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Bioremediations of different heavy metals by halotolerant bacteria isolated from Mangrove Rhizosphere Soils

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Abstract
Mangroves are coastal swamp forests that host halotolerant shrubs and trees, providing a productive ecosystem that acts as a carbon sink and supports diverse biodiversity, including fish, mollusks, birds, and crustaceans. They play a vital role in the local economy and protect coastlines from natural disasters like cyclones and erosion. This review focuses on halophilic and halotolerant bacteria in mangrove rhizospheres, which have adapted over millions of years to thrive in saline environments. Halophiles utilize either a compatible solute strategy, accumulating organic compounds to manage osmotic stress, or a salt-in strategy, maintaining high internal salt concentrations. These microbes produce unique enzymes and metabolites with potential biotechnological applications, including bioremediation of hydrocarbon and heavy metal-contaminated soils. Heavy metals like Cu, Cr, Pb, Cd, Hg, and Zn threaten mangrove ecosystems, often entering through human activities or natural processes. Metal-resistant microbes in mangrove sediments can remediate contaminated soil and protect plants from toxicity, suggesting a significant capacity for heavy metal tolerance in these organisms due to their adaptation to fluctuating salinity.
Keywords: **Heavy metals, mangrove, Biodiversity, bioremediations**

I. Mangrove Ecosystems

Mangroves in tropical and subtropical regions are salt-tolerant forest ecosystems, rich in biodiversity, including halotolerant and halophilic bacteria. These bacteria are valuable for biotechnological applications, producing enzymes and bioactive compounds with various beneficial properties, such as antioxidants and anticancer agents (Thatoi et al., 2020; Goyal, 2019). Covering approximately 152,000 km² across 123 countries, mangroves serve as critical coastal habitats between rivers, the sea, and land. They host diverse microbial communities that contribute to their complex ecosystems. Research has largely focused on specific tree compartments, revealing significant bacterial groups like Actinobacteria and Proteobacteria (Purahong et al., 2019). Both rhizospheric and endophytic bacteria from mangroves are important for producing industrially relevant enzymes (such as amylase and cellulase) and antibiotics. These bacteria also promote plant growth and survival. In some area, *Avicennia marina* is the primary mangrove species, providing ecological and economic benefits, including timber, fuelwood, and attracting eco-tourism (Friis and Burt, 2020; Purahong et al., 2019).

II. Biodiversity of halophilic microorganisms in mangrove habitat

Microorganisms are the most abundant living beings on the planet, found in every corner of the world. Reports indicate that only about 1% of the planet's total microorganisms have been identified, and many of these organisms thrive in unexplored environments. One intriguing group of microorganisms is extremophiles, which can survive in extreme conditions. These microbes have developed useful adaptations in their genetic and metabolic processes that allow them to flourish in hostile environments. Extremophiles are classified based on the specific conditions they require for growth (Arora and Panosyan, 2019). Among extremophiles, halophiles represent a notable class. Halophiles are organisms that thrive in saline environments and are often referred to as salt-loving organisms. They require at least 0.2 M NaCl for growth and can survive under hypersaline conditions. Depending on their sodium chloride requirements, halophiles are categorized as slightly, moderately, or extremely halophilic. Slight halophiles grow in salinity levels of 0.2-0.85 M NaCl (1-5%), while moderate halophiles thrive in concentrations of 0.85-3.4 M NaCl (5-20%). Extreme halophiles are found in environments with 3.4 - 5.1 M NaCl (20-30%) (Mukhtar and Mehnaz, 2020). Halophiles can be found across Archaea, Bacteria, and Eukarya. In recent decades, many halophilic reaction mechanisms under high-salinity conditions have been identified to produce a variety of important biomolecules, leading to their consideration for biotechnological applications (Liu et al., 2019). They are used in a variety of industries, including remediated and the production of enzymes. Heavy metals such as mercury, arsenic, cadmium, and other heavy metals are bio-remediated by halophilic bacteria from wastewater. Because of their power, availability, and ease of cultivation, microbial pigments are attracting a lot of attention. Natural pigment-producing microorganisms have been used in the food industry, and microbial pigments have a wide range of uses in the food, cosmetics, pharmaceuticals, textile, and medical industries. Halophilic enzymes have a high content of acidic amino acids in their amino acid mixing composition and have adapted to high salt concentrations (Sekar and Kim, 2020).

III. Strategies used for osmo-adaptation in halophilic Microorganisms

Water is one of the most essential elements for life to survive. Halophiles have evolved especially genetic and physiological modifications to survive in severe (hypersaline) environments (Mukhtar *et al.*, 2019). Halophilic bacteria and archaea adapt to hypersaline environments through two mechanisms. The accumulation of compatible solutes (osmolytes) to the imposed osmotic pressure and the preservation of high intracellular ionic (K$^+$) concentrations for the adaptation of the entire intracellular enzymatic machinery to function in hypersaline conditions (Mukhtar and Mehnaz, 2020).

