Microbes used as a tool for bioremediation of heavy metal from the environment

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Abstract: Heavy metal pollution poses a serious threat to all forms of life in the environment due to the toxic effects of long-term environmental pollution. These metals are extremely sensitive at low concentrations and can be stored in food webs, posing a serious public health risk. Different organic pollutants and metals are not degradable and remain in their environment for a long time. Remediation using conventional physical and chemical methods is uneconomical and produces large volumes of chemical waste. The balance of hazardous metals has shown a strong and growing interest over the years. The use of biosensor microorganisms is eco-friendly and cost-effective. Therefore, microorganisms have a variety of mechanisms of metal sequestration that hold greater metal biosorption capacities. Finally, we provide suggestion from microbial tools to remove, recover metals, and metal-loids from solutions using living or dead biomass and their components.

Subjects: Agriculture & Environmental Sciences; Microbiology; Bioinformatics; Biotechnology; Food Chemistry

Keywords: bioremediation; biosorbent; biosorption; heavy metals; microorganisms; remediation

ABOUT THE AUTHOR
I am Fikirte Zewdu (MSc) (the corresponding author), working as analytical chemistry at the University of Gondar, Department of Chemistry. Our group is mainly engaged in both basic and applied researches all on environmental issues. Although most of our reported works are the use of microbes and their application for monitoring the environment friendship. We still have works on assessing the microbes to the selected bioremediation heavy metals (carcinogenic) in discharged industrial effluent in general. In this regard, we are working in collaboration with national and regional environmental protection offices.

PUBLIC INTEREST STATEMENT
Heavy metal pollution poses a serious threat to all forms of life in the environment due to the toxic effects of long-term environmental pollution. These metals are extremely sensitive at low concentrations and can be stored in food webs, posing a serious public health risk. Different organic pollutants and metals are not degradable and remain in their environment for a long time.

Microorganisms, in particular, have the ability to degrade, detoxify, and even accumulate harmful organic as well as inorganic compounds. There are different sources of heavy metals in the environment. Activeness such as mining, electroplating, metallurgical smelting actions, and agriculture have contaminated wide areas of the world. Microbial resistance and tolerance toward pollutants, particularly heavy metals, are absolutely vital in the bioremediation processes as required microorganisms such as bacteria, fungi consortium organisms, and algae.
1. Introduction

The rapid increase in population and the increased demand for industrial establishments to meet human needs have created problems such as overutilization of accessible resources and increased pollution taking place in the land, air, and water environment. Heavy metal is of economic significance in industrial use and currently becomes a significant environmental problem throughout the world (Igiri et al., 2018; Siddiquee et al., 2015). Environmental pollution by heavy metals has become a serious threat to living organisms in an ecosystem (Deepa & Suresha, 2014; Hryniewicz & Baum, 2014; Okolo et al., 2016; Siddiquee et al., 2015; Su, 2014). According to EPA 2010 (Environmental Protection Agency (EPA), 2010), parameterization is defined as a spontaneous process in which the microbiological process is used to degrade, break down, or transform hazardous contaminants into less toxic or nontoxic forms, thereby remedying or removing and eliminating contaminants from environmental media. Microorganisms use chemical contaminants as an energy source through their metabolic processes throughout the microbiological process. However, excessive amounts of inorganic nutrients in soil cause microbial inhibition (Ahirwar et al., 2016).

Microorganisms, in particular, have the ability to degrade, detoxify, and even accumulate harmful organic as well as inorganic compounds. There are different sources of heavy metals in the environment, such as natural, agricultural, industrial solid waste, inland effluent, atmospheric sources, and more sources. Activeness such as mining activities, electroplating, metallurgical smelting actions, and the use of agriculture pesticides and fertilizers have contaminated wide areas of the world (Zhang et al., 2011).

Heavy metal (HM) pollution is a major environmental problem which reduces crop production and food quality due to excessive application of agricultural inputs like fertilizers, pesticides, and mulch have resulted in the heavy metal contamination of soils (Su, 2014). Most of the pesticides are organic compounds, and a few are organic—inorganic compounds or pure minerals, and some pesticides contain Hg, As, Cu, Zn, and other heavy metals (Arao et al., 2010). Unlike organic contaminants, metals are not degradable and thus remain in the environment for long periods of time; when present at high concentrations, metals can negatively affect plant metabolism (Ferraz et al., 2012). There is a need for innovative treatment technologies for the removal of heavy metal ions from soil, waterbodies, and wastewater. Different microbes have been proposed to be efficient and economical alternatives for the removal of heavy metals from soil and water (Ahirwar et al., 2016).

The microbes are biochemically discovered and their potential to resist heavy metals such as zinc and copper will be determined. In the past two decades, there have been recent advances in bioremediation techniques, with the ultimate goal being to effectively restore polluted environments in an eco-friendly approach, and at a very low cost. Researchers have developed and modeled different bioremediation techniques; however, owing to the nature and/or type of pollutant, there is no single bioremediation technique that serves as a “silver bullet” to restore polluted environments. Autochthonous (indigenous) microorganisms present in polluted environments hold the key to solving most of the challenges associated with biodegradation and bioremediation of polluting substances (Azubuike et al., 2016), provided that environmental conditions are suitable for their growth and metabolism. Environmentally friendly and cost-saving features are among the major advantages of bioremediation compared to both chemical and physical methods of remediation. Nevertheless, in some instances, the term biodegradation is used interchangeably with bioremediation the former is a term that applies to a process under the latter. In this review, bioremediation is defined as a process, which relies on biological mechanisms to reduce (degrade, detoxify, mineralize, or transform) concentration of pollutants to an innocuous state.
The process of pollutant removal depends primarily on the nature of the pollutant, which may include agrochemicals, chlorinated compounds, dyes, greenhouse gases, heavy metals, hydrocarbons, nuclear waste, plastics, and sewage. Apparently, taking into consideration the site of application, bioremediation techniques can be categorized as ex-situ or in-situ. Pollutant nature, depth and degree of pollution, type of environment, location, cost, and environmental policies are selected of the selection criteria that are considered when choosing any bioremediation technique (Smith et al., 2015). Apart from selection criteria, performance criteria (oxygen and nutrient concentrations, temperature, pH, and other abiotic factors) that determine the success of bioremediation processes are also given major considerations prior to the bioremediation project. Microbial remediation presents new techniques for addressing the problem of heavy metal pollution in the environment, and it has become a focus of new research and development in bioremediation technology. This review addresses microbes used as a tool for bioremediation of heavy metals from the environment.

