Chapter 2

2.1. Physicochemical parameters of river water

In the world, aquatic ecosystem is one of the most productive ecosystems that supports a large proportion of the earth's biodiversity (Danovaro et al., 2008). Living organism in the aquatic ecosystem from microscopic bacteria to large plants and animals, exhibit the different type of adaptations which allow them to survive and grow in water (Sharma and Saini, 2016). Among microscopic organism cyanobacteria plays an important role for the survival of other living components in the aquatic ecosystem and are responsible as a primary producer in the river ecosystem. Aquatic ecosystems, both lotic and lentic ecosystems are also the most biologically diverse eco zones of the world.

Freshwater resources including lakes, ponds, rivers and streams etc. are under severe and increasing environmental stress (Loucks and Van Beek, 2017). As a result of increased urbanization, agriculture and other anthropogenic activities, industrialization, freshwater become polluted with different harmful pollutants (Selvakumar et al., 2017). High concentration of organic, anthropogenic and industrial contaminants in the river is the main causes of the changing of physico-chemical parameters such as temperature, pH, alkalinity, salinity, total dissolved solids, total suspended solids, hardness, dissolved oxygen, biological oxygen demand, chemical oxygen demand etc. of river water (Singh et al., 2015). The water quality and biological component of the aquatic system depends upon the physico-chemical parameters. Microbes i.e. bacteria and fungi are an integral part of the aquatic ecosystem and have ecological importance in indicating the health of the water quality (Srivastava et al., 2017). Fungi are also sensitive to change in water quality parameters like pH, salinity, alkalinity, dissolved oxygen, biological oxygen demand, turbidity etc. A few investigations have been undertaken to assess the pollution status of the Subarnarekha river (Paria et al., 2018).

2.2. Geomorphology of Subarnarekha river

Subarnarekha river is originated from Nagri village of Ranchi (Chota Nagpur plateau) and confluences at the Bay of Bengal through Jharkhand, Odisha and West Bengal (Bera, 2019). The total river basin area is about 18951 km^2 and almost of it covers in the Chota Nagpur plateau region. The Subarnarekha river entered into the state of West Bengal at Brajapur (near Muri) of Purulia district. Then, it flows through (about 40 km) the undulating surfaces of the Purulia district maintaining the local landscape condition. Again, it enter into Jharkhand state for a way of about 135 km, then passes through West Bengal state near about 70 km stretch and finally mixed with Bay of Bengal near Talsari passing through the state of Odisha with about 55 km long way. With the diversity of territorial boundary, there is a vast diversity in valley landforms along with the changing fluvial dynamics of Subarnarekha river. After passing the Jamsola, the width of the river water speed is depending on the slope and topography. The watershed level erosion susceptibility of the upper catchment area is the most valuable aspect of the basin (Dhali and Sahana, 2017). Those eroded material from the upstream is carried by river course during the storm monsoonal discharge (Wilson and Goodbred, 2015). From the upper towards the downstream the sediment grain size is progressively changing from boulder, gravel, sand, silt and clay respectively (East et al., 2015). The climatic changeability of the region is the key factor to altering the river bed and floodplain geomorphology.

2.3. Biodiversity of fungi

Fungi are a naturally valuable group of entities, those are a useful group of organisms with an broad biotechnological perspective for industrial use. Fungi, as osmotolerant organisms, that can maintain the osmotic pressure of the atmospheres through collecting small organic molecules (i.e. glycerol, sugars, mannitol) and thus maintaining low ion intracellular concentrations (such as Na^{2+}, Mg^{2+}, Ca^{2+} etc.). In recent years the taxonomic identification of different types of aquatic fungi has been increased frequently (Pietryczuk et al., 2018). However, microbial diversity and community composition can also be affected by water pollutants, so they have been proposed as sensitive indicators of ecosystem health. The impurity of natural water bodies with agricultural or urban or industrial wastewater can potentially adjust structure, composition and microbial activity on a local and global scale (Ortiz-Vera, 2018). Aerobic aquatic fungi inhabit submerged plant litter in a variety of shallow stagnant to slow-flowing freshwater bodies (Borse et al., 2016). Overall, roughly 3000 fungal species have been reported from aquatic habitats (Zhang et al., 2014). Fungi are generally categorized as

1. The Ingoldian fungi are most common fungi, found in decaying leaves of streams and lakes in many countries around the world (Tsui et al., 2016).
2. The hyphomycetes and aquatic ascomycetes mainly found in Hong Kong and North America,occurring on submerged woody material (Dhanasekaran et al., 2006).
3. The oomycetes and chytrids fungi are important for the degradation of non-cellulosic materialbut are unable to degradation of cellulosic materials (Djemiel et al., 2017).

Beside those, more than 70% of the ascomycetes are reported from freshwater habitats (Raja, etal., 2012).

2.4. Ecological role of fungus

Aquatic fungi can play an important role in nutrient cycling. They can produce hydrolytic enzymes that degrade many compounds thus contributing to the changing of aquatic environments (Ortiz- Vera et al., 2018). Fungi are associated with almost every organism, often as parasites, sometimes as decomposers and of course as symbionts. Some aquatic fungi are able to break down most of the polymeric substances of plants (hemicelluloses, cellulose, starch, pectin and to some extent lignin) (Salehizadeh et al., 2018).

