

Novel Research in Microbiology Journal (2023), 7(4): 2034-2047 Review Article

Exploring the microbial dynamics for heavy metals bioremediation in the industrial wastewater treatment: A critical review

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Received: 6 June, 2023; Accepted: 9 July, 2023; Published online: 11 July, 2023

Abstract



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The purpose of this review article was to provide a concise overview of the current researches on the topic of *in-situ* microbial remediation of heavy metals (HM) in the industrial wastewater. Due to the ever-expanding industrial sector; groundwater contamination by HM is a global environmental crisis. Heavy metals; environmental pollution, and the adaptive mechanisms that allow the bacteria to thrive in the metal-contaminated environments, have all been linked to the dramatic shifts in the microbial diversity, which are observed during the microbial restoration. It has been suggested that in situ bioremediation (ISB) can help with the emerging contamination problems; as the bacteria can be used to clean up the polluted areas. In the future, the researchers should pay more attention to the assessment methodologies for determining the success of remediation using ISB technology. Bio-remediation is only effective if the polluted area is properly characterized; the appropriate microbial species is chosen, and the harmful metals are easily accessible for absorption. This new technology uses bacteria to remove the harmful metals from the environment at a low cost. This study analyzes the effectiveness of bioremediation using microorganisms; using unique methodologies and integrated assessment methods. In addition to providing an overview of ISB for pollutant(s) elimination; this review is useful for comprehending the primary functions of microorganisms in this process.

Keywords: Industrial wastewater, *In Situ* bioremediation, Heavy metals, Toxic metals, Environmental sustainability

1. Introduction

Industrial wastewater often contains high concentrations of HM that may be harmful to the environment and human health. Bio-remediation is a cost-effective and eco-friendly approach for treating the heavy metal-contaminated wastewater. Microorganisms play a crucial role in bio-remediation by metabolizing and transforming the HM into less toxic products (Rosca et al., 2021). The microbial dynamics of heavy metal bio-remediation involve several stages, including bioadsorption; biosorption, bioaccumulation. biotransformation, and bioadsorption biomineralization. During and biosorption; the microorganisms adsorb HM onto their cell surfaces and/or extracellular polymeric substances (EPS). However; in the bioaccumulation stage, microorganisms accumulate HM inside their cells or EPS (Choudhary et al., 2017). During biotransformation, microorganisms enzymatically transform HM into less toxic forms; by reducing or oxidizing them. Biomineralization involves the formation of metal oxides: sulfides, or carbonates, which are less toxic and more stable than the original heavy metal ions (Boughattas et al., 2019). The microbial communities involved in heavy metal bioremediation are diverse and complex, including bacteria: fungi, and algae. The different microorganisms have different mechanisms for heavy metal removal and tolerance (Privadarshanee and Das, 2021). For example, some bacteria can produce exopolysaccharides that can chelate heavy metal ions, while others can enzymatically reduce or oxidize the heavy metals. Understanding the microbial dynamics of heavy metal bio-remediation is essential for the development of effective bio-remediation strategies. Factors such as pH; temperature, nutrients, and the presence of other contaminants, can affect the microbial communities' structures and functions (Raj et al., 2021). Therefore, optimizing these factors can enhance the efficiency of heavy metal bio-remediation. The objectives of this review were to describe the bioremediation of wastewater produced by various industrial types, and to test the efficiency of ISB.

