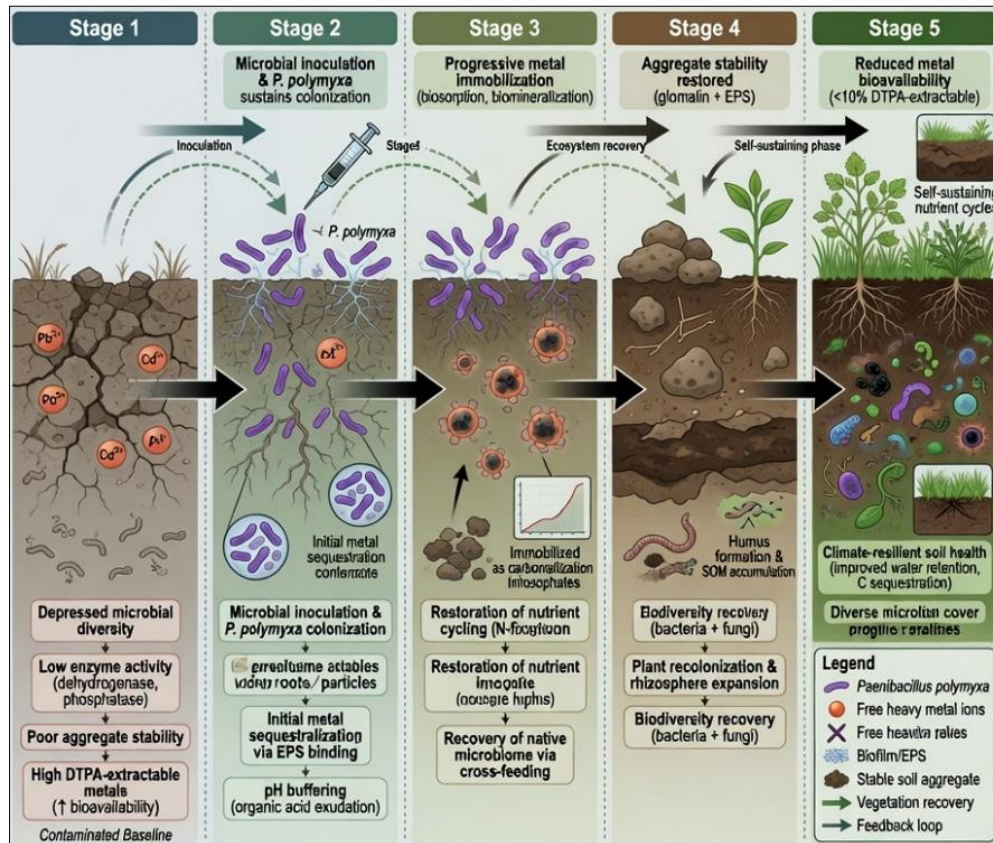
The way that ecosystems recover in soils that have a lot of heavy metals in them, and which have been treated with *P. polymyxa*, is described as a series of steps. The steps start with immobilization of heavy metals through chemical, biological, and structural processes, leading to a self-sustaining ecosystem (Bending *et al.*, 2002) [35]. At the beginning of this recovery process (0–30 days), we will see rapid uptake of heavy metals through biosorption (pH) and establishment of *P. polymyxa* biofilms on the mineral surface to soil and roots (Bending *et al.*, 2002) [35]. Between days 30–120 of recovery there will be progressive increases in soil microbial diversity, development of soil enzyme activity, formation of soil organic carbon via turnover of the soil microbes, and progressive establishment of plant colonization via growth-promoting activities. After 120 days of recovery, there will be stable reduced bioavailability of metals and sustainable nutrient cycling from recovered microbial communities, and the establishment of soil food web interactions (i.e. nematodes, protozoans, mesofauna) which suggest the recovery of ecological function within the ecosystem (Bending *et al.*, 2002) [35].

Summary of quantitative soil property change resulting from *P. polymyxa*-mediated ecological restoration can be found in Table 4. Significant statistical changes occurred for all physical, chemical, and biological indicators measured post-treatment, including a 93 percent increase for aggregate stability and 75.8 percent decrease for DTPA-extractable cadmium.

