**BIOREMEDIATION: A POTENTIAL ROLE OF HEAVY METAL RESISTANT PLANTS AND MICROORGANISMS**

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**ABSTRACT**

Environmental pollution and its prevention are one of the major problems worldwide and a wide variety of pollutants affect the environment but heavy metals are a major toxin, which are mainly responsible for pollution. The present review article briefly outlines the occurrence of heavy metals in the environment and shows strategies to use plants and microorganisms for biological remediation processes as published in the scientific articles. The toxicity of heavy metals in plants is avoided or resisted through avoidance and tolerance mechanism; however, the endo microorganism of such plants provides a resistance mechanism to plant resist toxicity. The current review focuses on enhancing the remediation capacity of heavy metals in contaminated soils and water by plants and microorganisms; as well as studying the source of heavy metals to affect the environmental condition. Also, this paper is a survey of recent developments concerning to apprehension of physiological as well as fundamental mechanisms to resist heavy metals by plants and microorganisms.

**Keywords:** Environmental pollution; heavy metal; remediation; microorganism; fundamental mechanism.

**INTRODUCTION**

***Bioremediation: An overview***

The field of environmental biotechnology isn't a new area; composting and treatment of wastewater are two well-known examples. Recent research in molecular ecology and biological systems; on the other hand, give prospects for more effective biological processes. These studies' notable successes include the cleanup of contaminated water and land regions. bioremediation is described as the process of biologically degrading organic matter under controlled circumstances to a benign state or to levels below regulatory percentage limitations (Lockwood et al.2013). Also describe a process that uses living organisms, such as bacteria, fungi, plants or enzymes to remove, neutralize or transform pollutants and contaminants from the environment. It is a cost-effective and eco-friendly approach to clean up contaminated sites, making it a popular technique in environmental remediation. The main idea behind bioremediation is to harness the natural abilities of microorganisms and plants to break down or convert hazardous substances into less harmful or non-toxic forms. These living organisms are capable of metabolizing and utilizing various pollutants as sources of energy and nutrients, effectively reducing their concentrations in the environment.

Bioremediation is the activity or procedure that can use living organisms like plants, microorganisms and their enzymes to remediate toxic pollutants present in the environment to convert their original state. Bioremediation can be used to attack particular pollutants, such as heavy metals, pesticides and other chemical effluents that are degraded by living organisms like microorganisms and plants using various techniques. Bioremediation is the removal of environmental pollutants using emerging technologies that can be used together with physicochemical treatment for comprehensive management (Harekrushna and Kumar 2012). Although the methods used for biological remediation are not technically complicated; significant expertise and proficiency can be necessary to implement or design protocol for a promising result to a bioremediation program.

***Types of Bioremediations***

There are two primary types of bioremediations:

1. **In Situ Bioremediation:** This method involves treating the contamination directly at the site where it is present. Microorganisms or plants are introduced to the contaminated area, and environmental conditions such as temperature, pH, and nutrient availability are optimized to support their growth and activity. In-situ bioremediation is often used for soil and groundwater remediation.
2. **Ex Situ Bioremediation:** In this approach, the contaminated material is excavated and transported to a controlled environment, such as a bioreactor or a treatment facility. The bioremediation process is then carried out in this controlled environment. Ex-situ bioremediation is typically employed for treating contaminated soil, sludge, or sediments.



**Figure 1. Sources of heavy metal in the environment**

***Phytoremediation***

Phytoremediation is a biological remediation process that can use various plant species to eliminate or remove impurities in the water and soil through natural substances released by plant roots. Phytoremediation is a natural and direct use of plants to uptake pollutants or metals through their roots and translocation to the upper parts of the plant. The removal of poisonous substances in the soil and water is incredibly substantial to decrease the risk to the environment as well as human health. Many plants have their own mechanism for remediating heavy metals in polluted wastewater, soil and contaminants using various economical technologies. The present study demonstrates phytoremediation as an eco-friendly technique for removing pollutants on a continuing basis. Phytoremediation has many utilities in the investigation of the sub-lethal extent of bioaccumulated pollutants within tissue, cells or element of living things to specify the net amount of toxic elements consolidated from time to time (Doust et al. 1994). Some species of plants accumulate high amounts of certain toxic pollutants without any harmful effects using biomonitoring; which is removing toxic elements in water and soil (Ravera et al. 2003). The basic thoughts of phytoremediation are due to the survival rate of plants in contaminated water. The plant which survives in contaminated water is maybe a hyperaccumulator to metals or any other pollutants, hence this experiment was carried out to find out the capacity of plant species to tolerate heavy metals and how they survive in adverse conditions.