3.1 Compatible solute strategy

The first form (salt-out cytoplasm) is more flexible and common in several microorganisms found in natural environments. Removing as much salt as possible from the cytoplasm and accumulating low-molecular-weight organic compatible solutes to provide osmotic equilibrium. Many halophilic bacteria can produce their compatible solutes like glycine betaine and ectoine (Abosamaha *et al*., 2022). Compatible solutes can accumulate to extremely high intracellular levels, and their cytoplasmic pool size is determined by the external salinity (Leon *et al.*, 2018) By accumulating osmolytes, mostly halophilic bacteria balance their cytoplasm with the high salt concentration of the medium (compatible solutes) (Mukhtar and Mehnaz, 2020). Trehalose, proline, glycine betaine, ectoine/5-hydroxyectoine, glucosylglycerol, and dimethylsulfoniopropionate are all important examples of compatible solutes used by both marine and terrestrial members of the bacteria **(**Figure 1). Compatible solutes can be released into the medium in the natural environment by the death of organisms or a decrease in external salinity (Abosamaha *et al*., 2022)

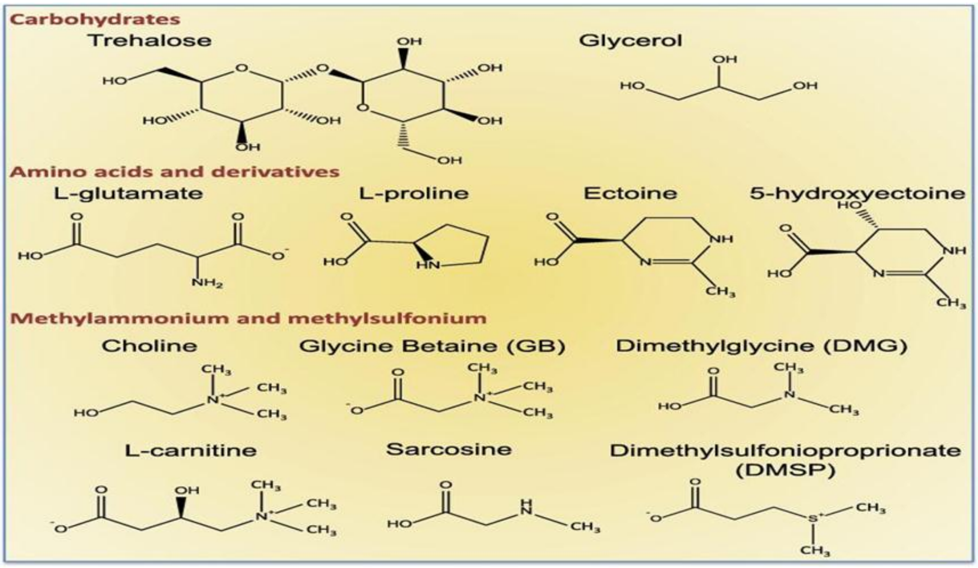

Figure 1. Structures of common compatible solutes include trehalose, glycerol (polyol), glutamate and proline (free amino acids), ectoine and hydroxyectoine, glycine betaine, and carnitine, dimethylglycine, and dimethylsulfoniopropionate.

3.2 Salt-in strategy

Many extreme halophiles are using the ' salt-in cytoplasm' method, which includes the accumulation of inorganic ions, especially Cl and K$^+$, in the cytoplasm to maintain osmotic balance (Abosamaha *et al*., 2022) Anaerobic halophilic bacteria (*Haloanaerobiales*) and aerobic halophilic archaea (*Halobacteriales*) all use the 'Salt in' strategy. To cope with external osmotic stress (high salt concentrations) these species use inorganic ions (K$^+$, Mg^{2+}, Na$^+$). The high salt concentration in the cell's external environment has adapted all intracellular machinery (Mukhtar *et al.*, 2019).

IV. Bacteria identification of halophilic microorganisms in mangrove habitat and Phylogenetic analyses

Molecular techniques enable the study of microorganisms by analyzing their proteins, DNA, and RNA. DNA analysis can identify specific genes or microbial species in a sample, while RNA levels indicate metabolic activity and provide functional information. Proteins can be analyzed through enzyme assays based on their amino acid sequence and structure (Saiz-Jimenez, 2017). The polymerase chain reaction (PCR) is a crucial molecular diagnostic tool that amplifies DNA segments, generating millions of copies quickly. Its versatility allows for applications in gene detection, forensic investigations, DNA fingerprinting, and genetic disease diagnosis (Malgwi et al., 2019). A typical PCR cycle involves three steps: denaturation, annealing with primers, and elongation, with copy numbers doubling each cycle.

The 16s rRNA gene, about 1500 base pairs long, is vital for bacterial analysis due to its conserved and variable regions, facilitating universal primer construction and taxonomic identification (Badnjevic et al., 2019). While 16s rRNA gene sequencing offers rapid identification of bacteria and archaea, it only resolves taxonomic classification to the genus level. Despite this limitation, it has been instrumental in phylogenetic comparisons (Byrne et al., 2018). Universal primers for the 16S rRNA gene are available online, with sequences stored in databases like the Ribosomal Database Project and GenBank (Raina et al., 2019). Bioinformatics plays a critical role in advanced molecular biology, providing tools for DNA alignments, phylogenetic studies, and database searches. Major DNA databases such as EMBL, GenBank, and DDBJ are essential for identifying microorganisms and are key resources for researchers.

