

MYCOREMEDIATION OF HEAVY METALS

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ABSTRACT

These days people are bending towards advanced things like in previous time's people uses organic and natural things and prefer more simple things but now a day's people are adopting industrial processes and coal mining which is creating great problems to the environment, as well as flora and fauna, live within. There are various anthropogenic sources due to which our natural environment is polluting day by day mainly the pollutants, which are responsible for this are heavy metals. So, dispose of these heavy metals and technologies are very costly to avoid the technologies there are other methods, which are cheap and eco-friendly and can treat contaminated water. If we cannot use the costly technique then another technique is obtained i.e. bioremediation which does not harm the mother-nature and sustainable in nature, during this technique fungi and microbes are used due to its ability of adsorption and accumulation of heavy metals and use it in a good manner. Bioaccumulation, bio adsorption, biomineralization, biosynthesis and bio-oxidation is the most important feature of fungi in bio mechanisms. The factors, which influence the bioremediation process, are the HMs pH, temperature, fungal biomass dose and rate of shaking as these features vary according to the heavy metals nature. In this review paper, we have discussed these terms briefly about fungi and microbes in which species fungi comes under how it works and the brief process of bioaccumulation, adsorption, absorption and others along with this we have also discussed the microbes and their characteristics.

Keywords: fungi, microbes, bioaccumulation, bioremediation, mycoremediation, heavy metals

INTRODUCTION

Now a days the increasing activities of industries and development areas affecting the mother-nature and it is reason foreign elements are getting introduced which include pollutants like explosive, hydrocarbons, solvents, chemicals originated from agriculture lands along with heavy metals like copper, arsenic, cadmium, nickel, lead, manganese, iron etc. they are the most harmful elements and released in very huge amount and these are the anthropogenic sources by which elements come directly or indirectly in the environment. As many metals act as micronutrients if they are applied inappropriate amount but if their amount increased then they become toxic and act as heavy metals but if they are in the proper amount they are very useful for the growth and development of the plant and also help in metabolic activity as metalloenzyme. Heavy metals have special properties like one of them is high solubility in the aquatic medium due to which it is taken by aquatic organisms but due to its other property like they are non-biodegradable and can cause a huge problem in future as it has high toxicity and long persistence. As by entering into the aquatic part of the environment they can easily interfere in the food chain by entering in drinking water or the crops by irrigation which can cause the problem in human beings as they affect their health as well as other flora and fauna. By continuous use of these heavy metals, it is causing great concern and its remediation is very important. Now after seeing emerging problem due to this heavy metals many scientists and researchers are taking steps to control this problem but first, it is important to develop a technique for contaminated water treatment, as they came up with some solutions like coagulation by chemicals, filtration of membranes, ion exchange which can treat wastewater polluted by heavy metals, but

as the amount of heavy metals increases this technique will not work because it can work only if the heavy metal is present less than 100mg/l. therefore, bioremediation is used to treat wastewater or any contaminated place as this method is eco-friendly and does not harm the environment because it is done by using fungi, microbes and various species of plants. as we all know there are various types and species of microorganism for different condition different microorganism are present which can cope up with conditions and they have the ability of tolerance as if there are the presence of heavy metals they can use that as their nutrients due to its special characteristic of absorption and adsorption, as they not only absorb this HMs but also reduce it from the environment and convert it into toxic to non-toxic nature. In bioremediation, process fungi are used because of their resistant and tolerant feature as in some aquatic places they become dominant then heavy metals this is the reason fungi are used in this field. The technique called mycoremediation in which growing fungi are used one of the examples is *Aspergillus niger* which acts as multi-tolerant fungi. To remove a different type of heavy metals they used different types of fungi like *Penicillium*, *Aspergillus*, *Trichoderma*, *Fusarium* etc. as they perform various strategies within the cell wall like cell surface precipitation, detoxification, accumulation, efflux and alterations. In this review paper, we have discussed various fungi and microbes how they reduce the effect and amount of heavy metals from an environment and the process. Earlier, we have mentioned about bioremediation below it is discussed in an elaborated manner as these organism actions also depends upon the behaviour of heavy metals so before using any microorganism it is important to know the properties of heavy metals.

TOLERANCE CAPACITY OF FUNGUS:- An organism has the tolerance ability even in the most adverse condition or environment but for most of the organisms heavy metals are toxic. Some of the fungi are metal tolerant as they continue to grow in an environment which is polluted by heavy metals. The fungi can tolerate or detoxify the heavy metals by some special process i.e. detoxification by enzyme, by the active or passive diffusion within the cell due to accumulation, permeability barriers exclusion, cell wall adsorption, capsule adsorption or slime adsorption, metalloids chelation and volatilization etc. (Zafar et al., 2007). But as per the production of an intracellular and extracellular chelating agent, some mechanism depends upon them. Under the presence of heavy metal much morphological response has been seen like colour morphology changes, elongation of cell or ruptured cells, growth of mycelia towards outwards direction whenever heavy metals are present and it has been observed in various studies (Abbas et al., 2016). Most of the metal tolerant fungi are isolated by contaminated soil and water. The heavy metals concentration, toxicity and their bioavailability is responsible for the response of fungi towards metal and also to their resistance, the fungi genetic background, the concentration of HMs, environmental conditions, availability of nutrients and various types of heavy metals is responsible for the fungi resistance nature. Some of the Heavy metals resistant fungi are *Trichoderma harzianum* and *Aspergillus flavus* CR500. The table 1 shows that most of the fungi have come under ascomycetes class and tolerant towards then heavy metals. In different habitats, these fungi class is distributed and also plays a major role in nutrient cycling and stabilizing of soil in an ecosystem, but there is no proper proof about the relation between them that how they came in same fungi class and highly tolerant to metals.

