Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
(Print copies up to 2016)
e-ISSN: 2395-3454

Vol. 23 No. 2 pp. 633-648 2024

Review Research Paper

https://doi.org/10.46488/NEPT.2024.v23i02.004

Open Access Journal

# Role of Biotechnology and Genetic Engineering in Bioremediation of Cadmium Pollution

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**Nat. Env. & Poll. Tech.**
**Website: www.neptjournal.com**

*Received:* 24-08-2023
*Revised:* 01-11-2023
*Accepted:* 02-11-2023

**Key Words:**
Bioremediation
Heavy metals
Cadmium
Anthropogenic
Environmental pollution

## ABSTRACT

Cadmium (Cd) is ubiquitous and an unessential trace element existing in the environment. Anthropogenic activities and applications of synthetic phosphate fertilizers greatly enhance the concentration of Cadmium in the environment, which proves to be carcinogenic. The long-term effects of heavy metals contamination on plants and animals have recently become a major public health concern. Thanks to the application of science and technology, new environmental initiatives can have a lower environmental impact significantly. The role of microbes is very well known and must be considered as potential pollutant removers. Microbial flora can remove heavy metals and oil from contaminated soil and water. In comparison to conventional techniques, bioremediation itself proved to be a more potent technique because the established mechanisms render it ineffective. Biotechnological advancements are inherently harmful to the environment because they have the potential to reduce metal pollution. Pollutants in the environment can be effectively removed using bioremediation. Both native and introduced species can thrive in a microorganism-friendly environment.

## INTRODUCTION

Currently, environmentalists are mainly worried about changes to biogeochemical cycles caused by the wide range of organic and inorganic contaminants generated by human activities (Vara et al. 2003). As industrialization spread, countries used a variety of remediation techniques for dealing with newly created pollution problems. Heavy metals are among the most pernicious of these environmental hazards. When compared to other organic contaminants like pesticides or petroleum by-products, heavy metals have a far longer half-life in nature. Because of this, the existence of heavy metals is cause for serious alarm. Variable quantities of heavy metals have increased over time alongside the expansion of the global economy, leading to environmental damage (Han et al. 2002). The presence of heavy metals severely harms all living things in the environment. Both the water supply itself and biomagnification can be a cause of heavy metal contamination. Air pollution is another potential cause of heavy metal contamination in mining regions (Santona et al. 2006). The Love Canal catastrophe at the American Niagara Falls (Fletcher 2002) described the devastating impact of heavy metals on the local human and animal population. There are now a number of traditional technologies in use for the elimination of heavy metals, but each has significant drawbacks, including a high initial investment and ongoing maintenance. In the process of removing heavy metals from the soil, beneficial soil components are also damaged by the chemical technique. Aside from the obvious health risks, chemical procedures produce a lot of slurry at a high cost per person (Hinchman et al. 1996).

Heavy metals can be classified as “essential” or “toxic.” Heavy metals such as Zn, Fe, Mn, and Cu are required for plant growth, whereas molybdenum, cobalt, and nickel are required for microbial cell maintenance. Some heavy metals, such as cadmium, lead, mercury, arsenic, and chromium, are harmful to plants, humans, and the microbial population and hence pose a risk to crops even in low quantities. Because these metals interfere with vital biological processes (photosynthesis, protein synthesis), plant development and productivity are hampered (Hall 2002). Cd causes Itai Itai, renal, and bone problems, but heavy metals, in general, cause cancer in humans (WHO 2007). Human actions and natural processes both have a role in the genesis of these heavy metals in the ground (weathering of rocks). Human activity includes but is not limited to, the extraction and processing of minerals and metals for use in manufacturing and everyday life. Human activity has resulted in a hundred times more Pb, Cd, and Zn emissions than those from natural causes.

Heavy metal contamination can be traced back to mining activities. Since their dangerous effects endanger human health, biodiversity, and ecosystems, they warrant severe environmental concern (Navarro et al. 2008).

The industrial revolution is directly responsible for the enormous growth in the prevalence of hazardous substances in the environment. Toxin build-up in the food chain has a negative influence on human health (Chaffei et al. 2004). Increasing concentrations of cadmium have a huge impact on the environment due to its toxicity and carcinogenicity. Human activities such as fertilizer application, sewage sludge disposal, and industrial waste disposal on land have all contributed to Cd accumulation (Naidu et al. 1997). As a result, Cd concentrations in food crops have increased. As a result, it alters the normal macronutrient-micronutrient ratio, slowing plant development. A range of cellular activities is affected when plants are exposed to even trace quantities of Cd (Hall 2002). Human health may suffer as a result of the rapid absorption of Cd from enriched soil by many crop species, resulting in high food consumption (Tripathi et al. 2005, Rani et al. 2008).

Furthermore, multiple Faisalabad businesses were discovered to be releasing effluent with high amounts of Cd, resulting in an increase of more than 200% in the amount of Cd observed in the land that was irrigated with this wastewater (Hussain et al. 2010). Plants serve as a key entrance point from the soil because they absorb it and then convey it to animals and people (Cheng et al. 2006). This heavy metal typically inhibits the growth of plants, decreases the availability of nutrients, and hampers the mechanism of absorption by plants (Ramon et al. 2003, Jun-yu et al. 2008). Even minute quantities of Cd in plants impact several biological processes (Hall 2002). Because Cd exposure reduces gas exchange reactions (photosynthesis and transpiration rates), plant growth is hampered (Januskaitiene et al. 2010, Wang et al. 2009, Ekmekçi et al. 2008, Wu et al. 2007, Sun et al. 2008).

Heavy metals in soil can be removed using different procedures. These procedures are depicted in Fig. 1. Biological methods have various advantages over physical and chemical approaches, including low cost, great efficiency, universal adoption, little environmental impact, and adaptability (it can be employed both ex- and in-situ). Gadd (2000) effectively used bacterial procedures to mobilize and immobilize heavy metals via different mechanisms such as biotransformation, complexation, sorption, sequestration, and other mechanisms. In recent years, the use of microbial technology to reduce toxic waste has grown in favor (Radhika et al. 2006). Park et al. (2008) reported the precipitation of Cd with sulfide by the activities of sulfate-reducing bacteria and achieved 99.7% results. According to Hadi and Bano, 2010 inoculating lead-polluted soil with diazotrophs dramatically boosted dry maize biomass. If plants are exposed to high amounts of Cd, the bacterial inoculants may aid in their growth. Plant growth-promoting bacteria can help mitigate the negative effects of environmental pressures such as salt, flooding, and metal stresses (Mayak et al. 2004, Grichko & Glick 2001, Burd et al. 1998).

