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REVIEW ON SOURCES AND EFFECT OF HEAVY METAL IN SOIL: ITS BIOREMEDIATION

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ABSTRACT

Heavy Metal (HM) contamination issues are becoming increasingly common in India and elsewhere, Heavy metals are natural constituents of the environment, but indiscriminate use for human purposes like agriculture, industrial, foundries, mining, smelters, coal-burning power plants and metallurgical has changed their atmospheric geochemical cycles and biochemical balance. This results in excess release of heavy metals such as chromium, mercury, lead, cadmium, copper, iron, zinc, nickel, etc. are major environmental pollutants, particularly in areas with higher anthropogenic activity. The exposure of humans to heavy metals can occur through a variety of routes, which include inhalation as dust or fume, vapourization, and ingestion through food and drink. Prolonged exposure and higher accumulation of such heavy metals can have deleterious health effects on human life, soil, air and aquatic biota. The role of plants and microorganisms in the biotransformation of heavy metals into nontoxic forms is well-documented, and understanding the molecular mechanism of metal accumulation has numerous biotechnological implications for bioremediation of metal-contaminated sites. The process of bioremediation uses various agents such as bacteria, yeast, fungi, algae and higher plants as major tools in treating oil spills and heavy metals present in the environment In view of this, the present review article details the range of heavy metals, there occurrences and toxicity investigates the abilities of microorganisms and plants in terms of tolerance and degradation of heavy metals. An assessment of the current status of technology deployment and suggestions for future bioremediation techniques and research has also been included. Finally, there is a discussion of the molecular basis of metal tolerance in plants and microbes, with special reference to the genomics of heavy metal accumulator plants and the identification of functional genes involved in tolerance and detoxification.

KEYWORDS: ***B****ioremediation,* ***B****iodegradation, Phytoremediation, Heavy metals, Toxicity*

INTRODUCTION

The soil is a very essential component for all the living organisms. Especially for plants, it's considered as the basic living factor. Soil serves as a nutrient media for the growth of plants. The soil is not essential for agriculture production but also towards maintained all life form. The quality of water and air is of immediate concern for most people because we all consume these natural resources on a daily basis. The importance of soil, the generally thin layer of unconsolidated material on bedrock, is more difficult to grasp for an average citizen or politician. Nonetheless, the soil is the "the biogeochemical engine of Earth's life support system". It provides us with food, fodder, fiber, and fuel. In addition to these readily rateable agriculture and forestry goods, soils deliver ecosystem services that cannot be easily traded in markets. These life-supporting functions include, for example, recycling of carbon and essential nutrients of all living materials, filtering, and storage of water, regulation of the atmosphere and biological control of pests.

Heavy metal appears to include all metals of the periodic table with atomic numbers greater than 20, generally excluding the alkali metal and the alkali earth. Heavy metals are metallic, naturally occurring compounds that have a very high density greater than $5g/cm^3$; compared to other metals at least five times the density of water. They are one of the most persistent pollutants in soil and water. Heavy metals can be divided into two categories: essential and non-essential on the basis of their role in living systems. Essential heavy metals such as Mn, Fe, Ni, Zn are needed by living organisms for their growth, development and physiological functions, while non-essential heavy metals such as Cd, Pb, Hg and As are not needed by living organisms for any physiological function (Gohre *et al.*,2006).

Abundant amounts of heavy metals present in soils cause the reduction in quality and quantity of food preventing plants growth, uptake of nutrients, metabolic and physiological processes. Heavy metals are toxic to humans. Even small doses can have serious consequences. Severe effects on animals may include reduced growth and development, cancer, organ damage, nervous system damage, and in extreme cases, death. To help mitigate the negative impacts of heavy metals on the health of humans, animals, and the environment, a variety of remediation processes exists.

However, anthropogenic activities such as mining have resulted in elevated levels of these contaminants in the environment. By definition, any toxic metal may be called a heavy metal, irrespective of its atomic mass or density. The classification includes some metalloids, transition metals, basic metals, lanthanides and actinides and metals of groups III to V of the periodic table Examples include As, Pb, Hg, Cd, Cr, Co, Ni, Cu, Zn, Se, Al, Cs, Mn, Mo, Sr, U, Be and Bi (Cairney et al., 1993).

In our previous studies, we have reported adverse effects of industrial pollution on the soil. It can be concluded that industrial pollution generally increases the heavy metal content of the soil. An assessment of the environmental risk due to soil pollution especially heavy metals is of particular importance for agricultural and non-agricultural areas. Because heavy metals, which are potentially harmful to plants, soil microorganisms and human health persist in soils for a very long time. When the heavy metals present in the natural condition they do not act as toxic up to certain extent. When the concentration reaches the maximum level or up to the final permissible level heavy metals will be converted in to toxic in nature and it will lead to the dangerous effects on the surrounding system (Ahirwar *et al.*, 2018). Some metals are essential to life and play irreplaceable roles as sources of vitamins, and minerals in the functioning of body organs. All living organisms require varying amounts of metals but become toxic at higher concentrations (NRC, 1993). Other metals have no useful role in the human physiology. Examples of such elements are arsenic, lead, and mercury. They may be toxic even at low levels of exposure. Once absorbed by the body, heavy metals continue to accumulate in vital organs like the brain, liver, bones, and kidneys, for years or decades causing serious health consequences (Norris *et al*., 1993).

These remediation processes are broadly classified into chemical and biological, although the latter is advocated in recent years. Biological remediation processes (microbial remediation and phytoremediation) are indicated to be very effective in the treatment of heavy metal pollutants in soil and wastewater. Microbial remediation is the restoration of the environment and its quality using microorganisms, such as bacteria, fungi, protozoan, and algae while phytoremediation is the use of plants to degrade or accumulate toxic metals, thereby leading to a reduction in the bioavailability of the contaminant in the soil or water.

The conventional techniques used for remediation have been to dig up contaminated soil and remove it to a landfill, or to cap and contain the contaminated areas of a site. The methods have some drawbacks. The first method simply moves the contamination elsewhere and may create significant risks in the excavation, handling, and transport of hazardous material. Additionally, it is very difficult and increasingly expensive to find new landfill sites for the final disposal of the material. The cap and contain method is only an interim solution since the contamination remains on site, requiring monitoring and maintenance of the isolation barriers long into the future, with all the associated costs and potential liability.

