ABSTRACT

Urbanization is taking place at a rapid pace resulting in an increased amount of pollution. Eventually, the extraction of precious metals and minerals releases hazardous metallic substances into the atmosphere increasing their existing quantity. Many health problems and illnesses in humans are associated with heavy metal toxicity. Plants, microorganisms and aquatic organisms are also affected. Toxic heavy metals includearsenic, zinc, etc. Physical and chemical methods of remediation have many drawbacks. Whereas the biological approach is a clean, inexpensive method, and a promising emerging field. This review article briefly summarizes the heavy metal impacts and their removal techniques.

Keywords: Mining; Heavy metals; Bioremediation; Physical Remediation; Chemical Remediation; Phytoremediation.

Highlights
Physical and chemical approaches to heavy metal remediation have already been in use. The biological approach considering it as a new technology has more advantages than them.

1.0 INTRODUCTION

Mining is considered the prime source of heavy metal contamination in the surrounding environment. Vigorous extraction of minerals produces a huge amount of waste minerals along with tailings and if not properly managed, they produce highly polluting acid mine drainage (AMD) that precipitates when exposed to water and can inflict serious environmental damage [1-3]. Tailings are substances that are left over after the separation process during mining and settle at the bottom due to their large size and heavy weight [4].

Heavy metals are described as metals that have high atomic weight and density, and in the field of biology, heavy metals are referred to the metals that are toxic for organisms even in a small amount. Metalloids are also sometimes considered in heavy metals; they are the chemical elements that have the characteristics of both metals and non-metals [5-7]. Some of the heavy metals that are of serious concern include Nickel, Zinc, Copper, Lead, Arsenic, Selenium, Cadmium, Chromium, Manganese, Cobalt and Mercury. Environmentalists are majorly focusing on heavy metal contaminated soil and water bodies. The past decade has seen a renewed importance in the elimination of heavy metal pollution as they are covert, persistent, and irreversible and degrade water quality, atmosphere, and agriculture and also raises a huge concern over the health of humans, plants, animals and microorganisms through contamination and accumulation in their food chain [8-12]. Due to these properties of heavy metals, they are non-biodegradable and remain in the environment for a very long time [6, 13]. [14] and [15] reported that magnetic pollution was related to heavy metal pollution concerning for to anthropogenic origin and transportation mechanism.

It is very difficult to degrade heavy metals from the ecosystem entirely, but there have been many conventional methodologies that reduce the heavy metal concentration from the ecosystem and include physical, chemical and physicochemical methods. They have certain disadvantages as they are not environment friendly, usually generate an unacceptable amount of secondary pollution, are expensive and sometimes fail during large scale implementation [16-22]. The biological method (bioremediation) is a cost-efficient and clean process for the degradation of heavy metals from polluted soil and water bodies [23-27]. Using microorganisms and plants for remediation purpose is gaining attention among scientists,
industries and environmental professionals in government [28-30]. Limitation of the biological method includes difficulty in isolation of microorganisms and growth of plants for bioremediation and adaptation abilities of those microbes and plants are not enough for practical application [31-33]. All remediation processes are used either individually or in combination for effective results [34]. Remediation approaches are performed either on the contaminated site or before the discharge of wastes from the sources, also known as source control [35].

This paper reviews research works on the harmful effects of heavy metal contamination on the surrounding locality of mining sites and discusses various methods to prevent its spread and create a safe environment.

2.0 IMPACT OF HEAVY METAL CONTAMINATION DUE TO MINING

As we see development in countries throughout the world, and industrial revolution took place which subsequently gives rise to huge amounts of wastes in the environment, coupling with a lack of pollution control; there has been an increase in heavy metal contamination [14, 36]. Some potential sources through which heavy metal contamination from the mining industries released in the environment are drainage and sediment run-off from mining sites, spills, dust, wind, abandoned equipment, land instability and accidental destruction of the site [37].

Heavy metal contamination comes from different sources in which mining is considered the major source. Mining industries extract precious minerals and other geological materials from the crust of the Earth [8, 38, 39]. Mining operations together with grinding and clustering of ores, and discharging of tailings in open which are transported through wind and flood lead to environmental contamination. Therefore, there is an increased volume of heavy metals within the encircling area of mining sites which is because of the discharge and disposal of waste materials into the environment in several forms like elemental, organic and inorganic [6, 40-42]. Rivers draining the mining wastes have deposition of heavy metals on their banks and streams [43, 44]. Heavy metals cannot be reduced into non-toxic harmless forms completely and then they leave an enduring negative impact on the environment. They are also carcinogenic, mutagenic and cytotoxic harming the organisms that are in contact [45]. They also affect the microbial population within the soil which ends up in the loss of species that
manage the nutrient cycling in the soil and resulting in the negative impact on ecosystem functioning [46, 47]. Therefore, health officials are concerned about the heavy metal effects on the ecosystem as well as on the health of organisms, more specifically human beings. With greater public awareness towards the environment and more strict regulations, there is growing interest among the scientific community to develop efficient and inexpensive remedial techniques to effectively remove wastes from the polluted sites [13, 48].