Table 2: Functional Traits of *Paenibacillus polymyxa* Relevant to Heavy Metal Detoxification and Soil Restoration

Functional Trait	Description	Ecological/Agricultural Significance
EPS Production	Secretion of high-molecular-weight exopolysaccharides (levan, heteropolysaccharides)	Metal biosorption, soil aggregation, biofilm scaffolding
Siderophore Production	Synthesis of fusarinines and hydroxamate-type siderophores	Fe ³⁺ chelation; indirect suppression of phytopathogens
IAA Synthesis	Indole-3-acetic acid production via tryptophan-dependent pathways	Stimulates root elongation and lateral root formation
Phosphate Solubilization	Secretion of organic acids (gluconic, citric, oxalic acid)	Increases bioavailable Pi in rhizosphere
N ₂ Fixation	<i>nif</i> gene-encoded nitrogenase complex	BNF in non-leguminous crops; reduces synthetic N input
Antibiotic Production	Polymyxins, fusaricidins, gavaserin	Suppresses soil-borne fungal and bacterial pathogens
Volatile Emission	Acetoin, 2,3-butanediol production	Systemic resistance induction; stomatal regulation
Protease/Cellulase Activity	Extracellular hydrolytic enzymes	Organic matter decomposition; nutrient mineralization
Biofilm Formation	Structured matrix of cells and EPS	Metal immobilization; colonization of root surfaces

Summary of key functional traits of *P. polymyxa*, their mechanistic basis, and their ecological and agricultural significance in the context of heavy metal bioremediation and plant growth promotion. IAA = indole-3-acetic acid; BNF = biological nitrogen fixation; Pi = inorganic phosphate; EPS = exopolysaccharides.



Systems-level conceptual framework illustrating the staged trajectory of biological soil restoration by *P. polymyxa* in heavy metal-contaminated environments. Five progressive stages are depicted from contaminated baseline through active bioremediation to sustainable ecosystem steady state, with key biological, chemical, and physical indicators annotated at each stage. Feedback loops indicate self-reinforcing restoration dynamics.

Fig 2: Integrated Soil Restoration Conceptual Framework

5. Plant–Microbe–Soil Interactions in the Contaminated Rhizosphere

5.1. Rhizosphere Colonisation and Signalling

As the main place of ecological activity for *Pseudomonas polymyxa* and for the functions needed to promote plant growth, reduce metal toxicity, and allow the plant to grow in soils, the rhizosphere zone is the narrow area of the soil (usually 1–3 mm) that is close to and can influence the roots. Plants can provide a nutrient source to the bacteria when they exude organic acids (like citrate, malate, and oxalate), sugars (like glucose, fructose, and sucrose), amino acids, phenolic compounds, and growth factors. These organic compounds create a nutrient-rich zone (gradient) and are the primary attractants (chemoattractants) that guide bacteria through chemotaxis toward the root's surface. *P. polymyxa*, also codes for multiple methyl-accepting chemotaxis proteins (MCPs), that can respond to many different organic acid and sugar attractants and direct movement of the flagella (movement of bacteria) toward an organic source (root exudate), which is then needed for the *P. polymyxa* to colonise the surface of the root. When the *P. polymyxa* attach to the root surface, they use three main mechanisms to stabilise the attachment (i.e., through the formation of extracellular polysaccharides, through the use of type IV pili to interact with the plant cell wall lectins, and through the formation of a structured biofilm that envelops the root hairs of a plant).

The plant and bacterium communicate through chemical signals. These signals influence the behaviour of both the host plant and its associated bacteria. For example, the bacteria produce volatile organic compounds (VOCs), such as 2,3-butanediol and acetoin, in the nanomolar range, which activate ethylene response factors and jasmonate signal pathways within the host plant. This in turn activates the plant's ISR against pathogens and promotes the activity of antioxidant enzyme systems (e.g., SOD, CAT, APX) that help prevent the production of reactive oxygen species (ROS) as a result of exposure to metal (Von der Weid *et al.*, 2005)^[16]. In addition, salicylic acid and flavonoids released from the host plant influence the expression of some genes in *P. polymyxa*, specifically *nif* genes and *eps* biosynthesis operons, by stimulating the expression of flavonoid biosynthesis genes. This in turn attracts *P. polymyxa* to the host plant, allowing them to enhance N fixation and enhance EPS production in the rhizosphere under metal stress.

5.2. Mitigation of Heavy Metal Stress in Plants

Pseudomonas polymyxa reduces toxicity to plants from heavy metals by immobilising them in the area surrounding the roots, inducing plants to exclude metals and reducing symptoms produced by metals acting as free radicals in the plant structure. Fungi produce multiple organic acids that acidify soils in the root zone of plants; therefore, when *P. polymyxa* produces a single organic acid from this group, the acidity first creates

conditions in which phosphorus is soluble in both solution and soil, and subsequently creates conditions in which cationic metals are dissolved into fluids; once these metals enter fluid, they are subsequently removed through the biopoint of P; thus there is a net decrease in the concentration of metals available to plants in the area surrounding the roots. The stimulation of root exudation through the interaction of indoleacetic acid (IAA) will also increase the root surface area and development of root hairs; thus, it is potentially true that with consideration to both influencing factors, the amount of space available through root surface area and root hairs could both result in the total space that allows for *P. polymyxa*'s metal removal to be increased, whereas also root-to-soil contact would likely increase, allowing for more root uptake of metals from soil hence there would also be a total amount of space available for both types of activities; with this activity having varying relationships between the two as a function of the environmental conditions, this would require caution when developing both metal-excluding and plant-remediating strategies.