2. Industrial wastewater's impact on the environmental and human health

Heavy metal toxicity in the surface environments is the primary cause of their numerous sources of pollution; lack of recyclables, and increasing tendencies. Growing amounts of metals in the agricultural systems are of significance (Mohammadi et al., 2020). Metals are seriously harming both the human and the environmental health. Metal contamination of the agricultural soil has grown to be a serious issue; as a result of the fast rise of industry and urbanization. The increasing concentrations of HM in the surface habitats can damage the soil; jeopardize human health, and affect the efforts exerted to preserve them. Several methods have been used to assess the contamination of HM in the different settings, including the danger gauge; accumulation gauge, pollutant load indicator (PLI), and the enhancement feature (EF). Kids who live in the manufacturing sectors have a greater probability to be close to the damaged urban soil; thus, putting their health at risk. Numerous serious human diseases, such as cancer; neurological problems, breathing ailments, and renal pathology, can be brought on by contact with the heavy metals. In the natural streams, there are limitations on all of these metals to the "US Environmental Protection Agency (US-EPA) and the World Health Organization (WHO) (Keshavarzi and Kumar, 2019). Municipal; industrial, and cement wastes include hazardous metals that endanger the biological and human existence. Expanding the manufacturing process, which is associated with urbanization, is bad for rivers because it raises the trace metal levels; especially for HM (Filote et al., 2022). Many toxic chemicals from the environment accumulate in the soil and water sediments. More than fifty HM are discharged into the water; seventeen of them are harmful, and the findings imply that they have a deleterious effect on the well-being. Cadmium (Cd) is also extremely dangerous and may bioaccumulate in the ecosystems and species; even at low quantities. Chronic absorption of Cd may also be harmful to the kidneys. Many countries and states regard "Cadmium" as pollutant organizations (Hadiani et al., 2018). The presence of lead (Pb), which is one of the most dangerous heavy metals in the consumed water, is a serious concern. Bones' calcium content can be altered by Pb; allowing for the creation of alternative areas in the future. The toxic effects induced from the excessive consumption of HM like copper (Cu) are possible. If the water's concentration of metal ions decreases or even becomes maintained below the tolerable values; this type of poisoning can be prevented (Lin *et al.*, 2017).

Table (1) demonstrates information on the harmful effects of exposure to the HM on the human and plant health.

Heavy metal	Exposure to human	Exposure to the plant	Reference
Cu	Liver and renal damage; fatigue, headaches, nausea, vomiting, and pain in the stomach	Hinder the root's development	<u>Kodama <i>et al.</i>, (2012)</u>
Hg	Problems of behavior and neurological conditions, such as loss of memory; tremors, and sleeplessness	Chlorosis; decreased growth of the seeds, and decreased plant height	<u>Engwa <i>et al.</i>, (2019)</u>
Со	Have an impact on the pulmonary and ocular functioning.	Lowered levels of sugars; proteins, and amino acids	Leyssens et al., (2017)
Cd	Harm the bones and kidneys	Influences the pace of germination	<u>Nordberg, (2004)</u>
As	Causes cancer; harm to the unborn child, and skin conditions	Decreased plant health and leaf health	Heck et al., (2014)

Table 1: Effects of metals on the human and plant health

Where; Cu: copper, Hg: mercury, Co: cobalt, Cd: cadmium, As: arsenic

3. Plant and human metal accumulation mechanisms

The globe has become increasingly polluted with HM; particularly in the underdeveloped countries. The presence of metallic metals (i.e., Cr; Cd, Cu, Ni, Zn, As, and Pb) in quantities of 5 g/cm^3 or more has been detected (Bharagava et al., 2020). Such pollution damages not only the air; water sources, and crops; but also accumulates in the important human organs like the kidney and bones; thus, endangering the wellness of humans. The human body does not require Pb to function: however. the communication and collaboration rigid; neurological, immunological, and endocrine systems, can be harmed by excessive amounts of this metal. Moreover, Pb can also block the enzymes, causing damage that can result in peripheral neuropathy; cutaneous lesions, skin cancer, and peripheral vascular disease. Pb poisoning may have negative effects on the human endocrine; neurological, immunological, circulatory, and enzymatic systems. Broken bones; renal disease, malignant pulmonary adenocarcinomas, and prostate proliferation disorders, have all been linked to Cadmium (Cd) toxicity. Metals enter the human cells and disturb a wide variety of cellular processes. The oxidation-reduction potential can be lowered and chromatin may be harmed if a metal enters into the nucleus, which can result in neoplastic changes (Wu *et al.*, 2018). Fig. (1) provides explanations on the human toxicity induced by the HM. Recent researches on HM levels in the soil focus mostly on the highly industrialized areas, including the populated areas; clusters of cities, and locations with linear and chronic contributors, such as landfills; manufacturing facilities, and roads. The functional harm brought on by Chromium (Cr) includes RNA polymerase arrest; DNA polymerase arrest, change in gene expression, and genotoxic effects. Cr-DNA adducts and collapsing replication forks can damage the DNA and impede proper repair of its lesions, which may result in genetic changes and detrimental cellular effects. Breaks in the double DNA strand can interfere with the signaling pathways; the cytoskeleton, and the cellular communication; by causing chromosomal instability. Cancer-causing potential has been connected to the bypassing of spindles and fabrication stages in the central nervous system (Briffa *et al.*, 2020).