V. Bioremediation process

Pollution refers to the introduction of harmful substances into the environment, affecting humans and other living organisms. Pollutants are detrimental solids, liquids, or gases that exist in higher-than-normal concentrations, leading to a decline in environmental quality (Manisalidis et al., 2020). Human activities, such as transportation, construction, and manufacturing, deplete natural resources while generating significant amounts of waste that contaminate soil, water, and air. These wastes also contribute to global warming, acid rain, and ocean pollution (Alzahrani et al., 2018). As a result of human actions, industrial processes, and heavy metals, the environment is experiencing rapid contamination. These pollutants are extremely harmful to animals and can cause cancer (Bibi et al., 2019). Through processes like bioconcentration, bioaccumulation, and biomagnification, heavy metals infiltrate the food chain and accumulate at higher levels, adversely impacting human health. Their harmful effects are observed in both adults and children, leading to disorders of the heart, kidneys, reproductive system, nervous system, and skeletal system. In severe cases, exposure can be fatal (Goyal, 2019). Recently, heavy metal contamination has become a serious issue; however, certain microbial isolates have shown potential for remediation, offering a glimmer of hope. The indiscriminate discharge of industrial effluents laden with heavy metals into our waterways and soil presents significant health risks to humans. Traditional heavy metal remediation techniques tend to be costly and ineffective at low concentrations. In contrast, microbial-assisted heavy metal remediation has emerged as a more affordable and efficient alternative (Goyal, 2019). Bioremediation, a process that utilizes microorganisms to degrade environmental pollutants, helps create a cleaner environment. Microbes perform bioremediation by leveraging their metabolic processes to eliminate contaminants. Given the high costs of chemicals, the significance of biodegradation is increasing, positioning bioremediation as the preferable choice for converting toxic substances into less harmful ones (Bibi et al., 2017b).

Bioremediation involves the degradation, elimination, alteration, immobilization, or detoxification of various chemicals and physical wastes by bacteria, fungi, and plants. Microorganisms require access to various materials and compounds for energy and nutrients to successfully combat pollutants. The effectiveness of bioremediation is influenced by the chemical nature and concentration of contaminants, the physicochemical characteristics of the environment, and the contaminants' availability to microorganisms. However, bioremediation is a complex process due to several factors, including the presence of a microbial population capable of degrading pollutants, the accessibility of contaminants, and environmental conditions such as soil type, temperature, pH, and the availability of oxygen or other electron acceptors and nutrients (Abatenh et al., 2017). Several methods exist for soil remediation, including physical, chemical, and biological techniques. While physical and chemical methods have drawbacks and harmful side effects, biological methods have proven beneficial and advantageous (Naeem et al., 2020).

5.1 The advantage and disadvantage of Bioremediation

It is a natural process that takes a little time, and it is an acceptable waste treatment process for polluted materials like soil. It takes very little time and effort, and it can also be done on-site without disrupting normal activities. This also removes the need to transfer large amounts of waste off-site, as well as the possible health and environmental risks that can occur during transportation. It is used in a cost-effective process because it loses less than other conventional methods (technologies) for hazardous waste cleanup. A significant treatment method for oil-contaminated sites. It does not contain any harmful chemicals. Fertilizers and other nutrients are applied to encourage active and rapid microbial growth. Their natural role in the environment makes them simple, less labor-intensive, and inexpensive. Eco-friendly and sustainable. Implementation is relatively easy (Abatenh *et al*., 2017).

Not all compounds degrade quickly and completely. Biodegradation products may be more persistent or toxic than the parent compound, according to some concerns. Biological processes are often very specific. The presence of metabolically capable microbial populations, suitable environmental growth conditions, and sufficient levels of nutrients and pollutants are all important site factors essential for success. Extrapolating from the bench and pilot-scale studies to full-scale field operations is hard.

Bioremediation techniques for sites with complex mixtures of pollutants that are not uniformly distributed in the environment need further research to develop and engineer. It takes much longer than other treatment methods like soil excavation and removal or incineration (Abatenh *et al.*, 2017) . Biodegradation is a very valuable and appealing option for remediating, cleaning, maintaining, and recovering contaminated ecosystems using microbial activity. Bioremediation has been used in a variety of areas around the globe with varying degrees of success. Generally, the benefits outweigh the drawbacks (Abatenh *et al.*, 2017).

VI. Types of bioremediations

Bioremediation is a pollution cleanup that is known to be environmentally friendly. Microbial cleanup may be performed in situ (at the source of contamination) or ex-situ (away from the source of contamination). In situ, remediation in the natural environment is thought to be slow and difficult to monitor and optimize the various parameters that affect bioremediation (Tekere, 2019).

The technique of ex-situ bioremediation is the method of bioremediation involves removing contaminants from a contaminated environment and transporting them to a new location for degradation. Bioremediation in situ These methods rely on the degradation of contaminated materials on the pollution site or place (Bibi *et al.*, 2017a, b). The ex-situ bioremediation are different types, Because of the low cost and low equipment requirements, land farming is one of the simplest bioremediation techniques. The main operations that stimulate the activities of autochthonous microorganisms to enhance bioremediation during land farming are tillage, which involves aeration, adding nutrients (nitrogen, phosphorus, and potassium), and irrigation (Azubuike *et al.*, 2016). The bioreactor is a vessel in which raw materials are converted into specific products by a series of biological reactions (Azubuike *et al.*, 2016).

