Effective microorganisms assisted biodegradation of heavy metals in polluted water bodies

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Abstract

The uncontrolled use of agrochemicals, the burning of fossil fuels, and the disposal of sewage sludge are only a few anthropogenic activities that have contributed to the rapid industrialization and severe heavy metal contamination of soils and waterways. Heavy metals are persistent in the environment and do not biodegrade. When these heavy metals enter the food chain and are present at a concentration just a little bit greater than what is needed for normal metabolic function, they have a deadly effect on human health. Even though the severity and degree of pollution vary from place to place, contamination brought on by the accumulation of these hazardous heavy metals has now become a global problem. Consequently, the prerequisite of sustainable living is the need for safe and efficient methods to remove these heavy metals. Heavy metals do not naturally degrade, in contrast to organic substances, and as a result, they accumulate in many significant places. The development of environmentally friendly cleaning methods is getting more attention as environmental protection becomes a more important issue. Bioremediation refers to the process of cleaning up contaminated areas by converting dangerous heavy metals into less dangerous forms using microorganisms or their enzymes. Hence, bioremediation can be thought of as the "green" fundamental method for the removal of heavy metals without releasing more metabolites into the environment. Due to its low costs and little waste production methods, the use of microbial scaffolds for the biodegradation of heavy metal ions is the favoured method. Despite the fact that these dangerous heavy metal concentrations are rising, microorganisms have developed a number of mechanisms to adapt to these poisonous heavy metals, such as lead, mercury, zinc, and nickel, etc., resulting in the immobilisation, removal, or detoxification of such heavy metals.

This review has gathered the information regarding sources of heavy metal pollution, , toxicity level, remediation strategies, mechanism involved in bioremediation followed by a detailed explanation of microorganism’s role in remediation of heavy metals.

Keywords- Agrochemical, Bioremediation, Immobilisation, Detoxification, Toxicity, Heavy Metals.

Introduction

Environmental contamination is one of the most pressing issues confronting modern society (Ali and Khan, 2017). Heavy metal pollution and contamination of the environment is a severe hazard to the environment (Ali *et al.* 2013, Hashem *et al.* 2017). Heavy metal contamination has resulted from rapid industrialization and urbanisation, and their rates of mobilisation and transit in the environment have considerably risen since the 1940s (Khan *et al.* 2004, Merian, 1984)

Industrialisation has increased consistently during the previous century. It has consequently raised the need for indiscriminate exploitation of the Earth's natural resources, exacerbating the world's environmental pollution crisis (Gautam *et al.* 2016). Several pollutants, such as inorganic ions, polychlorinated biphenyls, metalloids, radioactive isotopes, toxic gases, and nanoparticles, have severely impacted the environment (Walker *et al.* 2012)

Heavy metal pollution in the environment as a result of anthropogenic and industrial activity has wreaked havoc on aquatic ecosystems. Mining and smelting of ores, effluent from storage batteries and automotive exhaust, and the manufacturing and improper use of fertilisers, insecticides, and other chemicals are only some of the sources. Lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, silver, gold, and nickel are among the metals and metalloids that contaminate rivers and are widely found in the environment.

Heavy metals are classified as such due to their high atomic weights or high densities. The term 'heavy metal' is now used to refer to hazardous metallurgical chemical components and metalloids that are toxic to the environment and mankind. Some metalloids, as well as lighter metals including selenium, arsenic, and aluminium, can be harmful. While some heavy metals, such as gold, are normally not harmful, they have been called heavy metals (Tchounwou *et al.* 2012, Lenntech 2018, Duffus 2002 and Wang 2009)

Toxic heavy metal bioaccumulation in riverine ecosystem biota may have negative consequences for animals and humans. Elevated heavy metal concentrations in biosphere can have a negative impact on aquatic animal species' ecological health and contribute to population reductions (Luo *et al.* 2014). In fish, heavy metals are potent neurotoxins. Fish communication with their surroundings may be disrupted by heavy metals interacting with chemical stimuli.

The consequences of heavy metal on ecosystems and human health, as well as their possible implications for international trade, are causing concerns among health authorities in several regions of the world. Heavy metals are harmful to human health, hence heavy metal pollution in the food chain requires special attention. Numerous heavy metals are poisonous, and even at extremely low quantities, they can induce adverse effects and serious issues (Sanchez-Chardi *et al.* 2009). By forming free radicals, heavy metals induce oxidative stress. Oxidative stress is defined as an increase in the production of reactive oxygen species (ROS), which can exceed a cell's inherent antioxidant defences, resulting in cell death or damage. They can also substitute essential metals in enzymes or pigments, causing them to malfunction (Malayeri *et al.* 2008). As, Pb, Cd, Zn, Sn, Cr, Hg and Cu are the most hazardous heavy metals in terms of toxicity. Heavy metals such as Pg, Cd, As and Hg are non-essential, whereas Cu and Zn are essential (trace elements). Based on the heavy metal concentration and oxidation state, toxic heavy metals can lead to several health concerns (Ghosh, 2010).

Physical, chemical, and biological technologies are commonly used to remove metal ions from aqueous solutions. Chemical precipitation, sieving, ion exchange, electrochemical methods, membrane technologies, adsorption process, evaporation, and other conventional techniques for eradicating metal ions from wastewater solution have been suggested. Chemical precipitation and electrochemical treatment, on the other hand, are ineffectual, especially when metal ion concentrations in aqueous solution range from 1 to 100 mg L^{-1}, and generate significant amounts of sludge that are difficult to treat. When treating significant amounts of water and wastewater having low concentrations of heavy metals, ion exchange, membrane technologies, and adsorption techniques are too expensive to be applied on a wide scale. The merits and cons of various traditional metal removal procedures were described by Volesky (2001). The advantages of biological treatment, which is dependent on viable or non-viable microbes or plants, include minimal operating costs and greater efficiency.

High-dose heavy metals exposure, especially mercury and lead, can cause serious side effects like stomach colic, bloody diarrhoea, and renal failure (Bernhoft, 2012; Tsai *et al.*, 2017). Low-dose exposure, on the other hand, is a subtle and hidden threat unless it is repeated on a regular basis, and its complications, such as neuropsychiatric disorders such as fatigue and anxiety, as well as negative effects on intelligence quotient (IQ) and intellectual function in children, can be diagnosed (Mazumdar *et al.*, 2011).

The sources of heavy metal contamination in aquatic environments are discussed in this book chapter, as well as improvements in heavy metal bioremediation. It also covers the most prevalent heavy metal bioremediation methods, tactics, and biological approaches, as well as a broad overview of the role of microorganisms in heavy metal bioremediation in polluted environments.

Sources of Heavy metal pollution

Heavy metals (HMs) comprising cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), chromium (Cr), copper (Cu), iron (Fe), and platinum (Pt), which have a higher density and atomic mass and can harm people and the environment (Pt). One of the most critical environmental hazards impacting plants, animals, and humans is heavy metal poisoning of water (Gu *et al.*, 2018; Wang *et al.*, 2020). Since heavy metals are not biodegradable, they are toxic even at limited levels (Brodin *et al.*, 2017; Ferrey *et al.*, 2018).

Toxic heavy metal pollution is one of the most serious environmental problems, and it has accelerated considerably as a result of shifting industrial activities. Domestic garbage, car emissions, industrial processes (e.g., electroplating, dyeing, and mining), the haphazard dumping of electronic waste, agricultural fields, sewage sludge, and waste treatment plants can all introduce and build up pollutants in the environment (Tchounwou *et al*; 2012, Megateli *et al*; 2009) . Furthermore, when compared to the atmosphere (e.g., vapours or particles), heavy metals may be present at high quantities in aquatic and soil ecosystems (Wintz *et al*; 2002, Vhahanguiele and Khathutshelo, 2018). Heavy metals may originate naturally or as a result of human activity, resulting in their prevalence in soil, water, and air (RA and Okieimen, 2011, Assubaie, 2015, Szyczewski *et al.* 2009). (Fig.1). Human health is put at risk by polluted soil and ground waters through the ingestion of food grown in polluted areas, skin contact, and dust inhalation (Lasat, 1999, Gardeatorresdey *et al.* 2005, Olaniran *et al.* 2013).

