Innovations

Study of Heavy Metals Tolerance by Microorganism in Industrial Wastewater: A Review

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Abstract: *The contamination of the environment with heavy metals is a consequence of numerous human exercises, like mining and metallurgy, and the impacts of these metals on the biological systems are of huge financial and general wellbeing importance. Likewise, water contamination is on the ascent as a result of expanded human populace and exercises, unsustainable farming practices, and quick industrialization, and it is a significant worldwide concern. There are different microbial strains that are impervious to heavy metals and hence could be utilized in bioremediation. The utilization of naturally occurring microorganisms like bacteria, fungi, algae, or plants to remove such pollutant i.e., Bioremediation has proven to be effective and proficient. Microorganisms have adapted to the presence of heavy metal particles in their environments, and there are basically five fundamental mechanisms through which, the bacteria show resistance against the heavy metal pollutants: extracellular barrier, active transport of metal ions (efflux), extracellular sequestration, intracellular sequestration, reduction of metal ions. Moreover, such interaction among microbial strains and heavy metal particles are of extraordinary interest as it provides a fundamental process of heavy metals treatment i.e., advantageous, non-toxic, affordable, and natural processes. Moreover, such mechanisms to resist heavy metal toxicity involves production of specific genes and proteins by bacteria, such as, metallothioneins, enzymes, transporters, metal chelators, efflux pumps, and regulatory proteins. This article briefly discussed about the effect of heavy metals on the environment and human wellbeing, as well as the utilization of microbial strains for eliminating it.*

Keywords: *Industry effluents; Microorganisms; Bioremediation; Heavy metals; Bacteria; Mechanisms; Genes.*

Introduction

The group of metallic elements known as heavy metals includes transition metals, lanthanides, and metalloids, etc.While some of them are non-essential, others are necessary cofactors for various enzymes. The majority of them are derived from anthropogenic resources, primarily commercial and industrial in nature, and are discharged into the environment. They can be found in soils, water, as well as in rocks. As a result of their persistence in nature, they frequently build up in food chains. Heavy metals' hazardous properties have long been understood. The dangers linked to heavy metals usually surpass their advantages; for example, significant contact with antimony and chromium increases the risk of cancer. (Sundar and Chakravarty, 2010; Sun et al., 2015; and Coetzee et al., 2020), and lead poisoning results in intellectual impairments in infants (Hou et al., 2013). Additionally, heavy metals have a wide range of unfavourable effects on the environment. For instance, mercury's transformation into methylmercury in the presence of water results in highly poisonous sediments (Rice et al., 2014). The Environmental Protection Agency (EPA) mentioned the list of several metals that are considered to be the most hazardous for the environment, such as cadmium, arsenic, mercury, and lead (Goyer, 2004). In addition, although there are more than 20 heavy metals on the United States Agency for Toxic Substances and Disease Registry's (ATSDR) "top 20 list," arsenic is the most prevalent cause of acute heavy metal poisoning. Lead is rated second on the list (Fay and Mumtaz, 1996; Flora et al., 2011).

In addition to harming the skin and other organs of the body, heavy metals may also pose hazardous impact to the kidneys, nervous system, liver, heart, and other cardiovascular systems. Leads, cadmium, thallium, and antimony belong to the dangerous heavy metals often discovered in factories and contribute significantly to pollution of the environment.Thallium is more harmful than other heavy metals and contributes to human baldness (Karbowska, 2016). Itai-itai illness is caused by exposure to cadmium, while Minamata disease is caused by mercury poisoning. Therefore, it is advisable that the people must leave industrial regions where there are significant levels of heavy metal emission in order to avoid hazardous impacts (Trevors, 1985).

Sources of heavy metal pollution

These heavy metals have been present in the Earth's crust naturally ever since the planet's formation. However, there will soon be a surge in metallic compounds in both the terrestrial and aquatic environments due to the sharp growth in the usage of heavy metals. (Gautam, 2014). Due to anthropogenic activity, which is the main cause of pollution, heavy metal pollution has emerged. This is mostly due to the metal-based industries as well as the leaching of metals from a variety of sources, including garbage dumps, landfills, excrement, cattle and poultry dung, runoffs, cars, and road construction. One secondary cause of heavy metal contamination has been the usage of heavy metals in agriculture through the use of herbicides and fertilisers kind of chemicals. Other than that, there are presence of some natural factors as well, which involves release of heavy metals due to volcanic eruptions, soil erosion, corrosion from the metallic substances, and geological weathering, etc. (Masindi- He, Z. L).

In a similar vein, soil contamination can happen accidentally or on purpose. The contamination that occurs intentionally include the sources such as, use of leaded paint, animal manures, fertilisers, and pesticides; release of sewage sludge; petroleum distillates spills, residues from coal combustion, and wastewater irrigation (Aronsson, 2001). Heavy metal levels in our agricultural areas have grown due to use of untreated wastewater and sewage during irrigation, as the metals have been absorbed by crops. Moreover, the accidentsal seepage from hazardous material-carrying trucks and river and sea floods may be examples of the unintentional contamination to the land (Muchuweti, 2006). Similarly, urbanization and industrialization are to blame for air pollution. PMs, particularly tiny particles and dust, have been released due to anthropogenic and natural activity. Sandstorms, volcanic eruptions, soil erosion, and rock weathering all release the particulate matter that is present due to natural activities. While the production of particulate matter caused by human activity includes industrial processes, the burning of fossil fuels, automobile exhaust, smelting, and more. The particle matter can result in serious health issues, destruction of infrastructure, acid rain creation, corrosion, eutrophication as a result of particulate matter landing in the water during rain, and haze (Walker, 2005; Jessica, 2020).