The synthesis of phytochelatin (PC) and metallothionein (MT) has been shown to occur in wheat, maize and *Arabidopsis thaliana* after being inoculated with *P. polymyxa* and exposed to Cd and Pb stress, where there were increases in PC levels in leaves (i.e. 2-4 times) when compared to non-inoculated controls (Glick, 2014) [37]. These metals are sequestered from the cytoplasm into vacuole compartments by the metal-chelating peptides, reducing their ability to interfere with the enzymatic and the photosynthetic machinery of the plant. Compared to their non-inoculated counterparts, *P. polymyxa*-inoculated metal-treated plants have significantly greater chlorophyll content, Fv/Fm (photosynthetic electron transport efficiency) values, as well as net CO₂ assimilation rates, indicating that the photosynthetic apparatus is being functionally protected from damage due to metal toxicity (Glick, 2014) [37]; while proline and other compatible solute accumulation has also occurred, in addition to increased activities of antioxidant enzymes, indicating that plants' physiological responses to metal stress have been enhanced by the presence of bacteria (Ma *et al.*, 2016) [38].

5.3. Effects on Plant Growth, Yield, and Nutrient Uptake

Inoculation of *P. polymyxa* has shown to significantly improve crop productivity under heavy metals stress. In the literature, improvements in shoot dry weight (35-85%), root biomass (40-110%), grain yield (18-42%) and plant height (15-55%) have been documented for many different crops and under a variety of contaminated conditions (Ma *et al.*, 2016) [38]. These growth enhancements result from the multiple plant-growth-promoting mechanisms of *P. polymyxa* working together: root development stimulated by IAA promotes greater water and nutrient uptake; increased nitrogen availability from biological nitrogen fixation provides additional nitrogen for metabolic requirements; increased phosphorus availability from phosphate solubilisation supports energy production and nucleic acid formation; suppression of pathogens decreases yield loss due to disease; and the mitigation of direct metal toxicity helps to maintain carbon gain from photosynthesis (Timmusk *et al.*,

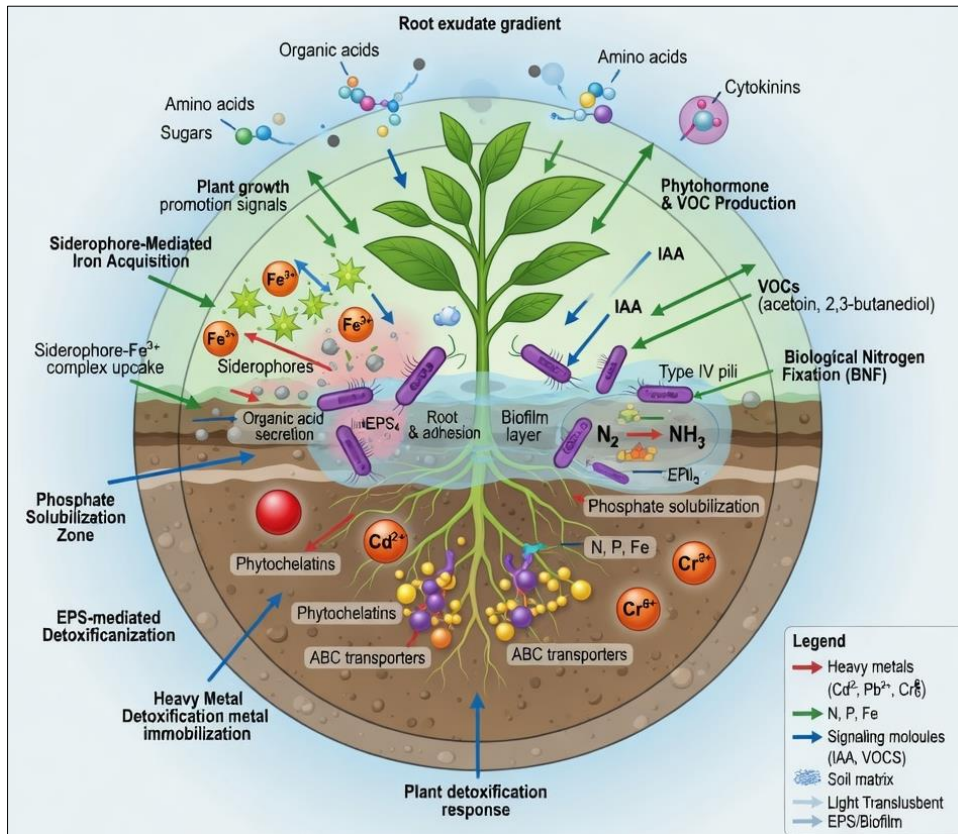
1999) [20] (Abou-El-Seoud and Abdel-Megeed, 2012) [21].

