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

Tarla Chudasama1, Kamlesh Gadhvi2, Rashik Sojitra3, Kiran Dangar4, Bindu Raigar5 and Suhas Vyas\*

1Department of Botany, Om College of Science, Junagadh, Gujarat

2Gujarat Forestry Research Foundation, Gandhinagar, Gujarat

3Department of Botany, Nobel University, Junagadh, Gujarat

4Department of Life Sciences, Bhakta Kavi Narsinh Mehta University, Junagadh, Gujarat

5Department of Microbiology, Om College of Science, Junagadh, Gujarat

\*Department of Life Sciences, Bhakta Kavi Narsinh Mehta University, Junagadh, Gujarat

Author 1: chudasamatarla685@gmail.com

Author 2: gadhvikj70@gmail.com

Author 3: sojitrark13@gmail.com

Author 4: kirandangar07@gmail.com

Author 5: bbraigar@omeducation.edu.in

Corresponding address: vsuhas.13@gmail.com

**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). The use of microbes in the latest bioremediation process is credited, in part, to George Robinson (US Microbics 2003). Bioremediation is explained as the use of microorganisms for the destroy or immobilize toxic materials (Shanahan 2004). The use of certain genetically modified microorganisms to check their ability to utilize specific contaminants such as carbons and pesticides uses increasing day by day. This method had an early mention in the late 1980s and early 1990s.

Bioremediation is 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 c clean up contaminated sites, making it 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.

***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 process of bioremediation that uses various types of plants to remove, transfer, stabilize and destroy pollutants in the soil and water. In this process, the plant releases the natural substance through its 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 (Rezania et al. 2016). The removal of toxic pollutants is extremely important to reduce the threat to human health and the environment; is achieved through various techniques such as reverse osmosis, ion exchange, chemical precipitation, adsorption and solvent extraction. 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. In addition, this article covers the potential use of aquatic plants in phytoremediation for the therapy of contaminated water.

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 pollutants consolidated over 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 plant’s capacity to tolerate heavy metals and how they survive in adverse conditions.

Water pollution by heavy metals is one of the worldwide environmental problems. heavy metal pollution due to increase in the industrialization and urbanization. toxic heavy metals such as cadmium, copper, lead, chromium, zinc and nickel are environmental pollutants; exactly in areas with high anthropogenic activity. They can’t be biodegraded so released into the environment and spreading lots of toxic effects even in lower concentrations on living organisms in the food chain. In many areas this waste is used by local farmers for irrigating their crops, hence introducing these pollutants to the crops (Waring et al. 1996). 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). Studies carried out by developing countries have reported heavy metal contamination in wastewater and wastewater-irrigated soil (Cao and Hu, 2000; Nan et al. 2002; Singh et al. 2004; Mapanda et al. 2005; Tiwari et al. 2008). The process of metal accumulation by different plants depends on the concentration of available metal in soils, solubility sequences and the plant species growing on the soil.

Around 40% population of the world is facing the problem of water deficiency due to climate change, urbanization, food requirement and increase in consumption of natural resources. during the past few decades’ rapid urbanization, industrialization, agricultural activities, discharge of geothermal waste and olive wastewater especially in olive-cultivating areas enhanced the discharge of polluted wastewater into the environment, industrial and domestic untreated wastewater; whenever it contains pesticides, oils, dyes, phenol, cyanides, toxic organics, phosphorous, suspended solids and heavy metals. commercial activities such as metal processing, mining, geothermal energy plants, automobile, paper pulp industries, pesticide manufacturing, tanning and dying are held responsible for the global contamination of heavy metals. The removal of heavy metals from the water is difficult because they exist in different chemical forms. Most metals are not biodegradable, and they can easily pass through different trophic levels to accumulate in the biota. The types of pollution or waste is depending on the area, people’s food habits, crop vegetation, used pesticides, industry etc.