Moreover, bacteria may serve as a disease defense for plants (Wang et al. 2000). Beta vulgaris's capacity to extract/accumulate Cd was greatly enhanced after being inoculated with Cd-tolerant bacteria like *Staphylococcus pasteuri* and *Agrobacterium tumefaciens* (Chen et al. 2013). When used for phytostabilization, plants with high Cd tolerance and root-holding capacity are ideal (Zhang et al. 2012). Strict crop species selection can boost plant metal stress resistance, although many soil microorganisms can also survive in metal-poor environments (Aleem et al. 2003). The uptake of Cd by the metal hyperaccumulator Salix caprea was reduced due to the presence of Burkholderia sp. bacteria in the rhizosphere, as reported by Kuffner et al. (2010). The bacterial strains show promise as inoculants to boost plant development even when exposed to hazardous levels of Cd in the soil.

Processes like global warming, depletion of the ozone layer, degradation of soil and water bodies via organic and inorganic activities, and other concerned environmental oddities have recently confronted humanity. These pose a threat to the world's existing biota because of the harm they can do to food supplies and human and animal health. Heavy metal pollution, in particular, is a major problem all over the world because of the harm it does to the environment and human health when it accumulates in the food chain. This review compiles expert opinions on the global concentration of Cd, the causes and consequences of Cd pollution, and new approaches to bioremediating Cd.

## HEAVY METALS

Phipps (1981) has developed the most widely used and well-known definition of heavy metals. Heavy metals are defined as those with an atomic number greater than 20 and a specific gravity of less than 5 g cm3. The production and utilization of several goods necessitate the use of heavy metals. Heavy metals are further categorized into -

a) **Essential/Non-Toxic:** All forms of life, whether plants, animals, or microorganisms, they are benefitted greatly from the presence of these essential metals, even in trace amounts. Iron (Fe), molybdenum (Mo), and manganese (Mn) are all required by all cells in the human body,

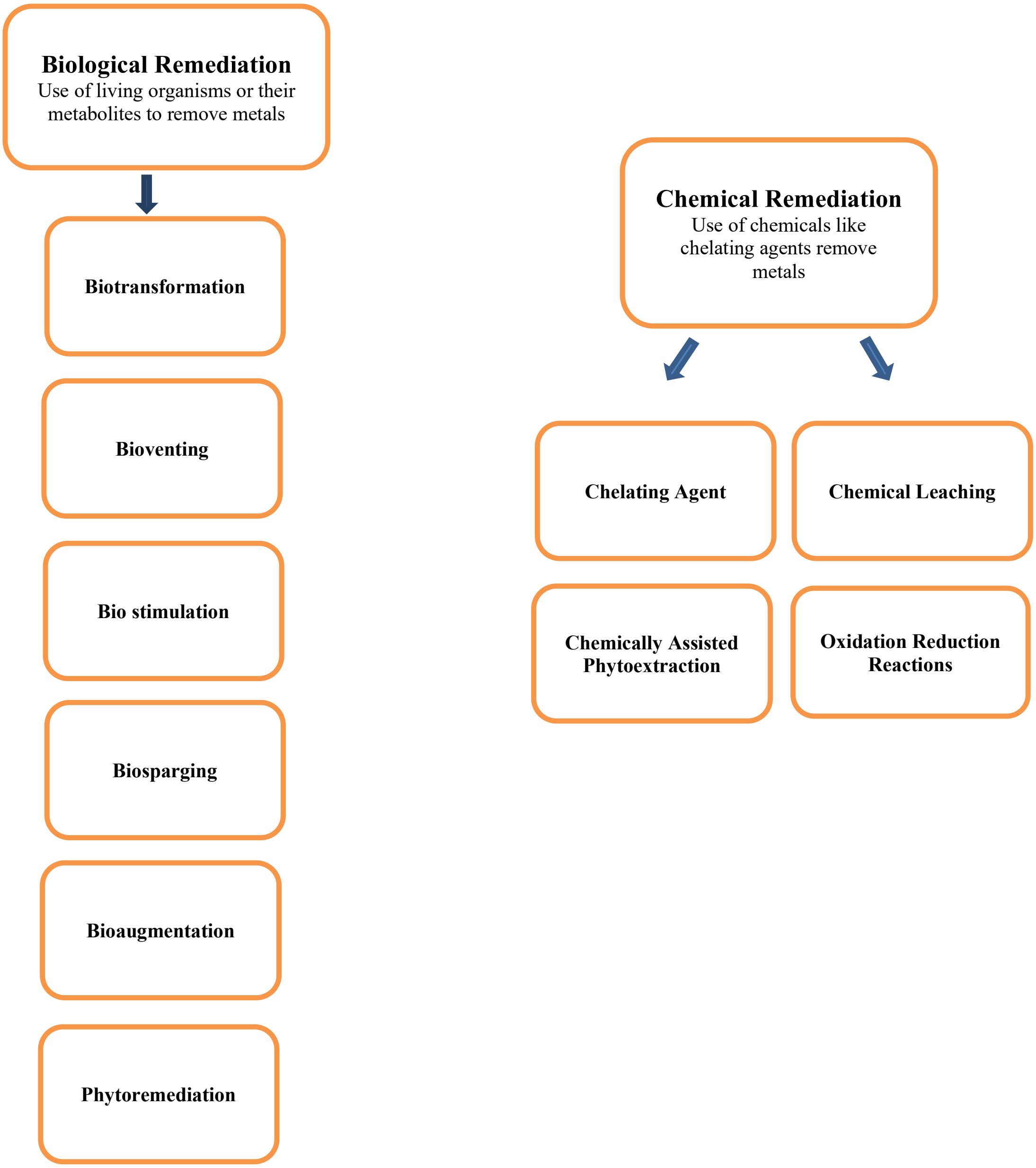

Fig. 1: General approaches used for metal remediation.

while animals require selenium (Se). Nickel (Ni), zinc (Zn), cobalt (Co), copper (Cu), and vanadium (V) are examples of heavy metals that are essential for life but also poisonous to cells (Meharg 2005).

b) **Toxic/Non-Essential:** These heavy metals are hazardous to any species that come into contact with them, even at minute concentrations, and yet they serve no biological purpose. Some examples of such metals are lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg), and chromium (Cr) (Sun et al. 2001).

## CADMIUM

At 4°C, Cd has a specific gravity that is about 8.65 times that of water. Natural cadmium is always bound to zinc; it never occurs by itself. It is mostly manufactured as a waste product during the smelting of zinc, lead, and copper ores. Although greenockite (CdS) is less well-known than zinc mineral sphalerite (ZnS), it is an extremely important mineral in its own right (Thomas 1992). Cd is found in the crust of the Earth at low concentrations (0.1 to 0.5 ppm) but in many different places. Approximately 5-110 ng L-1 of Cd has been found in ocean water, with the majority of this concentration being deposited in marine phosphates (Morrow 2001). Emitted through both natural and human-caused processes, Cd is most commonly found in the environment due to its use in Ni-Cd batteries, paint, electroplating, and solar cells, as represented in Fig. 2 (ATSDR 2008).