Table 1:- CLASS OF FUNGI USED FOR THE REMOVAL OF METALS/ POLLUTANTS

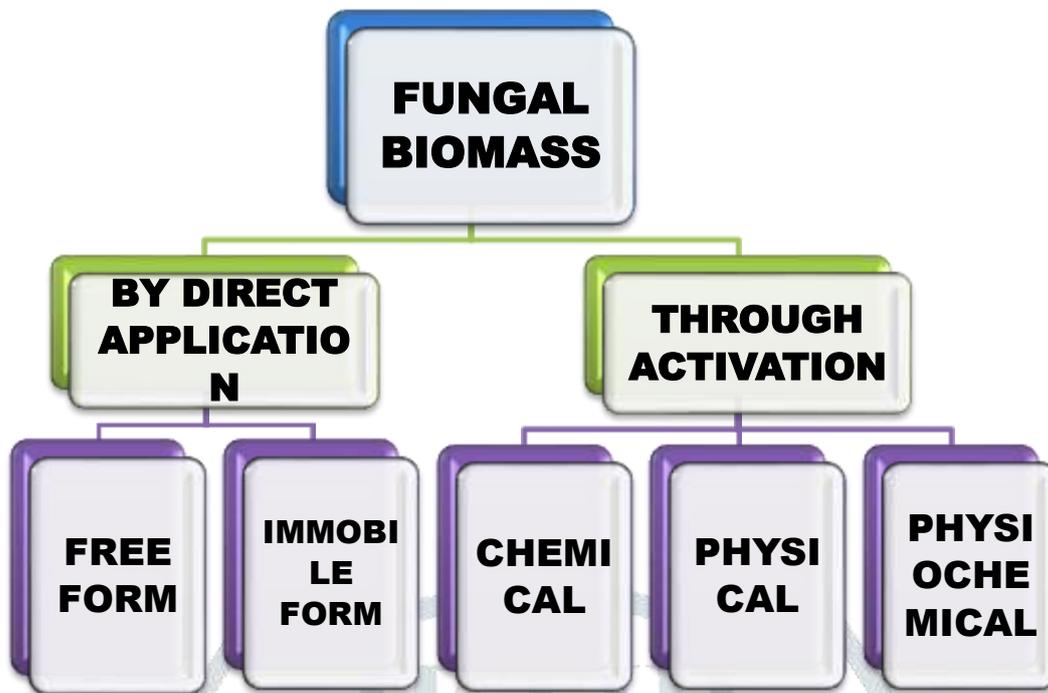
FUNGI	METAL	CLASS
<i>Aspergillus oryzae</i>	Chromium (Cr)	ASCOMYCOTA
<i>Aspergillus flavus</i>	Lead (Pb)	
<i>Botrytis cinerea</i>	Copper (Cu)	
<i>Fusarium oxysporium</i>	Lead (Pb)	
<i>Humicola sp.</i>	Cadmium (Cd)	
<i>Penicillium sp.</i>	Zinc (Zn), Chromium (Cr), Lead (Pb), Aluminium (Al)	
	Cadmium (Cd)	
<i>Trichoderma sp.</i>		

<i>Ganoderma sp.</i>	Zinc (Zn), Lead (Pb), Cadmium (Cd)	BASIDOMYCOTA
<i>Pleurotus sp.</i>	Cadmium (Cd), Chromium (Cr), Mercury (Hg)	
<i>Rhizopus oryzae</i> <i>Mucor sp.</i>	Zinc (Zn) Chromium (Cr)	ZYGOMYCOTA

To prove this one of the scientists Challaombe et al. (2019) investigated about the similarities between the genomic and secretomic to enhance their presence in the decomposition of biomass and parthenogenesis by different species of fungi in the arid environment i.e. Phoma CK108, Aspergillus CK392, Embellisc CK46, Coniocheeto CK134 and Chametomrum CK152, they all comes under ascomycetes (**Hua et al., 2012**). As per the analysis it has been observed after the test that all fungi can easily synthesized melanin due to the melanised structure due to these characteristics they can survive in arid-environment. Along with the fungi, there are some proteins which are also tested positive and have common nature. But again the mystery is unsolved that why theses HMs tolerant fungi majorly belong to ascomycetes class. There are two types of interaction between heavy metals and fungi i.e. positive and negative (**Ruley et al., 2006**). In positive interaction, there is no effect due to the presence of HMs but in negative interaction, there can be the death of fungus or growth inhibition due to heavy metal presence. The interaction of heavy metals and fungi positively can be a new step in the removal of heavy metals from a wasteland. It has been observed by many researchers that most of the fungi have multiple metal removing capacities in a viable form. Fungi have great potential and characteristics which can be used in bioremediation field and along with these fungi also act as decomposers having a high enzymatic activity (**Baker. A. J., 1987**).