Biopile-mediated bioremediation entails piling excavated contaminated soil above ground, accompanied by nutrient amendment and, in some cases, aeration, to increase bioremediation by enhancing microbial activity. The use of this ex-situ technique is becoming more common due to its beneficial advantages, which include cost-effectiveness and the ability to achieve successful biodegradation under the condition that nutrients, temperature, and aeration are all properly controlled (Azubuike *et al.*, 2016). Composting is characterized by microbial access to contaminants and the properties of the amending agents. This technique is environmentally friendly, has simple procedures, can manage large amounts of waste, and results in complete pollution mineralization. Bioremediation of soils polluted with petroleum hydrocarbons, solvents, chlorophenols, pesticides, herbicides, polycyclic aromatic hydrocarbons, and nitro-aromatic derivatives has been achieved using composting. Biopiles are more advanced composting systems that are more costly but allow for improved process control and performance (Dzionek *et al.*, 2016). Also, the engineered bioreactors are the preferred tool for doing this. Engineered bioreactors have been designed for use in bioremediation processes to achieve various remediation goals by providing optimal conditions for microbial growth and biodegradation (Tekere, 2019).

In situ bioremediation is classified into four techniques, bioventing is a technique that involves supplying oxygen to the unsaturated (vadose) zone of the airflow to enhance bioremediation by increasing the activities of indigenous microbes. The ultimate goal of bioventing is to achieve the microbial transformation of contaminants to a harmless state by providing nutrients and moisture to stimulate bioremediation (Azubuike *et al.*, 2016)**.** The biosparging method is similar to bioventing in that air is injected into the subsurface of the soil to stimulate microbial activity and promote pollutant removal from polluted areas. Unlike bioventing, however, the air is injected into the saturated zone, which can promote biodegradation by causing upward movement of volatile organic compounds to the unsaturated zone (Azubuike *et al.*, 2016). Also, Bioattenuation (Natural attenuation) is the elimination of pollutant concentrations from the surrounding area. Physical phenomena (advection, dispersion, dilution, diffusion, volatilization, sorption/desorption) may be used in biological processes. Chemical reactions, for example (ion exchange, complexation, abiotic transformation). Bioremediation would be supported by biostimulation or bioaugmentation if natural attenuation is not fast or enough complete (Abatenh *et al.*, 2017). Similarly, Bioaugmentation is the use of specific microbial strains or consortia as an efficient bioremediation technique for increasing bioremediation capacity (Hong *et al.*, 2020). Finally, Biostimulation is spiking nutrients to promote the proliferation of indigenous microorganisms. Carbon, nitrogen, and vitamin additions can all help bacteria degrade more efficiently (Yuan *et al.*, 2018).

VII. Heavy metals Toxicity

Anthropogenic activities, including urbanization, oil spills, pollutants, industrial waste, wastewater treatment plants, and harmful waste disposal strongly affect the mangrove habitats and they are highly susceptible to heavy metal accumulation from these sources. Heavy metal contamination in mangrove environments has been the subject of many studies around the world. When heavy metals have a specific density greater than 5 g cm^{-3}, they are considered dangerous contaminants because they can alter the structure of the environment and living organisms (Alzahrani *et al*., 2018). Naturally, heavy metals are present on earth as a high-density element. Cadmium, lead, mercury, zinc, chromium, and copper are the main dangerous metals. These heavy metals are highly toxic and can mainly contaminate soil water (Lata *et al*., 2019).

Heavy metal soil pollution is becoming a serious environmental problem. Not only do heavy metals destroy the soil's microbial ecology and decrease crop production, but they also threaten human health across the food chain (Lin *et al*., 2016). Via bioconcentration, bioaccumulation, and biomagnification phenomena, heavy metals enter the food chain and reach the top level, therefore adversely affecting human health. Their deleterious effects occur in both adults and children and include disorders of the heart, kidneys, reproductive system, nervous system, and skeletal system. It can also cause death in serious cases (Goyal, 2019).

At low amounts, heavy metals are cytotoxic and can cause cancer in humans. Heavy metals like cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), and molybdenum (Mo) are essential for living organisms in small amounts, but at higher concentrations, they can be harmful. Heavy metals are difficult to remove from polluted biological tissues due to their non-biodegradability, which is a serious concern for global health because of their lethal nature (Ojuederie and Babalola, 2017).

VIII. Heavy metals bioremediation

Coagulation, chemical precipitation, electrodialysis, evaporative recovery, floatation, flocculation, ion exchange, nanofiltration, reverse osmosis, ultrafiltration, and other methods are used in heavy metal degradation. Extraction, stabilization, immobilization, soil washing, and other Physicochemical methods Apart from the production of secondary noxious end-products, these methods are usually costly, even though they are successful. This is due to the high energy and chemical reagent requirements (Ojuederie and Babalola, 2017). Physical and chemical methods have many drawbacks and negative impacts, but biological methods have proven to be beneficial and have lots of advantages (Naeem *et al*., 2020).