2. Heavy metal

Heavy metals are defined on the basis of three different criteria, including density, atomic number, and their chemical properties. Heavy metals are of economic significance in industrial use and currently become a significant environmental problem throughout the whole world (Igiri et al., 2018; Siddiquee et al., 2015). Environmental pollution by heavy metals has become a serious threat to living organisms in an ecosystem, and it depends on the bioavailability of heavy metal and the absorbed dose (Deepa & Suresha, 2014; Hrynkiewicz & Baum, 2014; Okolo et al., 2016; Rasmussen et al., 2000; Siddiquee et al., 2015; Su, 2014).

Heavy metal toxicity involves several mechanisms, that is, breaking fatal enzymatic functions, reacting as redox catalysts in the production of reactive oxygen species (ROS), destructing ion regulation, and directly affecting the formation of DNA as well as protein (Gauthier et al., 2014). The physiological and biochemical properties of microorganisms can be altered by the presence of heavy metals. Different microbes have been proposed to be efficient and economical alternatives for the removal of heavy metals from soil and water (Ahirwar et al., 2016).

2.1. Pollution and diseases caused by heavy metals

The existing heavy metals in the environment and industrial wastewater increasingly pollute ecosystems and threaten human health in developing countries. Different concentrations of heavy metal elements commonly occur in all ecosystems. Several compounds have diverse properties, such as Zn, Cu, Ni, Fe, and Mn, are essential trace elements (Poli et al., 2009). The high-level accumulation of these metals or ingested in greater amounts than the required concentration can produce serious problems towards living things, including human beings. Alleviating heavy metal concentrations in water is vital to the quality of aquatic living organisms. In addition, heavy metals can cause severe toxic effects to expose plants, animals, and humans when exposed to excessive concentrations (Poli et al., 2009). The wastes containing metals is directly or indirectly being discharged into the producing serious environmental pollution and possess a major threat to human, soil, and sediment health (Siddiquee et al., 2011). Hussain et al. (Hussain et al., 2013) reported that heavy metals have the ability to accumulate in different parts of the human body. Heavy metal concentrations have the characteristic of having long biological half-lives as well as resistance to degradable processes and exhibit their chemical toxicity insoluble water. For this reason, heavy metals are considered a threat to humans and other organisms even though when present in low concentrations. When the concentration of heavy metals enters the human body through absorption, these metal ions can bind various biomolecules, such as proteins, nucleic acids, and interfere with their functions (Yu, 2001).

2.2. Contaminations of heavy metals in environments

Rapid developments in industrializations and urbanizations have led to the direct impact of the environment. The resultant degradation and contamination of the ecosystems have become a major threat to all living organisms worldwide, in particular, human beings. Globally, open
water and aquatic ecosystems are contaminated with several heavy metals through various human activities that indirectly or directly lead to these pollution (McEldowney et al., 1993). The most serious water pollution has occurred with some waterbodies, such as rivers, lakes, oceans, and groundwater. In addition, a high amount of materials can change the water properties and pollute the water, thus resulting in an unfit for intended uses. Water pollution can be classified into two distinct types that are point sources and nonpoint sources (McEldowney et al., 1993).

The point sources of pollution are single identifiable localized sources of water pollution. It can occur when harmful substances are emitted directly into the waterbodies. Examples of water pollution are the Exxon Valdez oil spills in 1989 that ran aground in the Prince William Sound, causing expelling of 11 million gallons of crude oil into the Alaska environment (Siddiquee et al., 2011). Non-point source pollution is affecting the waterbody from diffusion sources, such as polluted runoff from agricultural areas draining into the river and ocean waters. Besides, non-point source pollution is derived and comes from many different sources, which makes it difficult to find a specific solution to stop the pollution.

2.3. Major toxicity effects of heavy metals

2.3.1. Zinc (Zn)
Zinc is one of the major metals that can be found in effluents discharged from industries (Plum et al., 2010). Most of the industries are released zinc as their waste material, which includes electroplating, manufacture of batteries, galvanization, and metallurgical industries (Fosmire, 1990; Plum et al., 2010). Zn can play an important role in plant growth. The growth slows down when Zn is deficient in the environment. The functional group of Zn can increase the stabilization of the plants by altering its structure molecule and its membrane as well as act as a defensive mechanism against disease in plants. Basically, zinc in metallic form does not cause any harm to the environment and it has limited bioavailability. However, the presence of other chemicals, such as acids and oxygen can react with zinc to form a potentially toxic compound that can cause severe damage to biological systems (Fosmire, 1990).