There are three main types of metals and metalloid ions. Mercury, cadmium, and lead are among the first group of metals that are dangerous at low doses. The second group of metals (bismuth, indium, arsenic, thallium, and antimony) is less dangerous, while the third group consists essential metals like zinc, cobalt, copper, iron, and selenium, which are involved in a variety of physiological and biological processes in the body and are only toxic above a certain concentration (Odobaic' *et al.*, 2019).

As a result of absorption and, in some circumstances, inhalation, as well as accidents or mistreatment, HMs accumulate in the soil, human and animal tissues. Through regular biogeochemical cycles, metals have been present on the earth since the beginning (Dalziel, 1999; Masindi and Muedi, 2018). HMs were found in the soil as a result of the underlying weathering mechanism. The soil in the Mendip region of Great Britain is rich in cadmium, lead, and zinc due to mineralized veins and metal deposits in high concentrations in the bedrock. Bedrock weathering with a slightly high concentration of HMs can result in metal enrichment during soil formation. Human and anthropogenic activities are the primary causes of increased environmental toxicity caused by heavy metals. Wind-blown soil debris, forest fires, volcano eruptions, biogenic processes, and marine salt are among natural sources of HMs (Blaser *et al.*, 2000; Muhammad *et al.*, 2011). Mining operations, pesticides, fertilisers, and herbicides use, and crop field irrigation with industrial and sewage water are all anthropogenic drivers of HMs contamination (Sarkar *et al.*, 2018; Srivastava *et al.*, 2018) (Fig. 2). Heavy metal pollutants in our food come from trace quantities of HMs in fertilisers.

Numerous industries discharge mercury (Hg) into the atmosphere, including pharmaceutical manufacture, paper and pulp preservatives, agriculture, and the chlorine and caustic soda industries. Cadmium can be found in soils and rocks, as well as coal and mineral fertiliser. Cadmium (Cd) is commonly used in electroplating for a variety of purposes, including batteries, pigments, textiles, and metal coatings (Saini and Dhania, 2020). All of these activities are contributors in the rising environmental contamination with HMs.

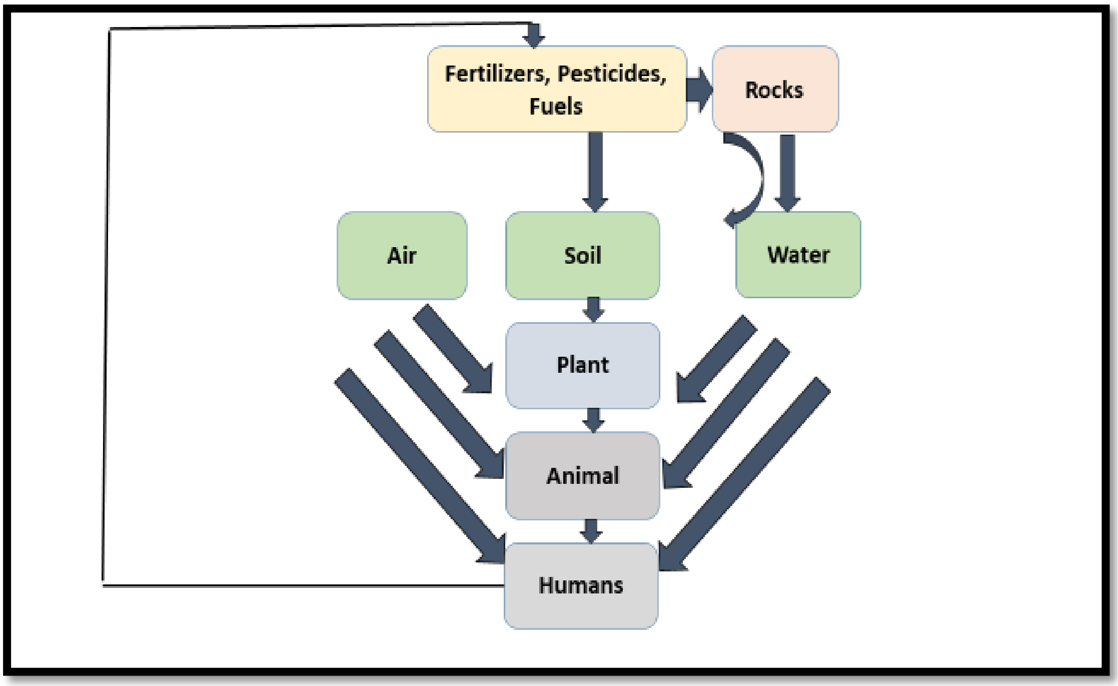

Fig. 1: Cycles and Sources of heavy metals in the ecosystem.

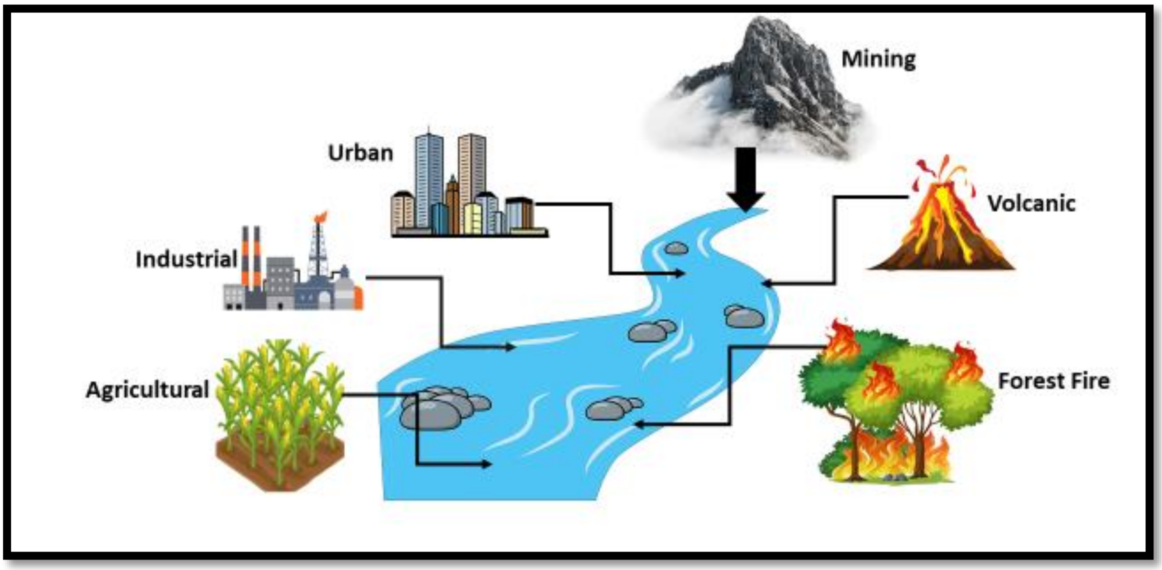

Fig. 2. Sources of Heavy metals into the water bodies

Pollutant entry and transport into the ecosystem

Pollutants can enter the environment through a variety of routes, including the hydrosphere, lithosphere, and atmosphere. Apart from entering through natural routes like as volcanic activity and rock weathering, manmade activity is a major source of contaminants entering the environment. They can occur as a result of an unexpected release, like in shipwrecks, spillages, mines, and explosions; in the specific applications of biocides, including vector control; and in waste disposal, such as industrial wastes and sewer systems. Temperature, movement and direction of rivers and streams, circulation of air masses, and wind speed all influence the migration of heavy metals and other contaminants.