A better approach than these traditional methods is to completely destroy the pollutants if possible, or at least to transform them into innocuous substances. Some technologies that have been used are high-temperature incineration and various types of chemical decomposition (e.g., base-catalyzed dechlorination, UV oxidation). They can be very effective at reducing levels of a range of contaminants, but have several drawbacks, principally their technological complexity, the cost for small-scale application, and the lack of public acceptance, especially for incineration that may increase the exposure to contaminants for both the workers at the site and nearby residents. Bioremediation is a natural process which relies on bacteria, fungi, and plants to alter contaminants as these organisms carry out their normal life functions. Metabolic processes of these organisms are capable of using chemical contaminants as an energy source, rendering the contaminants harmless or less toxic products in most cases. Thus, bioremediation provides an alternative tool to destroy or render the harmful contaminants through biological activity and this method is also cost-effective (Kamaluddin *et al*., 2003).

Bioremediation/ Phytoremediation and Rhizoremediation, Microflora associated with plants; endophytic bacteria, rhizosphere bacteria, and mycorrhizae have the potential to degrade heavy metals in association with plants and this process is termed rhizoremediation. Thus bioremediation, phytoremediation, and rhizoremediation contribute significantly to the fate of hazardous waste (heavy metals) and can be used to remove these unwanted compounds from the biosphere. Bioremediation processes can also beassessed through a multifaceted approach such as Natural attenuation, sensing environmental pollution, metabolic pathway engineering, applying phyto and microbial diversity to problematic sites, plant-endophyte partnerships and systems biology (Asha *et al*., 2013). Enhancement of these polluted soil residues with different organic amendments like manure compost, biosolids, MSW will lead to increased bioavailability which in turn will act as nutrients for microorganisms and also a conditioner to improve the physical properties and fertility of the soils (Jin *et al.*, 2011).

Phytoremediation, a fast emerging technology is an eco-friendly, low tech, cost -effective, green alternative to the problem (Meagher *et al.,* 2000). The specific plant and wild species that are used in this technique accumulate increasing amounts of toxic heavy metals by their roots and transport/translocate them through various plant tissues where they can be metabolized, sequestered and volatilized (Greenberg *et al.*, 2006, Doty *et al.*, 2000). These plants are known as hyper-accumulators. Phytoremediation can be done in different ways such as rhizofiltration, phytostabilization, phytovolatilization, phytodegradation (Long *et al.*, 2002) and phytoextraction (Jadia *et al.*, 2009).

HEAVY METALS

Heavy metals are natural elements in the environment. However anthropogenic releases, including industrial and domestic effluents, urban storm, water runoff, landfill leachate, atmospheric sources, and dumping of sewage sludge can give rise to higher concentrations of the metals relative to the normal background values. The term “heavy metal” refers to a metal or metalloid with a density exceeding 5g /cm^{-3} and is usually associated with pollution and toxicity, although some of these elements (essential metals) are actually required by organisms at low concentrations (Adriano, 2001). Several heavy metals, such as copper, zinc, and iron, are essential for the physiological functioning of living organisms, but they all become toxic at high concentrations. The toxicity of a metal depends on the metal itself, its total concentration, the availability of the metal to the organism, and the organism itself. Depending on the organism and the metal, different modes of action are recognized: binding to macromolecules (proteins, DNA, RNA), disruption of enzymatic functions, catalysis of radical formation, etc. For example, zinc (Zn) is a component found in a variety of enzymes (dehydrogenases, proteinases, peptidases), but it is also involved in the metabolism of carbohydrates, proteins, phosphate, auxins, and in RNA and ribosome formation in plants (Kabata-pendias et al., 2001, Mangel and Kirkby, 1982).

SOIL POLLUTION BY HEAVY METAL

Heavy metal pollution in soils constitutes a highly complex disruption of ecological equilibrium. Soils naturally contain a broad diversity of metallic elements, and each metal may be present at variable concentrations and as different chemical species. While some metals have no biological relevance, others are essential trace elements that become toxic when present beyond a certain concentration level. As metals often occur in ionized forms in the soil, they react with negatively charged soil particles, meaning that both their concentrations and their bioavailabilities are relevant. The result of this situation is that soil biota must permanently regulate their activities in order make essential metals available and take them up in the required concentrations, as well as to exclude or detoxify detrimental forms or concentrations.

In particular, soil microorganisms must display extensive physiological adaptivity. Considering the space and time variability of soils, selection pressure resulting from metal status in soils probably provides an impetus for the adaptation of physiological pathways in soil microorganisms and for their evolution. This is just one example of the complexity of soil, which may explain why the biodiversity of soil microorganisms is so high.

After estimation in 1995, a total amount of over 700 million kg of metals is being dumped in mine tailings worldwide annually (Warhurst, 2002). Depending on the metal (As, Cd, Cu, Ni, Pb, and Zn), the volume of tailing material ranges from 10,000 to 600,000 metric tons (ib.), illustrating the negative consequences of ore processing. When large volumes of the geogenic substrate are excavated, waste rock material is often still rich in metals after the extraction process. The reallocated geogenic material is prone to weathering and source of continuous metal release.

The leather industry is the major cause for the high influx of Cr to the biosphere, accounting for 40% of the total industrial use (Barnhart, 1997). In India alone about 2000 to 3200 tonnes of elemental Cr escape into the environment annually from the tanning industries, with a Cr concentration ranging between 2000 and 5000 mg L^{-1} in the effluent compared to the recommended permissible limit of 2 mg L^{-1} (Chandra *et al.*, 1997) Typical concentrations in natural soils are 1–1000 mg /kg soil (Frank, 1996, Lindsey, 1979).

Usually, the leached residues are dumped onto waste piles. Under irrigated and aerobic conditions, acid mine drainage ensues, often seen as seepage effluent with high-metal load and low pH. This contamination of the water path (often running through arable land) leads to soils with an increasing amount of metal and, subsequently, to a slow and continuous toxification of plants and animals, thus allowing for introduction in food chains and intoxication of humans through food or drinking water. In addition, the dilution leads to three-dimensional expansion of contamination which makes re-concentration and removal of metals impossible, resulting in both losses of metals and arable land.