Additionally, runoff from towns, cities, and factories carries the metals, which build in the water bodies' sediments. Typically, sewage systems receive both home and industrial wastes. Increased level of heavy metals are present in untreated sewage and are not broken down. Either the final effluent or the resulting sludge removes them. Treatment of the sewage affects the characteristics and contaminants of the sewage that enters the water. Moreover, jet engines and internal combustion engines are additional sources of atmospheric pollution. Along with refrigerators, aerosols, and radioactive pollution, pesticide spraying is another form of pollution (Sarkar, 2002; Masindi, 2018).

Bioremediation

To safeguard living things and the environment, the clearing of heavy metals in soil is crucial (Glick, 2010). Traditional remediation approaches include physical and chemical methods, which are less time taking but may be considered expensive and ineffective since they have adverse impacts on soil qualities and secondary contamination (Ullah et al., 2015; Singh and Prasad, 2015). On the other hand, biological approaches—such as phytoremediation and bioremediation—are thought to be efficient methods for heavy metal remediation. These methods use plants that may absorb heavy metals and use microorganisms to remove contaminants from soil (Adams et al., 2015; Doble and Kumar, 2005; Nakbanpote et al., 2016). The bioremediation approach is based on biological agents' strong metal-binding capacity, which facilitates the extraction of heavy metals from polluted environments very effectively. M. Robinson developed the method of bioremediation at first, utilising living microorganisms (Amin, 2013). It is a cutting-edge and developing technology because of its increased proficiency, sensitivity to the environment, and affordability (Deshmukh, 2016; Singh, 2020). In contrast to methods like burning, catalytic degradation, using adsorbents, physical removal, and ultimately pollutant annihilation, bioremediation is a natural process [Harekrushna, 2012]. In-situ and ex-situ bioremediation are the two forms of bioremediation. In-situ bioremediation, as opposed to ex-situ bioremediation, which takes place in bioreactors, bio-piles, and land farming, is more commonly utilised since it can lessen ecosystem disruption at heavy metal polluted locations. Bioremediation in-situ is substantially more effective and environmentally beneficial. Microbial bioremediation is an efficient, cost-effective, and environmentally safe method of treating heavy metal contamination that also reduces costs(Kumar Mishra, 2017). The main mechanisms through which the heavy metals could be removed by using microbes are biosorption, bio-oxidation, and biomineralization. Among them, the process of biosorption involves various mechanisms such as,ion exchange, chemical adsorption, surface precipitation, and complex formation, etc. Moreover, the mechanism of biomineralization includes bioleaching, that uses the process of dissolution or complexation to release of heavy metal ions from the insoluble ores (Jin et al., 2018). On the other hand, phytoremediation is a very sluggish and seasonally effective approach that frequently depends on the climate, water, and soil conditions (Chintakovid et al., 2008). In this situation, microbial techniques offer a beneficial substitute for removing heavy metals from soils.

Heavy metals in industry effluents

Massive amounts of hazardous heavy metals and other contaminants, such as As, Cd, Cr, Cu, Co, Hg, Ni, Pb, Sn, and Zn, can be found in industrial effluent. These toxins can affect any ecosystem. Toxic heavy metals can come from a variety of places, including waste from mines and hospitals, electroplating, sewage, smelters, battery factories, dye and alloy companies, and electronic factories. Natural or man-made sources of these heavy metals can contaminate water. Examples of natural causes include volcanic eruptions, erosion of soil, and rock disintegration; while manmade actions that can contaminate water include burning fossil fuels, mining, land filling, urban water runoff, irrigation, engineering processes using metal, printed circuit board manufacturing, colouring dye production, and more. Because of this, the water cannot be used by people. (Srivastav, 2020; Verma, 2013)

Toxic metals and metal chelate are present in both treated and untreated waste effluents from a variety of sectors. Thus, the quantity of heavy metals may got increased along the food chain and create their toxic effects at locations that are distant from the source of pollution due to their non- biodegradable nature (Tilzer and Khondkar 1993). Even if traces are carried to bodies of water, they may still be extremely harmful to people and other organisms. Numerous factors, such as the kind of metal present, its nature, its biological role, the organism exposed, and the duration of the exposure, all affect the toxicity of heavy metals. All the organisms in the food chain will be impacted if anyone is, and humans often come last in the food chain; therefore, this will have a greater impact on us because we would have absorbed more heavy metal as concentrations rise up the food chain. The heavy metals included in textile dyes' effluents have been discovered to be carcinogenic, and textile dyes are poisonous and highly stable (Tamburlini et al. 2002). According to APHA (1995), the majority of the chemical contaminants in tannery effluents are heavy metals like Cr, Zn, Fe, and Ca that cannot be eliminated using standard wastewater treatment techniques. Tannery and textile effluent may also alter the water bodies' physicochemical characteristics, such as pH, dissolved oxygen content, and electric conductivity. In addition to being hazardous to humans, chemicals present in industrial effluents have been discovered to be toxic to aquatic life (WHO 2002), posing major environmental issues and challenges to maintaining aquatic biodiversity (Das et al. 1997;Ghosh and Vass 1997). Heavy metals have been linked to many fatal disorders, including eyelid oedema, tumours, nasal and pharyngeal congestion, stuffiness, and gastrointestinal, muscular, reproductive, neurological, and genetic abnormalities (Tsuji and Karagatzides 2001; Das, 2010). In addition to making fish unsightly, dyes in surface and subsurface water are known to cause a number of water-borne illnesses, including dermatitis, ulceration of the skin and mucous membranes, bleeding, nausea, and severe respiratory tract irritation. Increased toxicant concentrations during summer paddy cultivation, when rivers have minimal discharge, may make the issue worse (Karim 1994). Sludge cannot be utilised as a soil conditioner or fertiliser in agricultural land because of the presence of heavily concentrated heavy metals in it (Islam et al. 2009).