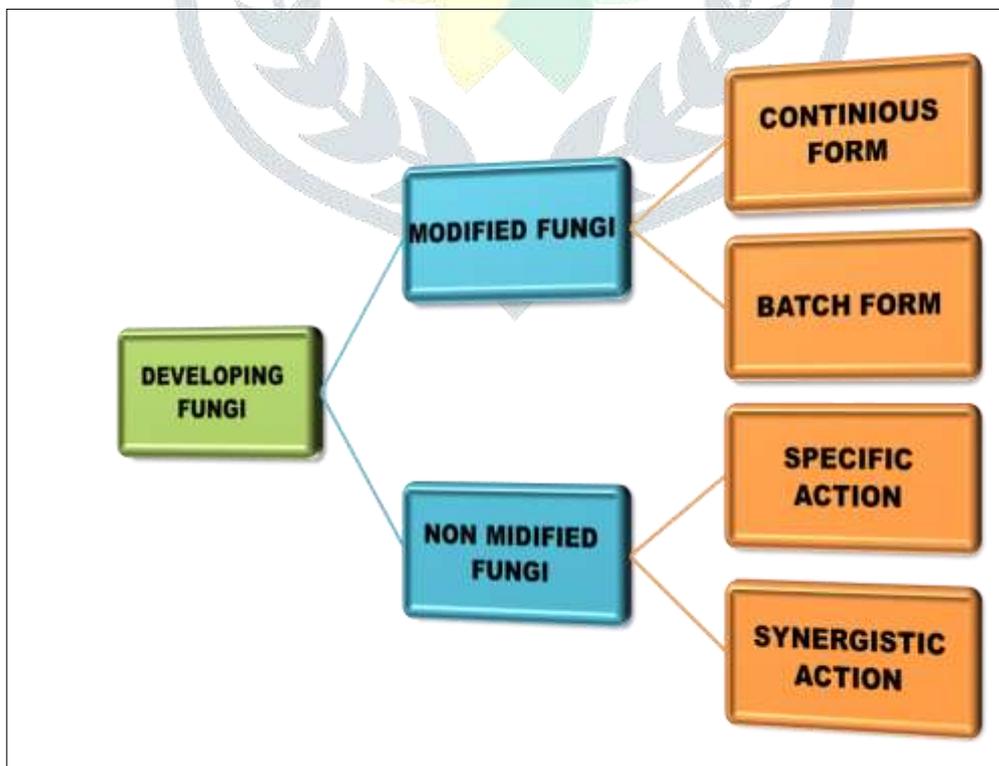
APPLICATION OF FUNGUS:- In an environment there are many problems to be a concern one of them is heavy metal pollution. Due to this major problem has been observed to mostly affect the environment as well as human health. After some test and research, the researchers observed some various group of fungi which can reduce the heavy metal from polluted water which include Fusarium, Trichoderma, Aspergillus, Penicillium species and can remove multiple types of heavy metal from any contaminated soil and water (**Chan et al., 2016**). While remediation there are two ways mainly fungi are used in the form of growing fungi as shown in fig. 1 and 2 and the form of dead biomass.

Fig.1:-MYCOREMEDIATION OF HEAVY METAL THROUGH FUNGAL BIOMASS.



If a growing form of fungi is applying then it should be tolerant towards heavy metal pollutant due to toxic nature of heavy metals the fungi growth can be inhibited, while on the other hand if you are using second from that is biomass form by a functional group of biomass present in soil heavy metal is driven, due to this tolerance characteristic of fungus is not needed and for a special type of pollutant (HMs), there is an important characteristic of fungus i.e. tolerance is needed to remove HM pollutant from wastewater or contaminated water (Prasad, R., 2017).

Fig. 2:- Mycoremediation of Heavy metals through Developing fungi



GROWTH OF FUNGUS:- The method in which growing fungi is used is the most important because it depends upon the metabolic rate and tolerance characteristics of fungi which work against specific fungi. Due to biosynthesis or bioreduction, the fungi are tolerance towards heavy metals and there are many other

reasons also like surface adsorption. It has been discussed in “effluent treatment process development” that fungi are applied by their mobile nature or by any medium which can support it while applying it continuous mode or along with contaminant solution as direct implementation (**Kumari, P., & Kumar, P., 2020**). There are specific fungi to remove a specific type of HMs but there are some multi-function fungi which can remove various metals at a time, for example, *Aspergillus niger* is an example of this which can remove lead, cadmium, nickel, chromium, zinc and manganese, and along with this several fungi that has tolerance capacities against heavy metals like copper, nickel, zinc and iron and it is also known as multi-metal adsorptive fungi (**Singh et al., 2016**). In aqueous system, some fungi can remove heavy metals which are listed in table no. 2. The dead and dried biomass is represented by fungal biomass of the fungi.