Pollution caused by heavy metals is also a major threat to the aquatic ecosystem, as it may cause chronic or acute toxicity to the organisms [49]. Generally, metals can be dissolved into the water bodies through lithogenic or anthropogenic ways. Lithogenic activities include weathering of rocks and soil, and metals are present in very low concentration in water bodies. Mining and smelting, municipal and industrial effluents, fertilisers and pesticides utilized in agriculture are some anthropogenic activities that are responsible for the mixing of toxic heavy metal pollutants in water bodies [50-54]. Water is taken into account as one of the important commodity and contamination of freshwater with industrial wastes and heavy metal contamination is of significant concern [55, 56]. Although, plants require some amount of metals for their growth through soil or water bodies, the increased concentration of these metals in their cell is toxic [45, 57]. The practice of irrigation using wastewater has been acknowledged to reduce soil pH and elevate the amount of organic carbon element, but it eventually increases the quantity of toxic metals within the soil which then accumulates in the crops [6, 58, 59]. Concern has increased over the reduction of beneficial soil microorganisms after the use of wastewater in the irrigation process as it results in the desertification of land with due course of time [60-62]. Groundwater is considered a valuable resource for drinking water universally and metal contamination in it is a major concern for everybody as the presence of metals even in very low concentration is toxic [63, 64].

Heavy metals are carcinogenic and mutagenic and can possess a great threat to humans and the flora and the fauna of receiving soil and water bodies [65, 66]. Metal ions accumulate into the body cells and interact with nucleic acids and proteins and induce changes, causing DNA damage thus destroying their biological functions and carcinogenesis [67-69]. Consumption of food contaminated with heavy metal can severely reduce the number of vital nutrients within the body that results in declination of immunological defence mechanism, growth delay and malnutrition [70]. There are several harmful effects of metal toxicity on the central nervous system of the human body leading to mental diseases, neurological deteriorative disorders like Alzheimer’s disease and Parkinson’s disease; damaging lungs, kidney, liver
and other essential body organs leading to several fatal disease conditions and in extreme cases, death [25, 65]. It can affect the capability of producing germ cells in both males and females. The amount of toxicity in the organism is related to the amount of metal taken by the organism and the duration of exposure. Consumption of aquatic organisms that originate in contaminated water can transfer metals from water to humans [71, 72].

Plants that grow on contaminated soil or water suffer a lot of damage. Heavy metals influence the yield and quality of the crops and plants growing on contaminated soil [73, 74]. These metals affect the seed germination, growth of plants, decreases enzyme activity and chlorophyll production and restrict photosynthesis. Direct effects of heavy metal toxicity on plant include inhibition of cytoplasmic enzymes and an increase in oxidative stress on the cell membrane which disrupts the cell structure. The indirect effect includes the substitution of vital nutrients in the metabolic sites of the plants. The heavy metal toxicity in the soil causes a reduction in beneficial microorganisms responsible for the decomposition of organic matter, thus depleting soil nutrients [75-78].

In the last few decades, companies that perform large scale mining have updated their techniques and are focusing on the radiation of the polluted land to satisfy regulatory orders and public pressure. However, pollution caused by small scale mining is neglected due to the existing economical, technological and administrative barriers [38]. Mining industries are focusing on sustainable development, their major factors being planning, environmental protection, remediation efforts, policy frameworks and management guidelines [79]. The increment in risk potential is caused due to mining activities and can be found out through comparison between the amount of pollution caused naturally and through mining [80, 81] and comparing uncontaminated and contaminated soil of the same locality for discovering the amount of contamination [82, 83]. Heavy metal pollution is evaluated through several indices like geoaccumulation index (Igeo), Nemerow Comprehensive Index, pollution load index (PLI) and enrichment factor (EF), whereas heavy metal pollution index (HPI) is employed to test the quality of water concerning heavy metals [41, 84, 85].

3.0 HEAVY METALS AND THEIR EFFECTS

In the environment, heavy metals are naturally present everywhere including the atmosphere, lithosphere, hydrosphere and biosphere. Both natural and anthropogenic ways are accountable for the discharge of heavy metals in the surroundings. During mining activities,
heavy metals are released from the ores, dumped in the soil or transported to other areas through air and water. As described by [4, 6, 18, 25, 47, 69, 86-88, 95, 96] some important heavy metals are discussed below.