Water contamination is caused by two key factors: urbanisation and industrialisation. Runoff from villages, towns, cities, and factories transfer the metals, which aggregate in the sediments of aquatic environment. Even if traces make their way into bodies of water, they might be extremely harmful to humans and other organisms. Heavy metal toxicity is determined by a variety of factors, including the type of metal present, the composition of the metal, the metabolic relevance of the metal, the organism exposed, and the time period during which the organism is exposed. If one species is harmed, it will have an impact on the entire food chain. Because humans are normally at the end of the food chain, it will have a greater impact on us because we will have absorbed more heavy metal as concentration rises up the food chain. In most cases, both industrial and residential wastes are discharged into the sewer system.

Heavy metals are present in high amounts in raw sewage and are not reduced during treatment. They are either eliminated in the effluent or in the resulting sludge. The properties and pollutants of sewage that enter the water supply are determined by sewage treatment. Considering the problems produced by sewage being discharged into waterbodies without being treated, a number of measures have been implemented. To reduce the amount of pollutants dumped into the seas, strict restrictions have been enacted, and improved technology has been developed. (Masindi and Muedi, 2018, Corrondo *et al.* 1978, Primary, Secondary, and Tertiary waste water treatment, 2019; Bonito, 2008)

Primary, secondary, and tertiary are the three steps in sewage treatment. After filtering the major contaminants in the wastewater, the primary stage includes the settling of solid debris found in the wastewater. The water is pumped through a series of tanks and filters that remove the impurities from the water, resulting in a sludge that is then put into a digester for further processing.

At this point, the sludge comprises around half of the suspended particles found in water. Secondary treatment includes the use of oxidation to assist purification of waste water and could be accomplished in three ways: biofiltration, agitation, or oxidized ponds.

The final phase is tertiary treatment, which includes removing of nitrates and phosphates out from water system. Charcoal and sand are commonly used in this technique to assist in the removal of pollutants. These are the main processes of sewage treatment, which vary based on the type of sewage or how it is processed. Contaminants can exist in a variety of states, including surface waters, solutions, and suspensions. They can be carried by water over a long distance, with particle materials sinking to the bottom. Droplets of liquid can either sink into the sediment or float to the surface.

Humans are omnivorous creatures. Toxic heavy metals can be found in a variety of foods, including seafood, cereals, and vegetables. Heavy metal contamination in freshwater bodies including rivers, lakes, and streams causes biomagnification of these components in freshwater fish, whereas heavy metal contamination in agricultural areas causes absorption of these materials in agricultural crops. Toxic heavy metal contamination of human food chains is a concern to human health. Contamination is a severe human health issue, as evidenced by cases from the twentieth century. In Japan, intake of Hg-contaminated fishes and Cd-contaminated rice produced Minamata disease (MD) and itai-itai sickness, respectively. The transmission of heavy metals from contaminated fish to humans is depicted in Fig. 3.

Although heavy metal biomagnification is a contentious topic in metal ecotoxicology, multiple studies have found heavy metal biomagnification in particular food chains. Organisms at various trophic levels in food systems are more vulnerable to biomagnification of certain metals in food chains. Biomagnification can result in increased concentrations of trace metals in creatures of higher trophic levels, posing a health concern to these organisms or their human consumers (Barwick and Maher, 2003).

To protect human health from the detrimental impacts of toxic heavy metals, the absorption and bioaccumulation of heavy metals in human food chains should be regularly monitored. To avoid biota loss owing to analysis, non-destructive sample procedures and the utilisation of environmental biomarkers should be used. Furthermore, untreated industrial and municipal effluents should not be discharged into ecological systems such as waterways and farmlands to avoid heavy metal pollution of food chains (Balkhair and Ashraf, 2016)

The duration of time a pollutant travels in a river is determined by the currants, stability, and physical state of the pollutant. Wind and currents convey the contaminant further once it reaches the ocean and seas. Persistent contaminants, such as heavy metals, can enter the food chain via marine life, affecting predators such as larger fish, avian, and primates, including humans, that migrate and carry the pollutant to various ecosystems (Walker *et al.*, 2010).

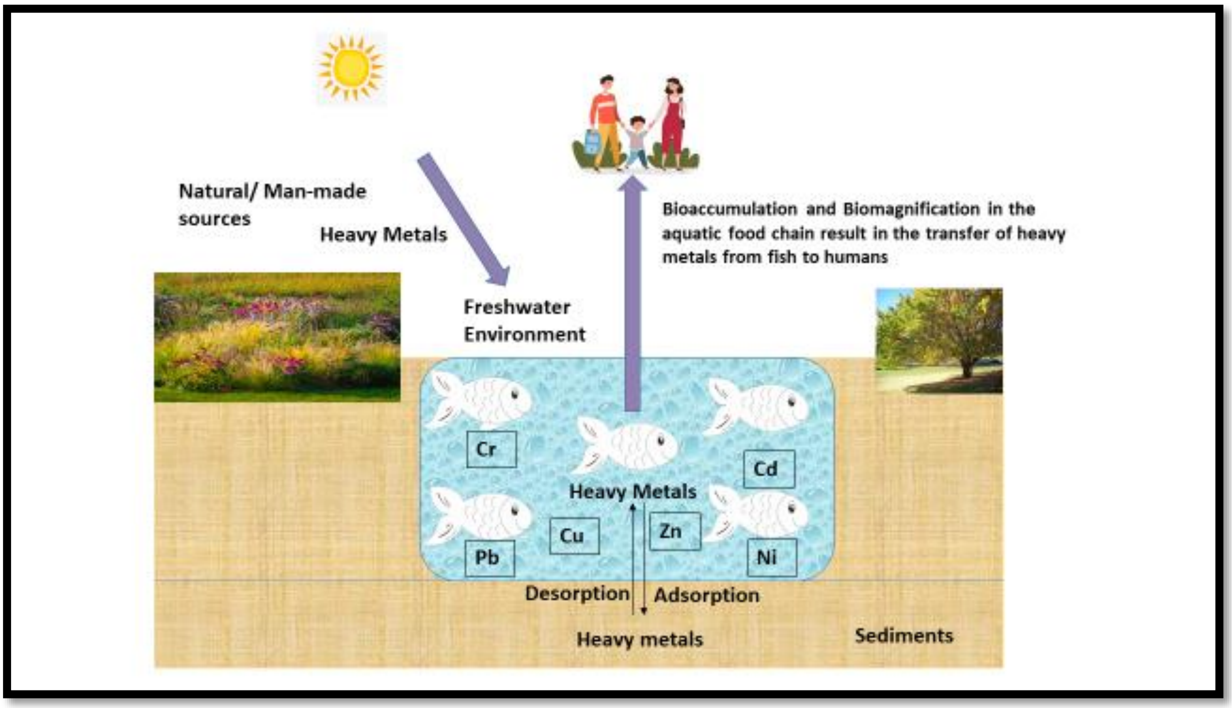

Fig 3: Heavy metals are transferred trophically from freshwater fish to humans in the food chain.

Heavy Metals Toxicity

As a result of multiple agricultural, residential, medicinal, industrial, and technical applications, heavy metals have become widely distributed in the environment, creating considerable direct concerns about their possible consequences on human health and the environment. The toxicity of heavy metals is influenced by a variety of parameters, including chemical species, dose, and route of exposure, and also the ages, gender, genes, and nutritional state of those who are exposed.

Mercury, Chromium, Arsenic, Lead and Cadmium are currently classified as priority metals because to their extreme toxicity, which is becoming a major public health concern. These metals are included in systemically toxic elements because they have been known to cause various organ harm even at low levels of exposure. As heavy metals enter the human body by meals, water, air, or skin absorption, they become poisonous and accumulate in mucous membranes if they are not digested by the body.