Bioremediation of industrial and domestic wastewaters

As it depends on microorganisms to remove toxins from waste, such as wastewater or soil, bioremediation is regarded as a valuable technique based on its safety and sustainability profile. Although microorganisms including bacteria, archaebacteria, yeasts, fungi, and algae help to make the bioremediation process possible, the biological treatment alone is insufficient to remove toxins. Numerous environmental factors influence the efficacy of bioremediating wastewater that is excreted after domestic and industrial uses. Due to their direct connection to the biological and metabolic activity, which serves as the foundation of bioremediation, some of these components are said to be the most important ones. The presence of nutrients, oxygen levels, temperature, pH, heavy metals, toxic compounds, the physiology and metabolic activity of biodegradative microorganisms, the effectiveness of mechanisms used for contaminants removal, and the type of microorganisms involved can all affect the efficacy of bioremediation process for both the domestic as well as industrial wastewater. Most of these elements work in tandem with the physicochemical interactions that happen between pollutants and bacteria during bioremediation. Further analysing these parameters reveals that the mix of biological, metabolic, and environmental variables covered by these criteria are the ones that affect the extent of success by which home and industrial wastewater may be treated via bioremediation (Bwapwa, 2022).

Adverse impact of heavy metals

When heavy metals are ingested by humans, they can become extremely poisonous. These have the potential to impair the immune system, harm the kidneys and liver, cause hypertension, alter genetic material, cause cancer, neurological abnormalities, and even death when present in high amounts. According to Goldhaber (2003), excessive manganese exposure can cause severe neurological difficulties like Alzheimer's and Parkinson's disease, by causing apoptotic cell death and affecting homeostasis (Harischandra et al., 2019). According to Garza-Lombó et al. (2019), arsenic poisoning affects the balance of neurotransmitters and synaptic transmission. Neurotoxicity caused by cadmium leads to neurodegenerative defects such as amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, and multiple sclerosis, as well as impairments in motor function and behavioural changes in both adults and children (Branca et al., 2018; Marchetti, 2014). (Wang and Du, 2013).

Besides, cadmium causes nephrotoxicity, which causes severe clinical symptoms as glucosuria, phosphaturia, Fanconi syndrome, and aminoaciduria (Reyes et al., 2013). An excessive amount of exposure can cause kidney related issues such as hypercalciuria, renal failure, and renal tubular acidosis (Jacquillet et al., 2007). Acute tubular necrosis is brought on by mercury exposure to the kidneys, and on long-term exposure, it may damage the epithelium and results in necrosis of the pars recta of the proximal tubule. Chronic kidney damage brought on by mercury exposure includes tubular failure, increased excretion of albumin and retinol-binding protein in the urine, and a nephritic condition with a membranous nephropathy feature (Lentini et al., 2017). Moreover, DNA is harmed by arsenic, which also induces epigenetic changes (Park et al., 2015, Martinez et al., 2011). Because it binds to DNA-binding proteins and slows down the DNA-repair process, arsenic exposure increases the chance of developing cancer. Lead is a carcinogen that damages the DNA repair process, genes that control cellular tumour growth, and chromosomal structure and sequence by releasing ROS (Silbergeld et al., 2000). Reactive oxygen species (ROS) are produced in large amounts by the peroxidative activity of mercury, and they can promote

protumorigenic signalling and the formation of malignant cells (Reczek and Chandel, 2017, Zefferino et al., 2017). Nickel is reported to affect various pathways that are responsible for causing cancer such as transcription factor control, free radical production, and gene regulation (Zambelli et al., 2016).
Lead's toxicity may cause an increase in oxidative stress, which damages the liver (Farmand et al., 2005, Malaguarnera et al., 2012). Chronic and acute cadmium-induced liver damage results in liver failure, which raises the risk of cancer (Hyder et al., 2013; Yu et al., 2019). The liver can be harmed by Cr(VI), as demonstrated by numerous investigations, and histological alterations such steatosis of hepatocytes, parenchymatous degeneration, and necrosis have already been observed (Hasanein and Emamjomeh, 2019). On the other hand, chronic lead exposure can result in heart disease, thrombosis, atherosclerosis, arteriosclerosis, and hypertension (Vaziri, 2008; Peters et al., 2010). Chronic exposure to arsenic encourages a variety of potential skin conditions, including hyperkeratosis, hyperpigmentation, and various forms of skin cancer (Huang et al., 2019). Furthermore, chromium exposure has been linked to a number of serious acute and long-term dermatological disorders, including systemic contact dermatitis, skin cancer, and contact dermatitis (Matthews et al. 2019, Uter et al. 2018, Yoshihisa and Shimizu 2012).
More than 10% of women are at risk of infertility due to their exposure to heavy metals, which are the most prevalent environmental contaminants that can result in reproductive abnormalities (Apostoli and Catalani, 2011) (Rattan et al., 2017). Arsenic consumption is linked to a higher risk of endometrial cancer in women (Salnikow and Zhitkovich, 2008). Teratogenesis and carcinogenesis are the two main mechanisms by which the genotoxic effects of human exposure to heavy metals alter genetic material (Young et al., 2008; Crespo-López et al., 2009). Arsenic's genotoxicity causes deoxyribonucleic acid to mutate, resulting in chromosomal abnormalities, mutations, the creation of micronuclei, deletion, and sister chromatid exchange (Roy et al., 2018).