2.5. River pollution

Urbanization along with industrial developement are closely related with rivers during availability of water. River is polluted by diverse mode such as sewage, fertilizer, pesticide, thermal power plant. Out of which heavy metals seem to be the chief source.

2.5.1. Heavy metals

Heavy metals are abundant contaminants that have convoyed by man from the most primitive periods, and distinct feature than additional environmental chemicals, heavy metals are natural elements that man does not generate or terminate. Heavy metals are well-defined on the basis of three different criteria i.e. density, atomic number or their chemical properties. Prior researchers has mentioned that heavy metals can be defined by the density of such metals (5 g/cm^3) which is denser than water by five times. Out of 118 elements 95 elements are metals and rest are non- metals. Metals are freshly prepared from chemical elements conducting heat and electric. Nonetheless, the metalloids have two types of characters of metal and non-metallic elements. Metalloids have a metallic appearance and suitable electric conductor. Chemically they act as non- metals (West, 2013).

Vernon suggested that a metalloid is a chemical element that, in its standard state, has (a) the electronic band construction of a semiconductor or a semimetal; and (b) an intermediate first ionization potential "(say 750−1,000 kJ/mol)"; and (c) a middle electronegativity (1.9−2.2) (Venger et al., 2007). However heavy metals have high atomic weight and mass and relatively high density (range from above 3.5 g/cm^3 to above 7 g/cm^3). Some heavy metals are more toxic to living organism's viz. mercury, cadmium and lead but ruthenium, indium but silver are less harmful to humans. While iron, cobalt and zinc are essential for the living system (Adkins, 2019).

Heavy metal pollution has become an important threat of environmental health. Metal contaminations largely effect to the decline of the water quality which is directly connected to the disease and ultimately to death living beings (Paria et al., 2018). Excessive discharge and increasing of heavy metals such as cadmium, copper, lead, nickel, zinc, mercury etc. from urban and industries were made into the soil and aquatic environments that get biomagnified with the food chain ecosystem and do a potential damage to human health (Sharifuzzaman et al., 2016).

Iron being the fourth most abundant element in the earth crust, the concentration of iron in water and sediments usually exceeds in amounts as compared to other metals. At some aquatic system, it is a cause of concern because of the elevated levels. It has registered owing to the impact of non-ore tailings associated with mining activities (Moreira et al., 2016). Increased Igeo values identified for Fe in the Mandovi estuary indicating that surface sediments are contaminated to some extent, most probably as a result of anthropogenic activities (Alagarsamy, 2006). This trace metal enrichment is attributed to iron ore processing in the higher reaches which contributes to the elevated levels of iron observed. This, however, doesn’t reflect upon all the estuaries as because not all estuaries are the receiving grounds of mine tailings. Iron has become a subject of study because of the multifarious uses. This subsequently leads us to the assumption that iron is inadvertently released into the environment in various forms which finally find its way to the estuaries and oceans. It is being concluded that industrial, domestic, and agricultural pollutant sources are likely to cause increasing problems in near future.

2.6. Fungi versus heavy metal

Fungi belong to the detritus ecosystem of earth. They can help from agriculture to biological based industries such as fermentation industries, waste water treatment plant, pharmaceutical industries etc. Besides fungi, many microorganisms are able to change the chemical structure of the metal ions by reduction, bioaccumulation, and immobilization process. For that purpose, they are important in the bioremediation of contaminated soils and water (Ayangbenro and Babalola, 2017). Among the microorganisms, fungi are very important for bioremediation due to their mycelial nature and well accumulation ability of all kinds of metals (Siddiquee et al., 2015). Fungi can resist heavy metals through various mechanisms, i.e., chelating of heavy metal to the cell wall, active transport of metal ions outside the cell, enzymatic transformation of metal ions, forming vacuoles in which metal ions are assembled and immobilization that produces melanin, and inside the cell produce of specific metal-binding compounds (Anand, 2018). Fungi can protect environmental biodiversity by the recovery of heavy metals through successive metabolic reaction. Among the several reactive compounds related to bacterial cell walls, the extracellular polymeric materials are of particular importance and are well recognized to have significant effects on metal ion adsorption (Palza, 2018). Microbial EPS can bind heavy metals by micro-precipitation and proton exchange mechanism (Cao et al., 2020).

2.6.1. Metal toxicity of fungi

Toxic metals have harmful effects due to their strong coordinating abilities with biomolecules (Anjum et al., 2012). Heavy metals have shown toxicity by various ways such as blocking of functional groups of molecules, the displacement of functional groups, the conformational change of proteins and enzymes (Tamás et al., 2014). Interactions between metals and fungi depends upon ionic state and concentration of metal ions and physico-chemical factors of the environment (Fernández et al., 2018). Although cell membrane is a primary site of toxic metal binding and membrane damage by loss of mobile cellular solutes (Macomber and Imlay, 2009). Organometallic compounds have environmental importance because of their use in petroleum and chemical industries. These are more deadly than equivalent free metal ions and the harmfulness of their compounds differs with the number of the organic groups (Duyck et al., 2007). This metal may also oxidize cell membranes by the production of free radicals (Wickens, 2001).