3.1 ARSENIC (As)

Chemical properties of As include: atomic number 33, atomic mass 75, density 5.7 g.cm\(^{-3}\) at 14°C, melting temperature 814°C and boiling temperature 615°C. It is the twentieth most abundant element present on the Earth. Arsenite and arsenate are the inorganic forms of arsenic that are fatal to organisms including humans. Arsenic intake can be through drinking water from the source where industrial wastes are dumped, eating contaminated food that may contain pesticides or originated from the arsenic-rich soil and other sources which include inhalation and dermal exposure. Health hazard to humans due to As contamination include: lung and bladder cancers, deterioration of red blood cells (RBCs), affects kidney, cardiovascular system and central nervous system. It also affects some vital cellular processes including oxidative phosphorylation and ATP synthesis. Arsenic is a protoplasmic poison (toxic substances that can damage or kill living cells) that causes malfunctioning of cellular respiration, metabolic pathway and mitosis.

3.2 MERCURY (Hg)

Mercury is the only metal that occurs in the liquid state at standard temperature and pressure. Mercury is odourless and has shiny silver-white colour in at the liquid state and turns colourless gas after heating. Mercury has an atomic number of 80, atomic mass 201, density 13.6 g.cm\(^{-3}\), melting point -13.6°C and boiling point 357°C. The most toxic form of mercury is its alkylated forms that are volatile in air and soluble in water. It forms a strong bond with both organic and inorganic compounds making it very soluble in the aquatic system. Methyl mercury is of greater significance as it is produced by microbes from Hg\(^{2+}\) in different environments. Mercury contamination is released through either coal combustion or from the manometers at gas/petrol station in gaseous form and can be inhaled by organisms. Mercury is a neurotoxin that damages nerve cells in the brain causing loss of memory, depression, insomnia, tremors and restlessness. It also causes lungs and kidney failure, autoimmune diseases, hair loss and vision loss.

3.3 LEAD (Pb)
Lead is a greyish blue metal found in the crust of the Earth. Although it is present in the environment, human activities like mining, burning of fossil fuel, manufacturing processes release lead at high concentrations. It has an atomic mass of 207, an atomic number of 82, a density of 11.4 g.cm\(^{-3}\), a melting temperature of 327.4\(^{\circ}\)C and a boiling temperature of 1725\(^{\circ}\)C. In the atmosphere, lead is released through industrial processes and vehicular exhausts and is then taken up by plants through the soil and water bodies, and hence human exposure can also be through ingesting food or drinking water. Humans may also be exposed through inhalation and ingestion. If children are excessively exposed to lead contamination, it hinders their brain development leading to reduced intelligence, memory lapses and disabilities in learning and problem-solving skill. Adults may have cardiovascular diseases, and their GI tract, kidneys, lungs and central nervous system are also severely affected. Lead enters microbial cells through uptake pathways of divalent ions and exerts its toxicity by inhibiting enzymatic activity, changing the conformation of nucleic acids and proteins, altering osmotic balance and disrupting the cell membrane.

### 3.4 ZINC (Zn)

Zinc is naturally present in the environment and is a trace element needed by organisms for their essential biological functions. The deficiency of zinc causes birth defects. Chemical characteristics of zinc are atomic number 30, atomic mass 65.4, density 7.14 g.cm\(^{-3}\), melting temperature 420\(^{\circ}\)C and boiling temperature 906\(^{\circ}\)C. Zinc is released into the atmosphere through industrial processes like mining, fossil fuel combustion and the processing of steel. Normally, it is utilized by plants, bacteria and humans as a micronutrient for many biological metabolic functions but if it exceeds normal physiological value then it is toxic for the organisms as it reacts with the sulfhydryl groups of amino acids and replaces other vital metals that are used in a wide range of proteins. Wastewater from industries pollutes the water bodies. Zinc is accumulated in the aquatic organisms and plants through contaminated water and soil, thus affecting the biological systems and food chain. Zinc also adversely affects microbes and insects living in the soil like earthworms, therefore inhibiting the degradation of organic matter. Excess zinc in humans causes dizziness, fatigue, and memory loss.

### 3.5 CHROMIUM (Cr)
Chromium occurs naturally as compounds (Cr (III) oxide, Cr (IV) oxide, Chromate and Dichromate) in the earth’s crust. Largely, chromium enters the ecosystem through industrial and anthropogenic processes as well as natural activities. It has an atomic number of 24, an atomic mass of 52, a density of 7.2 g.cm$^{-3}$, a melting temperature of 175°C and a boiling temperature of 2665°C. Chromium is used in petroleum industries, steel, fertilizers, and oil well drilling and in tanneries for metal plating. Industries play a major role in the contamination of chromium, as well as human anthropogenic practices, the treatment of wastewater and the use of fertilisers contribute to the release of chromium into the environment. It is associated with allergic dermatitis and hair loss in humans; inflammation of the nose lining is caused by inhalation of Cr. Chromium ingestion causes stomach and small intestine ulcers, anaemia, male reproductive system damage in animals.