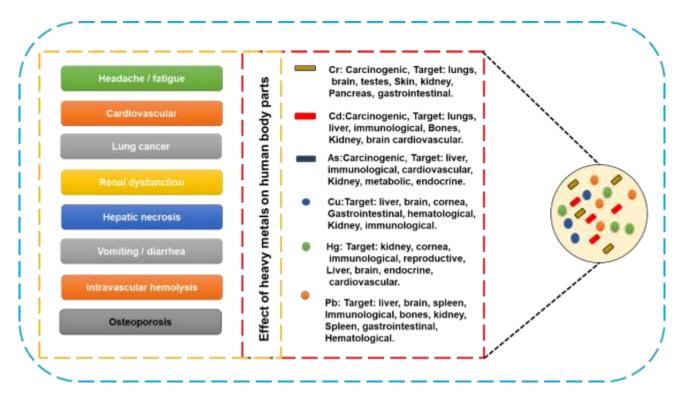


Fig. 1: Heavy metals toxicity on human body parts (Source: https://doi.org/10.1016/j.heliyon.2020.e04691)

4. Bioremediation microbial profiling

To quickly reduce the dangerous organic chemicals in groundwater; soil, substances, materials, and sediments to levels that are safe for the ecosystem; bioremediation is a technique to be used. Before now, several effective methods have been used to precipitate and immobilize the inorganic substances; where microbial neutralization has been devised to reduce the pollutants (Medfu Tarekegn *et al.*, 2020). By promoting the microorganisms; the biological molecules that serve as end donors for electrons and for nutrient administration, increase the biological remediation of the environmental pollution. In

bioremediation; sewage is employed as a source of energy and carbon (Osman et al., 2019). The pollutant substance is frequently broken down by the bacteria into the plant matter; fluid, and biogas or carbon dioxide. Biodegradation; is the method through which the chemical or collegial substances become deteriorated and changed into ecologically favorable substances (Ihsanullah et al., 2020). Bioremediation techniques can be improved by recognizing the microbes that are essential for detoxifying and profiling the bacterial communities. Acceleration of bioremediation may be achieved by encouraging the growth of the local microbial population and/or by importing the individual microorganisms with enough degradative capacity (Mohanty et al., 2021). Numerous bacteria have developed the ability to use or accidentally break down a wide range of contaminants and by-products. Total reduction of the harmful substances depends on the collective actions and interactions of the various microbial assemblies into benign end products; thus, examining the connections and interactions among the various microbial functional guilds and roles is vital (Ohoro et al., 2019). Additionally, you can gain the information required for more accurate assess and select the remedies before setup, in order to make immediate and precise enhancements when necessary. These are achieved by using the microbial community and evaluating their functions as diagnostic tools to spot the possible errors that may be encountered in the way treatment is being carried out (Roy et al., 2018). To more thoroughly analyze the microbiological data, studies on sewage treatment; subsurface treatment, and soil restoration, have all been made by extensive use of the high-speed sequencing technologies (Igiri et al., 2018). These technologies can assist in making the best use of the remediation approaches; by providing a tool for learning about the mechanistic interactions between the microbiomes; contaminants, and the removal methods (Escárcega-González et al., 2018). Quick decontamination of the environment by bioremediation may be achieved through an analysis that compensates for the lack of biological indicators. This is possible due the awareness of the dynamics and activities of the underlying microbial populations.