Using indigenous microorganisms with mechanisms capable of degrading toxic heavy metals, or genetically modified microorganisms to treat contaminated environments by turning toxic heavy metals into non-hazardous forms, is a valuable way of eliminating toxic metal pollutants from the environment and stabilizing the ecosystem. Only microorganisms with demonstrated ability to remediate and withstand high toxicity should be used in the bioremediation process. Microorganisms are important in heavy-metal remediation since they have a variety of ways of enduring metal toxicity (Ojuederie and Babalola, 2017). In addition, bacteria are effective in bioremediation heavy metals processes. Adsorption, uptake, methylation, oxidation, and reduction are all processes that microorganisms have evolved to protect themselves from heavy metal toxicity. Heavy metals are absorbed by microorganisms both actively (bioaccumulation) and passively (adsorption) (Abatenh *et al*., 2017) depending on the type of microorganism and the heavy metal species (Lin *et al*., 2016).

IX. Heavy metals resistant bacteria

Heavy metals such as Cu, Zn, Ni, Cr, Co, Mo, Fe, and Mn are important micronutrients for biological species, but they are harmful to health and the environment at higher concentrations. Lead has no useful biological activities and is harmful to plants, animals, and people even at low concentrations (Mohapatra *et al*., 2019) while cadmium is the most hazardous heavy metal, with high stability and toxicity even at low concentrations (Nufla and Henagamage, 2019). The ability of metal-resistant microorganisms to be used as an ecologically friendly way of treating heavy metal-contaminated soil and water has increased in importance (Nufla and Henagamage, 2019).

Hexavalent chromium is a human carcinogen that has a wide variety of toxicity. *Bacillus pumilus* was found to be resistant to all the heavy metals tested, including Pb, Cd, Ba, Cr, Fe, Cu, and F, out of a hundred and twenty-eight. At neutral pH, only *Bacillus sp*. could stand up to Cr and up to 700 ppm, eliminating nearly 86 % of Cr. *Bacillus* cells had high adsorption level of chromium metal ions at pH 7 and 8. The efficiency of bioremediation has risen from 86% at pH 7 to 96% at pH 8. The importance of bacteria in an eco-friendly system of reducing environmental contamination is emphasized by pH. Many other microbes that have bioremediation potential can be isolated in the mangrove ecosystem (Sahoo and Goli, 2010).

Cadmium (Cd) is a toxic heavy metal that, through different natural and anthropogenic sources, enters the environment and is a potential danger to most organisms, including humans (Saini and Dhania, 2020). Cadmium rarely occurs in pure form in the ecosystem, but it typically forms complex oxides with lead, zinc, copper ore, zinc, sulfides, and carbonates. In comparison to cadmium sulfate and cadmium chloride, cadmium oxide is the least soluble in water (Abbas *et al*., 2018). Cadmium is released into the environment by various sources classified as natural and anthropogenic sources. The natural resources include the crust and mantles of the earth, such as rock weathering and volcanic activity. Anthropogenic sources include raw materials such as extracts from fossil fuels, phosphate minerals, electroplating, leather tanning, cement manufacturing, pesticides, fertilizers, anticorrosive agents treated and recycled materials, in particular zinc and copper (Abbas *et al*., 2018). Cadmium is a non-degradable metal that remains in the ecosystem after it has been released. The amount of this polluting toxic metal is rising at an alarming rate because of progressive industrialization. By ingestion or inhalation, humans are exposed to cadmium. Overexposure to cadmium can lead to bone disease, renal damage, and several forms of cancer (Saini and Dhania, 2020) Also, the International Agency for Research on Cancer (IARC) has classified Cd as a human carcinogen as a group I (Kirillova *et al*., 2017). Contamination of heavy metals and metal ions is a threat to the environment. The electronic and metal industries are releasing their waste into the ecosystem without treatment, which contains high levels of toxic cadmium and is directly toxic to the environment (Anburaj *et al*., 2017). Because of Cadmium multiple biochemical and genetic pathways, the genus *Bacillus* has demonstrated remarkable ability in the bioremediation of cadmium in the environment among the various bacterial genera actively involved. Cadmium and nickel toxicity has also been reduced by this genus. It may use probiotic genera such as *Bifidobacterium* and *Lactobacillus* to perform bioremediation (Goyal, 2019)

Lead may result from a variety of anthropogenic sources as well as geochemical processes. Ceramics, batteries, printing pigments manufacturing, pesticide production, fuel additive, photographic materials, explosive manufacturing, coating, automotive, aeronautical, metal smelting plants, and incinerators are major sources of lead that can contaminate the environment (Mohapatra *et al*., 2017). At low concentrations, lead, cadmium, and mercury are toxic to the human body. Lead enters the human body via contaminated water, polluted air, especially from cars, paint peeling, and the food chain through cereals, vegetables, fish, and meat (Wahab *et al*., 2017). Accumulation of Lead in humans has serious consequences, including neurodegenerative disease, renal failure, reproductive damage, and cancer (Sahoo and Goli, 2020).

Mercury is one of the most toxic heavy metals and poses a significant danger to humans, animals, and plants when released into the environment. Because of its toxicity and potential to penetrate the biological system at very low concentrations, it is known as a serious environmental pollutant. Mercury contamination poses a threat to aquatic environments and wetlands. A continuous biosorption process using *B. thuringiensis* isolated from mangrove sediments efficiently removes mercury from mercurial solutions. The *B. thuringiensis* strain is a cost-effective and efficient way to eliminate mercury from a contaminated environment. As a result, this strain is suitable for the detoxification of industrial effluents containing high mercury concentrations (Saranya *et al*., 2019).