## CADMIUM POLLUTION

Heavy Cd contamination threatens the South and Southeast of the globe (Luo et al. 2012). Heavy metal pollution of China's agricultural soil has affected almost 20 million hectares, which might slow the country's food production (Wei & Chen 2001). Due to water scarcity, industrial effluents are increasingly being used for agricultural crop irrigation, contributing to soil contamination with hazardous metals (Luo et al. 2010). Cd is easily absorbed by living things due to its mobility and accumulation in the soil-plant system. While it serves no known biological purpose and is highly harmful to plants, animals, and humans (Lehoczky et al. 2000). The concentration of Cd, Ni, Cr, Zn, and Pb in urban soil was found to be higher than the allowable limit of surface soils (3 mg.kg$^{-1}$ soil) in a study by Malik et al. 2010, who argued for the urgent need for extensive baseline investigations of geographical distribution and remediation of heavy metals. Soil Cd limits vary by country, with Chinese limits set at 0.6 mg.kg$^{-1}$ (ESPA 2005), Dutch limits at 0.8-5 (Iram et al. 2012), and Indian limits at 3-6 mg.kg$^{-1}$ (ESPA 2005). (Awashthi 2000).

Shah Alam River is polluted by the trash from numerous factories in the textile, paper, ghee, tannery, sugar mill, and distillery sectors (Khan et al. 2010). In the Shah Alam River, the Cd concentration was observed to be ten times higher than the limit set by the WHO. Heavy metals (Cd, Cr, Co, Cu) were also shown to be polluting the Ravi River sediments by Rauf et al. (2009). Concentrations of Cd ranged from 0.99 to 3.17 micrograms per gram of dry matter. Metals like this accumulate in river sediments and then pollute the land above them. Kashif et al. (2009) studied heavy metal pollution in the Hudiara drain that runs near the Hudiara village in the region of Lahore in Pakistan. Due to the introduction of unknown polluted waste into the sewer, the metal pollution index increased. The accumulation of these metals in the food chain raises concerns about their potential hazard to human health.

Heavy metal contamination was also found in the municipal sewage water of Sargodha, Pakistan, as reported by Ahmad et al. (2011). Canola growth was stunted due to the high concentrations of Cd, Cr, and Pb in the wastewater. Rehman et al. (2008) analyzed the metal content of three industrial areas in Pakistan: Peshawar, Gujranwala, and Huripur. Industrial effluents with high concentrations of heavy metals are commonly utilized for irrigation by the local indigenous population, according to the paper. Crops that are subjected to this method absorb the metals, which then make their way into the human food supply. Another study used flame atomic absorption spectrometry to look for metal pollution in dust and soil samples obtained from several locations along the Islamabad Expressway (FAAS). Heavy metal buildup varied along the Expressway, although Cd was discovered at levels as high as 51 mg.kg$^{-1}$. Heavy metal pollution has increased as a result of urbanization, making its measurement essential (Faiz et al. 2009). In their study of 44

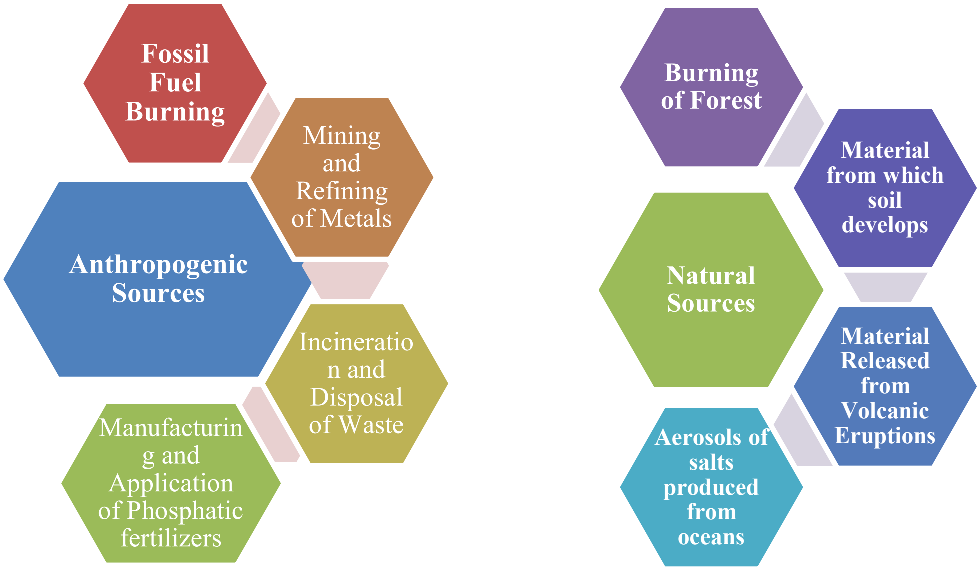

Fig. 2: Sources of cadmium.

samples, Ahmed et al. (2007) found that the Cd concentration in the blood of jewelers was 398 183 pg 100 $mL^{-1}$, whereas the Cd concentration in the blood of automobile workers was 768 180 pg 100 $mL^{-1}$. Cd levels were within WHO's tolerable range, but prolonged exposure could be harmful to those working in the jewelry and auto industries.

## CADMIUM CONTAMINATION AND OTHER HEAVY METALS

Heavy metal contamination in agricultural soils and crops is receiving increased attention in the south and southeast Asian countries like Pakistan, India, Bangladesh, China, Japan, Malaysia, and Indonesia due to their impact on human health, which will hamper the further life processes in these regions. Enhanced heavy metal contamination in northeast China's Phaeozem due to human and industrial activities was observed by Guo & Zhuo (2006). The accumulation of four metals (Pb, Cd, Zn, and Cu) was investigated, and a spectrum of heavy metal concentrations in soil was recorded (10-62, 0.01-3, 40-248, and 10-51 $mg.kg^{-1}$ soil, respectively). Previous data led them to believe that Cd, among other heavy metals, posed a unique environmental risk to the local area. Soil Cd is frequently found alongside Zinc, particularly in Zn and Cu ores. Plants may take up and translocate Cd as a result of a zinc deficiency (Koleli et al. 2004). In addition to raising Fe content, exogenous Cd treatment increased P, K, and Mn accumulation in wheat roots by limiting their transfer to the shoot (Zhang et al. 2002). Cd exchangeable percentages in soil were much greater than Cu and Pb exchangeable percentages. Cd was thought to be the most mobile element in the soil, making it easily available to crops and increasing the possibility that it would end up in human food sources. As a result, Cd pollution in agricultural soils became the region's top priority (Xiong et al. 2003). Cadmium (Cd) is a very poisonous metal that ranks eighth on the list of the most harmful elements, according to the US Environmental Protection Agency's (EPA) national priority list (HazDat 2008). Using edible crop components as metal accumulators causes spoilage, which limits food availability (Xiong et al. 2003). As previously discussed, Cd concentrations in soil and industrial effluent have exceeded the permissible level; nevertheless, the impacts of this increase on crop development, plant physiology, and biochemical properties have not been adequately examined.