2.6.2. Resistance and tolerance

Fungi can survive in the presence of toxic metals that depends upon biochemical and morphological properties of fungi. (Fashola, 2016). Resistance is the ability of an organism to survive against metal toxicity by environmental modification e.g. synthesis of metallothione ins or gamma-glutamyl peptides (Limón-Pacheco and Gonsebatt, 2009).

2.6.3. Environmental effect on heavy metal toxicity to fungi

The environmental physico-chemical properties define metal availability and toxicity. A pH can effect fungal responses by effects on metal specification and mobility. Increasing pH can result in the development and precipitation of metal hydroxides or oxides. In an aqueous solution, divalent metal cations form multiple hydroxylated species. The different hydroxylated forms have different toxicities (Sandrin and Maier, 2003). The hydrolysis product formation may also favor bio sorption. Clay minerals can absorb heavy metal cations and decrease their possible toxicity (Uddin, 2017).

2.6.4. Fungi in polluted habitats

A range of fungi may be found in metal-polluted habitats and they are able to survive and grow in the presence of possibly toxic concentrations. In general toxic metals are affected to fungal populations by reducing species diversity and selecting for a resistant/tolerant population. However, the effect of toxic metals on the microbial abundance in natural habitats varies with the types of metal and environmental factors (Gadd, 2016). In Figure 2.1 heavy metals are precipitated from upper surface of water by action of fungi and purified water released. Quantity of fungi such as *Penicillium sp.* and *Oidiodendron sp.* significantly decline at contaminated sites whereas in Zn and Cu polluted soil, *Paecilomyces sp* and *Geomyces sp.* has increased with growing pollution

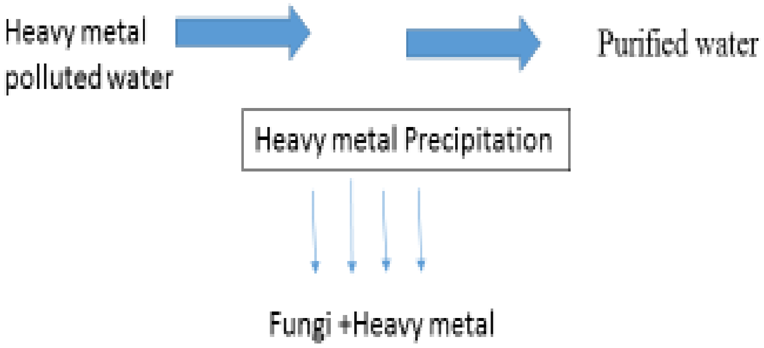

Figure 2.1: General scheme of Heavy metal removal by fungi

(Purohit, et al 2018). Mercury containing golf green contain *Trichocladiumasperum, Trichoderma hamatum, Zygorrhynchus moelleri* and *Chrysosporium pannorum* whereas *Chaetomum sp., Fusarium sp., Penicillium sp.* and *Paecilomyces sp*. were greatly decreased in untreated locations (Herrera-Estrella and Guevara-García, 2001). Nonetheless, some microbial metal tolerance is also found in the genus *Penicillium* such as *P. ochrochloron* can grow in saturated $CuSO_4$ isolated from industrial effluents whereas *P. lilacinum* isolated from soil polluted by mine drainage.

Heavy metal pollution is very common and while there may be significantly decrease in microbial diversity (including bacteria) but numbers of filamentous fungi and non-pigmented yeasts are generally less affected (Lotlikar, 2019). In fact numbers of *A. pullulans* showed a positive correlation with lead (Pb) concentrations (Hawkes, 2013). On the other hand, adaptation was not necessitated for the growth of *A. pullulans* on the polluted surface and the capability to bear high concentrations of lead occurred in isolates from the unpolluted site (Thomas, 2016). In distinction, the numbers of *Sporobolomyces roseus* and heterotrophic bacteria were very low in contaminated samples while numbers of *S. roseus* do not display a significant positive correlation with increasing lead concentrations (Rybakova et al., 2016.). Mercury tolerant fungi are generally found in the surfaces of the seeds treated with mercury compounds. These fungi are *Pyrenophora avenae, Penicillum crustosum. Cladosporium cladosporoides, Syncephalastrum racemosum* and *Ulocladium atrum* etc. (Iqbal et al., 2014).

Therefore concentrated heavy metals can affect the qualitative and quantitative composition of fungal populations. Phyllo plane micro-flora may be subjected to the influence of potential toxicants, e.g. SO_2 (Mandava, 2018). Generally polluted soils may be nutrient-poor, of variable pH and also contain additional toxicants. These factors may affect fungal populations. Furthermore, there are well known difficulties in obtaining meaningful assessments of fungal diversity. In spite of this, it is less apparent that certain fungi can show considerable tolerance towards toxic metals and can become dominant microbes in some polluted habitats. However, species diversity may be decreased in certain cases. Resistance/tolerance can be exhibited by fungi from both polluted and non-polluted habitats. Fungal populations were reduced and there were alternations in species composition in polluted soil near a zinc smelter (Laszczyca et al., 2004). Zn-tolerant genera are *Bdellospora sp., Verticillum sp.* and *Paecilomyces sp.* with *Penicillium sp., Torula sp.* and *Aureobasidium sp.* Cu and Ni tolerant fungi (defined as being capable of growth at approximately 16 mM Cu^{2+} and or Ni^{2+}) were isolated from both control and contaminated sites, the predominant genera being *Penicillium sp.* (60%) followed by *Trichoderma sp., Rhodotorula sp., Oidiodendron sp., Mortierella sp.* and *Mucor sp.* Previous studies revealed that in many cases survival must be dependent on intrinsic properties of the organisms rather than adaptive changes and physico-chemical properties of that environment (Zhang et al., 2019).