In 2008, 1.4 billion tons of metals were produced globally which is a production rate sevenfold higher than in 1950. In 1950, metal consumption was 77 kg per person and year, which increased to 213 kg in 2008, varying tremendously among countries. While the benefits of metal production are easy to recognize, the negative impact is less obvious. Global mining occupies a territory of approximately 37,000 km2 which equals approximately the area of Belgium -or 0.2% of the world's land surface (Dudka and Adriano, 1997). In addition, approximately 240,000 km^2 (approximately the size of the UK) is influenced by metals released from waste dumps and open mines (Furrer *et al.,* 2002). Estimates of the European Environment Agency listed 1.4 million contaminated sites (Prasad *et al.*, 2010). Since metal contamination cannot be detoxified by degradation, metal contaminated soils have to be either remediated by removal of the metals from the arable land with subsequent safe deposition, or by changing land use after metals have been immobilized on the spot.

An issue closely linked to the health hazards of metal contaminated land is soil erosion and land degradation. Estimations of the annual loss of farming land predominantly by industrial contamination, urbanization and desertification range between 70 and 140,000 km^2. 4.3 million km^2 of arable land became abandoned during the last 40 years. Globally, 100 billion tons of topsoil are lost every year (Doos, 2002). Natural pedogenesis proceeds five times slower than the devastation of soil. Especially, scarcely vegetated, metalliferous soils are prone to whatsoever mechanism of erosion.With the given numbers, it seems evident that soil protection, soil remediation, and soil recovery are of ultimate importance, especially when relating this to the growing world's population.

SOURCES OF HEAVY METALS IN SOIL

Since the beginning of industrialization, a great variety of anthropogenic chemical compounds have been synthesized for countless uses. The two main sources of heavy metals in soil are natural and anthropogenic/human. The natural factors include soil erosion, volcanic activities, urban runoffs and aerosols particulate while the human factors include metal finishing and electroplating processes, mining extraction operations, textile industries and nuclear power. The main natural sources of heavy metal pollutants in the soil are volcanic activities, soil erosion, urban runoffs and aerosol particles. It is reported that volcanic eruptions produce hazardous impacts to the environment, climate, and health of exposed individuals. Apart from the deterioration of social and chemical conditions and the gases (carbon dioxide, sulphur dioxide, carbon monoxide, hydrogen sulphide) released during eruptions, various organic compounds and heavy metals, such as mercury, lead and gold are also released. The presence of these heavy metals in soil and water bodies is known to significantly deteriorate the quality of such soil and waters. Several rocks and volatiles of volcanic origins are indicated to be responsible for the presence of metals in soils and waters. This is because the diffusion of acidic volcanic gases through water permeable rocks contributes to the hydrological material transfer in volcanic strata. The activities from volcanoes are reported to be responsible for the release of metals such as arsenic, mercury, aluminum, rubidium, lead, magnesium, copper, zinc and a host of others (Amarlal *et al.*, 2006).

Soil erosion is also indicated to be a source of heavy metal pollution in soil. The two main agents of soil erosion are wind and water. During rainfall, sediment-bound heavy metals are distributed to the soil. Water containing agrochemicals with toxic metal concentration drop this sediment-bound metal in the soil even as it causes erosion. In addition, some aerosol (fine colloidal particles or water droplet in the air, in some cases they can be gas) particles may carry different kinds of the contaminant; like smoke cloud and heavy metals. These heavy metal containing aerosols usually accumulate on leaf surfaces in the form of fine particulates and can enter the leaves via stomata (Sardar *et al.*, 2013).

Some of the human sources of heavy metals in soil are metal finishing and electroplating, mining and extraction operations, textiles activities and nuclear power. Metal finishing and electroplating involve the deposition of thin protective layers into prepared surfaces of metal using electrochemical processes. When this happens, toxic metals may be released into wastewater effluents. This may be either through rinsing of the product or spillage and dumping of process baths. It is also indicated that the cleaning of process tanks and treatment of wastewater can generate substantial quantities of wet sludge containing high levels of toxic metals (Cushnie, 1985).

Similarly, mining activities can release toxic metals into the environment. Metal mining and smelting activities are regarded as major sources of heavy metals in the environment. In environments where these activities take place, it is indicated that a large amount of toxic metals deposits are found in their water, soil, crops, and vegetable (Wei *et al.*, 2008). Additionally, textile industries are indicated to be major sources of heavy metal pollutants in soil and water. This is said to mostly originate from the dyeing process, which is a major process in such industries. The compounds used for these dyeing processes (coloration) include copper, chromium, nickel, and lead which is very toxic and carcinogenic. In some cases, nuclear-generating facilities have also been described as the source of discharge of heavy metals like copper and zinc to surface soil and water. In the nuclear plants, because a large amount of water is consumed for operation, after the operation, the nuclear effluent containing heavy metals are discharged into surface and groundwater bodies, which can pollute soil and aquatic systems (Hagberget, 2007 and Wuana *et al*, 2011).

Heavy metals occur naturally in the environment from pedogenetic processes of weathering of parent materials and also through anthropogenic sources (Figure 1). The most significant natural sources are weathering of minerals, erosion and volcanic activity, while the anthropogenic sources depend upon human activities such as mining, smelting, electroplating, use of pesticides and phosphate fertilizer discharge, as well biosolids (e.g., livestock manures, composts, and municipal sewage sludge), atmospheric deposition, *etc.* (Modaihsh et al., 2004, Sabiha-Javied *et al*., 2009). The disturbance of nature’s slowly occurring geochemical cycle of metals by man results in accumulation of one or more of heavy metals in the soil and waters, and above defined levels, this is enough to cause risk to human health, plants, animals and aquatic biota (Summer, 2002). The 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.