There are 35 metals that we are concerned about as a result of residential or occupational exposure, the majority of which are heavy metals (cobalt, cadmium, arsenic, gold, bismuth, cerium, iron, lead, mercury) and are commonly found in the environment (Showkat et al. 2017). Certain metals are essential for optimum health in small levels, but in larger quantities, they can be poisonous or hazardous to human health. These elements are necessary for optimal health in small levels, but in larger quantities, they can be poisonous or hazardous to human health. Heavy metal toxicity can lower energy levels and harm the functioning of the brain, kidneys, lungs, liver, blood composition, and other vital organs. Continuing to be exposed to certain metals may result in degenerative physical, muscular, and neurological processes that mimic diseases including multiple sclerosis, Alzheimer's disease, Parkinson's disease, and muscular dystrophy.

Some metals and their compounds may cause cancer if exposed to them often over time (Ghosh et al. 2007). As a result, having a thorough understanding of heavy metals is crucial for taking adequate protective measures against their excessive contact with the human body (Ferrara 2000).

Different types of toxicity caused by heavy metals (Fig.4) are elaborated below:-

Carcinogenicity

Arsenic causes epigenetic changes, DNA damage, changes in the expression of the p53 protein, histone modifications, DNA methylation, and a decrease in p21 expression (Martinez *et al.*, 2011; Park *et al.*, 2015). By adhering to DNA-binding proteins and inhibiting the DNA-repair process, arsenic exposure increases the risk of cancer (Garcia-Esquinas *et al.*, 2013).

Lead is a carcinogenic chemical that releases reactive oxygen species (ROS), which damages the DNA repair mechanism, cellular tumor-regulating genes, and chromosomal structure and sequencing. By removing zinc from particular regulatory proteins, it inhibits transcription (Silbergeld and colleagues, 2000).

Mercury's peroxidative action produces a large amount of oxidative stress (ROS), which can help protumorigenic signalling and the formation of malignant cells. ROS can contribute to carcinogenesis by causing cell damage by destroying cellular proteins, lipids, and DNA (Reczek and Chandel, 2017; Zefferino *et al.*, 2017).

Nickel acts as a carcinogen via influencing a number of carcinogenic pathways, including gene regulation, transcriptionally management, and the production of free radicals. It regulates the expression of large non-coding RNAs, mRNAs, and microRNAs in particular. It helps to boost the modulation of hypoxia-inducible factor-1 by methylating the promoter and downregulating gene 3 (MEG3), both of which lead to malignancy (Zambelli *et al.*, 2016; Zhou *et al.*, 2017).

Cardiovascular toxicity

Acute and chronic lead exposure causes a number of problems in the human. By increasing OS, reducing NO availability, rising vasoconstrictor prostaglandins, adjusting the renin–angiotensin system, disrupting vascular smooth muscle Ca2+ signalling, increasing inflammation and epithelial layer vasorelaxation, and adapting the vascular response to vasoactive agonists, chronic exposure to lead may cause hardening of the arteries and high blood pressure, thrombus formation, atherosclerosis, and cardiac disease. Long-term exposure also raises arterial pressure (Hertz-Picciotto and Croft, 1993; Vaziri, 2008, 2002).

Cadmium is a carcinogenic and poisonous metal. Cadmium causes renal disease, bone disease, and cardiovascular disease, in addition to its carcinogenic qualities (Toxicological Profile for Cadmium, 2002). Chronic kidney disease (Hellström *et al.*, 2001), high blood pressure (Tellez-Plaza *et al.*, 2008), diabetes (Schwartz *et al.*, 2003), carotid arteriosclerosis (Messner *et al.*, 2009), peripheral arterial disease (Navas-Acien *et al.*, 2004), myocardial infarction (Everett and Frithsen, 2008), brain hemorrhage, and heart failure (Peters *et al.*, 2010). Cadmium was related to an elevated risk of cardiac death in the general population of the United States in prospective studies (Tellez-Plaza *et al.*, 2013, Tellez-Plaza *et al.*, 2012).

In humans, mercury has been linked to neurotoxicity, nephrotoxicity, and hepatotoxicity. Recent research has also identified cardiovascular damage. Mercury levels in hair have been associated to high amounts of oxidised LDL in atherosclerotic lesions, abrupt cardiac failure, and osteoarthritis (Yoshizawa *et al.*, 2002).

Mercury also inactivates paraoxonase, an intracellularly antioxidative enzyme associated to HDL dysfunction (Gonzalvo *et al.*, 1997; Salonen *et al.*, 1999); this is interconnected to the development of atherosclerosis and the steadily increasing risk of heart attack, coronary heart disease, cardiovascular events, sudden cardiac death, and carotid artery stenosis (Gonzalvo *et al.*, 1997; Kulka, 2016).

Cobalt poisoning induces reversible acute cardiac depression, which can be separated from other types of cardiomyopathy. Cobalt cardiomyopathy is a gradual and fatal condition. Survivors' heart function, on the other hand, frequently improves (Packer, 2016).

Immunological toxicity

Lead exposure, both acute and chronic, has various harmful effects on the immune system and causes a variety of immunological responses, including increased allergies, infectious illnesses, and autoimmunity, as well as malignancy (Dietert *et al.*, 2004; Hsiao *et al.*, 2011). Lead exposure has been associated to an increased incidence of lung, gastrointestinal, and bladder cancer in different demographic groups (Rousseau *et al.*, 2007; Steenland and Boffetta, 2000). The generation of B and T lymphocytes, along with MHC activity, is triggered by lead exposure (Kasten-Jolly *et al.*, 2010). By changing the role of T-cells and increasing vulnerability to the establishment of autoimmunity and hypersensitivity (Mishra, 2009), it can impact cellular and humoral responses.

Occupational and environmental cadmium exposure can have immunosuppressive effects depending on the circumstances. Autoimmune immune responses are increased at low levels of exposure, but the effects at greater levels have yet to be determined. In most situations, however, ocytosis, innate immune cell function, and host resistance are significantly diminished in experimental infections.

Chromium has a number of negative impacts on the human immune system. Numerous experimental research have looked into the effect of chromium on the immune system. Lymphocytic growth is inhibited, thus according Faleiro *et al.*, who have used Co, Cr, Mo disc samples. High dosages of hexavalent chromium suppress alveolar macrophage phagocytic activity and the body's immune system (Glaser *et al.*, 1985). Furthermore, chromium causes two different types of hypersensitivity: type I (anaphylactic) and type IV (hypersensitivity) (delayed type). Many investigations have also indicated that chromium exposure causes allergic contact dermatitis (Bruynzeel *et al.*, 1988; Leroyer *et al.*, 1998).

Skin toxicity

Chronic arsenic exposure can lead to a variety of skin conditions, including hyperpigmentation, darkening, and several types of skin cancer. The most common skin change produced by persistent arsenic exposure is hyperpigmentation. Bowen's disease, a kind of early skin cancer, can be caused by arsenic exposure.

Arsenic hyperkeratosis affects the soles and palms of the hands, but it could also impact the legs, feet, fingers, arms, and dorsum of the hands. Some hyperkeratotic and Bowen's disease blemishes have the potential to turn into cancerous tumours (Huang *et al.*, 2019).

The body's outermost organ, the skin, creates a barrier against many pollutants. Contact with chromium can result in skin irritation, systemically atopic dermatitis, and skin cancer, among other serious dermatological complications.

Interaction dermatitis is a common skin condition marked by prolonged hypersensitivity as a result of repeated allergen contact on the dermis (haptens). Matthews *et al.*, 2019; Menné *et al.*, 1994; Uter *et al.*, 2018; Winnicki and Shear, 2011; Yoshihisa and Shimizu, 2012) describe a type of dermatitis caused by systemic exposure to an allergen, which leads the skin to become sensitive through direct dermal contact at first.