Plants that possess the capacity to accumulate or uptake in high quantities of metals than required for plant growth are termed ‘hyperaccumulators. The minimum concentration of a metal that a plant contains is to be termed a hyperaccumulator, which varies for each metal (Reeves and Brooks 1983). These plants can accumulate metals in concentrations 1,00,000 times greater than in the associated water. A maximum number of hyperaccumulators have been reported from families Brassicaceae, Lamiaceae, Scrophulariaceae, Cyperaceae, Poaceae, Apocynaceae, Euphorbiaceae, Flacourtiaceae, Fabaceae and Violaceae.

**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 component and gain their energy from available resources easily (Kensa 2011). The concept of bioremediation by utilizing microorganisms was first patent registered in 1981 for the degradation of petroleum oil by *Pseudomonas putida* (Prescott et al. 2002).

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). Because of this unique nature of microorganisms, they play an important role in bioremediation by interacting with heavy metals and utilizing heavy metals as their source of nutrition and survive in any habitat **(**Alvarez et al. [2017](https://bnrc.springeropen.com/articles/10.1186/s42269-023-01006-z#ref-CR10)). 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). They can also genetically adapt to degrade the contaminants.

Microorganisms and many other actinobacteria and rhizobacteria have been observed in many contaminated 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. *Corynebacterium, Rhodococcus, and Streptomyces* like actinobacterial species can also eliminate and neutralize heavy metals, such as Hg, Co, Cd, Cr, Zn and Ni (Oyetibo et al. 2010). 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 et al. 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). Volatilization of metal done by microbial species like some *Pseudomonas spp., Escherichia spp., Clostridium spp., and Bacillus spp.* They convert Hg (II), As and Pb to a gaseous methylated form by many cellular activities. Methylation plays a significant role in metal remediation (Ramasamy et al. 2007). 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.