2.3.2. Copper
Copper is recognized as another metal and contributes to heavy metal pollution or contamination. Copper (Cu) is mostly found deep inside the earth. It is a rare element that is found in nature in the uncombined state as well as in ores such as chalcopyrite (Igiri et al., 2018). According to Davies and Bennett (Davies & Bennett, 1983), copper is classed as the transition element in the periodic table and included among 25 elements that are found inside earth crusts that have with atomic numbers of 29 and atomic weights of 63.55. The pollution of Cu in the environment may have been caused by animal manure. After that, Cu is used as a supplement for the inhibition of parasites as well as in industrial factories, such as the point factory, and is disposed by electronic and electrical factories (Batey et al., 1972). The anthropogenic sources of a copper element are mostly found in pollution sites from landfills, mine sites, combustion of fossil fuels, and domestic wastewaters. It can be released by particles from volcanoes, dust, and forest fires into the atmosphere or dissolved compounds in water. Copper contamination can cause severe damage to the kidneys, liver, and even death when consumed at high concentrations. The accumulation of Cu in the organs and the toxicity inside bodies increased with a decrease in zinc element and sulfate ions (Lee, 2003) and also threaten the fish population. When the presence of heavy metals is highly found in the fish’s body, they can adversely alter the functional organ system (Shaw & Handy, 2011). Even though copper plays important activities in several enzymes for the production of hemoglobin, it has detrimental effects on some facts of the organisms. In living organisms, Cu is essential for living organisms as it acts as an antioxidant, participating in the electron transport chain as well as in collagen and elastin. This micronutrient is only required in certain amounts that mostly accumulate inside human tissues (Ahmadi-Vincu et al.).
2.3.3. Nickel
Apart from copper, nickel is known as a major environmental pollutant. It has possessed the potential of clastogenic, toxic, and carcinogenic effects. The different solubilities of nickel compounds have different carcinogenic potentials. Rani (Rani, 2017) stated that insoluble NiS₂ or NiO is a strong, while soluble nickel salts are present as weak carcinogen. Nickel is commonly an airborne contaminant in the form of nickel carbonyl Ni(CO)₄, an intermediate product of refining nickel activity, that enters the body through the respiratory system. It has a highly toxic volatile liquid that can cause death when inhaled and leads to several health problems such as pulmonary edema, pneumonia, and respiratory failure (Yu, 2001).

2.4. Heavy metal pollution cases
Accumulation of different types of heavy metals (e.g., Pb, Cd, Cu, Ni, Zn, and Mn) in the seawater not only contaminated the water but also occurred in the soil. It affects the sources of drinking and building up the dangerous concentration of heavy metals in grains and vegetables (Siddiquee et al., 2015). There are several cases involved with heavy metal contaminants that occurred in 1963 in Minamata Bay, Japan. Its tragedy is related to local people who consumed shellfish that contained a high amount of mercury concentrations near Minamata Bay (Siddiquee et al., 2015). The exposure of these diseases occurred due to the chemical substances were released and discharged without a controller by a chemical factory that operates near to bay (Blackmore & Reddish, 1996). A high amount of mercury concentration is discharged into the sea as wastewater and affects the marine food chains such as shellfish and other seafood, which can build a high concentration of mercury than become poisonous to the locals who are consumed (Siddiquee et al., 2015).

Heavy metal concentrations in the environment are increased continuously which can cause food chains in the environment and become a major human health hazard (Siddiquee et al., 2015). Heavy metals are one of the most serious environmental pollutants and can be derived from both direct sources, such as industrial effluents, sludge dumping, and indirectly through highway runoffs. As a result of these problems, the great interest in metal–microbe interactions has arisen by the researcher and also industrialists to find suitable methods for solving as, recovering, and stabilizing of the heavy metals in seawaters, soil, and effluents (Sani et al., 2001).

2.5. Conventional methods for removing heavy metal contamination
Numerous clean-up techniques have been suggested and practiced for the removal of heavy metals from contaminated or polluted areas using chemical, physical, and biological methods (Siddiquee et al., 2015). There are several conventional technologies, such as precipitation, ion-exchange, electrolytic technologies, chemical extraction, leaching, hydrolysis, polymer microencapsulation, and the most commonly practiced excavation and landfilling (Qazilbash, 2004). All of these chemical methods posed a serious health and ecological threat due to their toxicity and mutagenicity. Vapor extraction, stabilization, solidification, verifications, and membrane technology have been previously used to remove the heavy metal ions from the pollutant areas (Qazilbash, 2004). However, most of these techniques are very expensive for implementation large scale and also dangerous for constant monitoring and control because sometimes they cannot completely remove the heavy metals contaminated (Siddiquee et al., 2015).

2.5.1. Disadvantages of conventional methods
The high cost of technologies has always been used for entirely changing their manufacturing processes, or most of the industrialists do not implement clean-up technologies or replace their old systems with cleaner, safer, and environmentally friendly machinery (Siddiquee et al., 2015). The use of conventional chemicals for treating heavy metal pollution can be economically feasible, especially when dealing with low metal ion concentrations.

Yazdani et al. (2010) reported that conventional methods are expensive, especially for handling large amounts of water, and wastewater contains heavy metals at low concentrations. The need
for several innovative treatment technologies for the removal of heavy metal ions from wastewater is required. Khan et al. (1997) suggested that there are possibilities of employing the technology using biological treatments or bioremediation techniques as alternative methods for removing heavy metal ions from contaminated soils or waters.

