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A BRIEF SKETCH THROUGH MICROBIAL RESPONSE TO HEAVY METALS IN THE ENVIRONMENT

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Heavy metals are the metals having atomic density exceeding 5 g cm^{-3}. Accumulation of heavy metals above the threshold level is mainly due to anthropogenic activities including mining, chemical manufacturing, agriculture, hospital wastewater and electronic waste. Heavy metals can pose cytotoxic, carcinogenic and mutagenic effects and most heavy metal are hazardous to human even in low concentration. It was proven that the accumulation of heavy metal in the body has caused a severe effect in the heart, brain, kidney, bones and liver. The important heavy metals and their atomic number are given in Table 1.

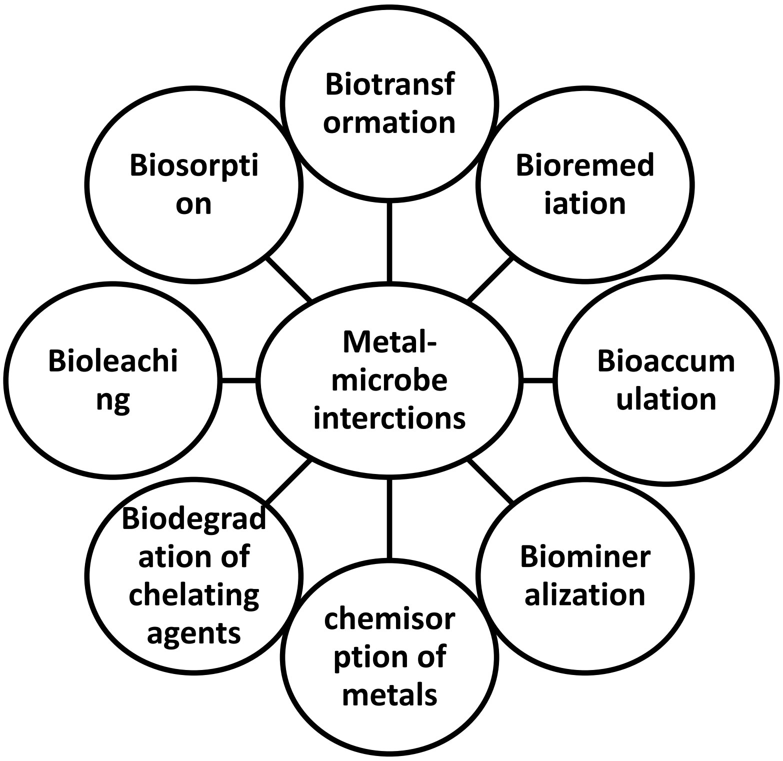
Table 1: Heavy metals with their atomic number

Metal	Atomic number
Mercury (Hg)	80
Lead (Pb)	82
Chromium (Cr)	24
Arsenic (As)	33
Cadmium (Cd)	48
Uranium (U)	92
Selenium (Se)	34
Silver (Ag)	47
Gold (Au)	79

Heavy metal pollution is considered as most severe environmental issue since the pollutant capable to infiltrate deep into the bed of groundwater sources and surface water, and affect public health. These heavy metals will end up in the food chain and form bio-accumulate and transfer from one food chain to another. Metals are able to exert their toxicity because it is non-degradable and are only transformable via methylation, sorption and complexation and alteration in a valence state which influence the bioavailability of metals and mobility. Urban areas with high population density and accelerated anthropogenic activities such as mining are considered as a reservoir of pollution commonly made up of heavy metals. Mine water pollution could cause severe impacts to biological systems as species diversity and total biomass composition in aquatic and terrestrial ecosystems can be affected due to acidity and heavy metal contamination. It has been reported that heavy metals contamination due to mining activities involved around million hectares out of 10 million hectares of heavy metals contaminated land in China. Another study that was conducted to evaluate chemical speciation of heavy metal in sediment of former tin mining area at Selangor, Malaysia proved that the sediment were contaminated with Cr, Zn, As, Cu, Pb and mainly with Sn.

Heavy metal – Microbe Interactions

Microbes are interacted with heavy metals in different ways as shown below in the pie diagram.



Biotransformation

Biotransformation is the chemical modification made by an micro-organism or chemical compound. Biotransformation of important heavy metals are given below;

a) Transformation of Hg

Metallic Hg is having high vapour pressure. Hence it is easily volatile from soil treated with Phenyl mercuric acetate (PMA), Ethyl Mercuric acetate (EMA), mercuric and mercurous ions. Coal contains about 1200-21000 ppb Hg. Burning of fuels releases Hg and other metals. The metallic Hg produced during electrolysis of brine to NaOH and bleach (Cl, OCl) and discharged into water resources.