2.6.5. Interactions between toxic metals and fungi

In common, microbes mediated metal exclusion or recovery from a contaminated site may include the following pathways:

a) Metal cations may bio-absorb on cell surface and bioaccumulate within the cell uptake through precipitation.

b) Metal ion may be passively transferred through metal-binding proteins inside the cell.

c) Heavy metals react with extracellular polymers produced by microbes and precipitate.

d) Enzymatic biotransformation and metal volatilization occurred in the cell.

2.7. Extracellular precipitation and complexation

Several extracellular fungal products can complex or precipitate heavy metals. Among organic acid, citric acid act as an effective metal-ion chelator and oxalic acid can interrelate with metal ions to from insoluble oxalate crystal around cell walls (Nahar, 2019). *Debaryomy ceshansenii* grown in Fe-deficient media or in the presence of copper, cobalt and zinc that produced riboflavin or a related compound.

2.7.1. Metal binding to cell walls

The cell wall is the primary cellular site of contact with metal species. Metal removal from solution may be fast though rates will depend on factors such as type of metal ion and biomass, the concentration of metal and environmental factors. Metabolism independent association of metal species to fungal walls may contain adsorption, precipitation, ion exchange, complexation, and crystallization.

The fungal cell wall has vital protecting properties acting as a barrier, controlling the uptake of solutes into the cell including potentially toxic metal (Emamverdian et al., 2015). It also indirectly affects the intracellular ionic composition by control of cellular water. The wall is mainly made of polysaccharides, some of which may have associated protein with other components with lipids and minerals. Variability of active sites may be involved in metal binding including carboxyl, amine, hydroxyl, phosphate and sulfhydryl groups although their relative implication is usually difficult to resolution (Paria et al., 2018). However primary relations probably involve binding to carboxyl and phosphate groups which may be induced by electrostatic interaction to other negatively charged functional groups. Metabolism independent biosorption is frequently rapid and unaffected over moderate ranges of temperature, e.g. 4-30^{0}C (Javanbakht et al., 2014).

2.7.2. Transport of toxic metal cation

The plasma membrane is the main cellular transporter of fungi. Most work on metal ion transport in fungi has concerned k+ and Ca+ largely due to their great importance in fungal growth, and metabolism. It now seems that the plasma membrane of fungi i.e. *Saccharomyces cerevisiae* may have at least 2 classes of K+ selective channels, the first of which is voltage-gated and the second is functioned via second messengers blocking by divalent cations. Yeast plasma membrane ATPase can be initiated by possibly toxic metal ions e.g. Cd^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+} and other organometallic compounds.

The filamentous fungi have been revealed the energy-dependent transport of many divalent cations. In count the metal concentration effect on the structural integrity of the cell membrane. Di-valent cation transport is dependent on the plasma membrane H+-ATPase activity (Hogan, 2016).

2.8.Metal transformations

Fungi as well as other microorganisms can effect chemical transformations of metals by methylation, de-alkylation, oxidation, and reduction (Siddiquee et al., 2015). Some enzymatic metal conversions may be involved in survival. In the meantime, certain transformed metal species are less lethal or more volatile than the original state of metals. Plasmid-mediated reduction of Hg^{2+} to Hg^0 is a well-characterized metal resistance system in bacteria and there is only a limited amount of evidence for the same process in yeasts and filamentous fungi. Metalloids and methylation of Hg and other metals can be catalyzed by several fungi and may be noticed as a detoxification mechanism. Meanwhile, methylated species are usually more volatile and may be absent from the environment (Ghimire et al., 2019).

2.9.Evolution of bioremediation

Bioremediation is considered an alternative method for removing heavy metals ions from the polluted area. Bioremediation is obviously living organisms to reduce the environmental pollutants into less toxic forms. It is followed by bacteria, fungi and plants to degrade or detoxify hazardousingredients to human health /or the atmosphere. The previous report has well-defined bioremediation as a procedure by which inorganic or organic waste transformed and degraded to innocuous materials. On the other hand it is a technology for eliminating pollutants from the environment for re-establishing the natural surroundings and avoiding pollution (Oyewole et al., 2020). Bioremediation can corroborate less costly than other technologies for spending clean-up of harmful waste. Maximum bioremediation procedures include oxidation-reduction reactions where either an electron acceptor (commonly oxygen) is added to excite oxidation of a reduced pollutant (e.g. hydrocarbons) or an electron donor (commonly an organic substrate) is added to reduce oxidized pollutants (nitrate, oxidized metals, chlorinated solvents, propellants and explosives) (Daghio et al., 2017).