**REFERENCES**

1. Aksu, Z., & Dönmez, G. (2006). Binary biosorption of cadmium (II) and nickel (II) onto dried Chlorella vulgaris: Co-ion effect on mono-component isotherm parameters. *Process Biochemistry*, *41*(4), 860-868.
2. Al-Garni, S. M., Ghanem, K. M., & Ibrahim, A. S. (2010). Biosorption of mercury by capsulated and slime layerforming Gram-ve bacilli from an aqueous solution. *African Journal of Biotechnology*, *9*(38), 6413-6421.
3. Alvarez, A., Saez, J. M., Costa, J. S. D., Colin, V. L., Fuentes, M. S., Cuozzo, S. A., ... & Amoroso, M. J. (2017). Actinobacteria: current research and perspectives for bioremediation of pesticides and heavy metals. *Chemosphere*, *166*, 41-62.
4. Amirnia, S., Ray, M. B., & Margaritis, A. (2015). Heavy metals removal from aqueous solutions using Saccharomyces cerevisiae in a novel continuous bioreactor–biosorption system. *Chemical Engineering Journal*, *264*, 863-872.
5. Atagana, H. I., Haynes, R. J., & Wallis, F. M. (2003). Optimization of soil physical and chemical conditions for the bioremediation of creosote-contaminated soil. *Biodegradation*, *14*, 297-307.
6. Ban, Q., Liu, G., & Wang, Y. (2011). A DREB gene from Limonium bicolor mediates molecular and physiological responses to copper stress in transgenic tobacco. *Journal of plant physiology*, *168*(5), 449-458.
7. Bazrafshan, E., Zarei, A. A., & Mostafapour, F. K. (2016). Biosorption of cadmium from aqueous solutions by Trichoderma fungus: kinetic, thermodynamic, and equilibrium study. *Desalination and Water Treatment*, *57*(31), 14598-14608.
8. Benazir, J. F., Suganthi, R., Rajvel, D., Pooja, M. P., & Mathithumilan, B. (2010). Bioremediation of chromium in tannery effluent by microbial consortia. *African journal of biotechnology*, *9*(21), 3140-3143.
9. Bhattacharya, A., Gupta, A., Kaur, A., & Malik, D. (2014). Efficacy of Acinetobacter sp. B9 for simultaneous removal of phenol and hexavalent chromium from co-contaminated system. *Applied Microbiology and Biotechnology*, *98*, 9829-9841.
10. Brown, J. C., & Ambler, J. E. (1974). Iron‐stress response in tomato (Lycopersicon esculentum) 1. Sites of Fe reduction, absorption and transport. *Physiologia Plantarum*, *31*(3), 221-224.
11. Cao, Z. H., & Hu, Z. Y. (2000). Copper contamination in paddy soils irrigated with wastewater. *Chemosphere*, *41*(1-2), 3-6.
12. Chaturvedi, M. K. (2011). Studies on chromate removal by chromium-resistant Bacillus sp. isolated from tannery effluent. *Journal of Environmental Protection*, *2*(01), 76.
13. Choi, H. J., & Lee, S. M. (2015). Heavy metal removal from acid mine drainage by calcined eggshell and microalgae hybrid system. *Environmental Science and Pollution Research*, *22*, 13404-13411.
14. Choudhary, S. P., Kanwar, M., Bhardwaj, R., Yu, J. Q., & Tran, L. S. P. (2012). Chromium stress mitigation by polyamine-brassinosteroid application involves phytohormonal and physiological strategies in Raphanus sativus L. *PLoS One*, *7*(3), e33210.
15. Congeevaram, S., Dhanarani, S., Park, J., Dexilin, M., & Thamaraiselvi, K. (2007). Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. *Journal of hazardous materials*, *146*(1-2), 270-277.
16. Cui, W., Li, L., Gao, Z., Wu, H., Xie, Y., & Shen, W. (2012). Haem oxygenase-1 is involved in salicylic acid-induced alleviation of oxidative stress due to cadmium stress in Medicago sativa. *Journal of experimental botany*, *63*(15), 5521-5534.
17. Dhokpande, S. R., & Kaware, J. P. (2013). Biological methods for heavy metal removal-A review. *International Journal of Engineering Science and Innovative Technology*, *2*(5), 304-309.
18. Díaz, E. (2004). Bacterial degradation of aromatic pollutants: a paradigm of metabolic versatility.
19. Dixit, R., Wasiullah, X., Malaviya, D., Pandiyan, K., Singh, U. B., Sahu, A., ... & Paul, D. (2015). Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. *Sustainability*, *7*(2), 2189-2212.