Methylation of Hg

Micro organisms in sediments transform metallic Hg to methylated forms (monomethyl and dimethyl Hg). Methylated forms are not only volatile and lipophilic but also toxic. Monomethyl Hg is water soluble and readily absorbed and retained in animal tissues – carcinogenic.

$$Hg^0 \longrightarrow CH_3Hg^+ \longrightarrow (CH_3)_2Hg$$

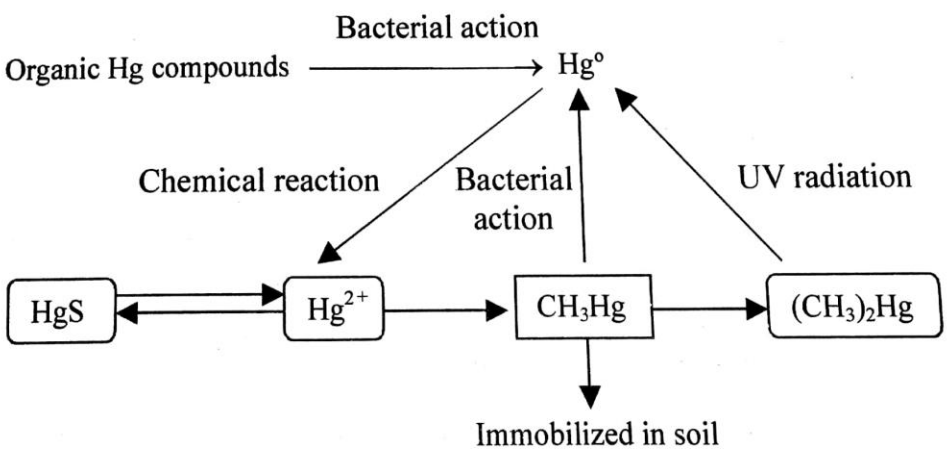
(elemental Hg) (Methyl mercury) (Dimethyl Hg)

Dimethyl Hg – insoluble in water and volatile, bio accumulates in tissues and difficult to eliminate.

- In soil, Hg metal con, <1ppm – mostly exist in cationic form of Hg (Hg^{2+})
- Reduction to metallic elemental form in soil is achieved by both biological and chemical reactions

$$Hg^{2+} \longrightarrow (CH_3)_2Hg \xrightarrow{\text{Photolysis}} Hg^0$$

Under anaerobic condition, soil microbes methylate Hg and form organo – mercury compounds that are bioavailable and toxic to microbes. At the same time, anaerobic conditions can convert Hg^{2+} into exceedingly insoluble HgS. Microbes involved in methylation are bacteria, fungi and yeast such as *Bacillus, Clostridium, Mycobacterium, Pseudomonas, Aspergillus* and *Neurospora.* Besides methylation, these microorganisms release organically bound Hg during their heterotrophic activity. Methyl Hg may be demethylated by many organisms resulting in the release of Hg.



Transformation of mercury in soil

The methyl sulphides, generated is insoluble but less toxic. Mercury accumulation in soils tends to correlate with organo metallic level. Acidic oxidizing conditions in soil tend to stabilize Hg^{2+} which complexes with phenolic and carboxylic functional groups. Adsorption of Hg^{2+} on silicate clays and oxides is more favourable at higher pH. In reducing soil environment strong association of Hg with sulphides renders it less mobile.

Transformation of As

Arsenite ($^{+3}$) [AsO_2^-] are the reduced state of arsenic and is present in anaerobic soil. May occur as $As(OH)_3$, $As(OH)_4^-$, $AsO_2(OH)^{2-}$ and Arsenate (+5) [$AsO_3{}^{3-}$] is oxidized state and most stable in aerobic soils. Soil microbes especially manganese oxidizers are able to promote the oxidation of arsenite to arsenate under aerobic anaerobic conditions. In anaerobic soils both arsenate and arsenite are released into soil solution by dissolution of iron and manganese oxides.

Fungal species like *Aspergillus, Mucor, Scopulariopsis, Fusarium, Paecilomyces* and *Candida humicola* synthesizes some volatile metabolite from arsenite and arsenate. Volatile arsenic compounds are often converted to methylated forms like those of mercury.

Arsenate As^{2+} → Methyl arsenate As^{3+} → Dimethyl arsenate As^{+} → Trimethyl arsine As^{3-}

Heterotropic bacteria that convert arsenile to more oxidising state. Pseudomonas and Alcaligenes reduce arsenate $(AsO_4)^{3-}$. Several fungi are capable of making trimethyl-arsine $(CH_3)_3$ from methyl arsenate and dimethyl arsenic acid.