In the late 19th century wastewater handling plants and biotreatment has become a better-engineered method (Mahimairaja et al., 2005). In polluted environments, native microorganisms have shown a significant role in bioremediation as well as biodegradation of contaminating materials at proper environmental circumstances (Varjani, 2017). The process of bioremediation was designed by George M. Robinson. In 1960s Robinson pioneered the idea of making mixtures of dried bacteria cultures for commercial use. In 1972 first commercial in situ bioremediation system was installed to clean up a Sun oil pipeline spill in Ambler, Pennsylvania. According to the Toxic Substances Control Act of 1976 genetically modified organisms are used in bioremediation under controlled conditions (Sharma et al., 2018). In 1979, Dr. Anand Mohan Chakraborty, an American scientist introduced recombinant DNA technology based bioremediation using a strain of *Pseudomonas putida* (Bharagava, 2017).

2.10. Bioremediation

2.10.1. Introducing microbes based clean up system

Soil inhabiting microbes like fungi, bacteria and actinomycetes are carried out biodegradation in optimal physico-chemical parameters. Bioremediation is the microbe-mediated process for control of the pollutants by all potential toxins like agrochemicals, hydrocarbons, and other organic toxicants. But for inorganic toxic compounds microbes are incapable to make simpler them into harmless compounds according to their specialization for the type of toxins.

Therefore the bioremediation policy for heavy metals depends on the vigorous metabolizing abilities of microorganisms. Several microbes are known to necessitate variable amounts of heavy metals as essential micronutrients for growth and improvement. For example, Fe^{3+} is essentially required by all bacteria while Fe^{2+} is important for anaerobic bacteria (Mergelsberg, 2017). There are anthropogenic sources of heavy metals -

1. As: wood preservatives, pesticides, ore mining, biosolids and smelting

2. Cd: Plastic stabilizers, electroplating, paints and pigments, phosphate fertilizers

3. Cr: Fly ash, Tanneries, steel industries

4. Cu: Fertilizers, pesticides, biosolids, ore mining and smelting

5. Hg: Medical waste, Au-Ag mining, coal combustion

6. Ni: Surgical instruments, effluent, automobile batteries, kitchen appliances.

7. Pb: Insecticide and herbicides, batteries waste, aerial emission from combustion of

leaded fuel.

Though, the adsorption capability depends on microbial total biomass and geochemistry of the microbial system. These reduction or oxidation reactions are happened due to enzymatic activity and biomass concentration of microbes. Microorganisms have a great deal of unexplored potential for bioremediation of soil pollutants and growing the crops manufacture with minimum input. Microorganisms as metal accumulators have an inherent novel remediation property for toxic metals in the soil. Detoxification and rehabilitation of contaminated soil with the use of microbes have occurred as the safest, easy and effective technology. Natural soil-based microbes have been explored and attached for their capability to eliminate or detoxify toxic products released due to human activities in the environment viz. pesticides, plastic, mining of ores, organic solvents, pigments, fuel and industrial processes etc. (Murad, 2019). The biological route for the redistribution of the organic pollutant in the soil starts from several physical, chemical and biological procedures resulting in adsorption by soil particles and root tissues, volatilizat ion, transportation through air, water and microbial breakdown and leaching, etc.

2.10.2. Fungal based bioremediation

Fungi are vital decomposers of organic substances and play a key role in carbon cycling and food chain dynamics in marine and terrestrial environments, also including mutualistic, pathogenic and parasitic taxa. In Figure 2.2 depicted that heavy metals are accumulated by fungal EPS, then degraded though aerobic or anaerobically and ultimately release less toxic metal or leached out from water.

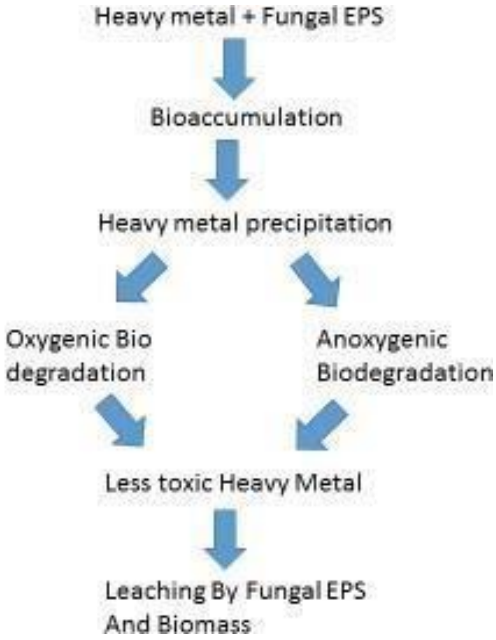

Figure 2.2: General scheme of Bioaccumulation of heavy metal by fungal system

The species of *Aspergillus niger* from estuarine residues can tolerate heavy metals such as Pb and Hg. The binding machinery between functional groups is the presence of cell surface of the biomass and metal ion that was considered by fourier transform infrared spectroscopy (FTIR), which confirms the existence of hydroxyl, amine, phosphate and carboxyl groups (Paria et al., 2018).