Table 2:- Medium used for the removal of heavy metals

FUNGUS	MEDIUM APPLIED	pH	METAL
<i>Penicillium sp.</i>	Aqueous	6	Cadmium (Cd)
<i>Aspergillus sp.</i>	<ul style="list-style-type: none"> • PDB medium coated with Iron • Aqueous • PDB medium coated with Manganese • Glucose medium 	9	Iron (Fe)
		9	Copper (Cu)
		9	Manganese (Mn)
		9	Lead (Pb)
<i>Trichoderma sp.</i>	Aqueous	5-9	Lead (Pb)
		9	Cadmium (Cd)
<i>Beauveria bassiana</i>	Composite media treated with Zinc	7	Zinc (Zn)
<i>Mucor indicus</i>	Sugar solution	6	Lead (Pb)

The mechanism known as adsorption is used to drive the heavy metal and it depends upon the functional groups of the surface of the cell, according to the composition of a molecule within the adsorbent the functional group availability of surface varies. The adsorption potential of pollutants is enhanced by the modification of physiochemical. There are two techniques which can enhance the biomass adsorption characteristics i.e. chemical treatment and physical treatment (**Tam, P. C., 1995**).

PHYSICAL AND CHEMICAL TREATMENT:- In fungal biomass this technique is usually done to create some difference or we can say uniqueness and to remove adsorbent moisture heat treatment is done in the reduction of an anti-adsorptive functional group. Biochar preparation is one of the ancient processes which means biomass activation by heat or thermal. Most of the time fungal biomass is converted into micro or Nanoscale absorbent which also helps in fast-growing bacteria, which can be cultivated easily, therefore, large scale production is done for biomass (**Gu et al., 2019**). It is preferred because it increases the surface area which is directly linked with active sites availability. It has been recorded that strain of *Acremonium strictum* produces a modified version of biogenic manganese oxides which works as an adsorbent for the removal of various metals like cadmium, copper and nickel which develop a new treatment in this field. To enhance the active sites and proper distribution on the adsorbent surface chemical treatment is used in heavy metals adsorption. To enhance the adsorptive characteristics researchers used various kinds of chemicals generally zero-valent iron in plant biomass, but on other hand NaOH, HCl, acetone and ether are used for activation of fungal biomass which also enhances the adsorptive characteristics of this. (**Zang et al. 2017**). It is much easier to cultivate biomass for fast-growing fungi as they are used for the adsorption of heavy metals. Some of the famous fast-growing fungal species are *Pleurotus ostreatus*, *Pleurotus eryngii* and *Agaricus sp.* which apply as an efficient adsorbent in the form of dead species. In some cases after the treatment fungal biomass is applied to expand the active sites.

One of the scientist name Zang et al. (2017) experiment, he has taken 0.5 N NaOH and boiled it with *Auricularia auricular*, to apply in Cr (VI) and it shows some difference in the Cr (VI) adsorption. To remove heavy metals from contaminated water fungal biomass has huge potential but, as per the types of pollutants, medium and pH adsorption is also varies and however, it has been discussed in “factors affecting removal of heavy metal”. And in another topic “fungal cell wall and its role in metal sorption,” the most important feature of biomass is discussed which affect heavy metals adsorption. To know the effect of time and heavy metal interaction with an adsorbent which has been applied several models are used like isotherm and kinetic. The model which is most suited in heavy metals adsorption is a kinetic model (Hassan et al. 2018; Rashid et al. 2016) on the other hand for the adsorption of heavy metals this model is much used (Shokoohi et al. 2020). Like the other model i.e. isotherm model is used to test the relationship between the concentration of adsorption and equilibrium, as the adsorbate behaviour of adsorbent can be determined by this model. Langmuir and Freundlich is the most used isotherm model which is used to determine the heavy metal adsorption on the biomass of fungal (Amin et al. 2016; Hassan et al. 2018; Manorama et al. 2016). It is assumed that the monolayer adsorption of heavy metals in langmuir isotherm theory. It has been assumed that whole sites of biosorption have the equal affinity for the molecules of adsorbate which supports the theory of single layer biosorption and no adsorbate transmigration on the surface, while on the other hand the heterogeneity of the system is described by the Freundlich isotherm. For the Cd (II) and Zn (II), biosorption Hassan et al. (2018) used species of *Neopesalotiopsis ASU1* and after this, they observed that for Hg(II) biosorption it is better to prefer Freundlich model. In fungal biomass, it is better to use these two models and it is also shown in table 3.