20. Doust, J. L., Schmidt, M., & Doust, L. L. (1994). Biological assessment of aquatic pollution: a review, with emphasis on plants as biomonitors. *Biological Reviews*, *69*(2), 147-186.
21. Favero, N., Costa, P., & Massimino, M. L. (1991). In vitro uptake of cadmium by basidiomycetes Pleurotus ostreatus. *Biotechnology letters*, *13*, 701-704.
22. Feng, D., & Aldrich, C. (2004). Adsorption of heavy metals by biomaterials derived from the marine alga Ecklonia maxima. *Hydrometallurgy*, *73*(1-2), 1-10.
23. Fracchia, L., Dohrmann, A. B., Martinotti, M. G., & Tebbe, C. C. (2006). Bacterial diversity in a finished compost and vermicompost: differences revealed by cultivation-independent analyses of PCR-amplified 16S rRNA genes. *Applied microbiology and biotechnology*, *71*, 942-952.
24. Gabriel, J., Kofroňová, O., Rychlovský, P., & Krenželok, M. (1996). Accumulation and effect of cadmium in the wood-rotting basidiomycete Daedalea quercina. *Bulletin of Environmental Contamination and Toxicology*, *57*, 383-390.
25. Gadd, G. M. (2010). Metals, minerals and microbes: geomicrobiology and bioremediation. *Microbiology*, *156*(3), 609-643.
26. Grujić, S., Vasić, S., Radojević, I., Čomić, L., & Ostojić, A. (2017). Comparison of the Rhodotorula mucilaginosa biofilm and planktonic culture on heavy metal susceptibility and removal potential. *Water, Air, & Soil Pollution*, *228*, 1-8.
27. Guiné, V., Spadini, L., Sarret, G., Muris, M., Delolme, C., Gaudet, J. P., & Martins, J. M. (2006). Zinc sorption to three gram-negative bacteria: combined titration, modeling, and EXAFS study. *Environmental science & technology*, *40*(6), 1806-1813.
28. Kenderešová, L., Staňová, A., Pavlovkin, J., Ďurišová, E., Nadubinská, M., Čiamporová, M., & Ovečka, M. (2012). Early Zn2+-induced effects on membrane potential account for primary heavy metal susceptibility in tolerant and sensitive Arabidopsis species. *Annals of botany*, *110*(2), 445-459.
29. Kensa, V. M. (2011). Bioremediation-an overview. *Journal of Industrial Pollution Control*, *27*(2), 161-168.
30. Kim, I. H., Choi, J. H., Joo, J. O., Kim, Y. K., Choi, J. W., & Oh, B. K. (2015). Development of a microbe-zeolite carrier for the effective elimination of heavy metals from seawater. *Journal of microbiology and biotechnology*, *25*(9), 1542-1546.
31. Kumar, R., Bhatia, D., Singh, R., Rani, S., & Bishnoi, N. R. (2011). Sorption of heavy metals from electroplating effluent using immobilized biomass Trichoderma viride in a continuous packed-bed column. *International Biodeterioration & Biodegradation*, *65*(8), 1133-1139.
32. Kumar, R., Singh, P., Dhir, B., Sharma, A. K., & Mehta, D. (2014). Potential of some fungal and bacterial species in bioremediation of heavy metals. *Journal of Nuclear Physics, Material Sciences, Radiation and Applications*, *1*(2), 213-223.
33. Leahy, J. G., & Colwell, R. R. (1990). Microbial degradation of hydrocarbons in the environment. *Microbiological reviews*, *54*(3), 305-315.
34. Ledin, M. (2000). Accumulation of metals by microorganisms—processes and importance for soil systems. *Earth-Science Reviews*, *51*(1-4), 1-31.
35. Lockwood, J. L., Hoopes, M. F., & Marchetti, M. P. (2013). Invasion ecology. John Wiley & Sons.
36. Ma, Y., Oliveira, R. S., Freitas, H., & Zhang, C. (2020). Phytoremediation of heavy metal(loid)s contaminated soils: A review of plant-Soil-microbe interactions and the mechanisms involved. Science of the Total Environment, 713, 136686.
37. Mangunwardoyo, W., Sudjarwo, T., & Patria, M. P. (2013). Bioremediation of effluent wastewater treatment plant Bojongsoang Bandung Indonesia using consortium aquatic plants and animals. *International Journal of Research and Reviews in Applied Sciences*, *14*(1), 150-160.
38. Mapanda, F., Mangwayana, E. N., Nyamangara, J., & Giller, K. E. (2005). The effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables in Harare, Zimbabwe. *Agriculture, Ecosystems & Environment*, *107*(2-3), 151-165.