2.10.3. Mechanisms of bioremediation

Microbes have mutual defense system viz. production of contaminant degradative enzymes as well as resistance to significant heavy metals. Various mechanisms of bioremediation are identified like biosorption, metal-microbe contacts, bio-accumulation, bio-mineralizat ion, bioleaching and biotransformation. Numerous pollutants are organic solvents which disturb membranes, but cells may grow defense machineries (Verma and Kuila, 2019). For example, plasmid-encoded metal efflux pump involving ATPase and ion/proton pumps are described for As, Cd and Cr resistance in many bacteria (Maitra, 2016).

a. *Bioremediation by absorption*

Among the numerous reactive compounds related with bacterial cell walls, the extracellular polymeric substances (EPS) have significant metal absorption capacity (Wei et al., 2011). Studies on the metal binding activities of EPS exposed a great aptitude to complex heavy metals through various mechanisms, which include proton exchange and micro-precipitation of metals (Cao et al., 2020). Recent studies have considered the proton and adsorbed metals on bacterial cells and bacterial EPS (Fang et al., 2011). This results in an incapability to perfect and envisage the process and progress a usual bioremediation procedure in the field (Lourenço et al., 2019).

b. *Bioremediation by Physico-Biochemical mechanism*

Saccharomyces cerevisiae acts as a bio sorbent for the elimination of Zn (II) and Cd (II) through the ion exchange procedure (Njikam and Schiewer, 2012). Heavy metal degradation includes energy for cellular metabolism. The combined effect of passive and active bioremediation can be named bioaccumulation (Dixit et al., 2015). Microbial biosurfactants can form stronger ionic bonds by metals and form complexes earlier being desorbed from soil matrix to water phase during little interfacial tension (Sarubbo et al., 2015).

Bioremediation are aerobic or anaerobic. Aerobic remediation frequently includes the presence of oxygen which is mediated by dioxygenases, monooxygenases etc. Anaerobic degradations of pollutant involve early activation reactions followed by oxidative metabolism mediated by anaerobic electron acceptors. Two main machineries for growth of resistance in bacteria are detoxification and active ion efflux of the toxic metal from cells (Ma et al., 2016). The microbe takings energy for growth through oxidizing the organic compound with Mn (IV) or Fe (III) as an electron acceptor (Lovley et al., 2011). Several defense schemes (compartmentalization, rejection, complex synthesis of binding protein or peptides) reduce the toxic metals (Pattanayak et al., 2014).

C. Molecular mechanisms of bioremediation process

Numerous mechanisms intricate in the elimination of heavy metals by microbes are known. Genetically engineered bacterium *Deinococcus geothemalis*, Hg decline has been stated at high temperatures for presence of mer operon from E. coli coded for reduction of Hg^{2+} (Ledwidge, et al 2005). Mercury resilient bacteria *Cupriavidus metallidurans* (MSR33) was altered genetically by presenting a pTP6 plasmid that providing genes (merB and merG) changeable Hg biodegradation alongside with the production of mercuriallyase protein (MerB) and mercuric reductase (MerA) (Ojuederie, & Babalola, 2017). Genetic engineering of *Deinococcus radiodurans* (radiation resistant bacterium) which generally reduces Cr (IV) to Cr (III) (Rashid et al., 2019). Bacterial metabolites like metal-bound coenzymes and siderophores mostly involved in the degradation path.

2.10.4. Bioremediation and biochemical entities of fungal EPS

Extracellular polymeric substances (EPS) are metabolic products. Their production by selected microorganisms was first reported in the 1880s (Gupta et al., 2020). Microbial EPS has several applications like sludge settling and dewatering. EPS have a huge number of the charged group that can efficiently remove the contaminant (Nouha et al., 2018). EPSs are a heterogeneous mixture of polymers comprised of polysaccharides, proteins, nucleic acids, and phospholipids (Morales- García et al., 2019). Besides that fungus EPS have been reported in cyanobacteria and marine microalgae. Generally, EPSs have often been reported in bacteria and cyanobacteria; however, they have also been reported in the marine microalga *Chroomonas sp*. *Dunaliella salina*, the medicinal mushroom *Phellinus linteus*, yeast, basidiomycetes and marine microorganisms (Jha, 2013). EPS-producing microorganisms have been isolated from various regular sources of both terrestrial and aquatic environments, viz. brackish and freshwater, wastewater, soils, and also stressful environment such as hot springs, hypersaline and halophilic environments, salt lakes etc. (Casillo et al., 2018). Microbial polysaccharides show biotechnological promise in pharmaceutical industries as immunomodulatory and healing**.** Research interest in the microbial EPS and its uses in bioremediation has increased due to their wide structural, physical and chemical diversity (Shukla, 2019).

2.11. Microbial exopolysaccharides: variety and potential applications

The chemical nature of EPS involved in flocculation is capable of binding with metallic ions to remove heavy metals from the environment. Microbial EPS can bind cations of toxic metals protecting and essential cellular components (Gupta and Diwan, 2017).

2.12.1 Anti-microbial activities of fungus

The *Aspergillus* genus is one of the dominant marine fungal genera and the marine fungal strains from *Aspergillus* sp. produced more new antibacterial and antifungal compounds than any other genus. The scientific community recently celebrated the 90th anniversary of Sir Alexander Fleming’s discovery of penicillin, which marked the starting point of the era of antibiotic chemotherapy. Most other commercial antibiotics are essentially derived from *Streptomyces sp*. or even from other prokaryotes. Antimicrobial resistance has been a major health issue and presents a threat to the health care system. Fungi have a close association with host plants and can produce asexual growth endorsing composite, competitiveness and defense of the host against herbivores and pathogens. Fungi, which used for medicines preparation (Paterson, 2008). These microorganisms are well known to produce bioactive secondary metabolites such as alkaloids, terpenoids, steroids, quinones, isocoumarins, lignins, phenylpropanoids, phenols, and lactones (Lingakumar, 2018).