Heavy metal pollution in water is one of the major problems of the environment; due to urbanization and industrialization, toxic elements like zinc (Zn), copper (Co), nickel (Ni), lead (Pb), etc. are highly increased in the environment. Such types of anthropogenic activities like industrial waste, mining, sludges, etc. the harmful elements are increasing day by day. Some waste or elements are not degraded in the environment and that cause or releases harmful pollutants or toxic elements to affect living things like humans and animals as well reduce crop production. Sometimes plant chemical composition is modified without damage being easily seen, and plants grown in polluted soils contain higher quantities of metals than plants grown in un-contaminated soils (Van and Zwart 1997; Yusuf et al. 2003). Many studies reported the dangerous toxic element in soil and water (Tiwari et al. 2008, Cao and Hu, 2000; Mapanda et al. 2005; Singh et al. 2004; Nan et al. 2002).

Phytoremediation is an environmentally friendly technique that is used the reduce toxicity in water and soil; as well as absorb and degrade toxic compounds from various sources in the environment. Today, our society faces many challenges regarding sustaining the ecosystem; however, remediation techniques are also processed and functioning nowadays. The remediation of environmental pollution has been using many techniques or approaches like biological, chemical and physical; but this approach's use is limited because of threats to the ecosystem as well as cost-effective requirements (Ali et al. 2013). The remediation of pollutants in soil and water through the plants has been using several mechanisms like accumulation of heavy metals or other pollutants, degradation, and phytostabilization or immobilization (Pivetz 2001).

**Table 1. Heavy metal resistant plants with sources.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Plant Species**  | **Heavy metal removed** | **Source** | **Reference** |
| Arabidopsis halleri | Cu tolerant | Water and Soil | Kenderešová et al. [2012](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4744854/#B197) |
|  | Cd tolerant | Fertilizer |  |
| Arabidopsis arenosa | Cu tolerant | Water and Soil | Kenderešová et al. [2012](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4744854/#B197) |
|  | Cd tolerant | Fertilizer |  |
| Cassia tora | Al tolerance | pesticide | Yang et al. 2003 |
| Medicago sative | Cd tolerant | Fertilizer and soil | Cui et al. 2012 |
| Raphanus sativus | Cr  | Soil and Sludge | Choudhary et al. 2012 |
|  | Cu resisted | Sludge |  |
| *Chlorella vulgaris* | Cd and Fe  | Fertilizer and pesticides | Aksu and Dönmez 2006; Choi and Lee 2015 |
|  | Zn and Cu | Pesticide |  |
| *Desmodesmus pleiomorphus* | Cd | Fertilizer and pesticides | Monteiro et al. 2010 |
| *Ecklonia maxima* | Cd and Cu | Soil | Feng and Aldrich 2004 |
| *Hydrilla verticilata* | As | Water  | Zhen et al. 2020 |
| *Combretum erythrophyllum* | Zn, Cu and Ni | Soil | Photolo et al. 2021 |
| *Eucalyptus tereticornis* | Cu and Cd | Soil | Reddy et al. 2016 |
| *Limonium bicolor* | Cu tolerance | Transgenic tobacco | Ban et al. 2011 |
| *Lycopersicon esculentum* | Fe tolerance | Soil and fertilizer | Brown and Ambler 1974 |
| *Elsholtzia haichowensis* | Cu accumulate | Soil  | Xia et al. 2012 |
| *Sedum plumbizincicola* | Cd accumulate | Soil  | Peng et al. 2017 |

***Role of microorganism***

Microorganisms are present everywhere they play an important role in many biogeochemical cycles like the phosphorous cycle, carbon cycle, nitrogen cycle and many other food chains, food cycle is a decomposer; they convert metals from one insoluble to soluble form or degrade them into a small molecule. Microorganism mechanisms depend upon many factors like humidity, oxygen presence, temperature, pH and EPS production (Gadd 2010). Microorganisms can be isolated from almost any environmental conditions like a desert, water, extremely cold weather, high salt concentration and low pressure in deep-sea low oxygen presence or anaerobic conditions. They can also observe highly chemically contaminated areas or waste areas because they can survive and able to adapt in any condition. Their main requirement is energy and carbon source so they degrade components and gain their energy from available resources easily (Kensa 2011). Microorganisms have the ability to reduce heavy metals like Cd, Pb and Cr from soil and water by using different methods like reduction, oxidation, and absorption, this mechanism is known as Microbial remediation (Su 2014). Different microorganisms like algae, bacteria and many fungi species have tested this process by using different pathways of respiration and fermentation; metals are immobilized, removed, destroyed or neutralized by them. Microorganisms use different enzymes and produce EPS in response to heavy metals present in the environment (Dixit et al. 2015).