4.1. Variety of microorganisms in the wastewater system

For a while and as a longer-term; the economical, ecologically responsible, and the flexible method of clean-up, depends on the local microbial ecology of the contaminated places. Wastewater treatment facilities are essential pieces of equipment for assuring the efficient wastewater management and fostering the long-term sustainability of our society (Hall and Mah, 2017). Methods for biological wastewater treatment make use of some genetically modified bacteria (Md Badrul Hisham et al., 2019). Engineers must first understand how the microbial populations are organized and how they react to the changes in the surroundings, to build and improve the ecological structures. Economically, environmentally, and in terms of human health; contamination is becoming more and more significant. The keys used to removing carbon dioxide; phosphorus, and nitrogen from wastewater in the context of biological wastewater treatment; offer several microbial operations that enhance the various beneficial microbes, such as denitrification: nitrification, heterotrophs, and polyphosphate accumulating microorganisms (PAOs). Several factors, including HM non-biodegradability; the environment, and their persistence; contribute to the metabolism of plastics in the food chain, as well as the severe health and environmental concerns. Investigation of novel technologies and ideas will contribute to the development of biological wastewater treatment systems; however, collaboration across the various disciplines is essential to attaining this objective (Wu and Yin, 2020).

4.2. Microorganisms' metal tolerance

Exploration of the microbiome prevalence was previously confined by our capacity to cultivate the majority of the bacteria, but this has been changed with the introduction of molecular tools, such as Polymerase chain reaction (PCR); methods of communication, and collaboration (Liang et al., 2019). The appearance of molecular tools like PCR techniques revolutionized the study of the microbial variety, which had previously been hampered by our inability to cultivate the majority of microorganisms. Researchers have studied the variety; manifestation, and presence of ribosomes and protein-coding genes in these settings for decades using PCR amplification and sequencing. This allowed for identification of the key microorganisms engaged in the processes of carbon breakdown and ammonia oxidation. Using PCR techniques provides insight into the range of important participants in the Biological Wastewater Treatment Plants (BWWTPs) and how inheritance is organized with respect to the particular environmental factors. Because of the intricate arrangement of microbes, there are several ways that metal may be consumed. According to the metabolic processes of the microbial cell; metal transport via the cell membrane is what causes intracellular storage. Metal transport is frequently connected to the microbe's effective defense system, which reacts to the build-up of dangerous metals. When a metal and a molecular structure on the microorganism surface bios collegial together; an absorption process takes place that is independent of the microbial metabolism (Sankarammal et al., 2014). Numerous heavy metals transport processes have been analyzed using several bacterial species; mainly Micrococcus: Pseudomonas. Flavobacterium, Enterobacter, and Bacillus spp. The majority of researches discovered that the active and dormant fungal cells contribute to adherence of the inorganic compounds (Tiwari et al., 2013).

Additionally, the recorded effective species for the detoxification of metals is *Aspergillus* sp., which has been used to get rid of Cr from the tannery wastewater. In a bioreactor system operating at pH 6; many bacterial spp. including *Flavobacterium*; *Enterobacter*, *Micrococcus*, *Pseudomonas*, and *Bacillus* spp., have been utilized to analyze the removal of different heavy metals (%) from the effluent. Table (2) displays a list of the most probable microorganisms with HM tolerance. Heavy metals focus on three processes;

mainly physical sorption; ion transfer, and chemical sorption, which are un-affected by the metabolism of the microbial cells. Microorganisms' cell walls are mostly consisting of substances that bind the metals, such as sulphate; phosphate, amino groups, and carboxyl, polysaccharides, proteins, fatty acids, and other substances. This type of metal absorption is quick and renewable, since it does not rely on the microbial metabolism (Dashti *et al.*, 2019).

5. Metals and microbial interaction

Through a variety of physicochemical and biological processes that microorganisms can use to influence the transformation of the dangerous metals in the environment; microorganisms have an impact on the changes among the insoluble and soluble phases of these hazardous metals. Some of these processes including reduction; oxidation, methylation, and collaboration, can be exploited to clean up the contaminated goods. Microorganisms are crucial parts of the natural biogeochemical cycles for the metals and metalloids, where they can alter the species of these metals and metalloids by reduction; oxidation, methylation process, and cooperation (Osman *et al.*, 2019).