### 3.6 CADMIUM (Cd)

In sedimentary rocks and marine phosphates, cadmium compounds are found naturally. It is used in several manufacturing activities such as the manufacture of alloys, batteries and pigments. Atomic number 48, atomic mass 112, density 8.7 g.cm$^{-3}$, melting point 321 °C and boiling point 765 °C provide the chemical characteristics of cadmium. The Cd content in the soil is raised by the disposal of industrial wastes. Cd enters the organisms' body via the food chain and persists for several years as it is bio-persistent and has toxicological properties. Cadmium is a non-essential metal, i.e., it is not involved in any known biological metabolic pathways, but it has been found to inhibit microbial DNA-mediated transformation and to interfere with the symbiotic relationship between plants and microorganisms. Several enzymes in the body accountable for the reabsorption of proteins in the renal tubules are impaired by Cd. It also influences the regulation of calcium in biological systems, raises blood pressure and twitching of muscles, damages lungs. The metabolic activities of soil microbes, such as nitrogen and carbon mineralization, the production of carbon dioxide and enzymatic activities are impaired by cadmium toxicity.

### 3.7 NICKEL (Ni)

Nickel is a transition metal having atomic number 28, atomic weight 59, density 8.9 g.cm$^{-3}$, melting point 1455°C and boiling point 2730. It is available in the atmosphere at very low levels and is crucial in very small doses, but if it reaches the maximum tolerable amount, it is harmful to the body. The nickel mining, electroplating and metal plating industries have
souces of nickel pollution in the soil. Different kinds of cancer are caused in organisms that live near mining sites or refineries. Nickel induces allergic skin diseases, inflammation of the mouth, immunotoxicity, neurotoxicity, lung cancer and affects fertility.

Table 1. Most hazardous heavy metals and their effects

| S. no. | Metal | Source | Effect |
|-------|-------|--------|--------|
| 1.    | Arsenic | Mining; Disposal of industrial wastes; Fossil fuel combustion; Pesticides | Affects cellular processes; Cardiovascular diseases; Kidney diseases |
| 2.    | Mercury | Mining; Fossil Fuel Combustion; Medical waste | Toxic to nerve cells, Autoimmune Disease, Hair loss, Vision loss |
| 3.    | Lead | Mining; Fossil Fuel Combustion; Industrial Processes; Batteries waste; Pesticides | Inhibits brain development in children; Cardiovascular diseases; Inhibits Metabolic Pathways if in excess |
| 4.    | Zinc | Mining; Fossil fuel combustion; Industrial Wastewater | Inhibits metabolic pathways and degradation of organic matter if in excess; Memory loss; Fatigue |
| 5.    | Chromium | Mining; Disposal of industrial wastes; Fertilisers; Flying ash; Tanners | Allergies, Hair loss, Ulcers, Anaemia |
| 6.    | Cadmium | Disposal of Industrial Wastes; Paints and pigments; Electroplating; Fertilizers | Damages lungs, kidney; Affects metabolic activities of microbes |
7. Nickel 
- Mining, Metal Plating Industries; Surgical Instruments; Kitchen appliances 
- Cancer, Allergies, Infertility, Immunotoxicity 

4.0 REMEDIATION TECHNIQUES OF HEAVY METAL CONTAMINATION

The main goal of remediation strategies is to create an ecosystem that is healthy for humans and other animals. The treatment method is influenced by the chemical and physical form of heavy metal [4]. Various methods of treatment are practised to avoid the spread of heavy metal contaminants in soils and bodies of water. By physical, chemical and biological processes, removal of heavy metals can be accomplished. Each technique has its pros and cons, which will be addressed in this section [19, 65, 89, 90]. Drawbacks of physical and chemical approaches include high cost, irreversible changes in soil properties, labour intensive, producing secondary pollution and destroying soil microflora [45, 91].

4.1 PHYSICAL REMEDIATION

Physical methods of remediation are said to be the most time taking and expensive. Commonly used physical remediation methods are explained below as described by [4, 6, 7, 13, 19, 52, 65, 90, 92-96].

4.1.1 SOIL REPLACEMENT

The system of soil replacement uses clean soil to completely or partially replace polluted metal soil [6]. It is also referred to as the ‘dig and haul’ process [96]. The key aim is to reduce the concentration of pollutants and increase the soil's natural potential, thus remediating the soil. This technique is very laborious, costly and only appropriate for smaller areas that are heavily polluted [96]. Again it is split into three types, namely soil substitution, soil spading and soil importing. Soil substitution is the process in which the polluted soil is removed and fresh soil is kept in its place. This approach is only sufficient for smaller polluted areas of land. It should be remembered that it only moves polluted soil to a lower risk area from a higher risk area. The replaced soil will cause second contamination, so it needs to be
carefully treated [92]. Instead of polluted soil, the soil importing method adds tons of fresh soil, covering the surface or mixing with it to reduce the concentration of contaminants.