Table 3:- MODELS USED IN THE REMOVAL OF HEAVY METALS

ISOTHERM MODEL	FITTED KINETIC MODEL	CONCENTRATION OF METAL	NAME OF METAL	FUNGAL SPECIES USED
Freundlich model	Pseudo first order	20mg/lit	Copper (Cu)	<i>Artemisis argyi</i>
Langmuir model	Pseudo second order	50mg/lit	Cadmium (Cd) + Chromium (Cr)	<i>Aspergillus niger</i>
Redlich model	Pseudo second order	55mg/lit	Nickel (Ni)	<i>Aspergillus niger</i>
Temkin model	Pseudo second order	250mg/lit	Chromium (Cr)	<i>TPBC300</i>
Langmuir model	Pseudo second order	100mg/lit	Zinc (Zn)	<i>Penicillium sp.</i>

During this research, it has been observed that biochar which is originated by plant biomass can also be used for biosorption of heavy metals can prove more benefitted for the removal of heavy metals. According to the nature of pollutants, the characteristics of bio sorbent varies which affect the biosorption and using biochar in contaminated water can affect by its cost of preparation because to prepare biochar around more than 400 °c temperature is needed for heavy metal biosorption (Chanley et al., 2007). On the other hand, even in the low cost and low graded material fungi can be cultivated very easily like only agro waste can be sufficient to make this fungus and in the treatment of wastewater the applicability of fungi can be enhanced due to this the cost of treatment also get low. There are many problems regarding the use of biochar along with fungal biomass that after the final application it has to be treated and disposed of which needed high cost, the researchers came up with the new idea to reuse these adsorbents where only 4 to 8 ph is needed to wash it again and the solution which is needed to wash this adsorbent it can again be reused (Rashid et al. 2016) but again if these solutions are used again and again they lose their efficiency of adsorption. And it can be harmful to the environment because of the corrosive property of solution again to avoid these

techniques there are other alternatives have been discovered and developed like composite production and other (Isaza-perez et al. 2020)

REMOVAL OF HEAVY METALS WITH FUNGUS:- In the bioremediation field it has been seen that single bioremediation agent is preferred it is more basic optimization will be simple as compared to other parameters. As only one fungus is used to remove the specific heavy metal as shown in table 1 or it can remove more heavy metals instead of the only one which is written in many papers. It has been observed in many studies that only one species of fungus is applied to remove one heavy metal because the toxicity can be very high if it is used to remove all heavy metals and the toxicity of metal can inhibit the fungal growth even if the concentration of heavy metal is very low. It has been observed that to remove the solution of binary metal tolerant fungus is used along with this several contaminated solutions of aqueous is also used to remove different heavy metals combine with an isolate of single fungal (Bano et al. 2018; Gola et al. 2016). Out of these isolates, few tend to remove the heavy metals more than two. Knowing that these effluent contains several heavy metals this field is all about exploring the industrial effluent treatment it contains *Beauveria bassiana*, *Aspergillus lentulus* etc. which can remove wastewater heavy metals. It is proven that fungal species have various types of metabolic activities even they cannot tolerate more than one type of metals but it can degrade organic pollutants of various kinds (Rauser. W. E., 1999). Our environment is affected by various types of activities by different fields like industrial and agricultural which gives rise to large numbers of organic pollutants for example; products of phenol, pesticides hydrocarbons, etc., which causes great concern to researchers for the biodegradation of contaminants and removal of heavy metals. This field has large things to focus and develop like it has been seen for the removal of reactive remazol red, Yellow 3Rs and indanthrene blue which are the type of dyes is removed by the help of *Beauveria bassiana* and by the help of mix solution heavy metals like Cr(VI), Zn(II), Cu(II), Cd(II), Pb(II), and Ni(II) is removed. For the removal of naphthalene, fluorine, anthracene which are the kind of hydrocarbons is removed by the *Acremonium* sp. P0997 and it is observed that this species is heavy metals resistance (Valix et al., 2001).

Synergistic application of two or more microbes

In this application, majorly two microbes are applied and it is one of the most novel ideas towards the removal of heavy metals. But as we know microbes produce several types of metabolites even in normal situation as well as stressed situation, therefore, the test of compatibility is needed. As microbes are applied they may affect the removal potential of heavy metals because it contains some negative effect on the synergist microbes due to which growth of synergists is inhibited. Therefore, to verify the survivability test of both microbes there should be a test of compatibility which is a very important method. So, the pair of should be the fungus-fungus or fungus-bacteria. In large plants, the mechanism of synergistic plays a very important role in fungi and microbes rhizosphere which directly increase the tolerance capacity of heavy metal. In the remediation of heavy metals, few researchers worked on the synergistic actions of a fungus with the species of various microbes and fungi (Herath et al. 2014; Huang et al. 2018; Migahed et al. 2017; Olubunmi and Bernard 2016). The fungi synergistic action is the best example of a fungal consortium that has the huge capacity to remove the heavy metals from contaminated water.