2.5.2. Toxicity of heavy metals to microorganisms
The toxicity of heavy metals is the ability of a metal to cause detrimental effects on microorganisms, and it depends on the bioavailability of heavy metals and the absorbed dose (Rasmussen et al., 2000). Heavy metal toxicity involves several mechanisms, such as, breaking fatal enzymatic functions, react as redox catalysts in the production of reactive oxygen species (ROS), destructing ion regulation, and directly affecting the formation of DNA as well as protein (Gauthier et al., 2014). The physiological and biochemical properties of microorganisms can be altered by the presence of heavy metals. Cr and Cd are capable of inducing oxidative damage and denaturation of microorganisms as well as weakening the bioremediation capacity of microbes. Cr (III) may change the structure and activity of enzymes by reacting with their carboxyl and thiol groups (Cervantes et al., 2001). Intracellular cationic Cr (III) complexes interact electrostatically with negatively charged phosphate groups of DNA, which could affect transcription, replication, and cause mutagenesis (Cervantes et al., 2001). Heavy metals like Cu (I) and Cu (II) can catalyze the production of ROS via Fenton and Haber–Weiss reactions, which from act as soluble electron carriers. This can cause severe injury to cytoplasmic molecules, DNA, lipids, and other proteins (Zhao et al., 2016). Aluminum (Al) can stabilize superoxide radicals, which are responsible for DNA damage (Booth et al., 2015). Heavy metals can stop vital enzymatic functions by competitive or non-competitive interactions with substrates, which will cause configurational changes in enzymes (Gauthier et al., 2014). Furthermore, it can also cause ion imbalance by adhering to the cell surface and entering through ion channels or transmembrane carriers (Chen et al., 2014). Cd and Pb pose deleterious effects on microbes, damage cell membranes, and destroy the structure of DNA. This harmfulness is generated by the displacement of metals from their native binding sites or ligand interactions (Olaniran et al., 2013). The morphology, metabolism, and growth of microbes are affected by changes in nucleic acid structure, causing a functional disturbance, disrupting cell membranes, inhibiting enzyme activity, and oxidative phosphorylation (Fashola et al., 2016).

2.5.3. Sources of heavy metal in the environment
Heavy metals occur naturally in the environment from the pedogenetic processes of weathering of parent materials and also through anthropogenic sources. There are different sources of heavy metals in the environment, such as natural, agricultural, industrial solid waste, inland effluent, atmospheric sources, and more sources. Activeness such as mining activities, electroplating, metallurgical smelting actions, and the use of agriculture pesticides and phosphate fertilizers discharge, as well biosolids (e.g., livestock manures, composts, and municipal sewage sludge), atmospheric deposition have contaminated wide areas of the world (Fuller et al., 2003; Zhang et al., 2011) in Table 1. The disturbance of nature’s slowly occurring geochemical cycle of metals by man results in accumulation of one or more heavy metals in the soil, waters, and above defined levels, this is enough to cause risk to human health, plants, animals, and aquatic biota (Doelsch et al., 2008), with heavy metals essentially become contaminants in the soil and water environment because of their excess generation by natural and man-made activities, transfer from mines to other locations where higher exposure to humans occurs, discharge of high concentration of metal waste through industries and greater bioavailability.

2.6. Bioremediation
Bioremediation is a technique that offers the possibility to destroy or render harmless various contaminants using natural biological activity in the ecosystem (Siddiquee et al., 2015). It is followed by bacteria, fungi, or plants to degrade, or detoxify hazardous ingredients to human health (Qazilbash, 2004) defines bioremediation as a process by which organic or inorganic waste biologically degraded or transformed usually to innocuous materials. The process can function naturally or can be enhanced by adding an electron acceptor, nutrient, or other factors. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site (Su, 2014).
Table 1. Sources of heavy metals resulting from anthropogenic activities

| Heavy metals | Anthropogenic activities |
|--------------|--------------------------|
| Chromium (Cr) | Mining, industrial coolants, chromium salts manufacturing, leather tanning |
| Lead (Pb)    | Lead-acid batteries, paints, E-waste, Smelting operations, cool-based thermal power plants, ceramics, bangle industry |
| Mercury (Hg) | Chlor-alkali plants, thermal power plants, fluorescent lamps, hospital waste (damaged thermometers, barometers, sphygmomanometers), |
| Arsenic (As) | Geogenic/natural processes, smelting operations, thermal power plants, fuel-burning |
| Copper (Cu)  | Mining, electroplating, smelting operations |
| Cadmium (Cd) | Zinc smelting, waste batteries, e-waste, paint sludge, incinerations, and fuel combustion |
| Molybdenum (Mo) | Spent catalyst |
| Zinc (Zn) | Smelting, electroplating |

For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them into harmless products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate (Su, 2014).

The microorganisms may be isolated from an indigenous contaminated area or elsewhere and applied to the contaminated site. Contaminant materials are transformed by living organisms through reactions that take place as part of their metabolic processes (Siddiquee et al., 2015). The principles of bioremediation can be divided into several techniques, including biofilters, bioventing, biosorption, composting, bioaugmentation, bioreactor, land farming, and biostimulation (Qazilbash, 2004). Khan et al. (1997) pointed out that the control and optimization of bioremediation processes are complex factors. These factors include the presence of a microbial population proficient in degrading pollutants, the availability of contaminants to the microbial population, and environmental factors as like as soil type, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients. Bioremediation is a unique method for cleaning polluted environments from the atmosphere (industrial emissions and soil vent gases), solids (soils, sediments, and also sludge), liquids (groundwater, industrial effluents), raw materials from industrial processing, and living, or non-living microorganisms can use their enzymes to accomplish this task (Qazilbash, 2004).

Several researchers have reported that some potential microbes are able to tolerate heavy metals either they able to remove them from the environment or break them down to less toxic or completely benign forms then utilize in their metabolic processes for growth (Qazilbash, 2004).

Microbial resistance and tolerance toward pollutants, particularly heavy metals, are absolutely vital in the bioremediation processes as required microorganisms such as bacteria, fungi Consortium Organisms, and algae—LRB – Table 2.