4.1.2 THERMAL DESORPTION

The technique of thermal desorption is based on the volatility of the metal compounds in the polluted soil [7, 92]. Polluted soil is first heated using steam, ultraviolet radiation or microwave to make the pollutant compounds volatile [6]. With the assistance of vacuum negative pressure or a carrier gas, the volatile contaminants are then extracted such that the pollutants are separated from the soil and the soil becomes uncontaminated. The thermal desorption technique can be categorized as high temperature desorption (320~560°C) and low temperature desorption (90~320°C) based on temperatures. The advantages of this process are that it is much simpler than the soil replacement method, the equipment is transportable and the remediated soil can be reused. However, its limitations include drastic changes in soil quality, expensive devices and long desorption time [7].

4.1.3 MEMBRANE FILTRATION

The technique of membrane filtration is given a lot of importance as it can eliminate suspended solids and organic and inorganic pollutants such as heavy metals [65]. Membrane filtration is categorized into ultrafiltration, nanofiltration and reverse osmosis based on particle size, which are used to extract heavy metals efficiently from water bodies. Ultrafiltration (UF) separates metal ions based on 5-20 nm pore sizes and 1-10 kDa molecular weight. With a metal concentration in the range of 10 to 112 mg/L, UF achieves more than 90% removal efficiency. Nanofiltration has a semi-permeable organic membrane with a small pore size of 0.1~10 nm to 1~2 nm and a molecular weight of <200 Da [93]. The driving force for the separation process in nanofiltration is the pressure difference over the membrane. Reverse Osmosis (RO) has the smallest pore membrane and is commonly used for water recycling and metal recovery [65]. It requires reversing a solution's osmotic process to push a solution through a membrane that retains the solution on one side and allows the membrane to pass through pure water [94].

4.1.4 ION EXCHANGE

Ion exchange is an effective process for the removal of heavy metals with low effluent concentrations used in the water treatment industry [65, 95]. In this process, the removal of
metal ions from the solution requires a special ion exchanger containing cations or anions. Synthetic organic ion exchange resins are typical matrices that are used in ion exchange [93]. Ion exchange resins are hydrophobic substances with properties to absorb ions from an electrolyte solution that are either positively or negatively charged and release the other ions into the solution [94]. Two kinds of ion exchange resins, cation and anion exchange resins, are available [65]. Nickel, copper and zinc ions can be absorbed by cationic resins, while anion exchange resins are ideal for low contamination of soil or water and are used for the absorption of chromate, sulphate, nitrate and cyanide. Valuable heavy metals can also be recovered through ion exchange after the separation of the resin from the elution.

4.2 CHEMICAL REMEDIATION

The chemical remediation approach is concerned with the reaction of heavy metal pollutants to the chemicals used to remove them. Few techniques of chemical remediation are explained below as described by [4, 6, 7, 13, 19, 22, 28, 52, 65, 90, 92-98, 115].

4.2.1 CHEMICAL PRECIPITATION

Due to its easy activity, chemical precipitation is commonly used to extract heavy metals from inorganic wastes [65]. Coagulants such as lime, alum, and other organic polymers are used to produce insoluble precipitates of heavy metals in the form of hydroxide, sulphide, carbonate and phosphate [28, 93]. Once the metal ions are precipitated and form clusters, they can be easily removed using sedimentation and filtration processes [13, 19]. By slightly changing important remediation parameters such as pH, temperature, initial concentration, ion charge, etc. the efficiency of this process can be improved. The limitations include the production of excessive sludge requiring secondary treatment [94], slow rate of precipitation and long term impact on the environment due to sludge production [95]. It also requires large quantities of chemicals to obtain an acceptable amount of remediation.

4.2.2 CHEMICAL LEACHING

In chemical leaching, freshwater, reagents, or other fluids or gases that can drain or leach the contaminant out of the soil are used to wash the polluted soil [92]. Heavy metals were moved from the soil to the liquid state by anion exchange, precipitation, adsorption and separation troughs, and then recovered from the leachate [6]. Inorganic eluents, chelating agents and surfactants etc. are primarily leachates used.
4.2.3 CHEMICAL FIXATION

The technique of chemical fixation applies reagents or chemicals to the polluted soil that interacts with heavy metals and forms low toxic substances that are insoluble or hardly mobile [6]. Such solid types of heavy metals could then not migrate to water, plants or any other environmental media, thereby completing the remediation process. This method is effective for soil with low contaminant concentrations. Conditioning agents used in the method include clay, metallic oxides and biomaterials, but these agents can affect the soil structure to some degree and can also have an adverse impact on the soil microbes [92].

4.2.4 IMMOBILIZATION

The technique of immobilization involves the capture of heavy metals from immobilizing agents such as clay, organic composts, minerals, calcium carbonate, manganese oxides, phosphates and zeolites, further reducing the solubility of metals and therefore their soil mobility [4, 96]. The main aim of this process is to aggregate metals inside the soil matrix into metal hydroxides. The downside of this approach is the presence of organic pollutants that when the immobilising agent is applied, may form a toxic organic vapour.