Methylation

The metabolic process of the methyl group and its synthesis and transfer is the most used (Bentley et al. 2002). In the metabolic process, the organic compounds are atoms like C, O, N and S are used as an acceptor of the methyl group. The formation of both methylated compounds i.e. volatile and non-volatile is known as biomethylation. Usually, in fungi, bacteria, algae and plants the methylation of arsenic has occurred but in *S. brevicaulis* fungus methylation occurrence is preferred to be the first. As in biovolatalization it has been discussed that at first arsenic is reduced as As(III) then after it is again reduced in As(V) (Kumari, P., & Kumar, P., 2019). At first, the methylation of antimony has occurred in the *S. brevicaulis* and *P. notatum* as same as the previous one for the phenylstibonic acid methylation to convert it

into phenyldimethylstibine by Sb(V) to Sb(III) by the process of methylation. Then later *Cryptococcus humicolus* a fungal species is reported as the methylation of Sb. It has been seen that the Sb(III) compounds potassium antimony tartrate and antimony trioxide transform by the *P.schweinitzii* into non-volatile species of dimethylantimony and trimethylantimony. By bacteria and fungi, the methylation of mercury takes place which is a metal. Genes are responsible for the methylation of Hg in bacteria along with transportation of Hg. For the methylation of Hg, various fungi are responsible such as *Coprinus comatus*, *C.radians*, *Candida albicans* and *Saccharomyces cerevisiae* but the least known mechanism is methylation of Hg (**Anahid et al., 2011**).

BIO OXIDATION OF METALS:- The lignin-degrading fungus produces manganese peroxidase which is a heme enzyme which is used for electron transfer reaction such as Mn²⁺ to Mn³⁺ oxidant. In the oxidation of manganese, the involvement of extracellular protein and usually it takes place in anamorphic ascomycetes strain like KR21-2 and *L.Discophora* SS-1 (**Hua et al., 2012**). There is a manganese oxidizer which is a type of protein known as phenylenediamine and 2, 2'-azinobis. In the presence of proper Mn(III) chelator the catalyzation of Mn(II) into Mn(III) takes place. Due to the action of *Stropharia rugosoannulata* the Mn(II) is catalysed into Mn(III) with the help of a special enzyme known as purified laccase (**Singh et al., 2016**).

Other mechanisms

The tolerance and accumulation of heavy metals in fungi occur because of many known and unknown mechanism, out of some divalent ions the exchange of ions is one of the major manner (**Sheng et al. 2010**). Behind the structure of microbial aggregate, there is an important intermolecular interaction between metal ions like divalent, Ca²⁺, Ni²⁺ and Mg²⁺. The exchange of ion involvement is indicated by the continuous and together release of Ca²⁺ and Mg²⁺ (**Yuncu et al. 2006**). In biofilms of extreme acidic eukaryotes, there is a strong capacity of binding can be seen especially for heavy metals like cobalt, nickel, copper, Arsenic lead and zinc which is produced by the materials of colloids like proteins. The H⁺ gradient driven system of transport is involved in some specific transport system they which plays an important role in the system of metal efflux at it maintains the salinity within the cell which has been proven that it enhances the efflux of zinc in *Suilus bovines* it acts as tolerance mechanism.

INVOLVEMENT OF GENES IN THE DETOXIFICATION PROCESS OF HEAVY METALS

At the time of heavy metal stress it is very important to explore the mechanism of basic molecule in the field of scientific stress. In the biological process gene expression has a very important role as it is responsible for the fungi growth and development during the stress condition of heavy metals. Under the stress condition there are many gene set which gets activated, as to set a link between signalling pathway and heavy metals there are several types of stressors are present (**He et al. 2013; Singh et al. 2016**). During the type of stress condition, there are mainly two types of genes which are responsible for the process i.e. regulatory and functional genes. If we talk about the regulatory genes group they are various transcription genes which are encoded by genes group, and it can easily establish a makeup system by regulating several genes which are responsive to stress. Therefore, compound likes sugar, amines, alcohol, amines etc. helps to tolerate the HMs which are encoded by gene group and the termed as functional gene group. It has been reported that CHR-1 protein is encoded by the exposure of Cr(VI) in *Neurospora crassa* as it is responsible for the accumulation inside the cell. In the fungus arbuscular mycorrhizal which consist of six *GintGst* genes encoded by the glutathione-S-transferase act at the cadmium, zinc, copper and zinc exposure, in the adaptation of *G. Intra radices* it confirms their role in the environment mainly which are contaminated. If the lead stress is developed it regulates all these *EpGSTs* but some of these acts differently if there is cadmium, copper or zinc stress. The heavy metals are bonded by the cysteinyl-thiolate but usually, metallothionein is responsible for creating the bond and active metal homeostasis (**Kumar. V, & Dwivedi, S. K., 2020**).