Mercury and mercury-containing compounds cause a variety of skin illnesses, including acrodynia (pink disease), a frequent cutaneous ailment in which the skin turns pink after exposure to heavy metals, notably mercury (Horowitz *et al.*, 2002). Within six months after getting tattooed with the red pigments cadmium sulphide and mercury sulphide, people may experience inflammation limited to specific locations (Boyd *et al.*, 2000). Acute contact dermatitis produced by mercury-containing chemicals is associated with mild swelling, cracking, vesiculation, and irritation. Mercury toxicity is the most common cause of dermatological issues, according to various research (Boyd *et al.*, 2000).

Hepatotoxicity

The hazard of lead on liver has long been known. It promotes oxidative stress, which leads to liver damage. Organic solvents, when mixed with lead, induce liver damage because they share many of the same features as lead (Farmand *et al.*, 2005; Malaguarnera *et al.*, 2012). Hegazy and Fouad (2014) found that persistent exposure to lead is potentially hazardous to liver cells, results in glycogen deficiency and cellular infiltration, that can lead to chronic cirrhosis.

The renal cortex and the liver are different human target tissues for cadmium (Bernard, 2004). It accumulates in the liver after acute exposure and has been associated to a range of hepatic dysfunctions. Cadmium leads to oxidative stress and hepatic damage by altering the cellular redox equilibrium (Zalups, 2000). Cadmium-induced hepatotoxicity, either acute and chronic, promotes liver failure and so raises the chances of malignancies (Hyder *et al.*, 2013).

Wilson's illness is known to cause copper accumulation in the liver. Hepatic copper accumulation is not only important in the pathogenesis, but also harmful, because increased copper levels can promote oxidative stress. Copper levels in the liver are also elevated in cholestatic liver disorders. Deering *et al.*, 1977; Gross *et al.*, 1985; Yu *et al.*, 2019) have shown that they are caused by decreased renal excretion of copper and are not a consequence of hepatic infection.

Cr(VI) has been found to injure the liver in numerous studies, and histological alterations such as hepatocyte steatosis, parenchymatous degeneration, and necrosis have already been documented. Cr(VI) hepatotoxicity is associated with increased ROS levels, lipid peroxidation, silencing of DNA, RNA, and protein synthesis, Damage to DNA, decreased antioxidant enzyme, mitochondrial dysfunction, including such mitochondrial dysfunction energy metabolism, cell growth arrest, and apoptosis (Hasanein and Emamjomeh, 2019).

Genotoxicity

Several studies have found significant inter individual diversity in receptivity to arsenicosis, with genetic variables identified as the primary driver of this variation.

Arsenic's genotoxicity causes deoxyribonucleic acid changes like chromosomal abnormalities, mutations, micronuclei formation, deletions, and sister chromatid exchanges (Roy *et al.*, 2018). The mechanism of arsenic's genotoxic effect, which includes the development of oxidative stress as well as the interruption of DNA repair, has been studied extensively (Pierce *et al.*, 2012).

Arsenic has been demonstrated to have no direct impact on DNA and is classified as a weak mutagen because it impacts the mutagenicity of other carcinogens despite its low mutagenicity. When arsenic is exposed to UV radiation in living cells, for example, its mutagenicity increases (Yin *et al.*, 2019)

The genotoxic effects of chemical chemicals on humans largely affect genetic material through two procedures: teratogenesis and carcinogenesis. Teratogenesis is the process by which a chemical molecule causes genetic material to alter. The first can cause congenital anomalies in kids, while the second can lead to the development of cancer in those who have been directly exposed. Certain mercury compounds, known as mutagenic agents, which are poisonous to the developing neurological system, have a particularly negative effect on the growth of the nervous system (Young *et al.*, 2008).

However, the link between mercury exposure and malignancy (one of the most significant consequences of DNA damage) is still a matter of discussion, as some tests appear to suggest that mercury has genotoxic activity while others have not (Crespo-López *et al.*, 2009).

The genotoxicity and carcinogenicity of chromium are being investigated in both yeast and mammalian cells. Workers in the mining and consuming industries who use Cr have also been found to be at risk of cancer (Li Chen *et al.*, 2012; Thompson *et al.*, 2012).

Cr(VI) induces DNA chromosomal protein crosslinks, inter-DNA strand crosslinks, and nucleotide strand breakage in living and cultured cells, according to researchers (Fang *et al.*, 2014).

Nephrotoxicity

Cadmium nephrotoxicity causes glucosuria, Fanconi-like disorder, phosphaturia, and aminoaciduria, among other symptoms (Hazen-Martin *et al.*, 1993; Reyes *et al.*, 2013). Direct exposure to the kidneys damages the proximal tubular epithelium, resulting in high levels of cadmium in the urine, aminoaciduria, 32-microglobulinuria, and glucosuria, as well as reduced renal tubular phosphate reabsorption (Goyer, 1989).

Excessive exposure can cause proximal tubules acidosis, kidney failure, and hypercalciuria (Friberg *et al.*, 2019; Jacquillet *et al.*, 2007).

Lead has negative effects on all organs, but the kidneys are the most vulnerable. Fanconi-like syndrome is caused by acute lead nephropathy, which results in proximal tubular dysfunction. Hyperplasia, interstitial fibrosis, tubule atrophy, kidney failure, and glomerulonephritis are all symptoms of chronic lead nephropathy.

Acute mercury poisoning of the kidneys causes abrupt tubular necrosis, which manifests as acute dyspnea, disturbed psychological state, stomach discomfort, profuse salivation, tremors, vomiting, chills, and hypotension, among other symptoms. Chronic mercury exposure, on the other hand, damages the epithelium and induces necrosis in the proximal tubule's pars recta. Tubular failure, increased protein and retinol-binding protein excretion in the urine, and a nephritic condition with membranous nephritis are all signs of mercury-induced chronic kidney disease.

Thallium sulphate excretion through the kidneys is slow, taking up to 2 months after ingestion. Albuminuria and hematuria are symptoms of toxic kidney damage. Thallium poisoning, on the other hand, does not result in a significant reduction in kidney function (Yumoto *et al.*, 2017).