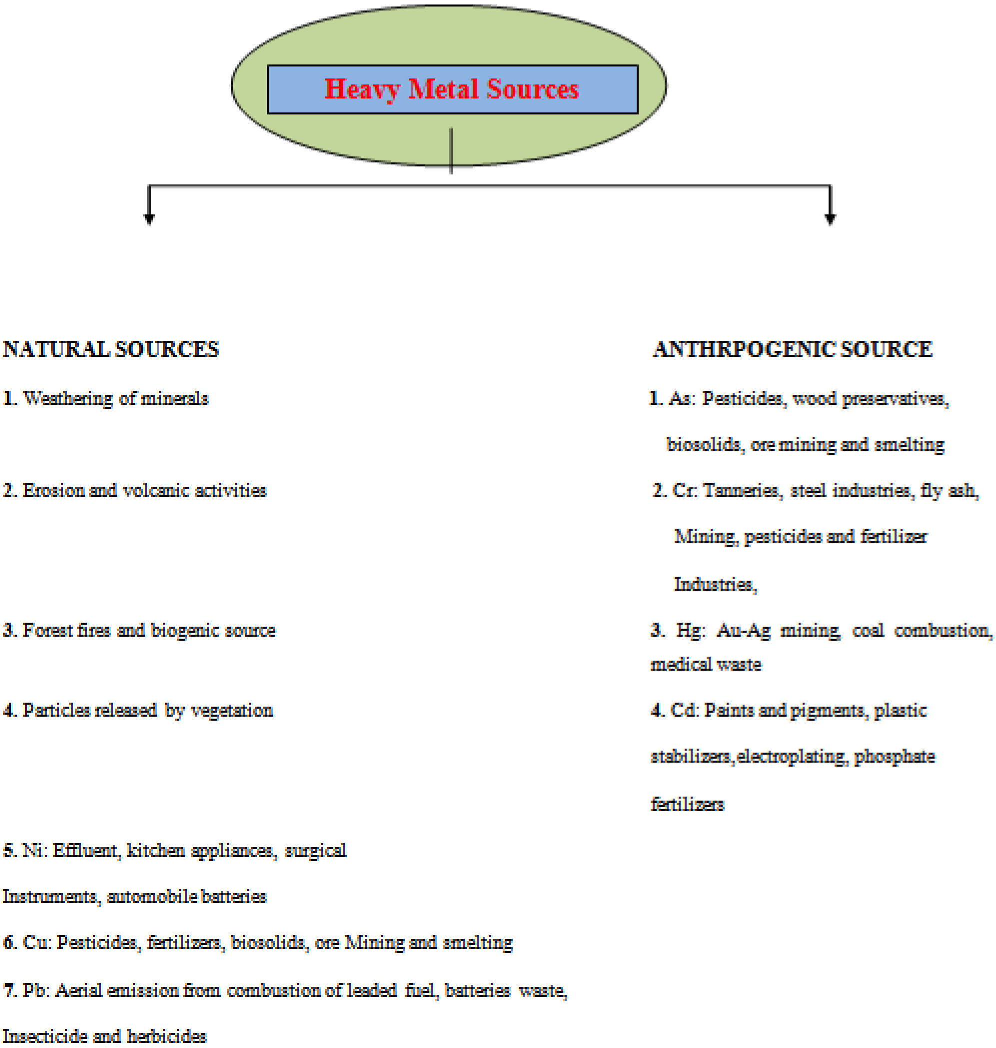

Figure 1: Sources of Heavy Metals in the Environment

HEAVY METAL POLLUTANTS SERVE AS GREAT THREATS TO PLANT AND HUMAN

Untreated or inadequately treated heavy metal contaminated wastewater effluents cause a variety of health and environmental impacts when released into receiving soil and water bodies. In aquatic ecosystems, heavy metals greatly depress the number of living organisms. Heavy metals have the negative effect on the growth of aquatic organisms and can cause serious upsets in biological wastewater treatment plants. The presence of heavy metal pollutants serves as great threats to soil and plants growing on such soils, with the consumption of such plants by animals and humans due to their entry into the food chain through biomagnification and bioaccumulation, leading to severe detrimental effects (Saidi, 2010). It is reported that the intake of toxic metals in vegetables and corn products accumulate in the kidney, leading to its dysfunction. Some reports have linked skeletal damage (osteoporosis) in humans to heavy metals, such as high levels of selenium (Abdullahi, 2013).

The nature of heavy metals polluted soil and wastewater effluents on humans may be toxic (acute, chronic or sub-chronic), neurotoxic, carcinogenic, mutagenic or teratogenic (Duruibe *et al.,* 2007). Although it is reported that individual metals exhibit specific signs of their toxicity, the signs associated with cadmium, lead, arsenic, mercury, zinc, copper and aluminium poisoning are gastrointestinal disorders, diarrhea, stomatitis, tremor, hemoglobinuria causing a rust-red colour to stool, ataxia, paralysis, vomiting and convulsion, depression and pneumonia, when volatile vapours are inhaled (Duruibe *et al.,* 2007, and McCluggage, 1991).

Although heavy metals are natural components of the earth crust that cannot be degradable, they are only toxic when they are not metabolized and synthesized by the body and when accumulated in the soft tissue of the body. As an example, lead is considered the number one health threat to children, whose effects can last a lifetime. Some of such effects include child's growth, damage the nervous system, and cause learning disabilities, but also it is now linked to crime and anti-social behavior in children (Salem *et al.,* 2000). It is indicated that the majority of ingested lead is removed from an individual's body through urine, there is still the risk of buildup especially in children. Also, toxicity due to lead accumulation may lead to a decrease in hemoglobin production, kidney, joint, reproductive and cardiovascular systems disorders and long-term injury to the central and peripheral nervous systems (Nolan, 2003 and Galadima *et al.,* 2012). Another highly toxic heavy metal, even when present in humans at low concentrations is cadmium. It is indicated to be carcinogenic and persistently cumulative poison (Lin *et al.,* 2005). A long-term exposure to cadmium in humans may lead to renal dysfunction; while high exposure levels could cause obstructive lung disease, cadmium pneumonitis, bone defects, osteomalacia, osteoporosis and spontaneous fractures, increased blood pressure and myocardial dysfunctions (Duruibe *et al.,* 2007). The level of exposure to cadmium compounds may determine the symptoms, which may include nausea, vomiting, abdominal cramps, dyspnea and muscular weakness. Severe exposure may result in pulmonary oedema and death (Duruibe *et al.,* 2007, McCluggage, 1991, Young, 2005, Madsen *et al*., 1990, INECAR, 2000).