Table 1: List of heavy metals, concentrations, toxic health effects, along with proposed mechanism

Name & symbol of Heavy Metal	Sources	Regulatory limit (ppm)	Human Health Disorders	Target Organs	Proposed Mechanism	Toxicity
Arsenic (As)	Pesticides, metal smelters, and fungicides	0.01	Dermatitis, Bronchitis, poisoning	Skin, Lungs, Kidney	Alter cellular processes (oxidative phosphorylation and ATP	Genotoxicity, Neurotoxicity, Carcinogeni

					synthesis)	city
Barium (Ba)	weathering of rocks and minerals, industries	2.0	Cardiac arrhythmias, gastrointestinal dysfunction, increased blood pressure	Lungs, Heart, GIT	Increase free radical that cause DNA damage	Carcinogenicity, Genotoxicity,
Cadmium (Cd)	Welding, electroplating, fertilizers, and pesticides	0.1	Renal dysfunction, lungs disorders, renal failure, bone marrow damage, defect on bones.	Lungs, Kidney, Bones, endocrine system, Brain	Endocrine disruptor along with altered calcium regulation. Attenuated depression, paranoia and memory loss	Cardiovascular toxicity, Mutagenic, Immunological toxicity
Chromium (Cr)	Mineral and mine sources	0.05	Hair loss, affects nervous system and cause irritability, fatigue	Brain, Hairs	Impaired activity of cytokines, altered biochemical parameters	Immunological toxicity, nephrotoxicity
Copper (Cu)	Mining, chemical industry, pesticide production	1.3	Brain and kidney damage, liver cirrhosis, anaemia	Brain, liver, kidney	Affect on neurotransmitter leads to neuron damage	Neurotoxicity
Mercury (Hg)	Batteries, pesticides, paper industry	2.0	Autoimmune diseases, memory loss, tremors, brain damage, gingivitis, spontaneou	Brain, teeth, kidney	Increased Nuclear factor kappa B (NF-κB) that cause inflammation	Immunological toxicity, carcinogenicity, nephrotoxicity, and cardiological toxicity

			s abortion			
Lead (Pb)	Paint, smoking, pesticides, automobile emission, mining, coal burning	15	Infant encephalopathy, chronic damage to nervous system, liver and kidney, mental retardation in children, delay in overall development, cardiovascular disorders	Kidney, liver, Heart, Brain	Increased inflammation and damage neurotransmitters	Immunological toxicity, carcinogenicity, nephrotoxicity, Heavy Metal-induced neurotoxicity, and cardiological toxicity

Microbial strains resistant to heavy metals

Microorganisms are essential to the environment as they help in the biogeochemical cycling of metals as well as act in the remediation of those habitats that got severely contaminated with heavy metals. They can survive in the presence of heavy metals despite the poisonous effects they have, thanks to a variety of mechanisms that lessen or tolerate their toxicity (Spain and Alm, 2003). Metals in water, soil, and industrial waste are resistant to many bacteria. By creating a range of resistance mechanisms, microorganisms have adapted to the presence of both nutritional and non-essential metals (Rehman, 2006). Several physical or chemical processes may also be involved in the concept of biosorption. In the cell walls of fungi, heavy metals are bound to various functional groups such amines, carbolic acid, sulfhydryl groups, carboxylic acids, amines, lipids, and phosphate (Wahyu, 2016). The carboxyl, hydroxyl, and amino groups in the biomass were involved in the lead ions' ability to bind to them (Parungao, 2007). Moreover, there are ion walls, that are having proteins, polysaccharides, and lipids as their constituents, and thus possess various functional groups such as COO^-, -OH, SO_4^{2-}, PO_4^{3-}, and $-NH_2$, that can bind with the metal ions (Gupta, 2016). There are numerous cases of heavy metal removal from industrial effluent and/or contaminated water using microbial biomass and the isolation of heavy metal-resistant microorganisms: removal of lead, chromium, and cadmium by the fungus *Pleurotus ostreatus* HAAS (Yang, 2009), nickel resistance of *Bacillus megaterium* and its removal capacity (Rivas-Castillo, 2017), susceptibility towards copper, mercury, and lead as well as their removal by *Rhodotorula mucilaginosa* (Grujić, 2017), the resistance and removal capacity of *Aspergillus niger* against chromium (VI) (Acosta-Rodríguez, 2017), different heavy metals removal by *A. niger* (Mukhopadhyay, 2011; Kapoor, 1999), bioleaching process via using *A. niger* (Yang, 2009), and the removal of different heavy metals such as, manganese, zinc, cadmium, lead, and iron, etc. by *A. niger* (Tsekova, 2010). In detail, the various microbial strains that are resistant to heavy metals and therefore could be used in bioremediation are as follows:

Algae

Algae are essential to the process of natural water purification. They may be used to adsorb radioactive and poisonous metal ions as well as recover valuable ions like gold and silver. Algae aid in nutrient removal by growing quickly and absorbing carbon, phosphorus, and nitrogen from wastewater. This offers a cost-effective and sustainable substitute for the treatment of sewage wastewater (Sharma, 2013). Several researchers have suggested using microalgae to extract nutrients from a variety of wastes in order to prevent further deterioration of wastewater's quality (Kshirsagar, 2013). Textile wastewater (TWW) contains organic dyes, which are a possible source of carbon, as well as the nutrients needed to grow algae (phosphate, nitrate, micronutrients, etc.) (Fazal, 2018). Microalgae use the nutrients and colours in wastewater for growth. Wastewater can be bioremediated by using the *C. vulgaris*, *S. quadricauda* culture. *C. vulgaris* is utilised in the wastewater sector to process the manufacture of dilute ethanol and citric acid. It helps in speeding up the process of decrease in BOD and COD effluent levels. With the help of *C. vulgaris* and *S. quadricauda*, nitrate removal was accomplished. *S. quadricauda* extracted the phosphate effectively. During the remediation, C. vulgaris uses phosphorus for growth in order to eliminate phosphate (Salgueiro et al. (2016). Wastewater from the textile industry (TWW) is bioremediated using Chlorella vulgaris strain UMACC 001. Both living and nonviable algae have been used to remove colour from dyes and wastewater.