Transformation of Selenium

Mobile form Se O4 $^{2+}$(6+), SeO3 $^{2+}$. Immobile form is elemental selenium. Volatile form is selenide HSe($^{-2}$). Selenite is stable under strongly oxidizing conditions over most of the ranges of soil redox conditions.

Soils contain, on an average, 0.05 to 1.27 ppm selenium. Plants can bioaccumulate selenium in their tissues so create a potential toxicity hazard to foraging animals. Organic forms of selenium are seleno-methionine and seleno-cystine. Organically complexed selenium is important fraction of soil selenium. Soluble organic selenium compounds are liberated through the decay of seleniferous plants. Organic seleniu1n is more soluble under basic than acid soil conditions.

$$Se(IV) \longrightarrow (CH_3)_2Se^+ \longrightarrow CH_3\,Se.SeCH_3$$

(Dimethyl selenide) **(Dimethyl diselenide)**

Up to 40% Se in soil is present in humus. Transformation is mainly caused by microbes like *Candida, Clostridium, Corynebacterium, Rhizobium* and *Micrococcus.* Final product of transformation is elemental Se and metal is deposited within the cell.

Tolerance of Soil Microbes to Heavy Metal

Micrococcus and Azotobacter rapidly immobilize lead (Pb) in cell wall, transfer Pb across the cell membrane. The excessive amounts of metals in soil reduce the diversity and distribution of microbial species in soil. More tolerant selective species appear in soil under constant metal stress. Microbial biomass and their enzyme activities usually get altered due to the presence of metal in organic matter. Under certain circumstances, microorganisms are capable of converting bound element to an easily assimiliable form in soil thereby help in purifying the soil.

Transformation of Vanadium

Low concentration of vanadium is beneficial for the growth of microorganisms and plants. Evidences show that vanadium can partially substitute for molybdenum in nitrogen fixation by microorganisms. It also functions in biological oxidation-reduction reactions.

Transformation of Cobalt

Cobalt is essential for the microorganisms having potential to fix atmospheric nitrogen. Cobalt is also required in the nodules of both legumes and older tree and in nitrogen fixing cyanobacteria and actinomycetes. It has role in vitamin B_{12} formation, the co-factor required for the production of heme compounds. Cobalt forms a complex with nitrogen atoms in the porphyrin ring structure that provides a prosthetic group for association with a nucleotide in the vitamin B_{12} coenzyme. This co-complex is called cobamide coenzyme

Bioavailability of Heavy Metal

The microorganisms are the first to be affected by any heavy metal than higher organisms. The bioavailability of heavy metals to terrestrial organisms is determined by the solubilization, adsorption and precipitation reactions occurring in soils and sediments. The extent to which these metals are adsorbed depends on the properties of metal concerned. Valency, radius, degree of hydration and coordination with $0_{2.}$ The physico-chemical properties of the environment (pH, redox status, soil water content, temperature. The nature of adsorbent (permanent and pH dependent charges, complex forming ligands, etc.). Other metals present and their concentration, and the presence of soluble ligands in the surrounding fluids.

Complexation of Chelation of Metals

All metals, except Mo, occur in soil primarily in cationic form. Cr and CrO_4^-, though initially anionic, are immediately converted to cationic $Cr_3\ ^+$ form in soil. The metals in soil either form complexes or are precipitated or chelated. The cationic micronutrients react with certain organic molecules to form organo-metallic complexes called 'chelates'. If these complexes are soluble, these are carried in soil solution so increase availability of micronutrients and protect them from precipitation reactions with inorganic soil constituents. However, insoluble complex formation decreases micronutrient availability. These may occupy spaces on organic matter complexes and are rendered less available for microbial action, mineralization and other processes.

Bioremediation

It is the process of using heterotrophic microorganisms to breakdown hazardous compounds to obtain carbon and energy. It is cost effective and ecofriendly technique that offers the possibility to destroy or render various contaminants using natural biological activity. The main biological agents used are bacteria and fungi. It is a combination of phytoremediation (plants) and rhizoremediation (plant and microbe interaction).

Most important parameters for bioremediation are nature of pollutants, pH, moisture content, nutritional state, microbial diversity of the site, temperature and oxidation reduction potential. Optimum moisture condition for bioremediation is 25-85% of water holding capacity and optimum C: N: P ratio is 100: 10: 1. The optimum oxygen concentration is >0.2 mg L^{-1} and > 10% air filled pore space for aerobic degradation.

Bioremediation is of mainly two types. *In situ* bioremediation and *ex-situ* bioremediation. *In situ* treatments include Bioventing, Biosparging, Bioaugmentation.