The metalloid oxyanions are reduced to several essential forms, such as the selenite (SeO₂₃) and selenate (SeO₂₄) ions. Moreover, the metalloids; metalloid oxyanions, and/or organic metals, are methylated to form methyl derivatives, including arsenite (AsO₂) and arsenate AsO₃₄ ions; while methylmalonic acid is methylated form to trimethylarsine [(CH₃)₃As]. The metal microorganism interactions include surface phenomena; biosorption using dead biomass, and absorption by live biomass. When the bacteria have different kinds of resistance; it represents a good survival method that allows them to transform the metals in the aquatic and in the different soil types; even if they exist in substantial amounts. The best possibilities for biosorption technology include creativity; affordable biomass renewal, and transformation of the recovered metal into an utilizable form (Ihsanullah et al., 2020).

Serial no.	Heavy metals	Microbes	Reference
1	Lead (Pb), Copper	Curtobacterium sp. NM1R1	Roman-Ponce et al., (2017)
	(Cu), Arsenic (As)	; Microbacterium sp. CE3R2	
2	Cu	Kocuria sp. CRB15	<u>Hansda et al., (2017)</u>
3	Cd	Klebsiella pneumoniae	Pramanik et al., (2017)
4	Cd	Enterobacter; Leifsonia,Klebsiella, and Bacillus	<u>Ahmad <i>et al.</i>, (2016)</u>
5	Cd	Rhodococcus sp. and Flavobacterium sp.	Belimov et al., (2015)

Table 2: The most probable heavy metal-tolerant microorganisms

Metal fermentation from the polluted soil requires a combined *ex-situ* and *in-situ* strategies. The microbial technologies may work because the metal-microbial interactions are physiological and genetic (Dave *et al.*, 2020). Enzymes alter the metal persistence and contaminants, and have biotechnology promise in bioremediation (Vázquez-Núñez *et al.*, 2020). Several microorganisms have developed metal resistance due to their early exposure to the harmful metals. Over the past fifty years, the surge in pollutants from the metals coverage has led to a rise in the metallic resistance (Ihsanullah *et al.*, 2020).

The microbial techniques used for detoxifying the harmful heavy metals are shown in Fig. (2). Additional researches on the microbial resistance to metals and its improvement are highly recommended, since the anthropogenic metal activities have harmed the ecosystem. Due to the presence of creative bacteria; the ecosystem has evolved wide metals detoxification and tolerance mechanisms. Greater concentrations of the metal-tolerant and resistant bacteria have been discovered in the polluted soil; water, and other resources. These bacteria have acquired tolerance and resistance to toxic amounts of the metals in diverse ways (Hadiani *et al.*, 2018). In this study, we have

exerted significant efforts to explain the recent changes in the relationship between the metals and the microorganisms; focusing on the metals' harmful impacts on the living beings, how microorganisms respond to them by developing metal tolerance and resistance mechanisms, and the potential applications of such microorganisms in the biological remediation of metals pollution.

6. Studying the varieties of proteins and secondary metabolites

Due to its bioaccumulation and lack of biodegradability in nature; metal poisoning is a significant issue on the global scale. Large numbers of metabolic and redox reactions and inorganic metals are necessary. Biosorption is a technique for treating the polluted environments; using bacteria or enzymes to change the hazardous chemicals into less hazardous forms (Zhao et al., 2023). Certain limits to the heavy metal bioremediation include non-biodegradability of heavy metals and production of hazardous metabolites microorganisms. by the То improve the bioremediation efficacy, it is innovative to directly microorganisms with various apply catabolic capabilities, such as enzymes and bio-surfactants.