## PHYSIOCHEMICAL METHODS FOR REMEDIATION

### Physical Methods

**On-site remediation:** It mainly involves the separation of contaminated soil from the uncontaminated one. But this procedure is not complete itself, it requires many further engineering measures (Herrero et al. 2005). With the help of this technique, we can create hindrances for the other contaminates in a specified area (Zheng & Wang 2002). We can use this technique when other methods are not economically feasible. Subsurface barriers are employed in this technique to remove the contaminated water from the soil which will help in the prevention of surface water and groundwater flow. The flow of uncontaminated water can be prevented from contamination after the utilization of these subsurface barriers (Zhu et al. 2012). Barriers can be used in the form of upstream, downstream, or surrounding the contaminated site. We can use these barriers in combination with the system where capping arrangements can be done properly which helps in maintaining the isolation of contaminated soil, and infiltration of contaminated water can also be restricted.

**Off-site remediation:** Before 1984, this method was commonly used for the remediation of contaminants from the soil. In this technique, the soil which is full of contamination can be replaced by uncontaminated soil. By doing so, it is going to enhance the efficiency of soil which will help in further dilution of the concentration of heavy metals (Khalid et al. 2017). This technique can be employed in 2 ways: 1. Soil spading 2. Importing new soil to the site.

### Chemical Methods

**Soil washing**: This is one of the widely considered methods of soil remediation. In this technique, different washing agents, such as inorganic, organic cheating agents, and surfactants, can be used. This technique also known as soil leaching or chemical extraction helps in the mobilization of organic and inorganic contaminants towards groundwater. A wide variety of reagents and chemicals can be utilized, such as EDTA, organic acids, cyclodextrins, and surfactants. Chelate EDDS was used for the chemical extraction ofZn and Pb (Hauser et al. 2005). Coal ash has become a popular material for the extraction of heavy metals. EDTA is well known for the effective removal of cationic metals rather than anionic metals.The soil wash method was developed for cadmium-contaminated paddy fields (Kimura et al. 2007). He performed it in three steps: (1) treating the soil with calcium chloride solution, (2) Eliminating Cd and $CaCl_2$ by washing of treated soil with $H_2O$, and (3) using of wastewater treatment system on site. The average cadmium concentration by two-thirds was reduced in rice grains. Soil fertility was affected but can be corrected afterward with this technique.

**Immobilization techniques:** This technique helps in the confinement of heavy metals with the help of immobilizing agents. Processes like adsorption, precipitation, and complexation have been employed. Immobilizing agents, which are organic and inorganic, can be used for the immobilization of heavy metals in contaminated soil (Shahid et al. 2013, Austruy et al. 2014, Ashraf et al. 2016). Heavy metals can get immobilized on solid surfaces, which helps reduce the bioavailability in soil. Various organic agents and amendments are widely used in this technique such as biosolids and animal manures. Organic agents such as biosolids have both positive and negative kind of impacts on this process. Positive impact means they are going to act as the best adsorbent for the stabilization of heavy metals in soil (Venegas et al. 2015, Shakoor et al. 2015). A negative impact was also reported for these organic agents (Cele & Maboeta 2016). The reagent di-ammonium phosphate was studied to be more efficient for stabilizing Cd and Zn in soil (Khan et al. 2015). Metals can easily form complexes with organic components (Shahid et al. 2013). Organic contents of the soil were increased by doing organic amendments (Bolan et al. 2014). Cellulose, lignin proteins, lipids, simple sugars, hemicellulose, and starch can form complexes with metals (Niazi et al., 2017).

## BIOREMEDIATION

Pollutants that are either organic or inorganic can be reduced to lower concentrations by using microbes, plants, or their metabolites. In the event of large volumes of effluents containing complex organic matter and low metal contamination, conventional Physico-chemical approaches for metal remediation are both prohibitively expensive and inappropriate. When the use of pure biosorption metal removal is not possible, a well-chosen consortium of metal-resistant cells in growth can guarantee improved removal by bio-precipitation, biosorption, and ongoing metabolic uptake of metals following physical adsorption (Gadd 2004). Researchers have recently discovered that bacteria, yeast, and fungi isolated from polluted environments may effectively scavenge metals. Some bacterial strains have a high tolerance for different metals, making them ideal for the simultaneous removal of those metals from wastes (Park et al. 2008). Everything is ready for metal collecting to be applied to growing microbial cells that are resistant to metals.

## BIO-AUGMENTATION

This subfield of bioremediation involves the external application of microorganisms that have been previously

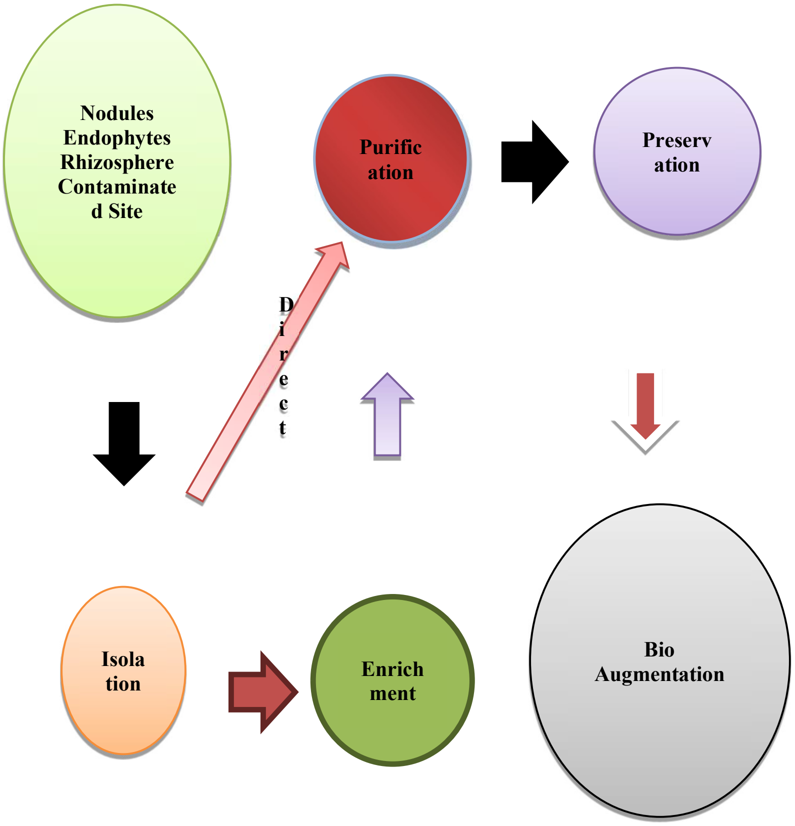

Fig 3: Schematic diagram of isolation and inoculation of microbes from source to end user.