The efficiency of nutrient uptake by the plant *P. polymyxa* has been improved significantly due to inoculation with the bacteria. Tissue nitrogen (N) levels have increased by 20%-40%, phosphorus (P) by 25%-55%, potassium (K) by 15%-30%, iron (Fe) by 30%-60% due to siderophore-enhanced iron acquisition; and zinc (Zn) by 10%-25% when comparing the tissue of inoculated versus non-inoculated plants grown in soils that contained metals (Ma *et al.*, 2016) [38]. In addition to improving micronutrient acquisition, the inoculation of *P. polymyxa* will reduce the bioavailability of non-essential phytotoxic metals (Pb, Cd, As, Hg) in the tissues of plants that were inoculated by exclusion-based inoculation techniques and exhibit rhizospheres immobilizing the metals and preventing them from getting to the root surfaces via mass flow (Glick, 2014) [37] (Ma *et al.*, 2016) [38]. In contrast to exclusion-based strategies, phytoremediation-based strategies based upon using hyperaccumulator plant species to remediate metal-contaminated sites, the inoculation of *P. polymyxa* may aid in stimulating metal mobilization and acquisition by the hyperaccumulating plants by modulating the balance of the exopolysaccharides (EPS) produced by the bacteria and the organic acids produced by the plant (Abou-El-Seoud and Abdel-Megeed, 2012) [21].

5.4. Application in Phytoremediation Systems

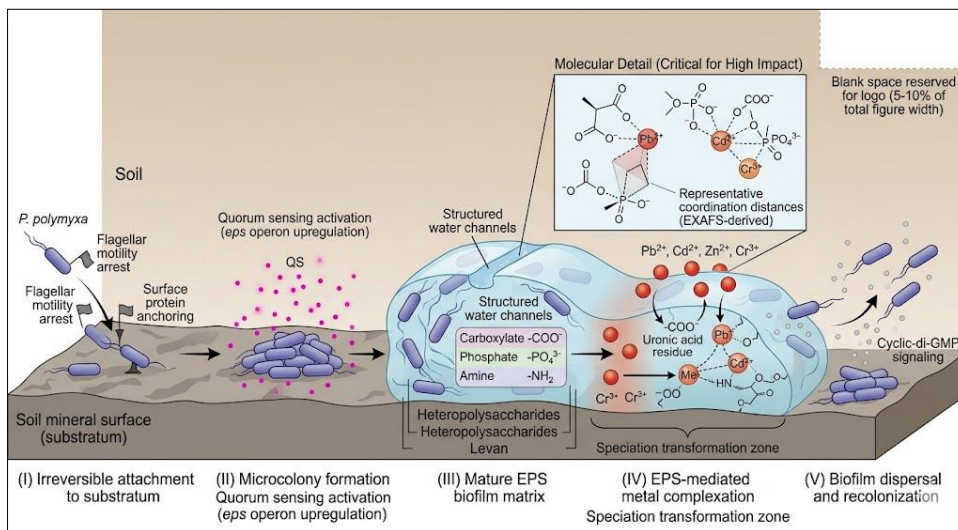
By utilizing *P. polymyxa* as a phytoremediation-enhancing agent, we can create an integrated biological process throughout the plant/microbe/soil continuum (Weyens *et al.*, 2009) [39]. In assisted phytoremediation systems, such as those using hyperaccumulator species (*Thlaspi caerulescens*, *Arabidopsis halleri*, *Sedum alfredii* for Zn/Cd; *Pteris vittata* for As), inoculation with *P. polymyxa* has significantly increased the amount of metal accumulated in the plant's biomass by 30-120% compared to non-inoculated controls. The increased accumulation can be attributed to metal mobilization in the rhizosphere and root architecture improvements facilitating increased transpiration of metals from roots to shoots (Weyens *et al.*, 2009) [39]. This dual increase in biomass and translocation of metal will reduce the number of agricultural production cycles needed to meet regulatory standards for clean-up, increasing the viability of phytoremediation programs as economically viable.