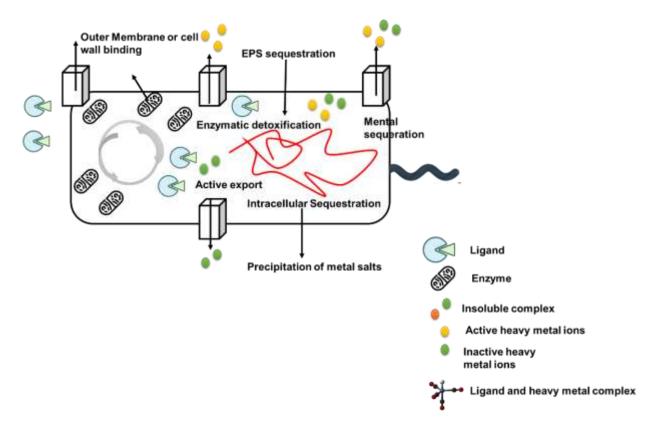


Fig. 2: Heavy metals detoxification (Source: https://www.mdpi.com/2304-8158/11/13/1905)

The bioremediation of heavy metals has limitations, which include the inability of heavy metals to degrade biologically and the production of harmful metabolites by the applied microorganisms. An innovative strategy to improve the bioremediation efficacy is through the direct use of several diverse catabolic-capable microorganisms, including substances and reactions (Upadhyay *et al.*, 2023).

The arrangement and physiological roles of the metal-exporting proteins are explored. The cation diffusion facilitator; RND (Resistance Nodulation Cell Division) superfamily, chromate efflux proteins, and P-type ATP-ases, are some of the molecules that control CBA (CBA: involves Cadmium, Cobalt, and Zinc) ATPase efflux pumps. These transport systems fall under the ABC family of ATP-Binding Cassettes (ABC); A-type ATP-ases, and P-type ATP-ases; as

ATP is utilized as a fuel for the transfer of heavy metals. In certain bacteria, this RND gene causes proton inflow and metal cation efflux. A protein component of the hydrogenase enzyme complex known as HoxN has been mentioned. The enzymes called hydrogenases are responsible for the reversible transformation of atomic hydrogen (H₂) into protons (H⁺) and electrons (e⁻). HoxN A as a subunit of several hydrogenase enzymes contributes to the stability and enzymatic activity of the hydrogenase complex. Magnesium ion (Mg²⁺) transporters; known as CorA proteins, are families that include the bacteria and archaea. By promoting the transport of Mg²⁺ across the cell membranes; CorA proteins play a critical part in preserving the magnesium homeostasis within the cells. HoxN: Cis-Regulatory Element Homology Region (CHR), the Cation Diffusion Facilitator (CDF), and the CorA proteins families, are investigated in the

absence of a recognized driving force technique for metals transport (Anuj *et al.*, 2019).

In a chemolitho-autotrophic way of life, NiCoT family of proteins transfers nickel (Ni²⁺) and cobalt (Co) ions across the cell membranes. NiCoT protein is necessary for the ingestion of Ni²⁺ by the hydrogenases, which do molecular hydrogen absorption. While the Gram-negative bacteria need ABC-transporter proteins for the same task: HoxNs is employed for the engagement-affinity Ni²⁺ extraction by the protozoan. Since HoxN transporters lack an ATP-ase activity; the chemiosmotic gradient favors their absorption of the divalent cations. Currently, several HoxN-family transporter proteins have been identified that are either involved in Co2+ or Ni2+ absorption. Still, only one of the two cations is linked to HoxN-proteins (Salunkhe et al., 2023).

7. Microbial signaling molecules

In quorum sensing; the autoinducers serve as signaling molecules, which the bacteria release to communicate with the other microorganisms of their species. As autoinducers; the smaller acyl-homoserine lactone (AHL) molecules or the bigger peptide-based compounds may be released, where every compound behaves differently. Numerous microorganisms produce extracellular polysaccharides that securely fasten the metals. Microbes are important in the cycle of metals, because they mobilize or immobilize the hazardous metals, such as lead; cadmium, and uranium, which all considerably are well-bound by the microbial exopolymers. It is generally accepted that the metal ions react with the exopolymers when the negatively charged functional groups are present. These groups include, uronic; succinyl, hydroxyl, and phosphate acids. The exopolymers of some bacteria spp., such as Azotobacer sp.; Micrococcus luteus, and Staphylococcus aureus, have been reported to immobilize lead (Netrusov et al., 2023). Exopolymers provide the biological function of concentrating the iron under low-iron settings and promote the cell infiltration.