4.3 BIOLOGICAL REMEDIATION

A recent emerging sector with an innovative and promising potential for the removal of heavy metals from contaminated water and soil is biological remediation. There is a long-term impact of bioremediation on the polluted site. In heavy metal environments, microorganisms have adapted to their existence and have developed their metabolic mechanisms so that they can easily detoxify heavy metals. This property of microbes is hence used in the bioremediation process. Bioremediation can take place using microorganisms or plant species. Bioremediation processes using different organisms and plant species are explained below as described by [4, 6-8, 32, 39, 45, 48, 63, 66, 67, 72, 77, 78, 88-90, 92, 99-114].

4.3.1 BIOREMEDIATION USING BACTERIA

There is a high potential for microorganisms to transform heavy metals into their non-toxic forms [101]. They do not degrade heavy metals, but instead, transform these metals by modifying their physical and chemical properties into non-toxic types [6]. Bacteria are important in bioremediation as biosorbents because of their universality, size, and ability to
reproduce under harsh environmental conditions. There are many functional groups, such as carboxyl, phosphonate, amine, and hydroxyl groups, in the bacterial cell wall. Bacteria are categorized into gram positives and gram negatives based on the structure of their cell wall. The ability of gram positive bacteria to bind metals is due to the inclusion in their cell wall of anionic functional groups found in peptidoglycan, teichoic acid and teichuronic acid [90]. The components primarily responsible for anionic characterization and metal-binding capacity are the existence of phospholipids and lipopolysaccharides in gram negative bacteria. Some bacteria used in the remediation process are Bacillus subtilis, Bacillus cereus, Brevibacterium iodinium, Alcaligenes faecalis Pseudomonas putida, Pseudomonas aeruginosa and Enterobacteria cloacae [72, 77, 78, 88]. Consortia of cultures are metabolically preferred for field application of bioremediation as bacteria survive better and are more stable when they are in a mixed culture.

Table 2. Bioremediation using bacteria and outcomes

| Bacterial species | Metal Ion | Condition | Outcome | Reference |
|-------------------|-----------|-----------|---------|-----------|
| Pseudomonas putida| Lead, Copper | Bioremediation procedure was performed in batch system by the bacterial specie with single component as well as binary system and differing the pH, temperature, kinetics and metal concentration of both single and binary mixture. | Bacterial biomass showed highest single and binary metal ion uptake at 25 and 30°C respectively, pH 5.5 and metal ion concentration 100 mg dm$^{-3}$. | [107] |
| Bacillus thuringiensis | Nickel | Bioremediation of nickel was performed on dried vegetative cell and spore-crystal mixture of bacterial specie as a function of pH, temperature and initial metal ion concentration. | Metal ion uptake of vegetative cell was 10% whereas of spore-crystal mixture was 15.7% at 250 mg/l of metal ion concentration and temperature of 35°C. | [108] |
Growing and resting cells of *B. circulans* were incubated in 50 ml of cadmium rich media (28.1 mg/l). Both growing and resting cells showed high uptake rate of cadmium, resting cells were markedly higher than growing cells. Maximum removals of both cells were at pH 7.

Effect of pH, metal ion concentration and contact time with the bacteria was studied. The process follows the Freundlich and Langmuirian isotherm model.

The optimum pH of chromium and aluminium was shown to be 4 and 5 at metal concentrations of 3 mg/g and 55.2 mg/g respectively.

### 4.3.2 BIOREMEDIATION USING FUNGI

Owing to their high proportion of cell wall content that has an exceptional metal binding property; fungi have the ability to extract toxic metals and are commonly used as biosorbents [90]. There are significant quantities of chitin, glucan, mannan and chitosan in the cell wall of fungi, which are abundant sources of metal binding sites such as carboxyl, amine, phosphate and hydroxyl groups [90]. There are many potential benefits of using fungus for bioremediation, including the ease of large-scale cultivation using inexpensive growth media, short multiplication phases and high biomass yields. Some fungi that are being used in bioremediation are *Aspergillus niger*, *Aspergillus awamori*, *Rhizopus oryzae*, *Coprinopsis atramentaria* and *Penicillium chrysogenum* [77]. Factors that need to be taken into consideration while using fungi as biosorbent are initial solute concentration, nature and concentration of biomass and physicochemical factors such as pH, temperature and ionic strength.