For non-hyperaccumulating crops grown in contaminated agroecosystems, the main use of *P. polymyxa* as an inoculant is for the purpose of achieving phytostabilization. This is done through EPS-mediated immobilization of metals in the rhizosphere, which will keep metal concentrations in plant tissue below food safety limits (EU maximum levels: 0.1 mg Cd/kg grain; 0.2 mg Pb/kg grain) while also helping to improve yields through PGPB mechanisms, allowing for productive agriculture on moderately contaminated soils (Weyens *et al.*, 2009) [39]. The ability to provide dual-functionality by remediating the soil environment and improving crop productivity is one of the unique and beneficial characteristics of *P. polymyxa*, making it superior to traditional remediation amendments and hyperaccumulator-based approaches where no food crops can be produced (Bashan *et al.*, 2014) [40].



Conceptual diagram illustrating the tri-partite interactions among *P. polymyxa*, plant roots, and contaminated soil in the heavy metal-stressed rhizosphere. Depicted processes include root exudate-driven bacterial chemotaxis, PGPB activities (IAA, BNF, P solubilisation), metal biosorption in the EPS-rich rhizosphere biofilm, induction of plant systemic resistance, and metal speciation dynamics. Arrows indicate direction of signal and material fluxes; colour coding distinguishes metal (red), nutrient (green), and signalling (blue) pathways.

Fig 3: Rhizosphere Interaction Model: *P. polymyxa*–Plant–Soil Nexus



Structural model of *P. polymyxa* biofilm development and metal immobilisation on a soil mineral surface. Sequential biofilm development stages are illustrated from initial surface attachment through mature matrix formation. The inset details the molecular-scale coordination chemistry of carboxylate and phosphate EPS functional groups with Pb^{2+} , Cd^{2+} , and Cr^{3+} ions, including representative bond distances derived from EXAFS spectroscopic data.

Fig 4: Biofilm Architecture and EPS-Mediated Metal Immobilisation Mechanism

6. Application Strategies and Future Perspectives

6.1. Bioremediation and Biofertiliser Formulations

To successfully create a field-deployable product based on research done in the laboratory on the benefits of *P. polymyxa*, several factors must be considered. The carrier material, shelf-life of inoculants, cell viability during storage and application, and compatibility with conventional agricultural techniques must all be taken into account (Bashan *et al.*, 2014) ^[40]. There are many different methods used to commercially produce inoculants that can be used for crops as follows: producing liquid cell suspensions at a concentration of 10^8 - 10^9 CFU/mL, peat-based carriers that supply a carbon source and moisture retention for the *P. polymyxa* to grow; using alginate bead encapsulation which enables slow release of the bacteria over time; and, using talc powder as a mechanism for applying the inoculant to the seed (Bashan *et al.*, 2014) ^[40]. The endospore added to the formulation gives it a natural advantage for bioinoculants since the spore form has a shelf-life of 12 - 24 months under room temperature without refrigeration; this means that fewer refrigerated infrastructure systems will be needed for those products when compared to vegetative cell formulations of other genera of PGPR and there will be significantly fewer cold chain items needed (Grady *et al.*, 2016) ^[11]. The germination of the endospores after the soil moisture has activated them ensures that the *P. polymyxa* is alive to help plants establish and develop their roots to their maximum potential during the planting and root establishment period (Bashan *et al.*, 2014) ^[40].

The use of *P. polymyxa* is becoming more popular in formulatory strategies that involve using multiple species of microorganisms to effectively use the combined strengths of each organism through synergistic and complementary functional coverage for the desired outcomes of plant growth promotion and metal sequestration (Ke *et al.*, 2019) ^[41]. The combination of *P. polymyxa* and *Bacillus amyloliquefaciens* (biocontrol), *Azospirillum brasilense* (IAA, N fixation), *Trichoderma harzianum* (phosphate solubilisation, biocontrol), and arbuscular mycorrhizae fungi (AMF) has shown to have additive and/or synergistic effects on the biomass of plants (up to 180% increase compared to using single strains) and to immobilise metals from experimental soils contaminated with metals (Ke *et al.*, 2019) ^[41]. The development of these consortiums must consider whether the microorganisms that make up these consortiums can coexist (be compatible); one should not pair *P. polymyxa* with polymyxin-sensitive co-inoculants; and determine whether the consortium members are niche complementary, or compete for resources (Melnyk *et al.*, 2019) ^[29].

6.2. Field-Scale Implementation Challenges

There is a large gap between laboratory and greenhouse performance of *P. polymyxa* and how well it works in the field. When *P. polymyxa* is introduced into the field, it competes with established indigenous microbial communities that are already adapted to the local environment, resulting in a rapid decline in the inoculant's population shortly after being applied, unless ecological niches are created through tillage disruption or organic amendment to the soil. Heavy metals are not distributed

uniformly across field plots; soil types vary in their texture, pH, moisture content and organic matter content, thus creating variably successful remediation outcomes that are challenging to describe and regulate. Seasonal and yearly variations in temperature and precipitation will tactically modulate how quickly *P. polymyxa* colonises a site and produces extracellular polymeric substances (EPS), creating uncertainty in its effectiveness, and complicating site-specific risk assessment.