Pseudomonas and *Bacillus* bacterial strains are commonly used to remove heavy metals from wastewater and soil due to their high metal binding affinities. The bacterial strain of *P. aeroginosa* isolated from mangrove soil is efficient in Zn solubilization (Kayalvizhi and Kathiresan, 2019). *Pseudomonas*, *Bacillus*, and *Enterobacter* are the most common bacterial genera identified for bioremediation of heavy metals through various biochemical and genetic pathways. These microbes provide us with effective tools for lowering heavy metal concentrations and thus toxicity in the environment (Goyal, 2019). When heavy metal concentrations increased in soil , the numbers of *Desulfococcus multivorans*, *lithotrophicum*, *Leptolinea tardivitalis*, and *Aminobacterium colombiense* increase. As metal levels are decreased, the abundance of *Nioella nitrareducens*, *Bacillus stamsii*, and *Clostridiisalibacter paucivorans* increases. These bacterial abundance aid in future monitoring of polluted mangrove sediment restoration programs. Bacteria, especially sulfur-related bacteria, may act as sentinels in polluted mangrove ecosystems, helping to diagnose and implement management and remediation programs (Fernández-Cadena *et al*., 2020).

X. Bioremediation Methods and protocols

Filtration, chemical precipitation, electrochemical treatment, oxidation/reduction, ion exchange, membrane technology, reverse osmosis, and evaporation recovery are some of the methods that can be used to remove heavy metals, including cadmium, from wastewater. Most methods, however, are costly, ineffective, and non-selective in the process of treatment. Many types of plants are used for heavy metal phytoremediation and the degradation and detoxification of pollutants. Many disadvantages that limit the use of Phytoremediation, it is more time-consuming than other methods of detoxification, and it is not feasible for fast treatment of heavy metal sewage (Anburaj *et al*., 2017).

Bioremediation is an innovative and promising technique for removing heavy metals from contaminated water and land. It is more appealing than physicochemical techniques because it is less expensive and more efficient at low metal concentrations (Saini and Dhania, 2020) Importantly, there is a potential for the use of metal-resistant microorganisms to treat soil and water polluted by heavy metals as an eco-friendly method (Nufla and Henagamage, 2019).

10.1 Bioaccumulation

The living and dead biomass of microbes has been used in the biosorption and bioaccumulation process for the effective removal of metal ions. Bioaccumulation is a process that is dependent, active, and partly reversible needs energy, and requires respiration (Wahab *et al*., 2017) Microbes have bioremediation processes that can take up cadmium ions and extract them from the solution. Understanding these processes is crucial for optimizing the bioremediation process's selectivity and capacity. Any bioremediation process's efficacy is determined by three factors: catabolic landscape, and physiochemical. The only biological process (metabolism-dependent) that is completely reliant on cell metabolism is catabolism. This involves actively transferring cadmium ions into the living cell via the plasma membrane into the cytoplasm, resulting in their accumulation in a cell compartment via sequestration or detoxification by the reductase enzyme (Jebril, 2020). Bioaccumulation is an active, energy-dependent mechanism that depends on microbial metabolism. It is a slow, irreversible, and complex process, as opposed to biosorption (Upadhyay *et al*., 2021).

10.2 Biosorption

Biosorption, on the other hand, is an independent, revisable process that does not need energy/respiration. Biosorption is one of the bioremediation processes in which biomass metal ion adsorption occurs due to interactions with functional groups of native proteins, lipids, and carbohydrates that make up the cell wall (Wahab *et al*., 2017). Biosorption is reversible, fast, and metabolically passive adsorption of metals process. Biosorption is a physicochemical process that involves several mechanisms (Upadhyay *et al*., 2021). The main advantage of biosorption is low operating costs, high capacity, metal recovery possibility, and efficient biosorbent revival (Chellaiah, 2018). Several biosorption mechanisms will be explained more below.

Physical adsorption, ion exchange, and chemical sorption between the ions and the microbial cell surface allow ion absorption. This cell surface adsorption occurs in both living and dead cells (Jebril, 2020). Carboxyl, hydroxyl, phosphate, and sulfhydryl are functional groups found on the bacterial cell wall that play essential roles in metal biosorption. In Gram-positive bacteria, active sites for metal binding processes include the peptidoglycan layer, which contains alanine, glutamic acid, meso diaminopimelic acid, teichoic acid, a polymer of glycerol, and other amino acids, and in Gram-negative bacteria, glycoproteins, lipopolysaccharides, lipoproteins, and phospholipids (Upadhyay *et al*., 2021).

A physical mechanism in which cadmium ions are adsorbed into the cell surface or chemical adsorption due to the specific component causes cell surface adsorption. Also, cell surface adsorption by the biological process of adsorption of cadmium ions to microbes that make an extracellular enzyme, lipopolysaccharides, or chelating polypeptides, which can precipitate cadmium ions by attaching them or decreasing them (Jebril, 2020). Metal-binding metabolites, such as extracellular polymeric compounds, are secreted by microorganisms. Polysaccharides, proteins, uronic acids, humic compounds, lipids, capsules, slimes, sheaths, and biofilms make up their structure. Biofilms serve as a binding matrix for heavy metals. Their most essential component is exopolysaccharide, also known as extracellular polymeric substances (EPS), which has the potential to sequester ions (Upadhyay *et al*., 2021).