**Table 3. Bioremediation using fungi and outcomes**

| Fungal species | Pollutant       | Condition | Outcome | Reference |
|---------------|-----------------|-----------|---------|-----------|-----------|
| *Bacillus circulans* | Cadmium | Growing and resting cells were incubated in 50 ml of cadmium rich media (28.1 mg/l). Both growing and resting cells showed high uptake rate of cadmium, resting cells were markedly higher than growing cells. Maximum removals of both cells were at pH 7. | [109] |
| *Chryseomonas luteola* | Chromium, Aluminium | Effect of pH, metal ion concentration and contact time with the bacteria was studied. The process follows the Freundlich and Langmuirian isotherm model. The optimum pH of chromium and aluminium was shown to be 4 and 5 at metal concentrations of 3 mg/g and 55.2 mg/g respectively. | [110] |
| **Aspergillus awamori** | Copper | Bioremediation procedure was performed in 250 ml Erlenmeyer flasks. 0.1 g of fungal biomass was added to 100 ml of heavy metal solution at 20°C. Pre-treatment with DMSO and Sodium hydroxide increased Cu(II) uptake capacity of fungus by 20.05% and 48.2%, respectively. |
| **Aspergillus niger** | Zinc | Experiment was performed in batch mode as well as in column mode followed by alkali treatment to increase pH. Results showed that the bioremediation was function of pH (increase in remediation with increased pH), biomass concentration (decrease in remediation with increased biomass concentration) and zinc concentration. |
| **Penicillin ochrochloron** | Copper | Mycelium was isolated from a plating solution containing 25% copper sulphate and 7% sulphuric acid. Isolate was grown at pH 2-8 and copper concentration of 5000 ppm. Culture studies showed metal uptake upto $4 \times 10^5 \mu g/g$ dry weight of biomass after a day. Recovery and removal of metal was seen in the experiments with lake water. |
| **Termitomyces clypeatus** | Chromium | Effluent from tannery industries was remediated using fungal specie. The process follows the Langmuirian isotherm model. *T. clypeatus* was best grown at pH 3 and showed prominent reduction of metal ion to a permissible limit. |

### 4.3.3 BIOREMEDIATION USING PLANT SPECIES

Phytoremediation is the process of bioremediation using plants and it involves techniques for immobilizing, degrading and reducing environmental toxins to clean up the contamination from soil, sludge, wastewater and groundwater. A variety of plants having different physical...
characteristics and metabolic processes are utilized in phytoremediation \cite{96, 99}. When the polluted area is wide and falls within the plant's root zone, this process is appropriate. Phytoremediating plants actively uptake large quantity of heavy metals and reduce them in less toxic form without showing any toxicity traits \cite{96}. Via different methods, including phytoextraction, phytofiltration, phytostabilization and phytovolatilization, the phytoremediation mechanism is achieved \cite{7}. Phytoextraction is the mechanism by which heavy metals are taken up by remediating plants and accumulate in their roots and shoots \cite{78, 90}. These plants are later harvested and destroyed. Rapid growth rate, high biomass, complex root system and the ability to withstand high concentrations of heavy metals are the properties of plants used in phytoextraction \cite{72}. Metal phytofiltration includes root filtration (rhizofiltration), seedling filtration (blastofiltration), and excised plant shoots (caulofiltration). In order to immobilize metals, phytostabilization is carried out thereby limiting their mobility and bioavailability, and preventing their migration to bodies of water and the food chain \cite{7, 89}. This procedure is carried out where it is not possible to conduct phytoextraction. In the phytovolatilization process, with the aid of special substances secreted by the roots, metal pollutants are taken up by the plants and converted into a volatile state or turning adsorbed metals into a gas state. Indian mustard (\textit{Brassica juncea}), Indian grass (\textit{Sorghastrum nutans}), Poplar tree (\textit{Populus deltoides}), Willow (\textit{Salix}) and Sunflower (\textit{Helianthus annus}) are examples of some plants used in phytoremediation \cite{89}. Phytoremediation technique is still in its testing stage and has not been used as a full scale application in many places \cite{4}.

Table 4. Bioremediation using plants and outcomes

| Plant species | Family | Metal Ion | Removal Mechanism | Reference |
|---------------|--------|-----------|-------------------|-----------|
| \textit{Chamomilla recutita} and \textit{Hypericum perforatum} \textit{L} | Cadmium | Asteraceae | Plant releases secondary metabolites which form complexes with cadmium ions resulting in less organo-metallic compounds. | [111] |
| \textit{Psychotria douarre} | Nickel | Asteraceae | High concentration of tannins present in leaves serves as detoxicant for the removal of nickel. | [112] |
**Eichhornia crassipes**  \[Mercury\]  \[Potederiaceae\]
Removal is achieved through ionic binding of mercury with oxygen ligand and carboxylate groups present in roots and sulphur groups present in shoots. \[113\]