isolated from their native environments (such as the rhizosphere, nodules, and polluted areas), purified, and enriched in a controlled laboratory setting (Fig. 3). The goal of introducing new microorganisms from outside the host organism is to increase the abundance of beneficial microorganisms in a given area. The toxicity of metals makes them an obstacle to the growth of microorganisms in both soil and water. What this means is that bio-stimulation or increasing a targeted population of microorganisms in a polluted place is necessary for decontamination (bio-augmentation). Some researchers have isolated bacteria from polluted places and then enriched them to be more effective against that particular pollutant (Kuffner et al. 2010) before applying them there. Inhibiting bacterial development, cadmium is a powerful metal (Prapagdee & Watcharamusik 2009). Cd-resistant strains were obtained from several contaminated locations and tested for Cd scavenging in a range of environmental settings by many researchers. Soil contaminated with metals may be more habitable for bacteria with a high tolerance for the elements. Heavy metal resistance, the mobilization or immobilization of metals in soil, and metal uptake by plants have all been studied extensively and found to correlate well (Kuffner et al. 2008, Gadd 2000, Aleem 2003).

## PHYTOREMEDIATION

Phytoremediation refers to the practice of using plants to remove and bind to pollutants. The phytoextraction of Cd from soil using three plant species was conducted with and without inoculation. The uptake and buildup of Cd in the plant's foliage from calcareous soil were dramatically enhanced by bacterial inoculation. Cd buildup in plants was inversely associated with a decrease in plant fresh weight. It was shown that under inoculation conditions, *Amaranthus retroflexus* accumulated the most Cd of any crop tested (including sunflowers and alfalfa) (Motesharezadeh et al. 2010). The potential for metal extraction by four bacterial species isolated from a radish plant (Chen et al. 2010). Improvements in both growth and Cd extraction from the soil to various plant sections were observed after bacterial inoculation. The inoculated bacteria produced siderophores, IAA, ACC-deaminase (inhibit ethylene synthesis), and solubilized inorganic P, all of which contributed to the enhanced growth. Microbial activity in soil increased cadmium's solubility and mobility, making it easier for the radish to absorb. Getting metals out of contaminated soil is a complicated process that relies heavily on bacteria. Pseudomonas and Bacillus, two common bacteria, increased Cd extraction from polluted soil by 58% to 104%, respectively. For the aforementioned bacterial strains, tomato cadmium absorption was increased by 92.1-113.0 percent compared to the non-inoculated control group (He et al. 2009).

Vegetables high in chlorophyll are less likely to contain metals than other vegetables. Two leafy vegetables were studied for their sensitivity to Cd stress and reported negative effects by Liu et al. (2007). Both plants absorbed significant amounts of Cd through their root cell walls, while *Brassica pekinensis* had larger Cd concentrations in their shoot. Protein levels rose in response to Cd stress and were associated positively with soluble Cd. Plants' metal accumulation is influenced by the metals' solubility, mobility, and velocity of transfer from the soil to the roots (Kumar et al. 2009). Vetches had the most metal uptake, while lufa had the lowest. In addition, heavy metals like Cd accumulate more in the leaves of vegetables than in the roots. Evidence was presented that vegetables have the potential to function as a hyperaccumulator for in-situ metals cleanup. Higher biomass and greater Cd extraction from polluted soil were observed in Solanum nigrum (Ji et al. 2011). Double-cropping this crop in contaminated soil was shown to dramatically lower Cd levels in field trials. These qualities are what made the Solanum nigrum a viable option for metal extraction.

The accumulation of Cd at higher trophic levels of the food chain has made the worldwide problem of remediating Cd-contaminated soil even more pressing. The ability of Cd hyperaccumulators to withstand and take up considerable quantities of heavy metal from soils makes them an interesting study subject. Species of plants vary in their ability to hyperaccumulate Cd. Since Cd has weak affinities with soil ligands, it can be readily taken up by the plant's roots and then distributed throughout the rest of the plant's aerial parts (Sanit`a Di Toppi & Gabbrielli 1999), pH, temperature, the content of Cd in media, and the concentration of other components are all responsible for plant remediation of Cd (Yang et al. 1998). Species of plants have been identified as Cd hyperaccumulators due to their ability to store up to 105 $mg.g^{-1}$ Cd in their shoot dry weight (Baker & Brooks 1989).

*Cd hyperaccumulation* in *Thlaspi caerulescens* was first documented in the early 1990s. The first signs of Cd toxicity in *T. caerulescens* appeared at a concentration of 200 M, indicating a substantially higher tolerance to the metal. Cd was notably concentrated in the shoots of *T. caerulescens*, suggesting that it was translocated from the solution to the upper parts of the plant (Brown et al. 1994). The *T. caerulescens* hairy root culture also demonstrated Cd remediation from an aqueous solution (Nedelkoska & Doran, 2000). "These findings verified the use of *T. caerulescens* as a hyperaccumulating plant for the removal of Cd contamination. Two species, *A. halleri* and *T. caerulescens*, were observed to hyperaccumulate Cd in addition to Zn (Cosio et al. 2004). Cadmium of *A. halleri* was found in the mesophyll of leaves (K¨upper et al. 2004). However, *T. caerulescens* and *A. halleri* were discovered to be low-biomass plants that could not adapt to a wide variety of environments. As a result, several alternatives to *T. caerulescens* and *A. halleri* were recommended, including *Calamagrostis epigejos*, *Sedum* species, *Brassica* species, and *Solanum nigrum* (Lehmann & Rebele 2004, Sun et al. 2009, Wei et al. 2006, Xiao et al. 2017, Li et al. 2018, Quartacci et al. 2006, Sheng & Xia 2006, Liu et al. 2011). *C. epigejos* grows quickly, even in poor, sandy soils or wet, marshy areas, and it can withstand harsh weather and high winds. Cd uptake was investigated, and limited root-to-shoot transfer was detected; this suggests that the plant can provide greater ecological value through phytostabilization than through phytoextraction, thanks to its high tolerance for heavy metals (Lehmann & Rebele 2004).