REMOVAL OF FUNGI:- Mainly, the high amount of metals is found in the polluted areas. The amount of heavy metals present in the earth atmosphere firstly relies on the surface of micro-organism. The cell surface of fungus is different from the cell surface of other micro-organisms (Abbas et al., 2016). The study of interaction in between the cell surface of fungus and the heavy metals is very important because the composition of the cell surface of fungus, their structure, adsorption and absorption mechanisms and the accumulation of heavy metals in the fungus varies from fungus to fungus and their composition also (Chan et al., 2016). The growth of the fungus is also one of the important factors which affect the thickness of the cell wall. To get a proper structure and to study their thickness scanning electronic microscope is used. With electronic microscopy, it is noticed that the internal thickness of negatively strained fungi is approximately 80-100 nm and the internal thickness of the yeast cell is about 200 nm (Kapahi & Sachdeva, 2017). The internal layer is very thick and is used to maintain the mechanical strength and is also made up of glucan and chitin. The surface of the cell wall is made up of carbohydrates and their side chain contains negative charge which is important for the electrostatic attraction process of positive charge metal ion adsorption (Rauser., 1999). The outer layer of the cell wall is made up of glycosylated nanoparticles which hinder the entrance of the foreign particles like pollutants, toxins, proteins and enzymes etc. The side chain of the cell wall is hydrophilic in nature and it helps in retaining water on the surface of fungus and this provides resistance from drought conditions (Cunningham, S. D., & Ow, D. W., 1996). The surface of the cell wall also contains the phosphodiester bridge which binds the metals through electrostatic attraction. The adsorption of heavy metals on the fungal cell surface is mainly due to the presence of functional groups and organic, inorganic ions, proteins, lipids, phosphatase, peptides, chitin and saccharides etc. (Tam, P. C., 1995). The adsorption of heavy metals on the surface of fungus is also known as myco adsorption and mycoremediation. The fungus-like *Aspergillus* sp., *Thamnidium* sp. etc. are used for the adsorption process of heavy metals like Cadmium, Mercury, Arsenic, Chromium and Lead etc. (Kumar. V., & Dwivedi. S. K., 2019a). The fungus is very useful to adsorb and extract heavy metals from the contaminated sites and this process is biologically safe. The living organisms like fungi i.e. mushrooms are used and are regarded as a very safe and environmentally friendly process for the removal of heavy metals (Singh, A., & Gauba, P., 2014).

EFFECT OF REACTIVE OXYGEN SPECIES:- The heavy metal stress shows some toxic effects on the growth and development of the living organisms mainly on the physiological, molecular and morphological levels (Zafar et al., 2007). Due to the heavy metal stress, the production of reactive oxygen species increases in plants. The reactive oxygen species effects also affect the fungus-like *Penicillium* sp., *Fusarium* sp., *Pleurotus* sp., *Aspergillus Fluxus* etc. The reactive oxygen species occurs in cell organelles like mitochondria, ribosome, chloroplast and peroxisome etc. (Valix et al., 2001). Due to heavy metal stress, there is a reduction in the functioning of electric transport chain which results in the production of reactive oxygen species. The reactive oxygen species consists of groups like singlet oxygen, hydroxyl group, hydrogen peroxidase and the superoxidase etc. (Walley et al., 1974). All these are highly reactive in nature and therefore all these four combined called reactively oxygen species. Among all these the hydroxyl radicle is responsible for high damage because of its highly reactive nature. Reactive oxygen species are sometimes useful and sometimes harmful and this also depends upon the type of organism (Kumari, P., & Kumar. P., 2019). It has both the negative and positive effects. Reactive oxygen species are very reactive in nature so that it can also react with the lipids, proteins and peptides etc. and causes oxidative effects which directly leads to the death of the cell (Ernst et al., 1992). It also shows some positive effects by acting like the signalling molecule and helps in the regulation of biomolecules. Reactive oxygen species also helps in the production of chelating, glutathione, thiolic compounds etc. which acts as a chelating agent (Gu et al., 2019).

NON ENZYMATIC REACTION OF DETOXIFICATION:- For the removal of heavy metals the uptake of essential elements and non-essential metals is important. The fungi can easily tolerate some metals with the help of certain mechanisms present in it. Every fungus has some special tolerance capacity

for some specific metals (Agarwal, M., & Singh, K., 2017). The presence of enzymatic and non-enzymatic antioxidants also helps in maintaining the tolerance capacity in fungus. For the antioxidant mechanisms, more than one antioxidant is present within a single organism (In yang et al., 2016). The enzyme glutathione first forms the metal glutathione complex in the Candida tropical plant species due to which the cellular oxidised glutathione level increases and thus helps in the detoxification of metals. Glutathione also helps in decreasing the levels of Cadmium toxicity by producing metallothionein due to which the tolerance level in fungi increases (Wu et al., 1974). In the plant species Aspergillus flavus there is an increase in the production of Thiol. Thiol is used for the signalling of cells and is regarded as the essential agent. Glutathione has two-tailed structure, among the two, the one-tail is of gamma glutathione and the other one is of Thiol group (Prasad, M. N. V., 2004). Here, the thiol group and the glutathione combined react with each other and forms Cadmium bisglutathionate. Under the Chromium stress, there is an increase in the proline, phenol, thiol and catalase content in the fungus Aspergillus flavus (Salt et al., 1998).