2.7. Bioremediation mechanisms of heavy metal-contaminated environment

2.7.1. Biosorption
Biosorption is the group of all processes, during which alive or dead biomass removes heavy metals or other pollutants from solutions (Kisielowska et al., 2010). Biosorption occurring with the participation of microorganisms may be conducted by surface adsorption concerning the gathering of metals on the cell surface and linking them with extracellular polymers. The other method relies
### Table 2. Remediation of heavy metals by microorganisms

| Microbial group | Bioremediation | Metals | Metal ion concentration (mg/L) | Sorption efficiency (%) | Reference |
|-----------------|----------------|--------|-------------------------------|-------------------------|-----------|
| Acinetobacter sp. | Cr             | 16     | 87                           | (Bhattacharya et al., 2014) |
| Sporosarcina saramensis (M52) | Cr | 50     | 82.5                         | (Ran et al., 2016)     |
| Bacillus cereus | Cr             | 1500   | 81                           | (Nayak et al., 2018)   |
| Bacillus circulans MN1 | Cr | 1500   | 96                           | (Chaturvedi, 2011)     |
| Bacteria | Bacillus cereus | Cr | 1 | 78 | (Singh et al., 2013) |
| Bacteria | Bacillus subtilis | Cr | 0.57 | 99.6 | (Kim et al., 2015) |
| Bacillus sp. B | Cr      | 50-37.06 | 47 | (Kumar et al., 2011) |
| Staphylococcus | Cr     | 4.108  | 45                           |                         |
| Pseudomonas aeruginosa (PCP2) | Cr | 6.4     | 72                           |                         |
| Pseudomonas aeruginosa (P) | Cr | 570-2   | 99.6                         | (Benazir et al., 2010) |
| Immobilized B.subtilis (B bead) | Cr | 570-2   | 99.3                         |                         |
| Bacteria | Cellulosimicrobiurn sp.(KX710177) | Pb | 50 | 99.3 | (Bharagava & Mishra, 2018) |
| Methylobacterium organophilum | Pb | 100 | 96.98 |                        |
| Bacillus firmus | Pb | 200 | 84.62 |                        |
| Staphylococcus sp. | Pb | 300 | 62.28 |                        |
| Streptomyces sp. | Pb | .183 | 82.6                         | (Kumar et al., 2011) |
|               |               | 0.286  | 32.5                         |                         |

(Continued)
| Microbial group | Bioremediation | Metals | Metal ion concentration (mg/L) | Sorption efficiency (%) | Reference |
|-----------------|----------------|--------|-------------------------------|-------------------------|-----------|
|                 | **Desulfovibrio desulfuricans (KCTC5768) (immobilize on zeolite)** | Cu Ni | 50 | 97.4 | (Congeevaram et al., 2007) |
|                 |                 |       | 100 | 98.2 |                     |
|                 |                 |       | 200 | 78.2 |                     |
| **Bacteria**    | **Desulfovibrio desulfuricans (immobilize on zeolite)** | Ni | 100 | 98.2 | (Kim et al., 2015) |
|                 | **Flavobacterium sp.** | Ni | 0.161 | 25 | (Kumar et al., 2011) |
|                 | **Bacillus sfirmus** | Cu | - | 74.9 | (Kim et al., 2015) |
|                 | **Desulfovibrio desulfuricans (immobilize on zeolite)** | Cu | 50 | 90.3 | (Kumar et al., 2011) |
|                 |                 |       | 100 | 90.1 |                     |
|                 |                 |       | 200 | 90.1 |                     |
| **Bacteria**    | **Micrococcus sp** | Ni | 50 | 55 | (Congeevaram et al., 2007) |
|                 | **Pseudomonas sp** | Ni | 1 | 53 | (Kumaran et al., 2011) |
|                 | **Acinetobacter sp. 89** | Ni | 50 | 68.94 | (Bhattacharya & Gupta, 2013) |
| **Bacteria**    | **Vibrio fluvialis** | Co | 100 | 8 | (Jafari et al., 2015) |
|                 | **Enterobacter cloacae** | Hg | 100 | 28.65 | (Al-Garni et al., 2010) |
|                 | **Klebsiella pneumoniae** | Hg | 150 | 29.83 | (Jafari et al., 2015) |
|                 | **Pseudomonas aeruginosa** | Hg | 5 | 90 |                     |
|                 | **Vibrio parahaemolyticus (PG02)** | Hg | 10 | 80 |                     |
|                 | **Bacillus licheniformis** | Hg | 0.1 | 70 | (Muneer et al., 2013) |
| **Bacteria**    | **Bacillus firmus** | Zn | - | 61.8 | (Salehizadeh & Shojasadati, 2003) |
| **Bacteria**    | **Pseudomonas sp.** | Zn | 1 | 49.8 | (Kumaran et al., 2011) |

(Continued)
| Microbial group | Bioremediation | Reference |
|----------------|----------------|-----------|
| **Consortium Organisms** | | |
| **Acinetobacter sp. & Arthrobacter sp.** | | |
| **Serratia marcescens & Enterobacter aerogenes (Y + P)** | | |
| **S. cerevisiae & P. aeruginosa (Y + P)** | | |
| **Fungi** | | |
| **Aspergillus sp.** | | |
| **Saccharomyces cerevisiae** | | |
| **Candida parapsilosis** | | |
| **Alga** | | |
| **Spirogyra sp.** | | |
| **Nostoc sp.** | | |
| **Metal ion concentration (mg/L)** | | |
| **C** | 16 | 16 | (De et al., 2008) |
| **Pb** | 570–2 | 570-16 | (Benesi et al., 2010) |
| **Cu** | 540–4 | 540-4 | (Benevolor et al., 2007) |
| **Hg** | 0.1 | 0.1 | (Benedict et al., 2013) |
| **Cd** | 0.1 | 0.1 | (Tajshin et al., 2010) |
| **Ni** | 0.1 | 0.1 | (Benedict et al., 2013) |
| **Pb** | 5 | 5 | (Benevolor et al., 2010) |
| **Cu** | 5 | 5 | (Benevolor et al., 2010) |
| **Hg** | 5 | 5 | (Benevolor et al., 2010) |
| **Cd** | 5 | 5 | (Benevolor et al., 2010) |
| **Sorption efficiency (%)** | | |
| **Cr** | 78 | 99.6 | (De et al., 2008) |
| **Cr** | 99.2 | 99.2 | (Benesi et al., 2010) |
| **Cr** | 97.2 | 97.2 | (Benevolor et al., 2007) |
| **Cr** | 95 | 95 | (Benedict et al., 2013) |
| **Cr** | 90 | 90 | (Tajshin et al., 2010) |
| **Cr** | 90 | 90 | (Benedict et al., 2013) |
| **Cr** | 80 | 80 | (Benedict et al., 2013) |
| **Cr** | 80 | 80 | (Tajshin et al., 2010) |
| **Cr** | 80 | 80 | (Benedict et al., 2013) |
| **Cr** | 80 | 80 | (Tajshin et al., 2010) |
| **Cr** | 80 | 80 | (Benedict et al., 2013) |
| **Cr** | 80 | 80 | (Tajshin et al., 2010) |
on metal infiltration to the middle of the cell (this term is close by meaning to intracellular accumulation). It is often when biosorption occurs as the first phase of the following intracellular accumulation and the process of surface adsorption occurring very fast – during several minutes may have a dominant role in metal linking or may lead to high metal accumulation in the middle of the cell in a longer time (Kisielowska et al., 2010).