Microalgae are used in two different ways to bioremediate TWW. The first process involves consumption of the colours by microalgae as a source of carbon and thereby changing them in the form of their metabolites, thus known as bioconversion. The other process involves adsorption of dyes on the surface of microalgae, which act here as biosorbent, thus known as bioaccumulation or biosorption. Both events may occur simultaneously during TWW bioremediation. Moreover, the tendency of microalgae to act as biosorbent may be due to their high surface area as well as strong azo dye binding affinity. It has been demonstrated that the Spirogyra possess considerable amount of non-viable biomass that further acts as an effective biosorbent and thereby helped in removing the Synazoldye from the textile effluent. Basic dyes can be eliminated through biosorption by living biomass of macroalgae like *C.lentillifera* and *C. scalpelliformis*. Besides, Phormidium kind of algaeare able to change the colours into their simpler chemical forms through biological process. Color is extracted from the surface of textile dyes using immobilised algae. Spirulina and chlorella are both useful for addressing wastewater disposal (Lim, 2010). *Scenedesmus bijugatus*, *Chlorella vulagris, Oscillatoria tenuisin*, and *Chlorella pyrenoidosa* are a few of the microalgae that can break down azo dyes into straightforward aromatic amines. (Mullai, 2017; Ojha, 2021).

Fungi

Fungi make up the majority of possible prospects and are crucial to bioremediation [Deshmukh, 2016]. Filamentous fungus eats heavy metals like cadmium, lead, zinc, nickel, iron, thorium, uranium, and silver. Biosorbents may leverage the capacity of fungal biomass to remove radionuclides and heavy metals from polluted waterways. Straw, sawdust, or maize cobs can all be destroyed by white rot fungus such Pycnoporus sanguineus laccase and Phanaerochaete chrysosporium [Amin, 2013; Trovaslet, 2007].Penicillium, Aspergillus, Rizopus, Mucor, Saccharomyces, and Fusarium all take up metal ions. Heavy metals (Cr, Ni, Zn, Pb& As) can be bioabsorbed using penicillium. Penicillium, Rizopus, and Saccharomyces are capable of biosorbing the radionuclides [Bishnoi, 2005]. High intensity phenolic waste can be broken down by white rot fungi [Strong, 2007]. Funaliatrogii, Coriolus versicolor, Pleurotus pulmonarius, and Phanerochaete chrysosporium may be utilised to decolorize and lower the chemical oxygen demand (COD) of molasses wastewater [Kahraman, 2003]. White rot fungi, including edible mushrooms like Lentinula and Pleurotus, as well as various yeast species, are effective in treating olive mill wastewater (OMWW). They act through the biological process and helps in decreasing thecolor, concentration of phenolics, and COD. Specific fungi such as Coriolus versicolor, Funaliatrogii, Geotrichum candidum, Lentinulaedodes, and Phanerochaete species have been used for OMWW remediation. Additionally, fungi such as Aspergillusterreus, Mucor thermohyalospora, Cladosporium oxysporum, Phanerochaete chrysosporium, Fusarium ventricosum, and Trichoderma harzianum can degrade endosulfan. Zygomycetes, along with Aspergillus, Mucor, and Penicillium species, are utilized to break down and detoxify textile effluent and crude oil. The polychlorinated biphenyls (PCBs) degradation taken place through the fungi such as Scedosporium apiospermum, P.chrysogenum, Fusariumsolani, and P.digitatum. Rhizopogonroseolus, Suillusbovinus, and Pinus are combined for cadmium extraction. A. nidulans, Bjerkanderaadusta, Trameteshirsuta, T. viride, Funaliatrogii, Irpexlacteus, and P. ostreatus are a few plant-related fungus that are employed to decolorize textile industry waste [Deshmukh, 2016; Gupta, 2017].

Yeast

The following factors make yeast cells a good alternative wastewater treatment method:

a. It is generally acknowledged as a safe microorganism and can be used without raising any concerns from the public; and
b. It has the capacity to aggregatevarious kind of heavy metals despite the presence of different external conditions (Wang, 2006).

A cheap and accessible source of biomass is yeast biomass. Under addition to high metal concentrations, yeast biomass can adapt to and thrive in a variety of extreme pH, temperature, and nutrient availability circumstances (Anand, 2006). According to certain studies, local microorganisms may withstand high levels of heavy metals in a variety of ways and may be crucial to the regeneration of contaminated sites (Carrasco, 2005; Ge, 2009). The most copper-resistant yeast, designated isolate ES10.4, was discovered in activated sludge at an industrial wastewater treatment facility in Rungkut, Surabaya, Indonesia. Yeast may absorb heavy metals as lead, thorium, zinc, nickel, cadmium, iron, and uranium, etc. (Amin, 2013). In OMWW bioremediation, yeasts including *Candida tropicalis*, *Trichosporon cutaneum*, and *Saccharomyces* sp. are employed. They were found to be additionally effective at decreasing COD levels and eliminating mono- and polyphenols (McNamara, 2008). They are employed to cleanse textile wastewater because harmful chromophores can be absorbed, accumulated, and degraded into simpler chemicals. They include enzymes for dye breakdown and can be employed as biosorbents for dye biosorption. To decompose colours and other materials, yeast such as *Candida krusei, Trichosporon beigelii, Galactomyces geotrichum, S. Cerevisiae*, etc. were utilised (Mullai, 2017).