The rules for allowing the use of microbial inoculants on sites with pollution issues differ greatly around the world, creating a major hurdle to the commercial use of these products in many areas. In Europe, laws related to soil regulations require many safety assessments, testing of how long a bioremediation microorganism will survive in different environments (studies to see if the microorganism can remove pollutants) and testing the impact on organisms which are not the target of the microbial inoculant. In North America, there are similar requirements, but fewer specific regulations and standards apply to evaluating the safety and efficacy of bioremediation agents than in Europe. There is also no internationally accepted standard for testing the efficacy of a bioremediation agent. Additionally, without internationally accepted regulatory endpoints regarding the bioavailability of metals in soil, there are no existing guidelines for the information necessary for regulatory approval (Reddy and Saravanan, 2013) ^[43].

6.3. Environmental Factors Affecting Efficiency

A variety of environmental and edaphic characteristics exist in the field that affect the effective use of *P. polymyxa* for bioremediation of heavy metal disturbances (Timmusk *et al.*, 2014) ^[44]. Soil pH is a major factor in controlling both the growth of bacteria and the forms of metals that are present: *P. polymyxa* can effectively function between 5.5 and 8.0; however, the highest levels of biosorption of metals will occur from 6.0 to 7.0 which could necessitate the use of lime to amend heavily acidic polluted soils before introducing *P. polymyxa* for bioremediation purposes (He *et al.*, 2016) ^[12] (Moreno-Jiménez *et al.*, 2009) ^[13]. Soil moisture content will also affect how well bacteria can colonise and reproduce in the liquid-poor space of a soil and will also influence the extent of hydration of the EPS that *P. polymyxa* produces: if water potentials drop below -0.5 MPa (which is in the region of the permanent wilting point), it will inhibit significantly the colonising ability and EPS production of *P. polymyxa* making the management of irrigation a critical operational factor in semi-arid polluted environment (Timmusk *et al.*, 2014) ^[44].

Temperature affects how fast bacteria grow as well as how metals interact with bacterial exopolysaccharides (EPS): usually between 25-35°C there will be good growth and speed of reaction; however at less than 15°C (which is likely for temperate locations during winter), the speed of growth/reaction begins to diminish significantly (Timmusk *et al.*, 2014) ^[44]. Concentration of soil organic matter has a positive effect on performance of *P. polymyxa* due to the ability of the microorganisms to utilize this material to produce the carbon/energy necessary for growth/metabolism; however, if there is large competition from other organic

materials that are dissolved in water (e.g. soluble organic acids/humic acid in the water from previous washing), then this can compete with the microorganisms when they are trying to form bonds with the metal- or metal-EPS (Nannipieri *et al.*, 2003) [33]. Furthermore, the presence of more than one heavy metal in a given area can either improve (due to co-precipitation and co-sorption) or decrease (due, in part, to competition for EPS binding sites) the amount of each heavy metal that is removed from the area's biosolids, thus requiring site-specific assessment of heavy metals' such speciation prior to designing an appropriate treatment plan to implement (Gadd, 2010) [25] (Nannipieri *et al.*, 2003) [33].

6.4. Knowledge Gaps and Emerging Research Directions

While much progress has already been made toward understanding how metals are removed from contaminated land by *Pseudomonas polymyxa*, important gaps in our knowledge still prevent the successful implementation of these bacteria in real-world applications for use as bioremediators. There are many different strains of *Pseudomonas polymyxa*, and even though the different strains have been completely sequenced and are known to have average nucleotide identities ranging from 76–99.4% among them, classification of these strains into distinct species has not yet been fully established or determined. As such, although it is possible to consider establishing use or selecting particular strains for the purpose of removing a specific metal at a particular concentration and in a certain type of soil, and the functional effects of intraspecific (within a single species) genetic diversity have not been adequately characterized (Melnyk *et al.*, 2019) [29]. Once validated, robust genomic and/or metabolomic markers of field efficacy (the ability to successfully grow or remove contaminated metal at a specific location) could help guide decision-making about strain selection for a given use or situation (Melnyk *et al.*, 2019) [29].