The sorption properties of microorganisms are the result of the outer cell shield. Metals are linked by active groups of compounds occurring in the surface layers of cells. Most often, this is the reaction of ion transfer between metal cations and active groups gifted with the negative potential of outer cell structures and the microorganisms belonging to various systematic units feature by the presence of various chemical groups in outer structures, active in metal linking (Kręgiel et al., 2008).

The practical application of biosorption to the removal or the recovery of heavy metals is mainly the result of the reversibility of this process. Desorption allows the recovery of metals (which is profitable in the case of more valuable heavy metals like gold, copper, and zinc) or their removal. During the desorption process of metals linked by microorganisms, solutions of weak mineral acid solutions (like 0,1 M HCl) or chelating compounds (like 10 mM EDTA) are applied (Skłodowska, 2000). In the pH of range 5–7, metal ions like Cu$$^{2+}$$, Cr$$^{3+}$$, Ni$$^{2+}$$, Pb$$^{2+}$$, Zn$$^{2+}$$, Cd$$^{2+}$$, and Ca$$^{2+}$$ are strongly linked to the microbial biomass. Lowering the pH to the value of 2 causes the liberation of metals from biosorbents. However, metal ions like Au$$^{3+}$$ and Ag$$^{+}$$ stay at this pH in biosorbents (Kisielowska et al., 2010). The biosorbents can be (Klimiuk & Lebkowska, 2003)

- Biomass of microorganisms is the secondary product in the sewage or pharmaceutical industry and in sewage treatment processes;
- Microorganisms from cultured and proliferated on a special base indicating the ability to efficiently metals;
- Sorbents of vegetable or animal origin (as nutshells, crust-rich tannins, sea plants, humus, moss peat, etc.)

2.7.2. Bioaccumulation
Bioaccumulation takes place when the absorption rate of the contaminant is higher than the rate of losing it. Thus, the contaminant remains contained and accumulates inside the organism (Chojnacka, 2010). Bioaccumulation is a toxicokinetic process that affects the sensitivity of living organisms to chemicals. Organisms can normally resist concentrations of chemicals up to certain levels, beyond which these chemicals become toxic and endanger the organism. The sensitivity of organisms to chemicals is highly variable depending on the types of organisms and chemicals involved (Mishra & Malik, 2013).

Bioaccumulation candidate organisms should have a tolerance ranging between one or more contaminants to higher levels. Furthermore, they may demonstrate superior biotransformational capabilities, changing the toxic chemical to a nontoxic form that enables the organism to reduce the toxicity of the contaminant while keeping it contained (Mishra & Malik, 2013). The bioaccumulation of metals may be practical and economically beneficial if they lead to a high concentration of metals. This process is compared to biosorption, features by the fact that metal removal from cells and its recovery is connected with the necessity of cellular structure transformation. This results in a lack of possibility of biomass applications in several cycles (Skłodowska, 2000). Many environmental bacteria species feature is the phenomenon of gathering large amounts of metals in cells, the cell wall itself, or in places bounded from the cytoplasm.

The amount of this deposit may be even 6% of the dry cell mass and, taking into account in the soil or water environment, this phenomenon may lead to the temporary lowering of heavy metal ion concentrations in the environment. It allows living for other organisms to be as of ecocommunion, including humans (Kisielowska et al., 2010).
2.7.3. Biotransformation

Microbiological transformations of heavy metals are reactions of oxidation, reduction, methylation, and demethylation. The enzymatic systems of microorganisms take part in reactions. Practically useful may be reactions of significantly toxic or valuable metal reduction, like bacteria Gram-positive isolated from tannery sewers, caused the reduction of highly toxic chromium (VI) to less toxic chromium (III), which may be removed from the environment (Kisielowska et al., 2010). Any bacteria, microscopic fungi, may conduct a reduction of metal ions (particularly valuable as gold or silver) to metallic form. This reaction may occur in vacuoles, on the cell surface, and in the extracellular environment, which is important from the point of view of this metal recovery (Kisielowska et al., 2010; Sklodowska, 2000).

2.7.4. Bioprecipitation and biocrystallization

As a result of microorganism’s activity, the precipitation or crystallization of heavy metal compounds may occur, which causes the transformation of metal into form sparingly, which lowers their toxicity at the same time. Some precipitation and biocrystallization processes take part in biogeochemical cycles, like forming microfossils, depositing of iron and manganese, and mineralization of silver and manganese. Precipitation of metals on the surface or inside of the cell may be the result of not only the direct activity of enzymes but also the result of the galactosis of secondary metabolites (Sklodowska, 2000).