As well as Cu and Zn, *S. nigrum* has been found to accumulate significant amounts of Cd (Wei et al. 2005). There was also a study that looked at how EDTA affected Cd absorption by *S. nigrum*. It was reported that only a small dose of EDTA (0.1 $g.kg^{-1}$) in soil could successfully promote phytoextraction of Cd. Still, a large dose (0.5 $g.kg^{-1}$) harmed plant growth and reduced biomass, rendering the phytoremediation approach less efficient (Sun et al. 2009). The ability of *S. nigrum* to enter a blooming stage has been investigated in a subsequent study (Wei et al. 2006). Collectively, these investigations demonstrate that *S. nigrum* significantly accumulates high levels of Cd and contributes to the regulation of pollution in Cd-contaminated soils. *Sedum alfredii*, another plant, also showed considerable promise for Cd clean-up. This research demonstrated that exposure to high Zn concentrations results in elevated Cd levels (Xiong et al. 2004). As Cd and Zn concentrations rise, the amount of both metals found in leaves and stems rises as well. This finding confirmed that *S. alfredii* functions as a hyperaccumulator of Cd and Zn. Soil DC supply and additions like humic acid and compost increased Cd extraction by *S. alfredii* by a factor of 2 (Xiao et al. 2017). By decreasing ion mobility in contaminated soil, *S. plumbizincicola*, another species, has been shown to increase Cd and Zn content when EDTA is added (Li et al. 2018).

Phytoextraction of Cd from the large-sized plant *Brassica juncea* (Indian mustard) was found to be on par with that from *T. caerulescens*. Unlike a Cd-sensitive species, *B. juncea* plants can withstand high levels of Cd stress (Quartacci et al. 2006). Lipid alterations in the cell membranes of *B. napus* were seen with direct exposure to cadmium, suggesting that this species of *Brassica* is more resistant to Cd than its relatives (Sheng & Xia 2006). It was discovered that six different kinds of the plant *B. pekinensis*, often known as Chinese cabbage, were able to extract a large quantity of Cd from soil (Liu et al. 2011).

More effective soil plants for Cd clean-up have been the subject of hydroponic system research. Arundo donax's phytoremediation capability was investigated through experiments in both soil and hydroponic systems. Because both the Bio Concentration Factor (BCF) and the Translocation Factor (TF) were greater than 1, the authors concluded that Cd was taken up significantly more efficiently in the hydroponic system than in soil cultures but that high exposure to Cd caused the plant to exhibit antioxidant stress (Khankhane et al. 2017). Garlic (*Allium sativum*) grown in a hydroponic system has been shown to accumulate high levels of cadmium (Cd) in its bulb, shoot, and root, proving that garlic has the potential to remove Cd from its solution and store it there. Increases in $Cd^{2+}$ concentration increase the quantity of Cd in garlic roots. Researchers observed that the plant extracted Cd at a rate 1826 times higher than the control but that only a trace amount of Cd is accumulated in garlic bulbs and shoots. (Jiang et al. 2001). Growing *Bidens pilosa* on soil resulted in 405.91 $mg.kg^{-1}$ of Cd accumulation in its shoots while growing it in nutrient solution resulted in 1651.68 $mg.kg^{-1}$ (Dai et al. 2017). There are a few species of hydroponics plants that are hyperaccumulators of Cd, and they include *Coronopus didymus* and *Abelmoschus manihot* (Sidhu et al. 2017). In *C. didymus*, TF is more efficacious than BCF. A. manihot's BCF values were found to be more than the reference value, and its TF values were also greater than 1 after being treated with Cd at 15-60 $mg.kg^{-1}$ (Wu et al. 2018). Superoxide anion amount, hydrogen peroxide content, and antioxidative activities were all shown to rise in both the roots and the shoots, all of which helped with the detoxification process (Sidhu et al. 2017, Wu et al. 2018). Because of this, *C. didymus* and *A. manihot* can be used as Cd hyperaccumulators to clear Cd from working-field environments.

## MECHANISMS OF CADMIUM REMOVAL

Soil contamination can occur when any pollutant, organic or inorganic, comes into contact with soil components (Fig. 4). But when heavy metals are bound to the organic matter and microbial cells in the soil, they are not destroyed. They can only be released slowly, limiting their bioavailability to plants.

### Biosorption

Metals can be bound to the surface of cells without expending any energy (passive biosorption) or to the inside of cells at the cost of energy (active biosorption) by the use of functional groups and proteins (Das et al. 2008, Goyal et al. 2003). Metals can be physically adsorbed on the surface of microorganisms and organic molecules in a quick process known as passive biosorption. In contrast, the more involved process of active biosorption might take a while. Biosorption is essential for both live and dead biomass. However, bioaccumulation is unique to the living organisms.

Biosorption is a complex process that is regulated by various factors such as temperature, pH, biosorbent concentration, and presence of contaminants. With the rise in the concentration of contaminants, biosorption tends to decrease. In this technique, the amount of biosorbent also plays an important role, which restricts the sorption process by bringing conformational changes in binding sites within the biosorbent (Das et al. 2008). The biosorption of Cd can be affected by temperature, pH, and time, which researchers have already reported. In general, it is the notion that with each degree rise in temperature increases the rate of reaction of the biosorption process by 10 times. Temperature factor plays a partial role in this process. When it is in the range of 25-30°C, the adsorption of cadmium occurs (Zeng et al. 2010), while at the range of 20-35°C, the biosorption process is not affected (Aksu et al. 1992). The influence of pH on the bioavailability of metals in the solution has been reported, which in turn affects the functioning of the carboxyl group. At higher pH, this carboxyl group gets activated and reduces metal availability by sorption-precipitation mechanisms (Kratochvil & Volesky 1998).

**Microbial biomass as biosorbent:** Heavy metals can be absorbed by microbes thanks to the presence of different functional groups. Cell walls are mostly composed of cellulose in algae, chitin and chitosan in fungi, and peptidoglycan in bacteria. These chemicals have a negative charge on microbial surfaces because of the presence of carboxyl, sulfhydryl, and sulfate functional groups (Das et al. 2008). To effectively bind positively charged metals, these active sites are required to be negative in charge. Cadmium can get adsorbed on the microbial and plant biomass by occupying active sites. pH influences the charges in soil. Acidic soils carry a positive charge, while basic soils carry a negative charge. In this case, the interaction between soil-metal and microbe would be a sandwiched kind of reaction for metals like cadmium. Soil health can be improved by the addition of organic matter, which provides negative charges to the particles that help in metal sorption by physical adsorption or ion exchange mechanisms. Metal bioremediation has been linked to a wide variety of tolerance and resistance mechanisms (Giotta et al. 2006).