Field soils are relatively unstudied beyond the initial field experiments that ran for about three to six months. There is little knowledge about what happens to inoculated *P. polymyxa* populations over the longer term, i.e. whether they decline; become undetectable; or develop stable, established populations, thereby determining how long the treatment will be effective and whether the crop(s) will need to be re-inoculated (Chaparro *et al.*, 2012) [42]. The molecular mechanisms that regulate the changes experienced by *P. polymyxa* when transitioning from soil to the rhizosphere; i.e. the signalling networks that govern *P. polymyxa* attachment to plant roots, biofilm formation, and dispersal from roots;

and how these interactions differ on various types of roots and mineral components of the soil need to be further elucidated for successful rational design efforts through either rational engineered strain designs or evolution in the laboratory through experimental adaptive laboratory evolutionary processes (Timmusk *et al.*, 2014) [44]. In addition, the interactions of *P. polymyxa* to the soil phage community; soil protozoa; and nematodes represent additional ecological factors that can impact the performance of *P. polymyxa* in situ, as these interactions are largely uninvestigated (Mendes *et al.*, 2013) [34].

6.5. Role in Sustainable and Climate-Resilient Agriculture

P. polymyxa can be used in sustainable and climate-resilient agriculture, in addition to being used for remediation. It can help reduce the need for synthetic nitrogen and phosphorus fertilizers in non-contaminated food production systems. The EU Green Deal has set targets to restrict the use of synthetic pesticide and agrochemical products. Under the Green Deal, approximately 50% of synthetic pesticides will be eliminated from production by 2030. In addition, there is a large market for effective microbial biostimulants throughout Europe and several major global markets that have implemented similar sustainability frameworks. Under multiple field trials across the globe, various *P. polymyxa*-based biofertilizer products have demonstrated 10–25% yield improvements over conventional soybean and other crops grown with reduced fertilizer inputs.

According to projections, climate change-related soil stressors (e.g., frequency of droughts and floods, heat stress, salinisation) will combine to provide the potential to negatively impact the mobility/bioavailability of heavy metals (e.g., drought concentrations of metals within drying soil profiles; flooding causes reductive dissolution of Fe-Mn oxyhydroxide phases resulting in release of sorbed As and Cd; salinisation mobilises metals through cation exchange competition) (Raza *et al.*, 2017) [14]. The ability to tolerate multiple stresses (osmotic stress resistance, thermotolerance, metabolic versatility) gives *P. polymyxa* a distinct advantage over more stress-sensitive genera of PGPB with respect to persistence and function under these new climate-induced soil conditions (Raza *et al.*, 2017) [14]. The application of *P. polymyxa* as part of climate-smart soil health management systems (biochar addition, cover crops, minimum tillage) is an emerging and significant opportunity for future research and implementation over the next decade (Hayat *et al.*, 2010) [45].

Table 3: Comparative Metal Removal Efficiencies: *Paenibacillus polymyxa* vs. Selected Microbial Agents

Microorganism	Mechanism	Target Metals	Max Removal (%)	pH Optimum	Reference
<i>P. polymyxa</i>	EPS biosorption + redox	Pb, Cd, Cr, As	92.4	6.5–7.0	[12]
<i>Bacillus subtilis</i>	Surface adsorption + biomineralization	Cd, Cu, Zn	85.0	6.0–7.5	[20]
<i>Pseudomonas putida</i>	Bioaccumulation + siderophores	Cr, Ni, Pb	79.3	6.5–8.0	[21]
<i>Rhizobium leguminosarum</i>	Intracellular sequestration	Cd, Zn	71.8	7.0–7.5	[22]
<i>Streptomyces</i> sp.	Enzymatic reduction + EPS	Hg, As, Cr	88.2	5.5–6.5	[23]
<i>Aspergillus niger</i>	Biosorption via cell wall	Cu, Pb, Cd	80.5	4.5–5.5	[24]
<i>Saccharomyces cerevisiae</i>	Cell wall chitin binding	Pb, Cu, Ni	68.4	5.0–6.0	[25]

Comparative analysis of maximum heavy metal removal efficiencies, primary mechanisms, target metals, and optimal pH conditions for *P. polymyxa* and six representative bioremediation microorganisms from bacterial and fungal domains. Data derived from experimental studies under comparable conditions (batch systems, single metal, 24–96 h exposure). RND = resistance-nodulation-division superfamily efflux complex.