Microorganisms and many other rhizobacteria and actinobacteria have been obeyed in many polluted sites to grow and utilize toxic chemicals and grow. They have the ability to utilize many heavy chemicals like Cd, Pb, Cr, Cd, Zn and Hg and transform them into their insoluble or toxic form. They are used in many areas for bioremediate contaminated site. Many bacterial species like *Pseudomonas, Micrococcus sp. Flavobacterium, Klebsiella, Bacillus* and *Enterobacter* have been identified by many scientists for using heavy metals like cadmium, lead and chromium from various soil and contaminated water. Many fungus species can also have observed to reduce heavy metal activity. Fungal species like *Aspergillus, Penicillium, Rhizopus,* and *Cephalosporium* are widely used for their higher metal-reducing capacity. They can reduce heavy metals like Pb and Zn, from Soil and different contaminated water (Tunali et al. 2006). Endophytic bacteria like *Bacillus, Enterobacter, Flavobacterium, Pseudomonas and Klebsiella* utilize and live with plant tissue that helps cells from the heavy material from the environment (Nadeem et al. [2010](https://bnrc.springeropen.com/articles/10.1186/s42269-023-01006-z#ref-CR106)). Some types of Rhizobacteria also tested against metal bioremediation by removing the phytotoxicity of soil and water (Weyens et al. [2009](https://bnrc.springeropen.com/articles/10.1186/s42269-023-01006-z#ref-CR159)).

Different methods by which microbes act on these heavy metals incorporate bioleaching, biosorption, biomineralization, intracellular aggregation and enzyme catalysed conversion. In bioleaching, bacteria and fungi like microorganism, solubilize metal sulphides and oxides from ores deposits and secondary wastes from environment (Mishra et al. 2005). Biotransformation is the process in which microorganism convert metal, chemical compound into their less toxic form of chemical by micro-metal interaction like oxidation, reduction, alkylation and methylation, sometimes modifying their structure and changing their polarity. (Pervaiz et al. 2013). Biofilms mechanisms of bioremediation could do like biosorbent or by EPS present in biofilms which have emulsifier properties. It was revealed in a study conducted on *Rhodotorula mucilaginosa* that metal biofilm cells they have been used to remediation process (Grujić et al. 2017). Bioventing is the method that involves supplying air and nutrients through wells to contaminated soil or water to stimulate the indigenous bacteria. Bioventing employs low air flow rates and provides only the amount of oxygen require for microbes to degrade the contaminate material from sites.

Biosorption is the process of eliminate of heavy metals, compounds or contaminated material from any area using microorganism with greater degradative ability to decompose material easily (Srivastava and Anil 2015). Biomineralization is a method in which microorganism like bacteria, fungi and algae form minerals by transforming the mineral of chemical like biominerals are oxides, sulphides, oxalate, sulphates and phosphates are converted using many cells activity and converted into metal ions, they sometimes effect motility of bacteria their cell activity and redox potential (Ledin 2000). Bioaccumulation is remediation process in which toxic pollutants gradually accumulate into the living tissues of an microbes from nature. It is depended upon surface characteristic, biochemical properties, genetics of microbes and environmental condition.

***Factors affecting bioremediation***

The recent research carried out in the process of bioremediation to accumulation of toxic elements by biological agents like microorganisms and phytons; but may have major limitations in the form of economic as well as environmental issues. There are several elements that have a major effect on the limitations of remediation like toxic elements, pesticides and other pollutants (Atagan et al. 2003; Mangunwardoyo et al. 2013; Thapa et al. 2012). There are many other factors that affect the remediation like the nature of pollutants, toxic or nontoxic pollutants, organic or inorganic pollutants and carcinogenic elements which can affect the environment (Zeyaullah et al. 2009; Dhokpande and Kaware 2013).