10.3 Accumulation, Precipitation and Transformation of heavy metals by bacteria

Through unique active transport processes, microbes can absorb essential metals from their surroundings. Metals can sometimes be collected with the help of specialized metal-binding proteins generated by organisms (Upadhyay *et al*., 2021). The functional groups on the surface of the microbial cells connect with the metal ions, resulting in the formation of insoluble metal precipitates (Upadhyay *et al*., 2021). The bacterial cell wall is the first efficient compartment for adsorbing heavy metal cations because it contains many anionic functional groups capable of binding to heavy metals (Lin *et al*., 2016). Microorganisms change metals and metalloids by various mechanisms such as oxidation, methylation, reduction, and demethylation (Upadhyay *et al*., 2021).

XI. Cadmium and Lead resistant bacteria

Many microbes have bioremediation potential for cadmium and lead can be isolated from the marine, mangrove and soil ecosystems (Table 1, 2). Many studies have been carried out in the past to isolate and identify heavy metal resistant bacterial strains (Sahoo and Goli, 2020). *Staphylococcus aureus*, *Bacillus subtilis*, *Lactobacillus plantarium*, *Staphylococcus lugdunensis*, *Ralstonia metallidurans*, *Alcaligenes eutrophus*, *Serratialique faciens*, and *Bacillus thuringensis*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Pseudomonas fluoroscenes*, *E. coli*, *Comamonas testosterone* and *Klebsiella planticola* were the most common and studied bacterial species for their ability to accumulate cadmium. *Pseudomonas putida* degrades more than 80% of cadmium in less than 5 min at pH from 5.0 - 7.5. *Alcaligenes eutrophus* CH34 bacterial strain degrades up to 90% of the cadmium while *P. aeruginosa* which is one of the most important bacteria present in almost all polluted places showed high capacity to remove 75 to 89% of cadmium. *Pseudomonas aeruginosa* is one of the versatile and high-tolerance cadmium- resistant bacteria isolated from various ecosystem regimens. It can be applied as a suitable biosorbent for the elimination of cadmium and other heavy metals from solution, polluted waste, water, and soil (Chellaiah, 2018). Also, *Bacillus laterosporus* and *Bacillus licheniformis* were two bacterial strains that remove cadmium from water using the biosorption process to eliminate the toxic metals that are present in water and streams (Lata *et al*., 2019).

Dried biomass of some genera of Cyanobacteria, could be used in the bioremediation of cadmium in polluted wastewater of several industries. Marine-derived microbial biomass as the potent and environment friendly for bio-removal of cadmium in contaminated wastewaters (Anburaj *et al*., 2017). *Rhodobacter sphaeroides* was applied to bioremediate soils polluted with cadmium and zinc and practically used in the bioremediation of soils co-contaminated by Cd and Zn (Peng *et al.*, 2018). Also, Lactic acid bacteria have been reported to remove Cd and Pb from solutions and so represent a valuable tool for the decontamination of food and beverages from heavy metals. *Lactobacillus plantarum* and *L. fermentum* strains were shown to remove Cd (Kirillova *et al*., 2017) while *B. safensis*, which was isolated from Mangrove sediments showed 83.5% of cadmium removal. The cadmium biosorption capacities of Red Sea isolate *Alteromonas macleodii* and *Nitratireductor basaltis* in aqueous solution were 53 and 50%, respectively. Biosorption capacity of *P. mendocina* strain E678 was up to 99% in cadmium chloride. The bacterial strains isolated from textile dye effluents, *Bacillus licheniformis* showed a cadmium removal capacity of up to 98.34% as compared with other bacterial strains like *Salmonella typhi*, *Pseudomonas fluorescence*, and *E. coli,* which showed a maximum cadmium removal of about 92.4, 94.8, and 92.06%, respectively. Bioreactor for cadmium resistance bacteria is not new to the environmental industry (Abbas *et al*., 2018). Several cyanobacterial and bacterial strains, for example, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas fluorescence*, *Synechococcus*, *Anabouena*, *Oscillatoria brevis*, *Bacillus megaterium*, *Salmonella choleraesuis*, and *Proteus penneri* has been reported for their activities in Lead remediation. *Bacillus pumilus* is a type of *Bacillus*. At neutral pH, *Bacillus sp*. could withstand 900 ppm of lead (nearly 96 %). The capacity of bioremediation has risen from 96.8% at pH 7 to >99% at pH 6 and 8. The role of bacteria in an eco-friendly system of reducing environmental contamination is affected by the pH value.