**Brassica juncea**  \[Zinc\]  \[Brassicaceae\]
Removal is achieved through the high production of shoot biomass. \[114\]

| Table 5. Different remedial techniques, their procedure along with pros and cons |
|---|---|---|---|---|
| S. no. | Remediation | Sub-types | Procedure | Pros | Cons |
| 1 | Physical Remediation | Soil Replacement | Replacement of polluted soil with clean soil. | Efficient removal of pollutant from contaminated area | Contaminants are not destroyed; requirement of additional procedure; costly; laborious |
| | | Thermal Desorption | Technique uses heat to make pollutant compound volatile and then extract it from soil. | Simpler process; equipment is transportable; reuse of remediated soil | Devices are expensive; long desorption time |
| | 1 | Physical Remediation | Membrane Filtration | Filtration of pollutants from polluted water using different pore size filters. | Pore size can be adjusted according to the pollutants; continuous operation can be performed | Lifetime of membrane is very short; production of concentrated sludge |
| | | Ion Exchange | Ion exchange resins are used to separate metal ions from polluted water and soil. | Wide range of heavy metals can be removed | Expensive; regeneration of resin after each cycle |
| 2 | Chemical Remediation | Chemical Precipitation | Coagulants are used to precipitate heavy metals and then they are removed through filtration. | Simpler process; less laborious | Production of sludge; requirement of secondary treatment; harmful to environment |
### Chemical Leaching
Leaching of contaminants using reagents.  
Wide range of heavy metals can be removed.  
Expensive; long term effect on environment.

### Chemical Fixation
Addition of reagents making heavy metals insoluble and immobile.  
Low contaminant soil can be easily remediated.  
Degrades soil quality; affects soil microbes.

### Immobilization
Technique involves capturing of heavy metals with immobilizing agents.  
Captured heavy metals can be reused in various other activities.  
Formation of toxic organic vapour due to presence of organic pollutants; expensive.

### Using Bacteria
Addition of remediating bacterial species which through their metabolic pathways degrade the heavy metals.  
Cost efficient; less material required; nil production of secondary pollutant.  
Not every heavy metal can be remediated; chances of contamination.

### Using Fungi
Addition of remediating fungal species which through their metabolic pathways degrade the heavy metals.  
Cost efficient; less material required; nil production of secondary pollutant.  
Not every heavy metal can be remediated; chances of contamination.

### Phytoremediation
Removal of heavy metals from contaminated soil and water.  
Preferable for large contaminated area; nil toxic production; easy to implement and maintain.  
Took many years to remediate a site; can be influenced by climate conditions.

### 5.0 CONCLUDING REMARKS
With an increase in urbanisation all over the world, the pollution rate in the environment is also increasing. Without proper remediation, these industrial wastes are discarded in the soil or water bodies which then harm the environment. Heavy metals are normally present at very low concentrations in the environment, but human activities in the ecosystem increase their quantities. Mining is regarded as a major source of heavy metal emissions. When any precious mineral or metal is extracted from the crust, it releases certain harmful metals that linger in the surrounding soil or travel to the bodies of water, including groundwater, rivers, streams, etc. Arsenic, chromium, mercury, zinc, nickel, etc. constitute heavy metals that contaminate the environment. Such metals are persistent and can remain for several years in the atmosphere. Ingestion of metals through food or inhalation through the atmosphere affects various organs of humans. It can cause inflammation in the throat, skin and nose lining, induces a variety of cancer, damage to the kidney and intestine and affects the cardiovascular system. Plants growing on contaminated soil encounter adverse effects such as inhibition of photosynthesis and decreased metabolic activity. Seed germination and plant growth are also affected. Microorganisms living in polluted soil are decreasing due to heavy metal exposure; thereby reduction in soil nutrients can be seen. There is a growing concern, therefore, about the impact of heavy metal contamination on human health and the biological functions of microorganisms and plants.

We concentrated on many techniques used for heavy metal remediation in this review article. A total of 115 scientific documents were reviewed for the preparation of this articles. Thirty-three (33) articles were studied on biological remediation, 18 articles were reviewed on chemical remediation and 13 articles were referred on physical remediation. There were significant limitations in the implementation of physical and chemical methods such as the development of secondary contaminants, as stated by different authors and also listed above; one has to invest more in their removal other than the process itself, which make those methods expensive. Industries are now opting for biological remediation techniques. The main concern with much of the bioremediation literature is that there is no precise and reliable method for remediating polluted rivers and streams. It is a much-anticipated necessity to eliminate heavy metal pollutants in large water bodies. Unfortunately, it is also a major concern that existing bioremediation techniques neglect the usage of immobilised microorganisms for heavy metal removal in large water bodies like rivers. For wastewater treatment in bioreactors, immobilized microorganisms are increasingly used as they provide benefits such as high cell densities, high durability, absence of cell washout, and multiple
Due to their significant importance, they can also be used to eliminate pollutants at a higher scale in large water bodies directly. More work needs to be done on methods that involve combination of different techniques in order to achieve better results.

By comparison of all remediation technologies, bioremediation is preferred overall. Besides, efforts of biotechnological approaches have seen some progress in biological methods which provides its successful application in the remediation of heavy metals.

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