- **Bioventing** : It is a technique which used indigenous microorganisms to biodegrade organic constituents adsorbed to soils in the unsaturated zone.
- **Biosparging** : It is a technique which used indigenous microorganisms to biodegrade organic constituents in the saturated zone. Here air (O_2) and nutrients are injected into the saturated zone to increase the biological activity of the indigenous microorganisms.
- **Bioaugmentation**.; When microorganisms are imported to a contaminated site to enhance degradation, process called bioaugmentation.
- ***Ex situ* bioremediation** : Here the soil is excavated and treated elsewhere. It can be of land farming, biopiling, composting, bioreactors.
- **Land farming:** It is the process in which the soil is excavated and mechanically separated via sieving. The polluted soil is placed in layers over the clean soil and allowing natural process to detoxify, degrade and immobilize the contaminants.
- **Biopiling :** It includes a treatment bed i.e. mound of contaminated soil, an aeration system, an irrigation/nutrient system and a leachate collecting system. Treatment time is 3-6 months
- **Composting:** Here organic wastes are degraded by microorganisms at elevated temperature of usually 55 to 65ºC.
- **Bioreactors** : Here the contaminated soil is mixed with water and nutrients and the mixture is agitated by a mechanical bioreactor to stimulate the action of microorganisms.

Advantages of Bioremediation

- It is a natural process and hence perceived by public as an acceptable waste water treatment for contaminated soil.
- Useful for complete destruction of a wide variety of contaminants.
- Environment friendly.
- Uses resources available in nature to clean up contamination
- Less expensive

Disadvantages of Bioremediation

- Sometimes it may lead to partial degradation of the contaminant which may result in toxic and potentially volatile products.
- Extensive monitoring is required throughout the process
- Labour intensive and may take several months to complete the process.

Biosorption

It is the property of certain types of inactive, dead, microbial biomass to bind and concentrate heavy metals from even very dilute aqueous solutions. Biomass exhibits this property, acting just as a chemical substance, as am ion exchanger of biological origin. It is particularly the cell was structure of certain algae, fungi and bacteria which was found responsible for this phenomenon.

Bioaccumulation

It is the accumulation of substances, such as pesticides, or other chemicals in an organism. Bioaccumulation occurs when an organism absorbs a substance at a rate faster than that at which the substance is lost by catabolism and excretion. Biotransformation can strongly modify bioaccumulation of chemicals in an organism.

Biomineralization

It is the process by which living organisms produce minerals, often to harden or stiffen existing tissues. Such tissues are called mineralized tissues. It is an extremely widespread phenomenon; all six taxonomic kingdoms contain members that are able to form minerals, and over 60 different minerals have been identified in organisms. Examples include copper, iron and gold deposits involving bacteria. Biologically-formed minerals often have special uses such as magnetic sensors in magnetotactic bacteria (Fe_3O_4), gravity sensing devices ($CaCO_3$, $CaSO_4$, $BaSO_4$) and iron storage and mobilization ($Fe_2O_3 \cdot H_2O$ in the protein ferritin).

Bioleaching

The extraction of metals from ores using microorganisms is known as 'bioleaching'. In bioleaching technique bacteria such as chemolithotrophs. (*T. ferrooxidans* and *T. thiooxidans*) are utilized to oxidize metal (iron, copper and zinc, etc.) sulphides to soluble metal sulphates. The elementary metals can then be readily extracted from solution. Bacterial leaching is currently used mainly for copper and uranium. Nearly 5% of metal production and 20% of copper mined world-wide comes from this low cost technology application to low grade mineral ores such as pyrite, metal sulphides, etc. Similar techniques are applicable to enrich uranium in soil. There is possibility of its application for the extraction of other metals in future. The microbial bioleaching agents are used to recover the metals from mined materials and in the up-gradation of ores. *Leptospirillum* is used to recover low grade ores. Bioleaching can be used to extract metals directly from deep underground ore bodies. Removal of sulphur from coal, prior to its combustion, would help to solve the problem of SO_2 production to atmosphere. Microorganisms offer solution to desulphurize sulphur containing coal in an economic manner. Various species of bacteria can degrade sulphur compounds in coal to water extractable compounds. Sulphur occurs both in organic and inorganic form. Bacteria such as *Thiobacillus ferroxidans*, *T. thiooxidans* and *Sulfolobus acidocaldarius* can oxidize sulphides to sulphates which can be drained from the coal.

Conclusion

Heavy metals are the metals having atomic density exceeding 5 g cm^{-3}. Understanding the relation between heavy metals and microbes will help mitigation and management of heavy metals from the soils to tolerable level. Among the microbes, bacteria plays a crucial role in elimination of heavy metals from the environment.

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