39. Marzan, L. W., Hossain, M., Mina, S. A., Akter, Y., & Chowdhury, A. M. A. (2017). Isolation and biochemical characterization of heavy-metal resistant bacteria from tannery effluent in Chittagong city, Bangladesh: Bioremediation viewpoint. *The Egyptian Journal of Aquatic Research*, *43*(1), 65-74.
40. Mishra, D., Kim, D. J., Ahn, J. G., & Rhee, Y. H. (2005). Bioleaching: a microbial process of metal recovery; a review. *Metals and Materials International*, *11*, 249-256.
41. Monteiro, C. M., Castro, P. M., & Malcata, F. X. (2010). Cadmium removal by two strains of Desmodesmus pleiomorphus cells. *Water, Air, and Soil Pollution*, *208*, 17-27.
42. Nadeem, S. M., Zahir, Z. A., Naveed, M., Asghar, H. N., & Arshad, M. (2010). Rhizobacteria capable of producing ACC‐deaminase may mitigate salt stress in wheat. *Soil Science Society of America Journal*, *74*(2), 533-542.
43. Nan, Z., Li, J., Zhang, J., & Cheng, G. (2002). Cadmium and zinc interactions and their transfer in soil-crop system under actual field conditions. *Science of the Total Environment*, *285*(1-3), 187-195.
44. Nanda, M., Sharma, D., & Kumar, A. (2011). Removal of heavy metals from industrial effluent using bacteria. *International journal of environmental sciences*, *2*(2), 765-780.
45. Nayak, A. K., Panda, S. S., Basu, A., & Dhal, N. K. (2018). Enhancement of toxic Cr (VI), Fe, and other heavy metals phytoremediation by the synergistic combination of native Bacillus cereus strain and Vetiveria zizanioides L. *International journal of phytoremediation*, *20*(7), 682-691.
46. Oyetibo, G. O., Ilori, M. O., Adebusoye, S. A., Obayori, O. S., & Amund, O. O. (2010). Bacteria with dual resistance to elevated concentrations of heavy metals and antibiotics in Nigerian contaminated systems. *Environmental Monitoring and Assessment*, *168*, 305-314.
47. Peng, J. S., Ding, G., Meng, S., Yi, H. Y., & Gong, J. M. (2017). Enhanced metal tolerance correlates with heterotypic variation in SpMTL, a metallothionein‐like protein from the hyperaccumulator Sedum plumbizincicola. *Plant, Cell & Environment*, *40*(8), 1368-1378.
48. Pervaiz, I., Ahmad, S., Madni, M. A., Ahmad, H., & Khaliq, F. H. (2013). Microbial biotransformation: a tool for drug designing. *Applied biochemistry and microbiology*, *49*, 437-450.
49. Photolo, M. M., Sitole, L., Mavumengwana, V., & Tlou, M. G. (2021). Genomic and physiological investigation of heavy metal resistance from plant endophytic Methylobacterium radiotolerans MAMP 4754, isolated from Combretum erythrophyllum. *International Journal of Environmental Research and Public Health*, *18*(3), 997.
50. Prescott L. M., Harley J.P. & Klein D. A. (2002) Microbiology. 5th International Edition
51. Rajendran, P., Muthukrishnan, J., & Gunasekaran, P. (2003). Microbes in heavy metal remediation.
52. Ramasamy, K., Kamaludeen, & Banu, S. P. (2007). Bioremediation of metals: microbial processes and techniques. *Environmental bioremediation technologies*, 173-187.
53. Ravera, O., Cenci, R., Beone, G. M., DANTAS, M., & LODIGIANI, P. (2003). Trace element concentrations in freshwater mussels and macrophytes as related to those in their environment. *Journal of Limnology*, *62*(1), 61-70.
54. Reddy, M. S., Kour, M., Aggarwal, S., Ahuja, S., Marmeisse, R., & Fraissinet‐Tachet, L. (2016). Metal induction of a P isolithus albus metallothionein and its potential involvement in heavy metal tolerance during mycorrhizal symbiosis. *Environmental microbiology*, *18*(8), 2446-2454.
55. Reeves, R. D., & Brooks, R. R. (1983). Hyperaccumulation of lead and zinc by two metallophytes from mining areas of Central Europe. *Environmental pollution series A, Ecological and Biological*, *31*(4), 277-285.
56. Shanahan, P. (2004). Bioremediation. Waste containment and remediation technology. *Massachusetts Institute of Technology*.