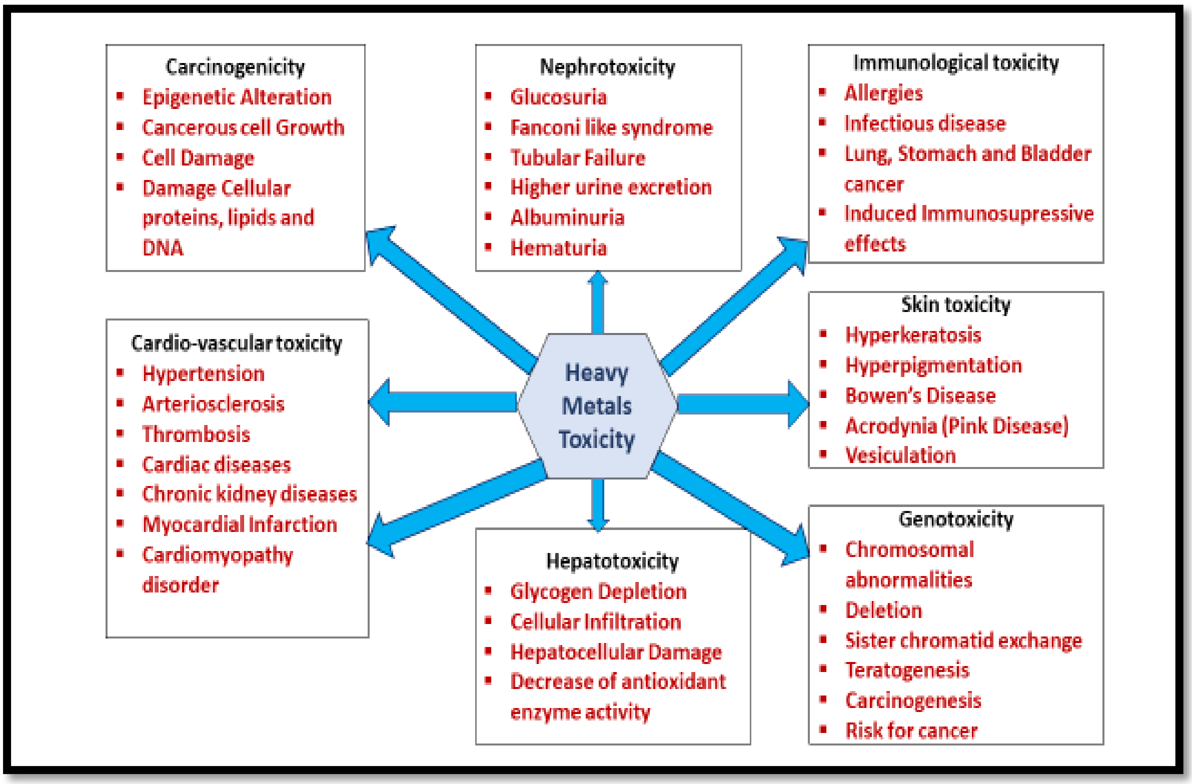

Fig.4 Various types of Heavy metals toxicity

Methods of Heavy Metal Remediation

Heavy metal clean up in polluted water and soil and water has used a variety of technologies and procedures in recent years. Physicochemical and biological approaches are among them; the latter is furthermore divided into in ex - situ and in-situ bioremediation.

Physico-chemical approaches- Processes that operate to remediate heavy metals of any polluted site are referred to as physicochemical approaches. They can take the form of metal particulate or metal-containing particles. Ion exchange, precipitation, ozonation, dehumidification restoration, solvent extraction, filtration, advanced oxidation processes, chemical leachate, electrokinetics, dumpsites, electrocoagulation, electrodialysis, ultra - filtration, soxhlet extraction, chemical coagulation, chemical oxidation, and isolation (mechanical) dissociation of metals are some of the physical and chemical processes that can be used for metal remediation (Lajayer *et al.* 2017; Lasat, 1999; Tang *et al.* 2007, Hia and Liyuan, 2002; Acheampong, 2010)

These procedures, on the other hand, may result in partial metal removal, as well as the use of a lot of solvent and the formation of hazardous waste products. They also have an intrinsic negative impact on the environment and are typically soil disturbing, in addition to being labour intensive and costly [10]. As a result, these approaches are constrained by their massive costs, highly energetic requirements, low efficiency, unexpected metal ion removal, and toxic sludge formation (Malaviya and Singh, 2011; Xu *et al.* 2013; Chen and Lin, 2010, Wen *et al.* 2010, Lyyra *et al.* 2007, Memon and Schroder, 2009).

Biological Remediation- Biological remediation, often known as biodegradation, refers to a variety of techniques for removing or degrading heavy metals via biological activity. These biological treatments can be used to remove heavy metals and can comprise aerobic (oxygen-containing) or anaerobic (oxygen-deficient) procedures. Microbial degradation is a technique in which a polluted environment is biologically degraded to levels below the dosage limits set by regulatory authorities under particular conditions (Pilon-Smits, 2005; Pilon-Smits and Le Duc, 2009; Audet, 2013). Based on the methodologies used in, bioremediation can be characterised as either in situ or ex situ.

In- situ Bioremediation- Methods of in situ bioremediation treat contamination on the spot without removing soil. The adoption of these specific procedures is determined by a number of parameters, including the contaminated region, the qualities of the chemicals involved, the contaminants' concentration, and the amount of time required to accomplish the bioremediation. This method is frequently advised because it requires less items to be moved and is less expensive. Intrinsic bioremediation and engineered in situ bioremediation are two types of in situ bioremediation. Bioventing, biosparging, biostimulation, bioaugmentation, and phytoremediation are only a few examples (Vidali, 2001, Atagan *et al.* 2003; Mangunwardoyo *et al.* 2013; Thapa *et al.* 2012).

Bioventing:- The bioventing technique is the most widely utilised in situ mechanism for supplying air and nutrients to polluted soil in order to promote microorganisms (bacteria). To release contaminants into the environment through biodegradation, bioventing necessitates a low air flow and low oxygen rate. Because it may simulate in situ biodegradation of simple hydrocarbons in the soil, contamination occurs well under the surface (Chipasa and Medrzycka, 2006). The difficulty to provide oxygen to polluted soil and poor aeration of superficial contamination limit bioventing (Azubuike *et al.* 2016).

Biosparging- Biosparging is the injection of low-pressure air beneath the water table to improve groundwater oxygen levels and speed up bacterial bioremediation of pollutants (Vidali, 2001). Both bioventing and biosparging procedures were used at the same time to ensure that soil contaminants were removed efficiently despite any adverse conditions. Biosparging can also combine soil and groundwater to lower the concentration of dissolved oil compounds in groundwater that is mixed with soil below the water table and inside the capillary fringe. It's a simple, low-cost surgery with a lot of options.

Biostimulation- To start the bioremediation process, biostimulation is used to boost bacterial growth. To begin, the contaminated soil is blended with enhanced nutrients and important chemicals to encourage microbial activity, allowing pollutants or harmful compounds to be rapidly degraded into carbon, nitrogen, and phosphorous sources (Medina – Bellver *et al.* 2005). Nature's initial recyclers are microorganisms like bacteria and fungi. The ability of microorganisms to convert chemical pollutants into energy and usable materials points to key biological processes that are both cost-effective and environmentally friendly.

Bioaugmentation:- Certain sites in bioaugmentation necessitate the use of microorganisms to remove pollutants. They also have the ability to outcompete native bacteria, allowing them to clean up the site quickly. Bioaugmentation has been used to remove hazardous compounds from the environment, such as soil and water. However, a number of drawbacks have been identified. Exogenous microorganisms, for example, have been found to decrease in quantity after being introduced to a contaminated region due to various biotic and abiotic pressures. Inadequate growth nutrients, such as substrates, temperature fluctuations, and pH, as well as competition between introduced and indigenous microbes, cause them (Stroo *et al.* 2013; Bouchez *et al.* 2000)

Phytoremediation:- Phytoremediation is a new technology that use plants to clean up polluted soil and water. It has the potential to be used in the decomposition of the organic pollutants, and it could be a viable option in the future. For sites with profound pollutants, this method is appropriate. Nonetheless, several studies have pointed up some of this technology's limitations, including contamination level, toxicity, bioavailability, plant type, and stress tolerance (Farraji *et al.* 2016).

Ex situ bioremediation – It refers to the process of excavation and treating soil before returning it to its original state. If the contaminated material is dug, it can be treated on or off site, which is usually a faster way to decontaminate the area. Ex situ bioremediation can be divided into two types: solid phase and slurry phase. Land farming, composting, biopiles, and bioreactors are among the most essential strategies (Vidali, 2001).

Land-Farming: It is a simple procedure that entails excavating polluted soil over a specified plot and tilling on a regular basis until pollutants are degraded by microorganisms, and the practise is confined to the remediation of a small portion of soil (Sivakumar *et al.* 2014). The procedure is simple and successful, especially when applied to soil that has been contaminated with petroleum. The approach, however, is confined to treating a small area of upper soil i.e. 10–35 cm.

Composting: Composting is a process that requires mixing polluted soil with non-toxic organic agricultural wastes to encourage the growth of a large number of microbes at high temperatures (40-65° C). The approach is used to test a combination of excavated soils and organic wastes (wood chips, animal and vegetal wastes) that have been polluted with organic compounds (hydrocarbons and pesticides) (Vidali, 2001, Sivakumar *et al.* 2014).

Biopiles- Biopiles are a hybrid between composting and land farming. Numerous microbes (both aerobic and anaerobic) thrive in enriched environments created by biopiles. Aqueous reactors are used to treat a contaminated environment ex situ, with reactors pumped up from a specific location. It entails utilising a specially built technique to bioremediate a contaminated environment (Vidali, 2001). Engineered cells are used to treat surface pollutants in order to control physical losses of contaminants through leaching, followed by volatilization (Sivakumar *et al.* 2014). For contaminated soils, biopiling is seen to be a realistic and cost-effective approach.