2.13 Eco- management and Eco-monitoring

Eco- management is entirely a dynamic and emerging conception that deals with the management for the environment encompassing a business. It represents the structure of organization, responsibilities sequences, and processes the improvement of an environmental corporate policy. The basic purposes of good eco-management are design, controlling, arrangement and planning of the environmental process and environmental management programs. Industrializat ion promotes economic development but environmental pollution will be an ecological nightmare. Hence, it has become vital to take into account the ecological consequences while setting up an industrial unit. Advance technology is obtainable today to decrease environmental pollution and it must be used to correct the excesses of ecological violence and to reduce the level of environmental pollution. For all these, a proper balancing and reporting of environmental information is a must which can lead to sound “Ecological Management”. The environment takes into consideration all conditions required for the survival of corporate sectors. Absence of environmental concern effects serious ecological damage. Poverty, lack of resources, population pressure and global inequity of resource using is generating unparalleled social and environmental problems at national and global levels. Sustainable development has a tendency to strike a balance between the demands of economic development and the need for management of environmental processes. Environmental management is especially valuable for internal managing initiatives with a specific environmental focus, such as bioremediation, biodegradation for sustainable ecological service. Several approaches to effective ecosystem management involve conservation efforts at both background and local that include natural resource management. Traditional supervision of ecological schemes emphases on goods or services preferred by the public, with prominence on profitable commodities. Resource managers only freshly have begun to appreciate the association of an ecosystem's condition and for sustainability to human. Allochthonous and autochthonous sources of nutrients influence the growth of aquatic fungi, which can control the level of pollution.

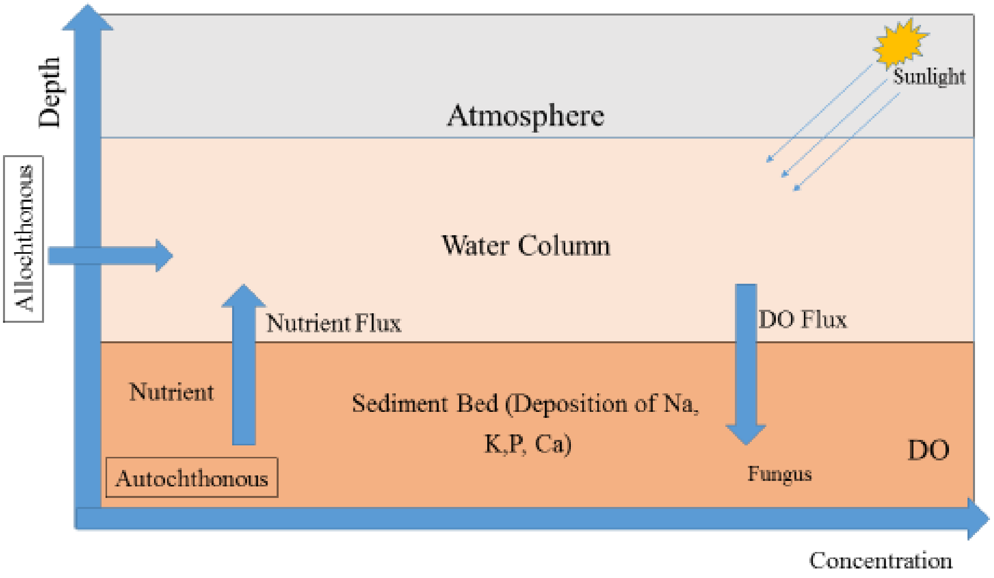

Figure 2.3. General scheme of eco-management and eco-monitoring by detritus food chain including fungi

As a result, eco-management supports corporate and other organizations to boost their public belief and confidence and are related to receiving a reasonable valuation. The already degraded environment demands its diligent management. Through eco- management, enterprises can enjoy the following benefits:

I. Pollution control being a hot subject of conversation, environmental management displays the degree to which pollution has been well-ordered by the communal.

II. Ecological management draws attention in another sense, which offers an idea about industrial development, a nation’s economic progress and social welfare and the fulfillment of responsibility towards society.

III. Eco-management is co-operative in discharging structural responsibility and increasing environmental pellucidity. Sustainable expansion is possible with the advantage of environmental management as it helps include ecological aptitude of innovativeness.

IV. Negotiation between management and society helps organizations seek to strategically manage a fresh and emerging issue with distinct users.

V. Ecological management supports green reporting to combat effectively all negative public opinions in the global economy where the existence of a strong environmental lobby against environmentally unfriendly industries is found.

VI. Ecological management improves performance through better management of environmental cost and thus, benefits the human and natural environments.

VII. Ecological managing services shared subdivisions to fulfill their promises towards introduction and change, and thus appears to be reactive to new factors. Countries giving significance to the ecological characteristic of activities are becoming more and more popular, predominantly in Western Countries.

VIII. Ecological management reflects unsound production and consumption patterns, misuse and scanty use of resources and assets like water.