Biosorption of Cd was effective for the photosynthetic bacterial species such as *Rhodobacter* and *Rhodovulum* depending upon their growth conditions, whether it is dark or light under aerobic and anaerobic processes. It was reported by Watanabe et al in 2003 that *Rhodovulum*s species had a maximum adsorption capacity of Cd in aerobic dark Kf= 17.44 and Kf= 1.27 for anaerobic light growing conditions. Biological strains were used for the biosorption of cadmium since concentrations were discovered to be too high for safety (Abou Zeid et al. 2009). Biosorption of Cd

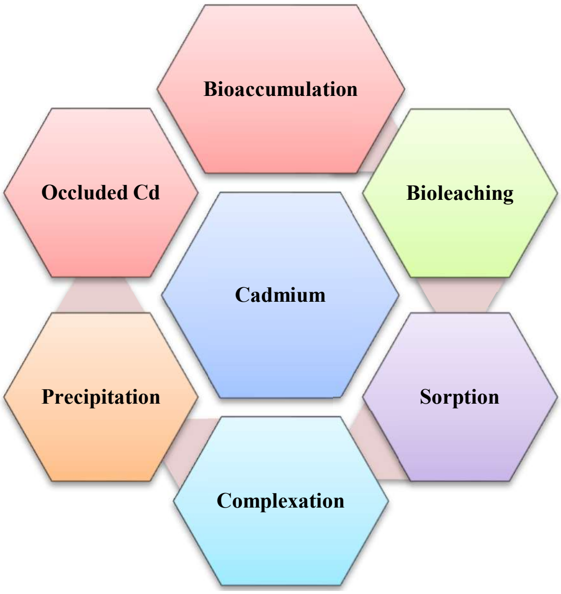

Fig. 4: Interactions of cadmium in soil.

by *Pseudomonas mendocina* proved efficient. Studying the optimal conditions for Cd uptake, researchers found that 2.5 ppm Cd, pH 7, and 30 °C were optimal. Different bacterial species, as described by Kumar et al. (2010), have been shown to reduce the stressors of numerous metals by a process called biosorption. Aspergillus niger reduced Cd stress by 50%, whereas *Pseudomonas* was the most effective metal-removal bacteria.

There was a positive association between Cd depletion and protein synthesis, suggesting that the protein generated by the implanted microorganisms plays a major role in Cd removal (Chovanova et al. 2004). Even though bacteria are abundant in soil and play a key part in biosorption, fungi efficiently adsorbed metals on their surface because of their higher biomass. Biosorption of Cd, Cu, and Pb by fungal cells was highly efficient. The results of the study's biosorption of metals were best explained by the Langmuir model. Dried fungal biomass was shown to be the most effective at absorbing heavy metals at a pH of 6. (Say et al. 2001). Yeast's ability to biosorb metals via cell

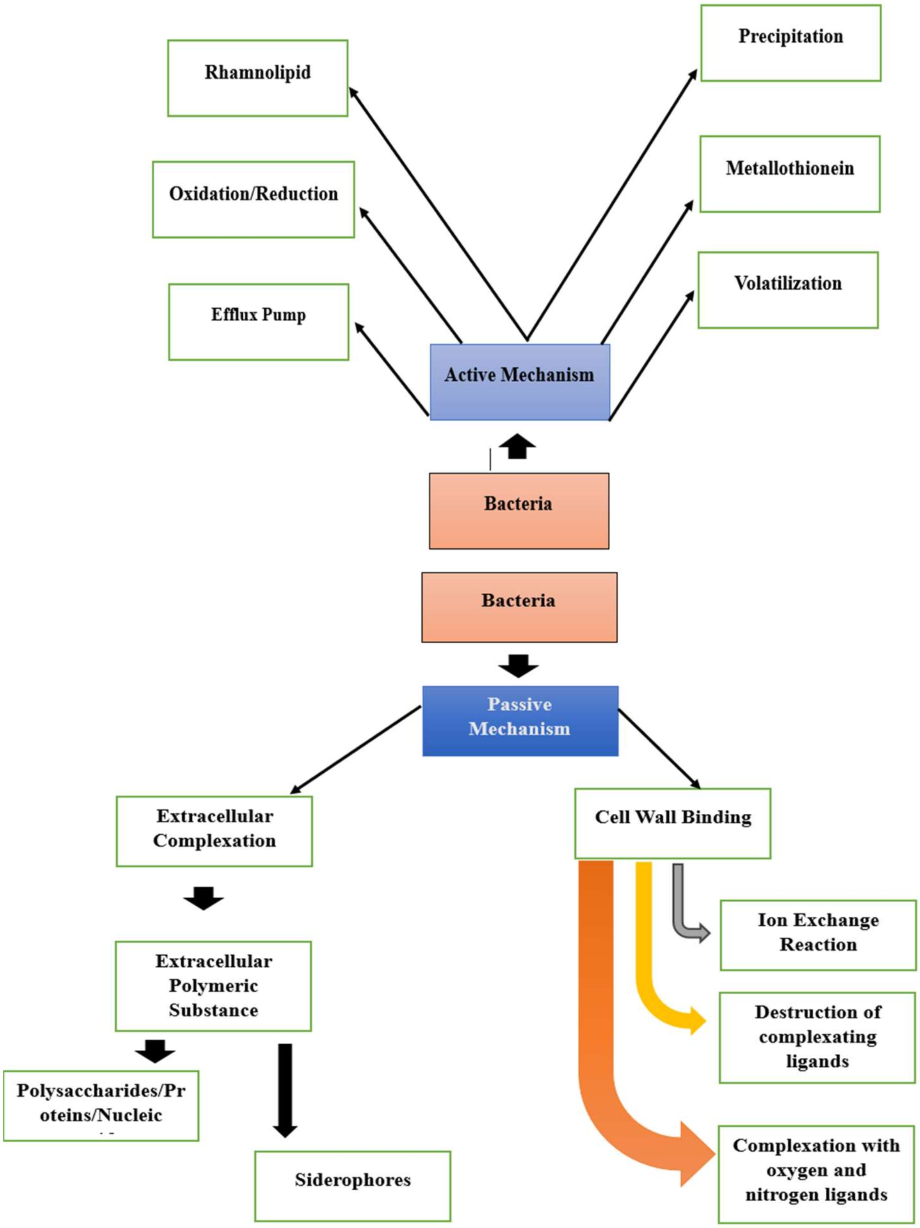

Fig. 5: General cadmium resistance mechanisms operating in bacteria.

surface adsorption was crucial. Cd uptake by exponentially growing yeast was greatest when inoculated at 30 degrees Celsius (Anagnostopoulos et al. 2010). The growth of *Rhodobacters phaeroides* was studied by Bai et al. (2008) to learn more about the kinetic characteristics and mechanism of Cd removal. With an R2 value between 0.9790 and 0.9916, the second-order equation was used to fit the data on the elimination process. In addition, bio-precipitation as Cd sulfide was revealed to be the primary route for Cd elimination, with bio-sorption only playing a secondary role.