Bacteria

Bacteria have a wide spectrum of bioremediation potential. They are considered highly effective from both an economic and environmental perspective (Das, 2014). Heavy metal discharges from industrial uses cause a serious environmental problem. The manufacturing industries related to textile dyeing, electroplating, tanning of leather, and metal processing frequently use the hazardous heavy metal chromium. *Arthrobacter, Desulfovibrio vulgaris, Serratiamarcescens, Pseudomonas sp., Ochrobactrum sp., Cellulomonas spp., Ochrobactrum,* and *Acinetobacter* are a few examples of the microorganisms that have been observed to reduce Cr (VI) to Cr (III), i.e., from highly soluble as well as toxic metal to comparatively less soluble and less toxic metal. ArthrobacterpsychrolactophilusSp 313 reduced the protein content of wastewater (Zahoor, 2009; Gratia, 2009). They are utilised for the sewage from industrial facilities. The technique of treatment involved variousbacterial species, including endophytes, *Pseudomonas*, and *B. subtilis* (Shah, 2020). CF-S9 strain of *Klebsiella pneumonia* was also used (Padhi, 2013). Similarly, *Cyanobacteri*a have been commonly used for the treatment of wastewater in terms of extracting the heavy metals, removing the pesticides and crude oil, as well as colour oxidation. Because *Cyanobacte*ria need nitrogen for their metabolic activities, they are usually considered very effective while eliminating nitrate. The strains of cyanobacteria such as, *Phormodiumtenueas* well as *Phormodiumbohneri* were found to be successful at extracting phosphate and nitrogen. Along with cyanobacteria strains, a combination of *Anabaena variabilis, Anabaena oryzae*, and *Tolypothrix ceytonica* was also used to treat home and industrial wastewater. Besides, the strains of *A. variabilis* and *A. oryzae* have been observed to remove organic materials. For solid removal, *T. ceytonica* and *A. variabilis* were utilised. Heavy metals like copper, cobalt, manganese, zinc, and lead have been extracted out from sewage wastewater by using some cyanobacteria species. Using Nostoc PCC 7936 and *Cyanospira capsulate*, copper was recovered (II), while Zn and Cu are eliminated using *T. certonic. Oscillatoriasalina, Aphanocapsu* sp. *Terenbans*, and *Plectonema* are found to successfully degraded crude oil. The studies reported the effectiveness of *Oscillatoriaformosa* NTDM 02 in the process of removal of dye from textile wastewater. Similarly, *R. sphaeroides* IL106 also eliminated phosphorus, while, *R. palustris* WS17 was utilised to degrade pesticides, and *R. Palustris* ASI.2353 is used in color disintegration (Idi, 2015).

Heavy metal tolerance mechanism in bacteria

There are five primary pathways of bacterial resistance to heavy metals (Choudhury and Srivastava, 2001, Bruins et al., 2000) :

1) Extracellular barriers: the plasma membrane, cell wall, or capsule can stop metal ions from entering the cell. Heavy metal ions are accumulated in the polysaccharides of bacterial capsules by the carbonyl group. For e.g., Pseudomonas aeruginous biofilm got resistant from zinc, lead, and copper metal ions.
2) Active transport: A technique to remove harmful metals from the cytoplasm is the active transport of metal ions (efflux), which is made up of proteins from three different families i.e., Resistance, Nodulation, Cell Division, P-type ATPases, and Cation Diffusion Facilitator. Metal ions are removed from the cell membrane through active transport, which can be either plasmid- or chromosome-based.
3) Extracellular sequestration: Through this process, there is the complex formation of metal ions in the form of some insoluble compounds or the metal ions may get accumulated by some cellular components in the periplasm or the outer membrane;
4) Intracellular sequestration: This method of preventing exposure to vital cellular components is based on the process of conversion of metals in their non-bioavailable for and getting accumulated within the cytoplasm. The examples involved, metallothionein production by *Synechococcus* sp. and cysteine-rich proteins by *Pseudomonas* sp. (Silver and Phung, 1996; Rouch et al., 1995) and
5) Reoxidation of metal ions: Oxidation of metals like Cu and As is another crucial detoxifying mechanism. For example, Cu(I) is converted to Cu(II)

by CueO, and As(V) is converted to As(III) before being effluxed from cells (Bruins et al., 2000; Barkay et al., 2003; Ianeva, 2009).

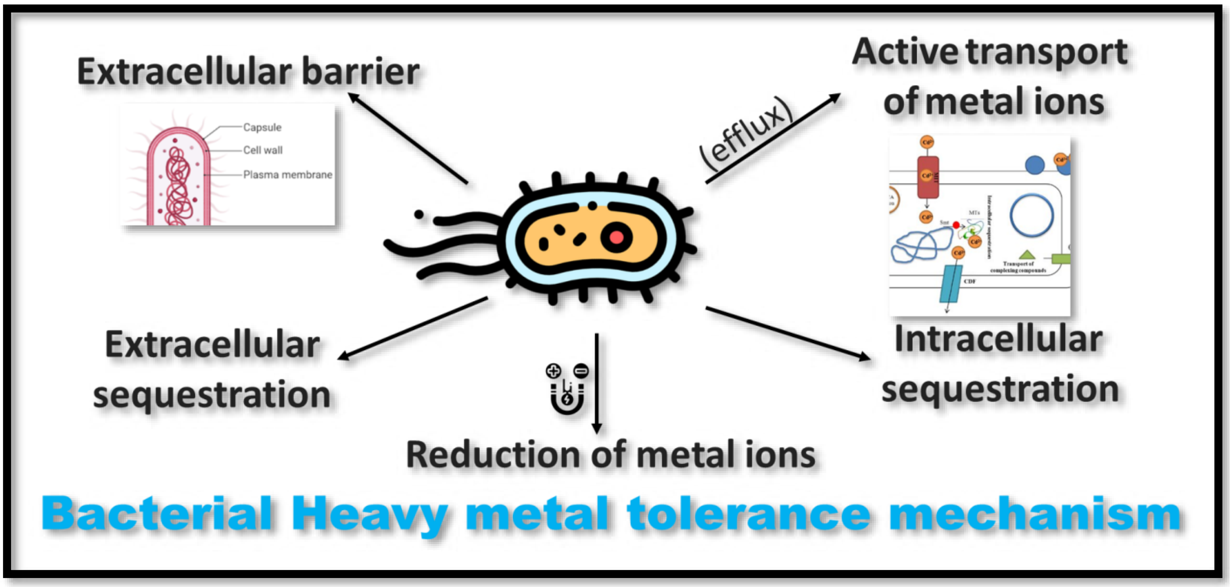

Figure 1: Types of bacterial heavy metal tolerance mechanism

Bacterial mechanisms can also be divided into biochemical and molecular types (Ma et al., 2016). The biochemical mechanism involved the interaction of microorganisms with metal ions that are extracellularly soluble through biochemical pathways. This mechanism helped in microbial resistance against these heavy metals through various processes such as detoxification, mobilisation, immobilisation, transformation, transport, and dispersion (Ma et al., 2016; White et al., 2016). Besides, the molecular mechanism through which bacteria showed resistance against heavy metals is based on the genetic determinants that might be located on chromosomes or extrachromosomal genetic elements (Ianeva, 2009). The example includes Gemella sp., Micrococcus sp., and Hafnia sp., that are the strains identified og genus level based on their traits corresponding to their morphology, physiology, and biochemical properties. Gemella sp. and Micrococcus sp. among these three isolates showed resistance to Pb, Cr, and Cd metals, but Hafnia sp. showed reactivity to Cd (Oladipo, 2017)