Two of the most typical kinds of siderophores produced by bacteria are the hydroxamate and catecholate. Ferric ions are strongly bound by the hydroxamate ions. Gallium; aluminum, and chromium, are examples of the trivalent metal ions that are comparable with iron in their dimensions. There may be a specific rival for catecholate siderophores in the form of aluminum. Beside iron; Md Badrul Hisham *et al.*, (2019) explored the form of interactions with the other metals; however, the humans are less drawn to these metals. Meanwhile, the recognition of this siderophore-metal complex by an uptake receptor determines the effects on metal intake and toxicity (Khan *et al.*, 2023).

Fig. (3) demonstrates an overview of the chemicals used by the microbes to signal. As a result of being exposed to the pollutants; the siderophores may have advantageous effects. Reduced concentrations of the free metals can result from the absorption receptor's discrimination towards the metal siderophore complex, which also has a protective effect (Rahman and Singh, 2020). Biosurfactants are produced by a large number of bacteria and released under stressful circumstances (Tiwari *et al.*, 2013). Metals are complexed with biosurfactants; even though the process may make them seem more soluble (Jasu and Ray, 2021).

Evidence suggests that a wider diversity of the biosurfactant-producing microorganisms can be extracted from the metal-contaminated than from the uncontaminated areas. Popular metabolic by-products that cause metal reduction may eventually have an impact on metal solubility. Under an aerobic environment and using an enzyme; copper and lead can be precipitated when *Citrobacter* sp. produces phosphate (Kumari *et al.*, 2021). However; under anaerobic environments, high H₂S concentrations from some sulfate-reducing bacteria such as *Desulfovibrio* sp. may easily lead to metal precipitation.

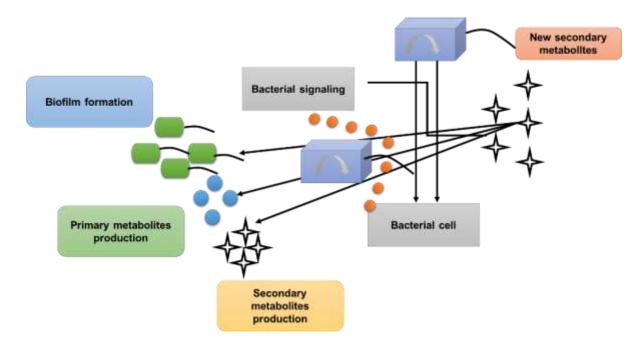


Fig. 3: Secondary metabolites production and bacterial signaling (Source: <u>https://biologyreader.com/difference-between-primary-and-secondary-metabolites.html</u>)

Conclusion

To avoid environmental contamination, this study examines the function of the microbial community in wastewater treatment plants, and also the significance of microbial compounds such as metabolites and proteins on the process of metal detoxification. Cellular processes can control the level of metal resistance in microorganisms. In general, the microbiological method prevents the accumulation of excess metal levels, and as a result; it initiates the release of impact energy. Metals have also been found to be mobilized and immobilized in a variety of ways through the microbe-assisted remediation techniques. The simultaneous spread of genetically engineered microorganisms (GEM) and gene transfer may be a viable tactics in case of environmental application. Additionally; using a biological method that genetically combines the modified bacteria with a specific polluted chemical may increase the effectiveness of bioremediation. Nanomaterials are said to be advantageous economically, because nanoparticles enhance the surface area and decrease the activation energy; thus reducing the toxicity of the pollutant(s) to the microorganisms. The optimal strategy of bioremediation may be determined by monitoring the pollutants; evaluating the effectiveness of the various bioprocesses, the lowest environmental impact, and the overall cost.

Acknowledgement

None.

Conflict of interest

No conflict of interests exists between the authors of this study.

Funding source

This research did not receive any fund from governmental, commercial, or nonprofit funding organizations.

Ethical approval

Non-applicable.

Author Contributions

Conceptualization, M.H. and P.S.; Data curation, M.H. and P.S.; Investigation, M.H. and P.S.; Supervision, M.H. and P.S.; Validation, M.H. and P.S.; Roles/Writing - original draft, M.H. and P.S.; Writing - review & editing, M.H. and P.S.

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