Bioreactors- It is a vessel that is used to carry out a biological reaction once an external environment has been optimised. To produce a high output of bioremediation, the system may comprise enzymes, tissues, microbes, animal and plant cells. Since the target environment is simple to handle, regulate, and anticipate, microbial degradation is faster in bioreactor systems than in other systems. Despite the benefits of reactor systems, it has been discovered that the polluted environment (for example, soil or water) requires physical extraction of the contaminant before being handled by a bioreactor (Vidali, 2001).

Bioremediation by microorganisms and the mechanisms involved

Bacteria, microalgae, fungus, and yeast are among the principal kinds of microorganisms usually utilised for metal bioremediation, as shown in Fig. 5.

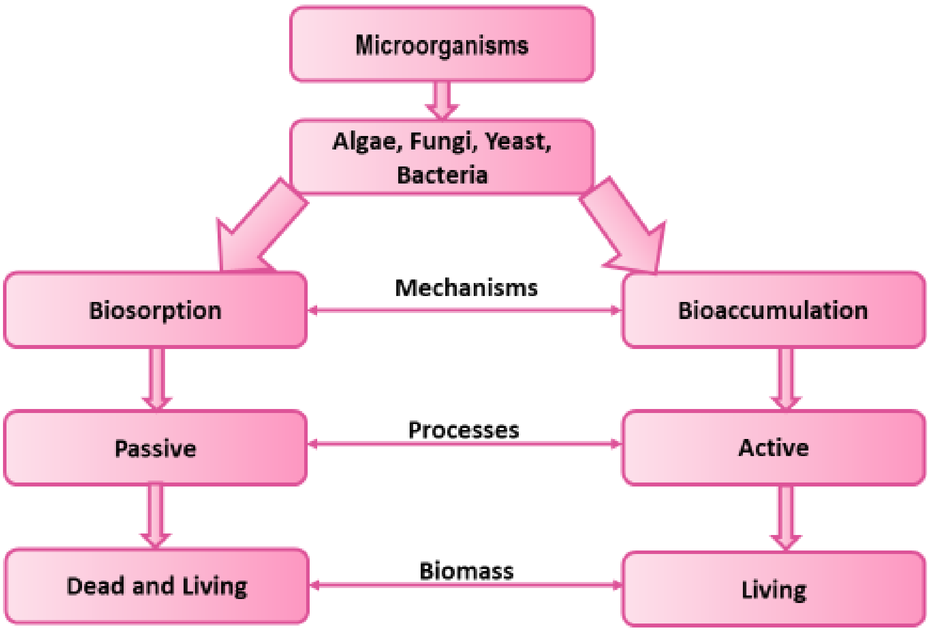

Fig. 5: Microorganisms used in bioremediation, as well as the processes and mechanisms involved in both dead and living biomass

Biosorption and bioaccumulation are two different types of bioremediation. Biosorption is a rapid and reversible passive adsorption mechanism (Gadd and White, 1993; Ahalya et al. 2003). Metals are retained by physicochemical interactions (such as ion exchange, adsorption, complexation, precipitation, and crystallisation) between the metal and the functional groups on the cell surface (Gadd and White, 1993; Gadd, 2009). pH, ionic strength, biomass concentration, temperature, particle size, and the presence of other ions in the solution can all affect metal biosorption (Volesky, 2004). Because it is not dependent on cell metabolism, both living and dead biomass can be used for biosorption. Bioaccumulation, on the other hand, encompasses both intracellular and extracellular mechanisms, with passive absorption playing a minor and ill-defined role (Gadd and White, 1993).

The essential characteristics linked with biosorption and bioaccumulation processes are compared in Table 1. Because the biomass may be sourced from industrial waste and recycled and regenerated in many cycles, the biosorption process requires a low cost. Bioaccumulation, on the other hand, necessitates a high cost because it happens in the presence of live cells, limiting reuse. Selectivity of metals and the possibility for regeneration are also key elements to consider. Because the bind happens only by physical interaction, biosorption has a low selectivity. It is possible to boost it by modifying the biomass. Bioaccumulation processes, on the other hand, outperform biosorption mechanisms in most cases.

A microorganism's cell wall contains a variety of macromolecules, including as polysaccharides and proteins that contain a large number of charged functional groups, such as carboxyl, imidazole, sulfydryl, thioether, phenol, carbonyl, amide, ester sulphate, amino, and hydroxyl groups (Zabochnicka et al. 2014; Bayramoglu et al. 2006; Akbar and Tunali, 2006). Adsorption happens when a positively charged metal in the solution gravitates toward these functional groups. The cell wall composition of microorganisms can be influenced by their cultivation method, which can be used to improve their adsorption capability (Gadd and White, 1993). Bacteria can remove heavy metals from wastewater using functional groups in their cell walls, such as ketones, aldehydes, and carboxyl groups, resulting in less chemical sludge (Qu et al. 2014).

Metals are taken up by both gram-positive and gram-negative bacteria. Algae such as green, red, and brown are also employed as biosorbents. Ion exchange can be performed by some functional components found in bacteria, such as uronic acid with carboxyl and sulphate groups, xylans, galactans, and alginic acid. The advantage of utilising algae as biosorbents is that, unlike other microorganisms such as bacteria or fungi, they rarely create hazardous chemicals (Das et al. 2008).

Adsorption is also done with fungi and yeasts. Fungi have the advantage of being widely diverse in size, ranging from mushrooms to minute moulds. They're simple to grow and produce a lot of biomass. Polysaccharides and glycoproteins, which include amine, imidazole, phosphate, sulphate, sulfhydryl, and hydroxyl groups, are abundant in the cell walls of fungi (Leitao, 2009; Volesky and Holan, 1993). Yeast cell walls, on the other hand, have a microfibrillar structure made up of more than 90% polysaccharides. Amine, hydroxide, carboxyl, sulphate, and phosphate groups are the most common groups found in these walls (Wang and Chen, 2009).

The majority of heavy metals are not biodegradable, therefore they begin to accumulate in microorganisms (Huang et al. 2014). Metal accumulation is influenced by a number of elements, including the level of exposure, metal content, temperature, and salinity, making it difficult to collect specific information on how it happens in bioremediation (Varma et al. 2011). The metal concentration regulates the accumulation process, which is complex and varies depending on the metabolic pathway (Fukunaga and Anderson, 2011). Surface binding and metallic ions penetrating the cellular membranes have been hypothesised as mechanisms for metal ion uptake (Al-Saraj et al. 1999; Acikel and Alp, 2009; Flouty and Estephane, 2012; Martin- Gonzalez et al. 2006).

Table 1: Difference between Bioaccumulation and Biosorption Processes

Attributes	**Biosorption**	**Bioaccumulation**
Expenditure	▪ Often Low ▪ Biomass generated from industrial waste ▪ Expenditure mostly related to production and transportation of biosorbent.	▪ Generally High ▪ The procedure takes place in the presence of viable cells that must be maintained.
pH	▪ The pH of solution excessively influenced the sorption rate of heavy metals.	▪ Substantial change in pH can have a significant impact on viable cells.