IX. Optimal distribution of scanty resources in the economy is possible with the support of environmental management.

X. Ecology is essential in measuring a nation’s economic development, social welfare, industrial development, pollution control and in fulfilling the needs of government, still the system is in its infancy and not all countries have been able to develop such a system. But with the passage of time, the system will gradually develop, research will be undertaken and it will fulfill the requirements for which it was originated.

XI. Impressive decision making through the application of environmental management reduces or eliminates many environmental problems.

The concept of water quality monitoring has been initiated with a view of water quality management in order to restore and maintain natural water bodies. Eco- monitoring is defined as the process of programmed and repeated observation measurement and recording for defined purposes of one or more parameters (physical, chemical and biological) of an environmental setup. It is usually done using physical parameters like temperature and other chemical variables like the concentration of key nutrients of a significant pollutant. Chemical monitoring is quite popular, being highly developed. However, it does not provide information regarding the long-term effects of pollution on the ecosystem. By taking merely measurements of abiotic parameters to assess river quality has several disadvantages, and does not wholly reflect river health (Crane et al., 2008).

Sometimes it is not so easy to detect pollution status by a physicochemical way and also it appears to be very expensive. Biomonitoring and establishment of indicator species is now a growing subject for research to combat increasing pollution threats. Many organisms are used as bioindicators to assess the effects of pollution on freshwater ecosystem viz. bacteria, fungi and algae.

The advantages of biomonitoring are: (1) biomonitoring reflects overall ecological integrity (i.e., physical, chemical and biological); (2) it provides a holistic measure of the environmental condition by integrating stresses over time; and (3) to create a community improved understand of alive organisms as measures of a "healthy" environmental condition (Balderas et al., 2016).

2.13 Metal uptake by living cells

Penicillium sp. can eliminate a variety of heavy metals from aqueous solutions. Spores of *Penicillium italicum* were revealed to accumulate copper (Abdulaziz and Musayev, 2017). The uptake was believed to be an ion-exchange reaction. *Penicillium spinulosum* has been shown to remove copper, gold, zinc, cadmium and manganese (Siddiquee et al., 2015). The metal uptake bynon-growing mycelia reached an equilibrium in 60-120 min. The uptake of heavy metals by fungalcells was usually very fast and about 90% uptake was attained in 10 min. The metal accumulationof growing cells varied with the cell age. The maximum metal uptake took place during the lag period or the early stages of growth and decayed as cultures reacheda stationary phase. *Aspergillus niger; P. spinulosum* and *Trichoderma viride* displayed a similar uptake pattern (Ahmad et al., 2011). The reduction in metal-uptake pattern may be a drop in pH during growth, but a reduction in metal uptake at constant pH can be described by the changes in cell-wall composition withgrowth and the release of metabolites binding with the metal ions. Copper uptake by living *S. cerevisiae* yeast cells was biphasic, consisting of an initial rapid surface binding of copper ions, followed by a second, slower, intracellular uptake of copper. The initial rapid uptake phase was completed in 5 s and the second, slower phase lasted for 150 min. Cadmium (II) and Pb (II) uptake was observed to be through surface binding only (Asuquo et al., 2017). Copper uptake by the fungus (*S. cerevisiae)* was observed to be slightly higher in the presence of glucose, indicative of an intracellular uptake mechanism (Wang and Chen, 2006). The higher uptake underaerobic conditions could be due to the presence of active copper-binding protein metallothione in. The removal of copper from aqueous solution by *Penicillium italicum* has been described by the Freundlich and Langmuir isotherms (Sarı and Tuzen, 2009) for *R. arrhizus* the copper removal was described by BET isotherm (Wen et al., 2015). The metal uptake by yeast for a range of metal ions did not follow the Langmuir or Freundlich isotherms and depended on cell density (Kapoor Viraraghavan, 1995). The uptake of mercury by *S. cerevisiae* was described by the Langmuirmodel for the initial phase; this was followed by a transition phase and then a multilayer or penetration phase was observed for metal-ion uptake (Daneshfozoun et al., 2018). The uptake of Ni, Zn, Cd and Pb by the mycelium of *Penicillium digitatum* was highly pH-sensitive and was inhibited below pH 3 (Zango et al., 2018). Huang et al. also observed that cadmium biosorption on various fungal strains was pH sensitive. *Aspergillus oryzae, Fusarium solani and Candida utilis* were found to perform better in the acidic range (Verma et al., 2017). The change in the sorption capacity with pH can be explained on the basis of proton-competitive adsorption reactions (Galamboš et al., 2012). The lower uptake at higher biomass concentrations can be attributed to the electrostatic interactions of the functional groups at the cell surfaces. The cells at higher concentrations in suspension attach to each other, thus lowering of the cell surface area in connection with the solution. The growth conditions also affect the metal uptake of the biomass. Several researchers observed that *S. cerevisiae* cultures grown on a synthetic medium had a higher uranium uptake rate than cultures grown on a rich, organic medium, while the uranium uptake rate was found to be the same for cultures grown under aerobic or anaerobic conditions (Leitão, 2009). It was observed that growth, uptake capacity and bio-sorptive yield (biomass concentration x uptake capacity) were enhanced by the addition of mineral salts to the growth media (Das, 2010). Growth media control the cell-wall chemistry (formation of various functional groups on the cell surface)and can lead to augmented metal uptake ability.