Apart from the molecular and biochemical mechanisms that helped the bacteria in developing the tolerance and resistance against heavy metals, their evaluation can also be done based on Minimum Inhibitory Concentration (MIC), which is considered as the minimum concentration of the metal that limits bacterial growth (Parameswari et al., 2010). Numerous researches have evaluated the MIC of microorganisms in relation to the tolerance of heavy metals (MejiasCarpio et al., 2018).

Besides, the process of biosorption mainly consists of the experience of bacterial cell wall against the metal ions. There are different functional groups found on the microbe's cell wall, including amine, carboxyl, hydroxyl, phosphate, and sulphate, that got coupled to or by the metal ions. The process of internalising metal ions into the cell protoplast occurs after metal ions have been bound to reactive groups on the cell wall. Due to the presence of glycoproteins in their cell walls, Gram positive strains acquire certain metal in greater quantities. Due to the presence of phospholipids and LPS in their cell walls, Gram negative strains are claimed to absorb fewer metals (Girdhar, 2022). Moreover, numerous factors, such as the methods of metal ion transport into the cell, the location of metal resistance genes and the function of metal ions in cellular metabolism, may also affect a bacterium's ability to tolerate metals (Ianeva, 2009).

Genes/Proteins responsible for heavy metal resistance in bacteria

Bacteria have evolved several mechanisms to resist heavy metal toxicity, including the production of specific genes and proteins (Silver, 2005- Turner, 2022). Some of the key genes and proteins responsible for heavy metal resistance in bacteria include:

1. **Metallothioneins:** Metallothioneins (MTs) are a group of small, cysteine-rich proteins available in various organisms, including mammals, fish, plants, and bacteria capable of binding and sequestering heavy metal ions, thus reducing their toxicity. These proteins have a high affinity for binding metal ions, particularly zinc and copper, and play a critical role in regulating the homeostasis of these essential metals. MTs are typically composed of 20-30 amino acids, with a conserved pattern of cysteine residues that form metal-binding sites. The cysteine residues are capable of binding metal ions in both reduced and oxidized states, allowing MTs to bind a wide range of metals with varying oxidation states. MTs are known to have a number of functions in cells. One of the most important functions is their role in regulating the intracellular concentration of metal ions. By binding to metal ions, MTs prevent the free ions from participating in harmful reactions that could damage cellular structures and biomolecules.

2. **Transporters*:*** Bacterial cells use specific transporters to move heavy metals across the cell membrane, either into or out of the cell. These transporters can be membrane-bound proteins that use ATP hydrolysis to pump metals out of the cell, or they can be passive transporters that allow metals to diffuse across the membrane. There are several families of transporters that are involved in heavy metal resistance in bacteria, including the P-type ATPases, the resistance nodulation cell division (RND) family, the cation diffusion facilitator (CDF) family, and the multidrug and toxic compound extrusion (MATE) family.

P-type ATPases are a large family of transporters that use ATP hydrolysis to transport metal ions across membranes. These transporters are involved in the efflux of a wide range of metals, including copper, zinc, and cadmium. One of the best studied P-type ATPases is CopA, which is involved in copper resistance in Escherichia coli. The CDF family of transporters includes proteins that are involved in the efflux of zinc, cobalt, cadmium, and other heavy metals. These transporters are found in both prokaryotic and eukaryotic cells and are thought to play an important role in maintaining intracellular metal homeostasis. The RND family of transporters includes proteins that are involved in the efflux of a wide range of toxic compounds, including heavy metals. These transporters are complex and typically consist of variety of proteins forming inner membrane periplasm, and the outer membrane. The AcrAB-TolC efflux pump is a well-studied RND transporter that is involved in the efflux of a wide range of toxic compounds, including heavy metals. The MATE family of transporters includes proteins that are involved in the efflux of a wide range of toxic compounds, including heavy metals. These transporters are found in both prokaryotic and eukaryotic cells and are thought to play an important role in maintaining intracellular metal homeostasis.

3. **Enzymes*:*** In addition to transporters, enzymes also play an important role in heavy metal resistance in bacteria. These enzymes can be involved in a range of processes, including detoxification, sequestration, and redox reactions. Some bacteria produce enzymes that are capable of transforming toxic heavy metals into less toxic forms. For example, some bacteria produce nitrate reductases that convert toxic nitrate into less toxic nitrite.

One of the most important classes of enzymes involved in heavy metal resistance in bacteria aremetalloproteins. These proteins contain metal ions as cofactors and help in various cellular processes, including energy metabolism, DNA replication and repair, and signal transduction. In some cases, metalloproteins can also be involved in the detoxification of heavy metals. For example, the mercury detoxifying enzyme MerB is a metalloprotein that contains a mercury-binding site.

Enzymes involved in redox reactions can also play a role in heavy metal resistance in bacteria. For example, glutathione reductase is an enzyme that is involved in the detoxification of reactive oxygen species and can also play a role in the detoxification of heavy metals such as arsenic. Bacterial enzymes can also be involved in the sequestration of heavy metals. For example, some bacteria produce siderophores, which are small molecules that bind to iron and other metals. By sequestering heavy metals, siderophores can prevent the metals from causing damage to cellular structures and biomolecules.