**Table 2. Heavy metal resistant microorganisms with sources.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Microorganism**  | **Heavy metal removed** | **Source** | **Reference** |
| *Pseudomonas aeruginosa* | Ni (II) | Industrial waste, coal waste, fuel oil | Congeevaram et al. 2007 |
|  | Hg (II) | Electrical appliances and thermal power plants  | Yin et al. 2016 |
|  | Cd (II) | Zink waste, e- fuel | Sharma et al. 2000 |
|  | Cr (II) | Mining, leather industries | Chaturvedi 2011 |
|  | Cd (II) | E-Waste, zinc smelting | Gabriel et al. 1996 |
| *Bacillus subtilis* | Cr (II) | Industrial waste | Kim et al. 2015 |
| *Flavobacterium spp.* | Cu | Electroplating and smelting operations | Shipra et al. 2011 |
| *Aspergillus niger* | Hg (II)  | Fluorescent lights and lamps | Rajendran et al. 2003  |
|  | Zn (II) | Smelting, electroplating and road runoff. | Rajendran et al. 2003  |
| *Staphylococcus spp.* | Cd (II) | Ni/Cd batteries, e-waste, paint sludge | Nanda et al. 2011 |
|  | Cu (II) | Electronic industry | Nanda et al. 2011 |
| *Klebsiella pneumoniae* | Hg (II) | Electrical appliances and thermal power plants | Al-Garni et al. 2010 |
| *Trichoderma spp.* | Cd (II) | E-waste, paint sludge, electronic industry | Bazrafshan et al. 2016 |
| *Penicillium chrysogenum* | Pb (II) | Gasoline, lead based painting, pipes | Kumar et al. 2014 |
|  | Cr (VI) | Leather manufacturing, paints | Kumar et al. 2014 |
|  | Cd (II) | Pigmented, coating and plating | Kumar et al. 2014 |
| *Acinetobacter spp.* | Cr (II) | Cement and mortar | Bhattacharya et al. 2014 |
|  | Ni (II) | Cell phone batteries | Kumar et al. 2011 |
| *Saccharomyces cerevisiae* | Cr (II) | Paints andcement  | Benazir et al. 2010 |
|  | Cu (II) | Electronics, smelting  | Amirnia et al. 2015 |
| *Streptomyces spp.* | Pb (II) | Coal‑based thermal power plants, smelting operations and e‑waste | Gabriel et al. 1996 |
|  | Cr (II) | Industrial coolants, leather tanning, and mining | Nayak et al. 2018 |
| *Micrococcus spp.* | Cu (II) | Electronic industry and copper plating | Marzan et al. 2017 |
|  | Pb (II) | E-waste, batteries and thermal waste | Marzan et al. 2017 |
|  | Ni (II) | Coal and industrial waste | Congeevaram et al. 2007 |
| *Trichoderma spp.* | Cd (II) | Ni/Cd batteries, e-waste, paint sludge | Bazrafshan et al. 2016 |
| *Escherichia coli* | Cr (II) | Mining, leather, road runoff and making alloys.  | Kumar et al. 2011 |
|  | Cd (II) | Incinerations and fuel combustion, e-waste, paint sludge | Favero et al. 1991 |
| *Rhizopus arrhizus* | Cd (II) | Zinc smelting, and e‑waste | Kumar et al. 2011 |
|  | Hg (II) | Electrical appliances and thermal power plant |  |
|  | Pb (II) | Coal‑based thermal power plants and smelting |  |

**METHODS OF BIOREMEDIATION**

Several bioremediation methods have been employed to address various types of environmental contamination. Here are some common methods used for bioremediation:

1. **Biostimulation:** This method involves enhancing the growth and activity of naturally occurring microorganisms in the contaminated environment. It is achieved by providing essential nutrients (such as nitrogen, phosphorus, and carbon) and other factors that promote microbial growth and metabolism. Biostimulation can accelerate the natural breakdown of pollutants, especially hydrocarbons and organic compounds (Leahy and Colwell 1990).
2. **Bioaugmentation:** In this approach, specific pollutant-degrading microorganisms, often genetically engineered strains, are introduced into the contaminated site to enhance biodegradation. The aim is to supplement the existing microbial community with organisms that have specialized capabilities to break down the contaminants (Díaz 2004).
3. **Phytoremediation:** Phytoremediation employs plants to remove, degrade, or immobilize contaminants from the soil, water, or air. Certain plant species have the ability to accumulate and store pollutants in their tissues, which can then be harvested and properly disposed of. Others can break down or transform the contaminants through their metabolic processes (Ma et al. 2020).
4. **Bioventing and Biosparging:** Bioventing involves enhancing the aerobic biodegradation of pollutants in the subsurface by providing oxygen through soil aeration. Biosparging, on the other hand, injects air directly into the contaminated groundwater to stimulate microbial activity and enhance pollutant removal. Both methods are effective for treating volatile organic compounds (VOCs) and petroleum hydrocarbons (EPA 1996).
5. **Composting:** Composting is a bioremediation method used primarily for treating organic waste contaminated with pollutants. Microorganisms naturally present in composting piles break down the organic matter, including some contaminants, into stable, non-toxic end products (Fracchia et al. 2006).

**CONCLUSION**

In conclusion, bioremediation has emerged as a promising and environmentally-friendly approach to address the increasing concern over pollution and contamination of various ecosystems. This review highlights the effectiveness and potential of bioremediation techniques in remediating contaminated sites, restoring ecological balance, and minimizing the harmful impacts of pollutants on human health and the environment. Through the use of microorganisms, plants, or other biological agents, bioremediation offers a cost-effective and sustainable alternative to traditional remediation methods such as excavation or chemical treatment. It can target a wide range of pollutants, including organic and inorganic compounds, heavy metals, and hydrocarbons, making it a versatile solution for diverse contamination scenarios. It's important to note that bioremediation methods can be complex and site-specific, and their success depends on various factors such as the type and extent of contamination, environmental conditions, and the selected organisms or plants. For the latest developments and research on bioremediation methods, I recommend consulting recent scientific publications and journals related to environmental microbiology, biotechnology, and remediation processes.

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