Selectivity	▪ Usually low ▪ Modification/Biomass transformation can however, boost this figure	▪ In comparison to biosorption better in bioaccumulation.
Rate of Removal	▪ Majority of mechanism occur instantly	▪ As intercellular accumulation takes a long period, the rate is slower than in the context of biosorption
Regeneration and Reuse	In many cycles, biosorbents can be reused and regenerated.	Due to intercellular accumulation, reuse is limited.
Recovery of metals	The removal of heavy metals is feasible with adequate extractant.	Even if it were feasible, biomass could not be used for different purposes.
Energy Demand	Generally Low	Necessary for cell growth

Factors Affecting Bioremediation

Heavy metal removal by microbes may have environmental and economic limitations. When choosing an appropriate bioremediation, several aspects should be taken into account. There are a few factors that have a significant impact on the rate of biodegradation. First, nutrients like nitrogen, phosphorus, sulphur, iron, and potassium found in contaminated environments can stimulate and sustain vigorous microbial growth, cell metabolism, and microorganism proliferation (Atagan *et al.* 2003). These nutrients are essential for bacteria to develop the enzymes they need to break down pollutants. Second, the cost of remediation may play a key part in the long-term success of bioremediation, thus the expense should be minimal to ensure financial viability.

Third, the nature of pollutants, such as whether they are solid, semisolid, liquid, or volatile in form, or whether they are poisonous or nontoxic, organic or inorganic contaminant, heavy metals, polycyclic aromatic hydrocarbons, pesticides, and chlorinated solvents, may have an impact on the process. The nature of the polluted environment is also crucial, as it might influence bioremediation quality. Fourth, the bioremediation process is influenced by pH, temperature, and other physicochemical parameters. The optimal range of these parameters can also have a significant impact on the rate and extent of biodegradation, as it affects microbial development and, as a result, the removal of pollutants (Vidali, 2001). Fifth, moisture content (water) is an important component in biological growth and bioremediation efficiency.

Sixth, microbial diversity capable of biodegrading any contaminant, such as *Pseudomonas*, *Aeromonas, Flavobacteria, Aeromonas, Chlorobacteria, Corynebacteria, Acinetobacter, Mycobacteria,* *Streptomyces, Bacilli, Macrobenthos,* and other aquatic plants such as E. crassipes and L. hoffmeisteri, can degrade turbidity Seventh, oxygen is mostly employed in contaminated areas for the early decomposition of hydrocarbons, but it can also be used for aerobic and anaerobic bioremediation (Thapa *et al.* 2012).

Role of Microorganisms in Remediation of Heavy Metals

As microbes are nature's original recyclers, they can be employed for bioremediation. They may also convert chemicals into energy and raw materials for their own growth, resulting in a low-cost, environmentally beneficial biological process. Heavy metals have become a worldwide genuine environmental hazard as a result of their widespread industrial use. Due to industrial operations and fuel consumption, toxic heavy metals accumulate in the food chain, causing environmental and health problems. These heavy metals are poisonous to living cells (mercury, silver, lead, cadmium, and arsenic).

In their DNA, many bacteria have resistance genes against a variety of heavy metal cations and oxyanions. To cope with the ingestion of heavy metal ions, bacteria employ a variety of strategies. Biosorption, trapping, efflux, reduction, precipitation, and complexation are examples of these mechanisms. As a result, microorganisms may be a viable and limitless resource for new environmental biotechnologies. Natural microorganisms are used in bioremediation to decompose or detoxify dangerous compounds to human health and/or the environment. The microorganisms can be employed in their natural habitat or isolated from other resources at the polluted site (Singh, 2014; Adenipekun and Fasidi, 2005)

Table 2 includes microorganisms that are implicated in biodegradation, such as the following examples: *Acinetobacter, Alcaligenes, Arthrobacter, Bacillins, Beijerinckia, Flavobacterium, Methylosinus, Nitrosomonas, Nocardia, Xanthobacter, Penicillium, Phanerochaete, Pseudomonas, Rhizoctonia, Trametes, and Serratia* (Tanmoy and Nimai, 2019; Dhokpande and Kaware, 2013). Most bioremediation operations are carried out in aerobic circumstances, although running a system in anaerobic conditions may allow microbial organisms to digest compounds that would otherwise be resistant to degradation (Singh, 2014). During their growth process, aerobic organisms rely on oxygen. These are ongoing processes known as cellular respiration, in which oxygen is used to oxidise substrates such as fatty acids from oil to produce energy.

Pseudomonas, Sphingomonas, Rhodococcus, Alcaligenes, and *Mycobacterium* are examples of degradative aerobic bacteria. Aside from hydrocarbons, microorganisms can be employed to breakdown hazardous compounds like insecticides (Vidali, 2001). The pollutant serves as a metabolic substrate for a large number of microorganisms (carbon and energy). An anaerobic bacterium differentiates from aerobic bacteria in that it does not require oxygen for its metabolism. Biphenyl bioremediation, dechlorination, and chloroform bioremediation have all been done with anaerobic bacteria (Vidali, 2001).

Table 2: Heavy metal dispersion in the environment and Biodegradation Microorganisms

Heavy metals	**Distribution**	**Microorganisms Involved**	**References**
Arsenic	Volcanic Activity, Soil	*Sporosarcina ginsengisoli*	Tanmoy and Nimai (2019) Ojuederie and Babalola (2017)
Cadmium	Water, Rocks (Sedimentary), Soil	*Bacillus* sp. *Klebsiella* sp. *Pseudomonas* sp.	Sharma *et al.* (2000) Rani *et al.* (2010) Kapahi and Sachdeva (2019)
Chromium	Water, Soil, Volcanic Activity Rocks	*Bacillus cereus* *Bacillus subtilis* *Enterobacter* sp.	Dong *et al.* (2013) Kanmani *et al.* (2012) Balamurugan *et al.* (2014) Rahman *et al.* (2015)
Lead	Soil	*Rhodobacter* sp. *Leclercia* sp.	Rigoletto *et al.* (2020)
Mercury	Air, Soil, Water	*Enterobacter* sp. *Acinetobacter* sp.	Pushkar *et al.* (2019)
Copper	Rivers, Oceans, Lakes, Earth's crust	*Kocuria flava*	Coelho *et al.* (2015)
Zinc	Rock, Soil, Surface water	*Pseudomonas putida*	Pardo *et al.* (2003) Chen *et al.* (2005)
Nickel	Water, Soil, Air, Sediments	*Bacillus licheniformis* *Pseudomonas putida*	Kim *et al.* (2015) Zhou *et al.* (2007)
Cobalt	Soil, Air, Water	*Bacillus* sp. *Rhodopseudomonas palustris*	Gao *et al.* (2017) Mani and Kumar (2014)

Conclusion

Large amounts of waste waters containing hazardous metals are produced by both natural and human-made activities. In recent decades, numerous studies have been carried out with the objective of lowering metal concentrations produced from natural resources. Also, a lot of work has gone into creating technologies that are successful and affordable and applying them to the treatment of industrial wastewater. It has been demonstrated that microorganisms have the capacity to eliminate metals from solutions through both active and passive mechanisms, and the effectiveness of such processes depends on the experimental settings, the target pollutant, and a number of other factors.

Although it is yet difficult to apply this kind of bioremediation technology on a wide scale, prevention of metal contamination issues is advocated. It is necessary to conduct additional research to characterise biosorbents, identify the mechanisms at play, and advance genetic engineering.

Several microbes have the ability to naturally break down metals, yet this is not an adequate worldwide solution. Hence, genetic engineering can be used to create designed microorganisms as a solution to this issue. Future studies will be more effective in addressing this particular environmental problem if they have a better grasp of the detoxification pathways and how both eukaryotic and prokaryotic microorganisms digest heavy metals.

On an industrial scale, it is vital to choose the most feasible type of biomass while also considering its cost and availability. The microorganisms ought to be simple to obtain and grow. Industrial-scale application, for instance, wouldn't be desirable if the microbe was challenging to grow, a rare species, or a species that was in risk of becoming extinct.

Even if there has been considerable development in the understanding of the significance of microbes for the detoxification of contaminated waters, there are still some crucial issues that need to be resolved. Yet science now faces a brand-new obstacle. Hence, future research should concentrate on creating new, ecologically friendly technologies that are also commercially viable.

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Conflict of interest- The authors declare no confict of interest.

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