2.7.5. Bioleaching of metals

Bioleaching based on the application of microorganisms, bacteria, and fungi their metabolism products to transfer the metal contained in mineral to solution in relation to sulfide materials has become a known industrial technology. The basis of this process is based on the transformation of compounds of metals present in the environment in the form of sparingly soluble substances (most often sulfides) into forms easily soluble, where removal of metals is an easy task (Kisielowska et al., 2010). The ability of fungi to bioleach, mobilization of metals from indigent ores, and industrial waste is connected mainly with two processes: the creation of various organic acids in the living environment (citric acid, gluconic acid, oxalic acid) and secretion of complexion agents. For such fungi, the following types may be included: Aspergillus sp., Penicillium sp., Rhizopus sp., Mucor sp., Alternaria sp., and Cladosporium sp. in metal leaching because of their biochemical abilities and relatively high resistance to bad factors such as pH and temperature. It is used mainly when it is not possible to apply classical methods of chemical or bacterial leaching with an application of A. ferrooxidans and A. thiooxidans (Blaszczyk, 2007).

Biological methods of leaching are mainly used in biohydrometallurgy. Microbiological processes may be applied to metal leaching from sulfide and oxide minerals. There is a possibility to recover such metal arsine, antimony, bismuth, zinc, cobalt, gold, lead, copper, molybdenum, nickel, vanadium, and uranium, thanks to biohydrometallurgical methods. For now, the industrial application of these methods in the world is limited mainly to copper, gold, and uranium leaching (Blaszczyk, 2007).

2.7.6. Factors affecting microbial remediation of heavy metals

The propensity of heavy metals to be stimulatory or inhibitory to microorganisms is determined by the total metal ion concentrations, chemical forms of the metals, and related factors such as redox potential. Environmental factors like temperature, pH, low molecular weight organic acids, and humic acids can alter the transformation, transportation, valence state of heavy metals, and the bioavailability of heavy metals towards microorganisms (Rasmussen et al., 2000).

Heavy metals tend to form free ionic species at acidic pH levels, with more protons available to saturate metal-binding sites. At higher hydrogen ion concentrations, the adsorbent surface is more positively charged, hence reducing the attraction between adsorbent and metal cations, thereby increasing its toxicity. Temperature plays a significant role in the adsorption of heavy metals. An increase in temperature increases the rate of adsorbate diffusion across the external boundary layer (Bandowe et al., 2014). The solubility of heavy metals increases with an increase in temperature, which improves the bioavailability of heavy metals (Bandowe et al., 2014). However, the
actions of microorganisms increase with a rise in temperature within a suitable range, and it enhances microbial metabolism and enzyme activity, which will accelerate bioremediation. The stability of the microbes-metal complex depends on the sorption sites, microbial cell wall configuration, and ionization of chemical moieties on the cell wall. The outcome of the degradation process depends on the substrate and the range of environmental factors.

3. Bioremediation capacity of microorganisms on heavy metals
The uptake of heavy metals by microorganisms occurs via bioaccumulation which is an active process and/or through adsorption, which is a passive process. Several microorganisms like bacteria, fungi, and algae have been used to clean up heavy metal-contaminated environments with the application of metal-resistant strains in single, consortium, and immobilized forms for the remediation of heavy metals have yielded effective results, while the immobilized form could have more chemisorption sites to adsorb heavy metals. Remediation of heavy metals by microorganisms (Kim et al., 2015).

3.1. Bacterial remediation capacity of heavy metal
Microbial biomass has different biosorptive abilities, which also vary significantly among microbes. However, the biosorption ability of each microbial cell depends on its pretreatment and experimental conditions. Microbial cells must adapt to the alteration of physical, chemical, and bioreactor configuration to enhance biosorption (Ayangbenro & Babalola, 2017). Bacteria are important biosorbents due to their ubiquity, size, and ability to grow under controlled conditions and resilience to environmental conditions (Srivastava et al., 2015).

Several heavy metals have been tested using bacterial species like Flavobacterium, Pseudomonas, Enterobacter, Bacillus, and Micrococcus sp. Their great biosorption ability is due to their high surface-to-volume ratios and potential active chemisorption sites (teichoic acid) on the cell wall (Mosa et al., 2016). Bacteria are more stable and survive better when they are in mixed cultures (Sannosi et al., 2006). Therefore, consortia of cultures are metabolically superior for the biosorption of metals and are more appropriate for field applications (Kader et al., 2007). De et al. (2008) reported a 78% reduction of chromium (Cr) using a bacterial consortium of Acinetobacter sp. and Arthrobacter sp. at 16 mg/L metal ion concentration. Micrococcus luteus was used to remove a large quantity of Pb from a synthetic medium. Under ideal environments, the elimination ability was 1965 mg/g (Puyen et al., 2012).

Abioge and his coworkers (Abioge et al., 2018) investigated the biosorption of Pb, Cr, and Cd in tannery effluent using Bacillus subtilis, B. megaterium, Aspergillus niger, and Penicillium sp. B. megaterium recorded the highest Pb reduction (2.13 to 0.03 mg/L), followed by B. subtilis (2.13–0.04 mg/L). A. niger showed the highest ability to reduce the concentration of Cr (1.38–0.08 mg/L) followed by Penicillium sp. (1.38–0.13 mg/L), while B. subtilis exhibited the highest ability to reduce the concentration of Cd (0.4–0.03 mg/L) followed by B. megaterium (0.04–0.06 mg/L) after 20 days. Kim and his coauthors (Kim et al., 2015) designed a batch system using zeolite-immobilized Desulfovibrio desulfuricans for the removal of CrVI, Cu, and Ni with removal efficiencies of 99.8%, 98.2%, and 90.1%, respectively. Abbas et al. (2014) reported efficient removal of chromium, zinc, cadmium, lead, copper, and cobalt by bacterial consortia at approximately 75% to 85% in less than 2 h of contact duration.