### Bio-precipitation

The precipitation mechanism could be used to get rid of cadmium. When there are more carbonates, phosphates, and sulfides in the soil (Wang et al. 2002, 2001), chemical precipitation is a major contribution. When bacteria exist that can hydrolyze the aforementioned chemicals and release the appropriate anions for metal precipitation, bio-precipitation becomes the most common method. As a result of the widespread interest in the recently published study on metal precipitation by microbial manipulation, other investigations on the topic have been reported (Seetharam et al. 2009, Diels et al. 2006, Wang et al. 2001, 2002, Sharma et al. 2000, Bang et al. 2000). Metals are removed via bio-precipitation after being reduced in concentration via sulfate reduction, which is aided by the use of a variety of organic waste as a carbon source for SRB (Diels et al. 2006). It was discovered that Pseudomonas aeruginosa was responsible for the removal of Cd from hydrothermal vents through precipitation; the bacteria hydrolyzed thiosulfate, releasing sulfide ions that interacted with Cd to create cadmium sulfide precipitates (Wang et al. 2002).

## MECHANISM OF CADMIUM RESISTANCE IN BACTERIA

For the detoxification of cadmium and other heavy metals, eukaryotic microorganisms exhibit a special mechanism that binds with polythiols. The presence of plasmids in bacteria promotes resistance against heavy metals. Resistance mechanisms in bacteria against cadmium employ various mechanisms, which are as follows (Fig. 5).

### Efflux Mechanisms

A number of efflux mechanisms have been studied so far. The presence of integrated membrane proteins in the efflux system helps in the protrusion of contaminants from inside of the microbes into the environment. A very well-known efflux mechanism has been reported in *S. aureus* (Andersen 2015). With the advancements in the field of bioinformatics maximum number of efflux pump proteins were identified in *S. aureus,* which were encoded by either plasmids or chromosomes (Schindler et al. 2015). These efflux pumps were further categorized into 5 different families.

1) the resistance-nodulation-division (RND) superfamily,
2) the ATP binding cassette (ABC) superfamily,
3) the multidrug and toxin extrusion (MATE) family,
4) the small multidrug resistance (SMR) family, and
5) the major facilitator superfamily

There are various RND transporters, like the AcrAB–TolC complex and the MexBA–OrpM complex, which are present in *E. Coli* and *P. aeruginosa*. *B*oth are gram-negative. With these transporters, periplasmic adaptor proteins, transmembrane pumps, and the outer membrane channels were also present, which helps in the efflux of metals. Gram-negative bacteria that possess RND transporters are responsible for the resistance against multidrug. Protein AcrB, which is present in *E. Coli,* has a homologous protein that was identified in the *S. aureus* named FarE, which is also a part of the RND efflux system. Thus, the resistance against metals in *S. aureus* was identified due to the presence of FarE when it combines with fatty acids, which act as the substrates (Alnaseri et al. 2015).

*Alcaligens eutrophus* strain CH34 was found in sediments or soils with a content of heavy metals. This strain grows at the expense of a variety of organic substrates with the exclusion of sugars, oxidative metabolism, facultative chemolithotrophy, and the presence of megaplasmids conferring resistance towards heavy metals (Collard et al. 1993). Plasmid pMOL 30 is responsible for the resistance towards $Cd^{2+}$, $Co^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Hg^{2+}$and $Ti^{2+}$ (Diels & Mergeay 1990). In this plasmid, the CZC gene cluster ensures resistance to these metals. This gene cluster includes the operon (structural genes), upstream of which a regulatory region is located. When compared with the sequences of corresponding putative proteins, the strongly hydrophilic czcA emerges as a transmembrane protein, which ensures a cation/protein antiporter efflux. In association with czcB and czcC, czcA allows the efflux of heavy metal ions. A cadmium-resistant micro-organism identified as an *Azomonas agilis* PY101 has been isolated from a contaminated stream, which accumulated high levels of cadmium by the formation of cadmium sulfide in the cell (You et al. 1996, You & Park 1998).

**Enzymes responsible for the impermeability of bacterial cell walls to the cadmium:** Cadmium makes its way into the cell as a toxic substrate of zinc and manganese transport systems, which are present in Gram-negative and Gram-positive bacteria. Both transport systems are encoded by chromosomes (Filice et al. 2016). The concept of impermeability was best shown in B. Subtilis, which is associated with chromosomal mutations. As a result of these mutations, the entry of cadmium is restricted by membranes of the transport system (Zheng et al. 2016).

**Enzymes catalyze the biotransformation of cadmium into non-toxic forms:** For the remediation of heavy metals many organisms, such as bacteria and fungi, employ different strategies of detoxification processes. During the detoxification process, changes in the valence state of organometallic metals or compounds occur (Dixit 2015). This biotransformation results in valency change, which further results in the production of less volatile and toxic compounds. For example, there is the reduction of mercury ions into metallics mercury and the oxidation of arsenite to arsenate. In comparison to other heavy metals, the actual mechanism required for the conversion of cadmium to $Cd^0$ is still unknown (Guo et al. 2016). Methylation is a widely used and most common detoxification mechanism that results in the transformation of metals into their organometallic compounds. Mercury and lead are metals that can undergo methylation, which produces free metal forms that are less toxic than the actual ones, such as methyl mercury and dimethyl mercury are more toxic than mercury. Methylated products form volatile compounds following chemical and microbial degradation mechanisms. There is only one report that showed the biotransformation of cadmium and lead (Pavic´ et al. 2015).

**Cadmium ions binding:** Metallothionine, metallo chaperons, cell wall components (exopolysaccharides), surface factors, and their binding to intracellular binding proteins provide an important strategy for bacterial species to combat cadmium-increasing concentrations. In *Arthobacter viscosus* and *Klebsiella aerogenesis,* cadmium can bind with the capsular surface, while in other bacteria, cadmium binds to insoluble cell-bound $CdHPO_4$ (Coelho et al. 2015). The binding potential of intracellular binding proteins helps in the sequestration and storage of nutrients, which enhances the resistance potential. Small cysteine-rich proteins such as metallothionines can bind with $Cd^{2+}$ (Naik & Dubey 2017). The presence of this small cysteine-rich protein, metallothionine, helps in the reduction of the concentration of free ions in the cytoplasm and cytoplasmic metal cation binding proteins.

## CONCLUSION

Bioremediation is an important part of environmental clean-up because it allows metals like lead, mercury, and cadmium to be removed without releasing harmful pollutants. Bioremediation occurs when contaminants and essential supplements are degraded by microorganisms such as nitrogen, phosphorus, and trace components. Treatment of degraded wastewater and soil has proven to be an affordable option in comparison to other clean-up methods and pollution control technologies. To develop new methods of cleaning up polluted areas, collaborative efforts are required. Microorganisms are essential for the preservation of natural resources and environmental management. Even though fungi, algae, and protists can all help remove persistent pollutants, more research is needed to determine the best microbial genetic and expression system so that these agents can be the most effective bioremediators in the future.

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