4. **Metal chelators*:*** Bacteria can produce small molecules known as chelators that bind to heavy metal ions and prevent them from interacting with other cellular components. One example of a bacterial chelator is siderophores, which are small molecules that bind to iron and other metals. In bacteria, metal chelators play a critical role in heavy metal resistance by sequestering and detoxifying excess metal ions. One of the most well-known metal chelators in bacteria is EDTA (ethylene diamine tetraacetic acid), which is a synthetic compound that has a high affinity for binding to divalent metal ions such as zinc,

calcium, and magnesium. EDTA is often used as a chelating agent in laboratory experiments to remove metal ions from solutions and is also used in some industrial applications to treat heavy metal pollution.
Bacteria also produce a variety of natural metal chelators, including siderophores, which are small molecules that are able to bind to ferric iron (Fe3+) ions with high affinity. Some siderophores can also bind to other metal ions, such as copper and zinc. By sequestering metal ions, siderophores help to maintain metal homeostasis in the cell and prevent the toxic effects of excess metal ions. Other metal chelators produced by bacteria include phytochelatins, which are small peptides that are synthesized in response to heavy metal exposure and are able to bind to a range of metal ions, including cadmium, lead, and copper. Phytochelatins are thought to play an important role in detoxifying heavy metals in both bacteria and plants.

5. **Efflux pumps*:*** Bacteria can use efflux pumps to remove heavy metals from the cell. These pumps are often ATP-dependent and can transport a wide range of heavy metal ions out of the cell. These pumps use energy to move metal ions across the cell membrane and into the extracellular environment, thereby reducing the concentration of metal ions inside the cell and preventing their toxic effects. There are several types of efflux pumps involved in heavy metal resistance in bacteria, including the Czc system in *Pseudomonas aeruginosa*, the Cus system in Escherichia coli, and the P-type ATPases in a variety of bacterial species. These pumps are able to transport a range of heavy metals, including copper, zinc, cadmium, and mercury.
The Czc system in *P. aeruginosa* is one of the best-characterized efflux pumps in bacteria. It consists of three components: CzcA, CzcB, and CzcC. CzcAis a membrane transporter that binds to metal ions and transports them out of the cell. CzcB is a membrane fusion protein that connects CzcA to CzcC, which is an outer membrane protein that forms a channel for metal ions to pass through. Together, these components form a complex that is able to pump out excess metal ions and confer resistance to heavy metal toxicity.
The Cus system i.e., found in E. coli is another well-characterized efflux pump involved in heavy metal resistance. It consists of three components i.e., CusA, CusB, and CusC, which are present in the membrane, the periplasm, and the outer membrane, respectively. The Cus system is able to pump out copper and silver ions and therefore mightact via protecting the bacteria from heavy metal's toxic effects.

6. **Regulatory proteins*:*** Bacteria use specific regulatory proteins to control the genetic expressions which play main role against heavy metal resistance. Regulatory proteins play a critical role in heavy metal resistance in bacteria by controlling the expression of genes involved in metal detoxification and other cellular processes. These proteins are able to sense changes in metal ion

concentrations and activate or repress gene expression in response to heavy metal exposure. One of the best-characterized regulatory proteins involved in heavy metal resistance is the MerR family of transcriptional regulators. These proteins are able to bind to metal ions such as mercury and activate the expression of genes involved in mercury detoxification. The MerR family of regulators includes proteins such as MerR, MerD, and MerR-like protein (MlrA), which are found in a variety of bacterial species.
The ArsR family of transcriptional regulators is another group of proteins involved in heavy metal resistance. These proteins are able to sense the presence of arsenic and activate the expression of genes involved in arsenic detoxification. The ArsR family of regulators includes proteins such as ArsR, ArsD, and ArsR-like protein (AlpA), which are found in a variety of bacterial species. Other regulatory proteins involved in heavy metal resistance include two-component systems, which consist of a sensor kinase and a response regulator. These systems are able to sense changes in metal ion concentrations and activate or repress gene expression in response to heavy metal exposure. Two-component systems involved in heavy metal resistance include CzcRS in Pseudomonas aeruginosa, CusSR in Escherichia coli, and CadRS in Staphylococcus aureus.

Conclusions

The accumulation of heavy metals in the earth's crust leads to serious pollution. Numerous disorders that put human health at risk and raise serious ecological problems can be brought on by surroundings that accumulate large quantities of heavy metals. Some heavy metals are toxic to organisms even at extremely low concentrations, including mercury, arsenic, lead, silver, cadmium, and chromium. Due to mining, urbanisation, volcanic eruptions, rock weathering, industrialization, etc., heavy metal pollution is growing steadily. Different physical and chemical techniques have been explored to remove heavy metals during the past few years, but they are expensive, need a laboratory, and are ineffective. Studies show that bioremediation and biosorption procedures are far more advantageous, affordable, non-toxic, and natural processes. A desirable alternative has been suggested in the form of bioremediation technologies because of their affordability and effectiveness. In order to tolerate heavy metals in polluted environments using environmentally acceptable methods, several microbial strains have developed very effective and distinctive mechanisms. In various contaminated environments, bacteria, fungi, and algae all aid in preserving tolerance to heavy metals. Bacteria have a wide spectrum of bioremediation potential. They have evolved several mechanisms to resist heavy metal toxicity, including the production of specific genes and proteins. Some of the key genes and proteins that helps the bacteria against impact of heavy metal include metallothioneins, transporters, enzymes, metal chelators, efflux pumps, and regulatory proteins. In conclusion, a variety of biotechnological and bioremediation procedures employ bacteria that are resistant to heavy metals.

Metals have been extracted through bioleaching for hundreds of years, and this area of study is currently quite active. The most promising procedures include biosorption, and the employment of metal-resistant bacteria has a significant deal of potential in the rehabilitation of metal-contaminated environments.

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