With respect to copper, although copper is an essential nutrient to humans, its presence in high concentration in drinking water is indicated to cause liver cirrhosis in patients, anemia, liver and kidney damage. Exposure to water contaminated with copper can lead to the development of anemia, liver and kidney damage and diarrhea, abdominal pain, vomiting, headache and nausea in children (Salem *et al.,* 2000, Nolan, 2003, Bent and Bohm, 1995). In addition, although zinc is a component of several enzymes (alkaline phosphatase, superoxide dismutase, alcohol dehydrogenase, carbonic anhydrase) in humans when taken at high concentrations can lead to system dysfunctions, which may result in growth and reproduction impairment. The clinical signs of zinc toxicosis include diarrhea, vomiting, icterus (yellow mucous membrane), bloody urine, anemia, kidney failure and liver failure (Duruibe *et al.,* 2007, Nolan, 2003, INECAR, 2000).

On the other hand, mercury is known as one of the most dangerous metals for human consumption, for it has no known biochemical function. It is reported that toxicity symptoms of mercury are dependent on the chemical form ingested. The ingestion of its inorganic forms cause spontaneous abortion, congenital malformation, and gastrointestinal disorders while ingestion of its organic forms may lead to erethism (abnormal irritation or sensitivity of an organ or body part to stimulation), gingivitis, stomatitis, neurological disorders, brain and central nervous system damage, acrodynia (pink disease, characterized by rash and desquamation of the hands and feet) and congenital malformation (Duruibe *et al.,* 2007; LTAP 2004; Simone et al., 2012). Furthermore, exposures to high levels of arsenic can cause death, since it is known to coagulate protein, form complexes with coenzymes and inhibit ATP production during respiration (INECAR, 2000).

Table 1: Toxic Effect of Some Heavy Metals on Human Health

Heavy Metal	EPA Regulatory Limit(ppm)	Toxic Effects	Ref.
Ag	0.10	Exposure may cause skin and other body tissues to turn gray or blue-gray, breathing problems, lung and throat irritation and stomach pain.	ATSDR, 1990
As	0.01	Affects essential cellular processes such as oxidative phosphorylation and ATP synthesis	Tripathi *et al.*, 2007
Ba	2.0	Cause cardiac arrhythmias, respiratory failure, gastrointestinal dysfunction, muscle twitching and elevated blood pressure	Acobs *et al.*, 2002
Cd	5.0	Carcinogenic, mutagenic, endocrine disruptor, lung damage and fragile bones, affects calcium regulation in biological systems	Degraeve, 1981
Cr	0.1	Hair loss	Salem *et al*, 2000
Cu	1.3	Brain and kidney damage, elevated levels result in liver cirrhosis and chronic anemia, stomach and intestine irritation	Salem *et al*, 2000, Wuana and Okieimen 2011
Hg	2.0	Autoimmune diseases, depression, drowsiness, fatigue, hair loss, insomnia, loss of memory, restlessness, disturbance of vision, tremors, temper outbursts, brain damage, lung and kidney failure	Neustadt and Pieczenik, 2007; Gulati *et al*.,2010
Ni	0.2 (WHO permissible limit)	Allergic skin diseases such as itching, cancer of the lungs, nose, sinuses, throat through continuous inhalation, immunotoxic, neurotoxic, genotoxic, affects fertility, hair loss	Salem *et al*, 2000,Khan *et al* 2007: Duda *et al* 2008
Pb	15	Excess exposure in children causes impaired development, reduced intelligence, short-term memory loss, disabilities in learning and coordination problems,a risk of cardiovascular disease	Salem *et al*, 2000, Wuana and Okieimen 2011, Padmavathiamma *et al.*, 2007
Se	50	Dietary exposure of around 300 µg/day affects endocrine function, impairment of natural killer cells activity, hepatotoxicity and gastrointestinal disturbances	Vinceti *et al.*, 2001
Zn	0.5	Dizziness, fatigue *etc.*	Hess *et al.*, 2002

Heavy metals are also known to have impacts in soil ecosystems. The impact of heavy metals pollution on soil is mostly felt by plants that grow in such environments. Some of these impacts include decreased seed germination and lipid content, decreased enzyme activity and plant growth, inhibition of photosynthesis, reduction of seed germination, reduction of chlorophyll production and plant growth; which may be caused by cadmium, chromium, copper or mercury, nickel and lead, respectively (Gardea-Torresdey et al., 2005). The presence of large amounts of heavy metals in a soil could also lead to the prevention of plants' growth, uptake, physiological and metabolic processes, chlorosis, and harm to root tips, minimized water and uptake of nutrients and impairment to enzymes (Sardar *et al.*, 2013). Furthermore, the potential detrimental effects of heavy metal polluted wastewater effluents on the quality of receiving water bodies are numerous, although it may depend on the volume and composition of the effluent that is discharged (Owyli 2003, Akpor *et al.*, 2011). As an example, in aquatic ecosystems, the concentration and availability of lead can lead to decreased dissolved oxygen, which may make young aquatic organisms, such as young fishes vulnerable to lead than the adult fish. The presence of lead may also cause blackening of the tail region and spiral deformity to young fishes (Peplow 1999, European Commission 2002).

BIOLOGICAL REMEDIATION APPROACHES FOR HEAVY METAL POLLUTANTS

Biological removal of heavy metals in soil involves the use of biological techniques for the elimination of pollutants from soil. It is a selective technique that utilizes the operational flexibility of microorganisms and plants. Microbial remediation may entail ex-situ and in-situ application. In phytoremediation, plants play a great role in the biological process as they break down, reduce, degrade and remove these contaminants using various parts, such as the root, leaves, stomata, cell wall and the shoot (USEPA 2004; Rajendran *et al.*, 2003; Sharma 2012).