57. Sharma, P. K., Balkwill, D. L., Frenkel, A., & Vairavamurthy, M. A. (2000). A new Klebsiella planticola strain (Cd-1) grows anaerobically at high cadmium concentrations and precipitates cadmium sulfide. *Applied and environmental Microbiology*, *66*(7), 3083-3087.
58. Shipra, J., Dikshit, S. N., & Pandey, G. (2011). Comparative study of agitation rate and stationary phase for the removal of Cu2+ by A. lentulus. *International Journal of Pharma and Bio Sciences*, *2*(3).
59. Singh, K. P., Mohan, D., Sinha, S., & Dalwani, R. (2004). Impact assessment of treated/untreated wastewater toxicants discharged by sewage treatment plants on health, agricultural, and environmental quality in the wastewater disposal area. *Chemosphere*, *55*(2), 227-255.
60. Srivastava, S; Anil Dwivedi, K. (2015). Biological wastes the tool for biosorption of arsenic. J.Bioremed. Biodegrad., 7, 2.
61. Su, C. (2014). A review on heavy metal contamination in the soil worldwide: Situation, impact and remediation techniques. *Environmental Skeptics and Critics*, *3*(2), 24.
62. Thapa, B., Kc, A. K., & Ghimire, A. (2012). A review on bioremediation of petroleum hydrocarbon contaminants in soil. *Kathmandu university journal of science, engineering and technology*, *8*(1), 164-170.
63. Tiwari, K. K., Dwivedi, S., Mishra, S., Srivastava, S., Tripathi, R. D., Singh, N. K., & Chakraborty, S. (2008). Phytoremediation efficiency of Portulaca tuberosa rox and Portulaca oleracea L. naturally growing in an industrial effluent irrigated area in Vadodra, Gujrat, India. *Environmental monitoring and assessment*, *147*, 15-22.
64. Tunali, S., Çabuk, A., & Akar, T. (2006). Removal of lead and copper ions from aqueous solutions by bacterial strain isolated from soil. *Chemical Engineering Journal*, *115*(3), 203-211.
65. U.S. EPA. (1996). Introduction to in situ bioremediation of groundwater. EPA/625/R-95/005.
66. USMicrobics. (2003). Annual Report FY-2003. http://www.bugsatwork.com/USMX/BUGS%20Report%20PRIN T%20(07-13-04) %20Hawaii%20(paginate%201-8).pdf
67. Van Lune, P., & Zwart, K. B. (1997). Cadmium uptake by crops from the subsoil. *Plant and Soil*, *189*(2), 231-237.
68. Waring, C. P., Stagg, R. M., Fretwell, K., McLay, H. A., & Costello, M. J. (1996). The impact of sewage sludge exposure on the reproduction of the sand goby, Pomatoschistus minutus. *Environmental Pollution*, *93*(1), 17-25.
69. Weyens, N., van der Lelie, D., Taghavi, S., Newman, L., & Vangronsveld, J. (2009). Exploiting plant–microbe partnerships to improve biomass production and remediation. *Trends in biotechnology*, *27*(10), 591-598.
70. Xia, Y., Qi, Y., Yuan, Y., Wang, G., Cui, J., Chen, Y., ... & Shen, Z. (2012). Overexpression of Elsholtzia haichowensis metallothionein 1 (EhMT1) in tobacco plants enhances copper tolerance and accumulation in root cytoplasm and decreases hydrogen peroxide production. *Journal of Hazardous Materials*, *233*, 65-71.
71. Yang, Z. M., Wang, J., Wang, S. H., & Xu, L. L. (2003). Salicylic acid-induced aluminum tolerance by modulation of citrate efflux from roots of Cassia tora L. *Planta*, *217*, 168-174.
72. Yin, K., Lv, M., Wang, Q., Wu, Y., Liao, C., Zhang, W., & Chen, L. (2016). Simultaneous bioremediation and biodetection of mercury ion through surface display of carboxylesterase E2 from Pseudomonas aeruginosa PA1. *Water research*, *103*, 383-390.
73. Yusuf, A. A., Arowolo, T. A., & Bamgbose, O. (2003). Cadmium, copper and nickel levels in vegetables from industrial and residential areas of Lagos City, Nigeria. *Food and chemical toxicology*, *41*(3), 375-378.
74. Zeyaullah, M. D., Atif, M., Islam, B., Abdelkafe, A. S., Sultan, P., ElSaady, M. A., & Ali, A. (2009). Bioremediation: A tool for environmental cleaning. *African Journal of Microbiology Research*, *3*(6), 310-314.
75. Zhen, Z., Yan, C., & Zhao, Y. (2020). Influence of epiphytic bacteria on arsenic metabolism in Hydrilla verticillata. *Environmental Pollution*, *261*, 114232.