3.2. Fungi remediation capacity of heavy metal
Fungi are widely used as biosorbents for the removal of toxic metals with excellent capacities for metal uptake and recovery (Fu et al., 2012). Most studies showed that active and lifeless fungal cells play a significant role in the adhesion of inorganic chemicals (Tiwari et al., 2013). Srivastava and Thakur (2006) also reported the efficiency of Aspergillus sp. used for the removal of chromium in tannery wastewater. Eighty-five percent of chromium was removed at pH 6 in a bioreactor system from the synthetic medium, compared to 65% removal from the tannery effluent. This could be due to the presence of organic pollutants that hinder the growth of the organism. Coprinopsis atramentaria was studied for its ability to bioaccumulate 76% of Cd²⁺ at
a concentration of 1 mg/L of Cd²⁺, and 94.7% of Pb²⁺, at a concentration of 800 mg/L of Pb²⁺. Therefore, it has been documented as an effective accumulator of heavy metal ions for mycoremediation (Lakkireddy & Kües, 2017). Park and his coauthors (Park et al., 2005) reported that dead fungal biomass of A. niger, Rhizopus oryzae, Saccharomyces cerevisiae, and Penicillium chrysogenum could be used to convert toxic Cr (VI) to less toxic or nontoxic Cr (III). Luna et al. (2016) also observed that Candida sphaerica produce biosurfactants with removal efficiencies of 95%, 90%, and 79% for Fe, Zn, and Pb, respectively.

Biosurfactants have gained interest in recent years owing to their low toxicity, biodegradability, nature, and diversity. Mulligan et al. (2001) assessed the viability of using surfactin, rhamnolipid, and sophorolipid for the removal of heavy metals (Cu and Zn). A single wash with 0.5% rhamnolipid removed 65% of Cu and 18% of Zn, whereas 4% sophorolipid removed 25% of the Cu and 60% of Zn. Several strains of yeast such as Hansenula polymorpha, S. cerevisiae, Yarrowia lipolytica, Rhodotorula pilimanae, Pichia guilliermondii, and Rhodotorula mucilagare have been used to bioconvert Cr (VI) to Cr (III) (Chatterjee et al., 2012).

3.3. Heavy metal removal using biofilm

There are several reports on the application of biofilms for the removal of heavy metals. Biofilm acts as a proficient bioremediation tool as well as biological stabilization agent. Biofilms have a very high tolerance against toxic inorganic elements even at lethal concentrations that are lethal. It was revealed in a study conducted on Rhodotorula mucilaginosa that metal removal efficiency was from 4.79% to 10.25% for planktonic cells and from 91.71% to 95.39% for biofilm cells (Goher et al., 2016). Biofilms mechanisms of bioremediation could either be via biosorbent or by exopolymeric substances present in biofilms that contain molecules with surfactant or emulsifier properties (El-Masry et al., 2004).

3.4. Algae remediation capacity of heavy metal

Algae are autotrophic and hence require low nutrients and produce enormous biomass compared to other microbial biosorberts. These biosorbents have also been used for heavy metal removal with a high sorption capacity (Abbas et al., 2014). Algae biomass is used for bioremediation of heavy metal-polluted effluent via adsorption or by integration into cells. Phycoremediation is the use of various types of algae and cyanobacteria for the remediation of heavy metals by either removal or degradation of toxicants (Chabukdhara et al., 2017). Algae have various chemical moieties on their surface, such as hydroxyl, carboxyl, phosphate, and amide, which act as metal-binding sites (Abbas et al., 2014). Russian and Napiórkowska-Krzebietke et al. (Abbas et al., 2014) used dead cells of chlorella vulgaris to remove Cd²⁺, Cu²⁺, and Pb²⁺ ions from aqueous solutions under various conditions of pH, biosorbent dosage, and contact time. These results suggest that the biomass of C. vulgaris is an extremely efficient biosorbent for the removal of Cd²⁺, Cu²⁺, and Pb²⁺ at 95.5%, 97.7%, and 99.4%, respectively, from a mixed solution of a 50 mg/dm⁻³ of each metal ion could be considered as the agents in the bioremediation process.

4. Final considerations

This review revealed that the interest in processes involving heavy metal uptake by microorganisms has increased considerably in recent years due to the biotechnological potential of microorganisms in removing and/or recovering metals. Conventional methods, such as synthetic ion exchangers are considered as mature technologies. Biosorption is still in infant stages, and additional improvements in both performance and costs can be expected. Besides the contributions of the various biosorbents, those are potentially effective and readily available for heavy metal removal. These biosorbents present attractive opportunities as a low-cost means of protecting the environment from pollution. In the future in the area researchers should explore novel species that have great potential. The speed of unwanted waste substance degradation is determined in competition with biological agents, inadequate supply of essential nutrients, uncomfortable external abiotic conditions (aeration, moisture, pH, temperature), and low bioavailability of the pollutant. Bioremediation can be effective
only when environmental conditions permit microbial growth and activity, and a very safe and obli- ging technology because it depends on microbes that occur naturally in the soil and pose no hazard to the environment and the people living in that area. Even though numerous sources of bio- remediation, for instance, bacteria, archaeabateria, yeasts, fungi, algae, and plants are accessible, but, biological treatment alone is not adequate enough to treat the pollutants or contaminated sites. A comprehensive study of an area-wise and pollutant-type database is much desirable to finalize the priority area and the need for the operative elimination of contaminants from contaminated sites. As regular resources are major assets to humans their adulteration resulted in long-term effects of pollution (noise and radiation), global warming, ozone depletion, and greenhouse gases. The sanitization of these natural resources is important for the preservation of nature and the environment using a bioremediation process.

Bacteria are one of the most vital microbial candidates that need to be widely explored for bioremediation ability with however, a few studies have been carried out in the said area, more inclusive and complete studies need to be conceded out for extracting the best out of bacterial systems as “heavy-metal contamination alleviators”. However, more studies are needed regarding the exact and clear mechanisms involved in the removal of heavy metals by bacteria, fungi, and algae.

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