MICROBIAL REMEDIATION OF HEAVY METAL

The term biodegradation is often used in relation to ecology, waste management and mostly associated with environmental remediation (bioremediation). Bioremediation process can be divided into three phases or levels. First, through natural attenuation, contaminants are reduced by native microorganisms without any human augmentation. Second, biostimulation is employed where nutrients and oxygen are applied to the systems to improve their effectiveness and to accelerate biodegradation. Finally, during bioaugmentation, microorganisms are added to the systems. These supplemental organisms should be more efficient than native flora to degrade the target contaminant (Marinescu 2009). A feasible remedial technology requires microorganisms being capable of quick adaptation and efficient uses of pollutants of interest in a particular case in a reasonable period of time. In recent years, considerable interest has been paid to rhizobacteria, which are aggressive root colonizers and produce siderophores. Siderophores provide an advantage in the survival of both plants and bacteria (Narendra *et al.*, 2015). Many factors influence microorganisms to use pollutants as substrates or metabolize them, like, the genetic potential and certain environmental factors such as temperature, pH, and available nitrogen and phosphorus sources, then, seem to determine the rate and the extent of degradation (Fritschu *et al.*, 2008). Therefore, applications of genetically engineered microorganisms (GEM) in bioremediation have received a great deal of attention. These GEM have a higher degradative capacity and have been demonstrated successfully for the degradation of various pollutants under defined conditions. However, ecological and environmental concerns and regulatory constraints are major obstacles for testing GEM in the field (Menn *et al.*, 2008).

In microbial remediation or bioremediation, microbial communities are of primary importance. The process is cost-effective process, with non-hazardous end products (Ahmedna *et al.*, 2004). During pollutant removal, the microbe(s) alter the metal chemistry and mobility through either reduction, accumulation, mobilization or immobilization (Faryal and Hameed, 2005). Previous studies we have identified five bacterial isolates based on the high level of heavy metal resistances. On the basis of morphology, biochemical revealed that the isolates were identified as *Proteus vulgaris* (MR1), *Bacillus cereus* (MR2), *Bacillus decolorationis* (MR3), *Pseudomonas fluorescence* (SS4) and *Pseudomonas fluorescence* (SS5). The soil isolates showed optimum growth at pH 7.0 and 30°C. The identified isolates were resistant to cadmium (Cd), nickel (Ni), lead (Pb), arsenic (As), and chromium (Cr). The minimal inhibitory concentration (MIC) of soil isolates against Cd, Cr, Ni, Pb and As was determined in solid media (Narendra *et al.*, 2016). The identified heavy metal resistant bacteria could be effective and useful for the bioremediation of heavy metal contaminated soil. The major groups of microorganisms that have been implicated in heavy metal remediation are bacteria (such as *Anthrobacter*, *Bacillus* sp, *Citrobacter, Cupriavidus metallidurans, Cyanobacteria, Enterbacter cloacae, Pseudomonas aeruginosa, Streptomyces* sp, *Zoogloe aramigera*, *Alcaligenes*, *Sphinganonas*, *Rhdococcus*, *Mycobacterium* and *Arthrobacter*) and fungi (such as *Aspergillus tereus*, *Penicillium chrysogeum*, *Candida utilis, Hamsenula anomala* and *Rhodotorula mucilaginosa*) (Ahirwar *et al.,* 2016, Dias *et al.,* 2002). Besides bacteria and fungi, certain protozoa, such as *Euplotes mutabilis* and algae, such as *Oscillatoria* sp, *Chlorella vulgaris,* and *Chlamydomonas* sp have been reported to possess metal reducing capabilities (Ramasamy *et al.,* 2006)

The microbial remediation of toxic metals is said to occur in two ways: direct and indirect reduction (Sinha *et al.,* 2009). Microbial remediation can be in the form of bioaugmentation, biosorption or sparging. Bioaugmentation entails the introduction of microbial strain, which has high degradation factor to assist the indigenous microbe in the active degradation process of the contaminated environment. It is mostly used in municipal wastewater to restart activated sludge bioreactor (Rajiv *et al.,* 2009). Soil microorganisms vary widely in their tolerance to heavy metal contamination, and the proportion of culturable resistant microorganisms can range from 10% to nearly 100%. The activities of enzymes in soil may serve as indicators of heavy metal contamination, as there are generally high correlations between reduced enzyme activities (of, e.g., dehydrogenases, acid phosphatases and ureases) and increased heavy metal contamination (Ahirwar *et al.,* 2018). In our previous studies, we have reported that the higher reduction of chromium for lower initial concentrations by *Bacillus cereus, Bacillus decolorationis,* and *P. fluorescence.* The seed germination and plant growth ability were analyzed in different experimental groups using *Pseudomonas fluorescence, Bacillus cereus,* and *Bacillus decolorationis*. *Pseudomonas fluorescence* (95%), *Bacillus cereus (90%)* have shown maximum seed germination and plant growth ability noted compared to *Bacillus decolorationis* (84%) inoculated strain in Cr contaminated soil (Ahirwar *et al.,* 2013).

In biosorption, there is the immobilization of metals by microbial cells. Its technique involves the sequestration of a positively charged heavy metal ions to the negatively charged microbial cell membranes and polysaccharides, which is secreted (Sinha *et al.,* 2009). The mechanisms of heavy metal removal from soil by microorganisms can be based on microbial precipitation, complexation, ion-exchange and intracellular accumulation. During biosparging, also known as air sparging, there is the injection of air by pressure to the water to enhance the activation of oxygen concentration by the microorganism, which can increase biological degradation of contaminant. Apart from the promotion of aerobic bacterial growth, air sparging also leads to the volatilization of contaminants from the liquid to the vapor phase (Sharma 2012).

A wide variety of synthetic organic compounds contaminate the environment from chemical and industrial processes. In many instances, organic loads entering receiving waters add to the existing organic pools and cause perturbations in the natural degradation processes of the aquatic microbial community. Many chemicals employed in industrial processes are both refractory and toxic, and removal of these pollutants from the aquatic environment occurs primarily by microbial activities. Microbial degradation is dependent upon physical and chemical environmental variables, as well as on the toxicity of the chemical. Physical and chemical factors may render a given compound more or less susceptible to microbial degradation. For example, irradiation in the visible and ultraviolet ranges can aid in the degradation of polymerized plastics and dechlorination of halogenated substrates and, perhaps, in the cleavage of alkylated biphenyls and fused aromatic ring systems. Photodegradation has also been implicated in the potential formation of chlorinated dibenzofurans from chlorinated biphenyls producing more toxic compounds of unknown biodegradative potential (Crosby *et al.,* 1973). Especially attractive is the potential for early warning of environmental change since microbiological responses are rapid and can be detected within hours or days. The microbial potential, perhaps measured as a community structure index, or other mathematical formulation, should be more fully investigated as an ecotoxicological yardstick of health. Clearly, the microbial aspects of ecotoxicology should be explored since here lies, indeed, a fertile ground for discovery and application in environmental pollution.