XII. Importance of Marine Halophilic/Halotolerant Bacteria

Most polluted areas have high or low temperatures, alkaline or acidic pH, high pressure, and high salt concentration. Marine bacteria are classified as halophilic/halotolerant because they are naturally exposed to unfavorable conditions such as temperature, pH, salinity, conductance, seawater temperature, water currents, precipitation regimes, and wind patterns. They are well adapted to extreme environmental conditions and have complex adaptation characteristics, bacteria obtained from marine sources are believed to be better exploited in the bioremediation of hazardous metals and many other resistant xenobiotic substances (Amoozegar et al., 2005, Mohapatra *et al*., 2017). *Halomonas* can be isolated from saline soils, solar saltern, marine water, hypersaline and/or alkaline lakes, and non-saline hydrothermal vents, sewage, oilfields, sand, food material, plants, mural paintings, and other habitats. Many *Halomonas* species can detoxify various heavy metals via bio-adsorption, either directly with the cellular boundaries or via secreted molecules like extracellular polymeric substances EPS (Biswas *et al*., 2022).

Table 2.1 Cadmium (II) remediation by Halophilic/Halotolerant bacteria.

Cadmium resistant bacteria	**Isolation source**	**Tolerance**	**pH/NaCl**	**References**
Halomonas sp.	Hypersaline soil and water, Iran	5 mM	pH: 3.0, NaCl: 1%	Amoozegar *et al.* (2012)
Alcaligens sp., *Enterobacteriaceae* sp., *Kurthia* sp., *Staphylococcus* sp., *Vibrio* sp.	Sediments of Vembanad Lake, Kerala, India	0.05– 4.0 mM	NaCl: 5-15%	Sowmya *et al*. (2014)
Halomonas elongate Tetragenococcus halophilus	ATCC 33173 ATCC 33315	3 mg/l	pH: 7, NaCl: 10-20%	Siripongvutikorn *et al*. (2016)
Pseudoalteromonas sp. SCSE709–6	Deep sea sediment, south China	100 mg/l	pH: 6.5-7.5, NaCL: 3%	Zhou *et al*. (2013)
Vibrio harveyi 5S-2	Sediment, Alexander eastern harbour, Egypt	> 60 mg/l	pH: 7.2	Abd-Elnaby *et al*. (2011)
Pseudoalteromonas sp. CD15	Coral tissue, Awur Bay, Jepera waters	5 mg/l	pH: 7.0	Sabdono (2011)
Alteromonas macleodii ASC1	Sediment, Hurghada harbour, Red Sea	150 mg/l	pH: 6.0	El-Moselhy *et al*. (2013)
Bacillus sp. NT-1 *Enterobacter* sp. NT-5 *Aeromonas* sp. *Pseudomonas* sp.	Water and sediment of industrially polluted estuarine	400-500 mg/l	pH: 8.0, NaCl: 0.5%	Mathivanan and Rajaram (2014b)
Pseudomonas stutzeri Pseudomonas mendocina Alcaligens faecalis	Surface water of Cuddalore coastal	250 - 400 mg/l	pH: 7.0	Mathivanan and Rajaram (2014a)

Acinetobacter baumannii Bacillus licheniformis Lysinibacillus fusiformis	ecosystem, Tamil Nadu, India			

Table 2.2 Lead (II) remediation by Halophilic/Halotolerant bacteria.

Lead resistant bacteria	Isolation source	Tolerance	pH/NaCl	References
***Halomonas* sp.**	Hypersaline soil and water, Iran	5 mM	pH: 3.0-6.0, NaCl: 5%	Amoozegar *et al*. (2012)
***Bacillus* sp. Pb15**	Marine sediment, Sarno river mouth, gulf of Naples, Italy	4.82 mM	pH: 7.0, NaCl: 2.4%	Pepi *et al*. (2016)
***Alcaligens* sp., *Enterobacteriaceae* sp., *Kurthia* sp., *Staphylococcus* sp., *Vibrio* sp.**	Sediments of Vembanad Lake, Kerala, India	0.1–12 mM	NaCl: 5-15%	Sowmya *et al*. (2014)
Halomonas elongate*, *Tetragenococcus halophilus	ATCC 33173 ATCC 33315	1 mg/l	pH: 7, NaCl: 10-20%	Siripongvutikorn *et al*. (2016)
***Micrococcus luteus* DE2008**	Microcoleus consortium	3 mM	pH: 6.5-7.0. NaCl: 8%	Puyen *et al*. (2012) Maldonado *et al*. (2010)
Alcanivorax consortia	Sepetiba Bay, Brazil	6 µg/ml	Sea water	da Costa Waite *et al*. (2016)
***Acinetobacter* sp. THKPS16**	Sediment, meiliang bay, Taihu lake, China	100 mg/l	pH: 5	Ma *et al*. (2015)
***Klebsiella* sp. 3S1**	Wastewater treatment plant	3.4 mM	pH: 5	Muñoz *et al*. (2012)

XIII. Conclusion

Mangroves are salt-tolerant forest ecosystems, found in tropical and subtropical regions, often affected by heavy metal pollution that impacts plants, animals, and microbial diversity. This pollution necessitates attention due to its toxicity and accumulation in the food chain. Industrial effluents typically have high salt concentrations, rendering non-saline bacterial biosorption ineffective. However, mangrove microorganisms, particularly halophilic bacteria, are better equipped to tolerate these conditions and are promising candidates for bioremediation. Several halotolerant bacteria have been successfully isolated to remove heavy metals in high-salinity environments. Hence, exploring new bacterial species from mangroves or innovative techniques for heavy metal removal is vital, aligning with Saudi Arabia's future objectives.

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