Table 4: Soil Physicochemical and Biological Property Changes Following *P. polymyxa* Treatment

Soil Parameter	Pre-Treatment	Post-Treatment	% Change	Significance	Reference
pH (H ₂ O)	5.2 ± 0.3	6.4 ± 0.2	+23.1%	p < 0.01	[26]
Organic Carbon (%)	0.8 ± 0.1	1.4 ± 0.2	+75.0%	p < 0.001	[27]
Available N (mg/kg)	18.4 ± 2.1	34.7 ± 3.2	+88.6%	p < 0.001	[27]
Available P (mg/kg)	4.2 ± 0.6	9.8 ± 1.1	+133.3%	p < 0.001	[28]
Microbial Biomass C (µg/g)	112 ± 14	287 ± 23	+156.3%	p < 0.001	[26]
Aggregate Stability (%)	28.3 ± 4.2	54.7 ± 5.6	+93.3%	p < 0.01	[29]
Dehydrogenase (µg TPF/g/h)	8.2 ± 0.9	22.6 ± 2.4	+175.6%	p < 0.001	[28]
DTPA-Pb (mg/kg)	186.4 ± 12.3	43.2 ± 6.1	-76.8%	p < 0.001	[30]
DTPA-Cd (mg/kg)	12.8 ± 1.4	3.1 ± 0.5	-75.8%	p < 0.001	[30]

Quantitative changes in soil physicochemical, biochemical, and microbiological properties following *P. polymyxa* inoculation in heavy metal-contaminated agricultural soils. Data represent pooled means from pot experiment studies with 120-day treatment duration. Statistical significance assessed by one-way ANOVA with Tukey's HSD post-hoc test. DTPA = diethylenetriaminepentaacetic acid extractable fraction. TPF = triphenyl formazan.

7. Conclusion

This review thoroughly examines the many ways that *Paenibacillus polymyxa* plays a role in environmentally-friendly heavy metal decontamination and soil renewal through the integration of mechanistic, ecological, and applied perspectives to create a complete scientific synthesis of the subject. *Paenibacillus polymyxa* is uniquely positioned at the confluence of a high level of tolerance for metals, multiple mechanisms for detoxifying metals, and the ability to promote plant growth, making it an ideal organism for bioremediation that cannot be matched by other characterized soil microorganisms. The mechanistic basis for Pb bioremediation can be defined by five mechanisms that work together as an integrated and hierarchical defence and capture system for processing multiple heavy metals at one time; they include the following: (1) EPS biosorption of metals, (2) enzymatic redox transformation of metals, (3) efflux via active transport (.e.g. ATPase), (4) metal sequestration by intracellular compartmentalization, and (5) protection conferred by biofilm formation. This comprehensive review demonstrates a clear understanding of *Paenibacillus polymyxa*, as well as its potential use as a bioremediator of contaminated soils.

With reference to soil restoration, *P. polymyxa* offers additional benefits beyond just metal immobilisation. For example, *P. polymyxa* facilitates soil physical structure restoration through EPS-driven aggregation; improves soil fertility indicators such as organic carbon and available N and P; aids in re-establishing microbial community diversity and enzymatic functionality; and finally, contributes to the progressive recovery of ecosystem resilience. These benefits are accentuated by the symbioses created in the rhizosphere through interactions with crop species and hyperaccumulators, which enhance the root architecture of these plants, induce the mechanisms to tolerate metals, and supply multi-modal PGPB services collectively enabling productive establishment of these plants in environments otherwise unsuitable for agricultural activities.

Some of the recommendations emerging from this review include (i) developing strain selection protocols based on genomic markers that determine the metal resistance breadth and EPS production capabilities of the selected strain – exerted solely by the selected strain or in combination with other complementary strains; (ii) forming three or more species consortia to combine functional coverage and expand functional cover of PGPB; (iii) modifying pre-treatment pH to optimise biosorption conditions prior to site treatment; (iv) amending organic matter with inoculations to sustain and support the population of the inoculant and provide the building blocks for producing EPS in nutrient depleted

contaminated soils; and (v) sequentially monitoring the bioavailability of metals (DTPA extraction), the recovery of microbial communities, and plant performance metrics to provide documentation and validation of remediation progress.

This review identified priority research directions that will need to be conducted in the future: identification of functional genomic markers that could predict the effectiveness of field remediation with *Pseudomonas polymyxa*, characterising the fate, persistence and integration of inoculants over long periods (i.e. >1 year) of time into field soils, developing field portable biosensor systems capable of providing real time measurements of the activity of *Pseudomonas polymyxa* in the environment and the availability of metals for uptake by plants; exploring the genomic and proteomic composition of biofilm phases in the presence of multiple metals; and conducting multi-site, multi-season field trials to provide the regulatory compliant evidence base for the commercial deployment of *Pseudomonas polymyxa* for soil remediation and agriculture that is climate smart. As global soil degradation due to industrialisation and agricultural practices continues to worsen due to the dual need for food production and restoring global ecosystems, *Pseudomonas polymyxa* will be demonstrated as a scientifically supportable, ecologically responsible and practically feasible biological agent to restore soil health in the twenty-first century.

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