BIOREMEDIATION STRATEGIES

Different techniques are employed depending on the degree of saturation and aeration of an area. *In situ* techniques are defined as those that are applied to soil and groundwater at the site with minimal disturbance. *Ex situ* techniques are those that are applied to soil and groundwater at the site which has been removed from the site via excavation (soil) or pumping (water). *Bioaugmentation* techniques involve the addition of microorganisms with the ability to degrade pollutants

Table 2: Summary of Bioremediation Strategies

Technology	Examples	Benefits	Limitations
In situ	*In situ* bioremediation	Most cost efficient	Environmental
	Bioaugmentation	Natural attenuation processes Treats soil and water	Time Monitoring difficulties
	Bioventing	Relatively passive	Extended treatment
	Biosparging	Noninvasive	Constraints
Ex situ	Biopiles	Can be done on site	Need to control abiotic Loss Mass transfer problem Bioavailability limitation
	Landfarming	Cost efficient	Space requirements
	Composting	Low-cost	Extended treatment time

DEGRADATION BY GENETICALLY ENGINEERED MICROORGANISMS

As mentioned above, bioaugmentation and biostimulation are methods that can be applied to accelerate the recovery of polluted sites. In the late 1970s and early 1980s, bacterial genes encoding catabolic enzymes for recalcitrant compounds started to be cloned and characterized. Soon, many microbiologists and molecular biologists realized the potential of genetic engineering for addressing biodegradation (Cases *et al.,* 2005). A genetically engineered microorganism (GEM) or modified microorganism (GMM) is a microorganism whose genetic material has been altered using genetic engineering techniques inspired by the natural genetic exchange between microorganisms. These techniques are generally known as recombinant DNA technology. Genetically engineered microorganisms (GEMs) have shown potential for bioremediation of soil, groundwater and activated sludge, exhibiting the enhanced degrading capabilities of a wide range of chemical contaminants (Sayler *et al.,* 2000). As soon as the prospect of releasing genetically modified microorganisms for bioremediation became a reality, much of the research effort in the field was aimed at biosafety and risk assessment (Cases *et al.,* 2005).

PHYTOREMEDIATION OF HEAVY METAL

In phytoremediation green plants are employed technique in the in-situ treatment of contaminants. Such plants have the advantage of accumulating and degrading components of such contaminants. The commonest phytoremediation processes are rhizofiltration, phytostabilization, phytoextraction, phytovolitazation, phytodegradation and rhizodegradation (Rober *et al*. and Ana *et al.,* 2009*).*

Remediation of heavy metals polluted soil could be carried out using physical-chemicals processes such as ion-exchange, precipitation, reverse osmosis, evaporation and chemical reduction. However, the measures require external man-made resources and therefore are very costly (Mangkoedihardjo and Surahmaida, 2008). Phytoremediation is an emerging technology that can be considered for remediation of contaminated sites because of its cost-effectiveness, aesthetic advantages, and long-term applicability. For a country like India, phytoremediation is best suited as it requires low investment, and relies on plants' natural capability to take up metal ions from soil (Ghosh and Singh 2005). Identification/selection of plant species for phytoremediation is a continuous process and till date, many plants have been found as remediation plants but there are very few reports about the use of ornamental plants for phytoremediation purpose [Liu *et al.*, 2009; Ramana *et al.*, 2008a & 2008b, Ramana *et al*., 2009]. During rhizofiltration (phytofiltration), both aquatic and terrestrial plants are used to sorb, concentrate and precipitate toxic metals and an organic compound from wastewater effluents. The technique involves the breakdown of the organic contaminant by enhanced microbial activity in the plant root zone and is absorbed by the root surface or by the plant root. The technique is based on the effectiveness of a plant root to synthesis chemicals. Both the root exudate and a change in pH of the rhizosphere can cause a biogeochemical condition, which may result in the precipitation of this metal to the surface of the root (Vineeta 2007). In phytostabilization (in placed inactivation or phytoimmobilization), a plant root is used to limit a contaminant mobility and bioavailability by providing a barrier mechanism against direct contact with contaminated soil (Schnoor 1997). It is indicated that plants that are best suited for phytostabilization include trees, which transpire large amounts of water for hydraulic control and grasses with fibrous roots help to bind and hold (Sinha *et al.*, 2009).

In the case of phytoextraction, metal-accumulating plants are used for the translocation and concentration of metals, radionuclides, and non-metals in the root of the plant, before they are translocated to the shoots or leaves (Asha and Sandeep 2013). The biological processes involved in phytoextraction are metal acquisition and transport and shoot accumulation. In some instances, some heavy metals can be removed by binding to soils and root masses through rhizofiltration, while others may require the addition of chelating agents, such as ethylene diamine tetraacetate (EDTA) to the soil. Sunflower and mustard are examples of plants that have been implicated to have phytoextraction ability for heavy metals (Robert *et al.*, 1997). Similarly, in phytovolatilization, a contaminant is removed by transforming it from its original medium to the atmosphere. The technique entails the ability of a plant to take up a contaminant that is water soluble and release it to the atmosphere without the need of harvesting or disposal.

The accumulation of Cr in soil is of great concern because of its movement into the food chain. Therefore, researchers have proposed safe, economically feasible and eco-friendly approaches for phytoremediation using non-edible plants (Ramana *et al.*, 2013; Khajanchi *et al.*, 2013). In our previous studies, we have studied the phytoremediation of soils contaminated with Cd and Pb with some popular floriculture plant species. Explored the possibility of phytoremediation of soils contaminated with Cr using three varieties of Tuberose (Polianthes tuberosa) (Ramana *et al.*, 2012), chrysanthemum, calendula, aster and dahlia (Ramana *et al.*, 2012). From these studies, it was found that majority of the plant species could tolerate at the most 10–15 mg Cr/kg soil. However, the contaminated sites would have very high levels of Cr and at times would even be unfit for cultivation of the crops. The potential of an ornamental shrub Crown of thorns (*Euphorbia milli*) was evaluated for remediation of soil contaminated with Cr. The plant could tolerate well up to 75 mg of applied Cr and beyond that, there was mortality of plants. Though the plant could not be classified as a hyperaccumulator, the plant was still very efficient in translocating Cr from roots to shoots as evident from the data on uptake and translocation efficiency values. The translocation efficiency of over 80% in our study demonstrates that a large proportion of Cr has been translocated to the harvestable biomass of the plant and therefore, this plant could be effectively recommended for the remediation of soils contaminated with low to medium level of contamination i.e., up to 50 mg/kg soil (Sivakoti *et al.*, 2015). Previously we have conducted to evaluate the ability of an high biomass producing, drought-tolerant succulent plant Mauritius hemp (*Furcraea gigantea Vent.*) for its tolerance to different levels of Cr (0, 25, 50, 100 and 200 mg Cr kg soil^{-1}) and its potential for phytoremediation purposes. Based on the data on inhibition of the growth of plants with Cr, tolerance index and grade of growth inhibition, it was observed that the plant could tolerate up to 50 mg Cr kg $^{-1}$ soil. Absorption of Cr from soil to plant and its translocation into plant tissues were discussed in terms of bio-concentration factor (BCF), transfer factor (TF), and translocation efficiency (TE%). Cr was mainly accumulated in the roots and exclusion of Cr was found to be the principal physiological tolerance mechanism followed by a marked increase in proline, ascorbic acid, total free amino acids in the leaf tissue and malic acid in the rhizosphere samples to counter Cr stress. Based on the tissue concentration of Cr ($< 300\ \mu g\ g^{-1}$ in the leaves and TF<1), it was concluded that, *Furcraea gigantea* could not be considered a hyperaccumulator and therefore unsuitable for phytoextraction of Cr. Nevertheless, *Furcraea gigantea* could be a suitable candidate for phytostabilization of Cr contaminated soils (Ramana *et al.*, 2015)

Certain metals, such as selenium and mercury have been reported to form the volatile molecule, which may be released to the atmosphere by some plants (Ghosh and Singh 2005). However, during phytodegradation (phytotransformation), there is the breakdown of organic contaminants taken up by a plant into simpler molecules. The breakdown is carried out by the plant enzymes, which metabolize the contaminant and release it in the rhizosphere, which may then undergo further active transformation (Sinha *et al.*, 2009). Also, in phytostimulation (rhizodegradation), the technique involves the release of natural substances by the plant through its roots, thereby supplying nutrients to microorganisms, which may in turn, enhance biological degradation. In this technique, the plant may secrete exudate (amino acid, organic acid, fatty acid, sterol, growth factors and other compounds) that can lead to an increase in the number and activities of microorganisms (Meers and Tack 2004; Akpor and Muchie 2010).

Table 3: Overview of Phytoremediation Applications

Technique	Plant Mechanism	Surface Medium
Phytodegradation	Enhances microbial degradation in rhizosphere	Soils, groundwater within rhizosphere
Phytoextraction	Uptake and concentration of metal via direct uptake into the plant tissue with subsequent removal of the plant's	Soils
Phytotransformation	Plant uptake and degradation of organic Compounds	Surface water, groundwater
Phytostabilization	Root exudates cause the metal to precipitate and become less available	Soils, groundwater, mine tailing
Phytovolatilization	Plants evaportranspirate selenium, mercury, and volatile hydrocarbons	Soils and groundwater
Rhizofiltration	Uptake of metals into plant roots	Surface water and water pump

CONCLUSIONS

This review, which was aimed at discussing the sources, impacts and remediation processes for heavy metals pollution in wastewater effluents revealed that the two main sources of heavy metals in wastewater are natural and human, with the natural factors being soil erosion, volcanic activities, urban run-offs and aerosols particulate while the human factors include metal finishing and electroplating processes, mining extraction operations, textile industries and nuclear power.

The entrance of untreated or inadequately treated heavy metal contaminated wastewater to receiving water bodies pose a variety of health and environment impacts on humans, animals, and plants. In aquatic ecosystems, heavy metals greatly depress the number of living organisms. Also, heavy metals have the negative effect on the growth of aquatic organisms and can cause serious upsets in biological wastewater treatment plants.

To safeguard the health of living organisms and for environmentsustainability, a variety of biological treatment processes are employed for the removal of heavy metals from wastewater effluents, with the most common being microbial and phytoremediation. Biological removal of heavy metals in wastewater is a selective technique that utilizes the operational flexibility of microorganisms and plants for the elimination of pollutants from wastewater.

Microbial remediation may entail ex-situ and in-situ applications. In phytoremediation, plants play a great role in the biological process as they break down, reduce, degrade and remove these contaminants using various parts, such as the root, leaves, stomata, cell wall and the shoot.

The microbial remediation of toxic metals is said to occur in two ways: direct and indirect reduction. Microbial remediation can be in the form of bioaugmentation, biosorption or biosparging. In phytoremediation green plants are employed technique in the in-situ treatment of contaminants. Such plants have the advantage of accumulating and degrading components of such contaminants. The commonest phytoremediation processes are rhizofiltration, phytostabilization, Phytoextraction, phytovolitazation, phytodegradation, and rhizodegradation.

FUTURE PROSPECTS

Rapid industrialization and technology development have adverse side effects like soil contamination and degrading soil health. Due to the complexity involved in the conventional methods for remediation of soil, the use of microbes has arisen as a time-saver for bioremediation. However, bioremediation technology has limitations; several microorganisms cannot break toxic metals into harmless metabolites, and these have inhibitory effects on microbial activity. Modification in the outer membrane proteins of bacteria with potential bioremediation properties for improving metal binding abilities is the likely way to enhance their capacity for biotransformation of toxic metals. Future studies should focus on the factors involved in improving *in situ* bioremediation strategies using genetically engineered microorganisms (GEM) and also the applicability and adaptability of these GEMs in all the possible adverse/stress conditions and multiple-heavy-metal-polluted conditions. The reluctance among the public to accept GEM for bioremediation also needs to be considered in future studies, and they must be proved non-toxic to the environment.

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