USE OF PROTEINS IN MYCOREMEDIATION:- In certain fungi, the production of some proteins is high which can help in the accumulation and detoxification of heavy metals. Even the over the synthesis of such proteins also leads to the stress condition like heat shock (Verkleij, J. A. C., & Schat, H., 1990). The organic acids are also used to remediate heavy metal stress. The secretion of organic acids provides resistance from the stress of heavy metal occurs in plants. The organic acids like citric acid, malic acid, gluconic acid, oxalic acid and pyruvic acid etc. found in the fungus Penicillium sp. and this helps in the detoxification and remediation of metals like Cadmium, Chromium, Copper, Manganese, Lead, Arsenic and Zinc etc. (Kumari, P., & Kumar, P., 2020). These acids have intercellular, intracellular and extracellular metabolites. For the phytomining of metals, these extracellular organic acids help in the extraction process from the low graded mining ores. The intracellular organic acid causes the precipitation of all these metals (Dekock, P. C., & Mitchell, R L., 1957).

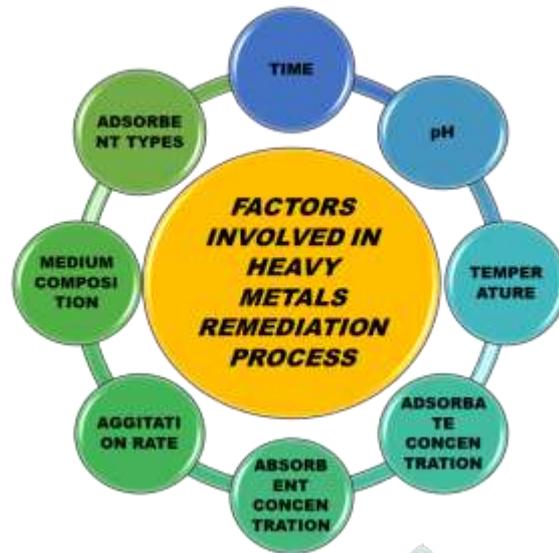
METALS TRANSPORTATION:- Metals are easily available in the earth's environment and there they come in the contact with another organism. After the contact with each other, the organisms can accumulate the heavy metals from the outer environment to the inner part of the cell wall with the help of mechanisms like active and passive (Anderson et al., 1999). The active mechanism is metabolic dependent and this process is completed with the help of some transporting agents which are commonly known as transporters. In a passive mechanism, the metals are on ion exchange with the cell and the osmotic mechanism occurs here (Hua et al., 2012). These transporters help in the transportation of metals from the cytoplasm of the cell to the other cell organelles like the vacuoles and other plasmas present within the cell. Various types of transporters are present in the cell for the transportation of heavy metals from the outer side to the inner side of the cell and also from the inner side to the outer side of the cell (Kulshreshtha et al., 2014). The transporters like metal transporting ATPase, Manganese transporter, Cadmium transporter, Iron transporter, Copper transporter and ATP binding cassette (ABC) etc. Mainly the ATP binding cassettes are used for the import and export of the solute material. In this, the trans-membrane is present which have a nucleotide-binding site and hydrolyser ATP molecule to function the transportation cycle (Singh et al., 2016). The copper transporters are also used and they have three trans-membranes with N-terminal and C-terminal present in the cytosol of the cell. The copper transporters are used for the extraction of heavy metals like copper from the cell with the help of cell Saccharomyces cerevisiae. These types of transporters belong to the family P-type ATPase family having eight trans-membranes (Kumar, V., & Dwivedi, S. K., 2021).

USE OF CHELATORS:- The Phyto chelating belongs to the family of peptides which are cysteine-rich and have thiol group which can easily bind the metals together. Some activators are required for the production of phytochelatin so they can ultimately synthesize the reactive oxygen species (Joo, J. H., & Hussain, K. A., 2012). There are various agents which are used for chelation like phytochelatin, metallothionein etc. are used and the process used for the removal of heavy metals are chelation, bio-

oxidation and bio complexation etc. are used (Matagi et al., 1998). These chelating agents are present in the internal cell of fungus and help in the removal of heavy metals from the atmosphere, plants and other areas etc. The chelating agents are secreted by the fungus and can bind with the metal ions and also they can assimilate within cell organelles like cell vacuoles (Aley. P., & Kumar, P., 2018). The metallothionein is firstly found in the kidney of human beings. These are cysteine molecules having very low molecular mass and are made up of polypeptides. Metallothionein has poly glycin structure (Chen et al., 2017). The metallothionein assembled with the Cadmium is used for the detoxification of heavy metal Cadmium. But there is the sequestration of some metals and it depends upon their electronegativity and the radius of respective metal ions (Jia-Wen et al., 2013). The detoxification of metal ions with the soluble organic ligands like peptides, organic acids and alkali etc. is stored by the complexation in the vacuole. The ATPase molecules and the vacuolar proton pump enhance the metal uptake in the plant system. The fungus species *Laccania* sp. and *Paxillus* sp. helps in the detoxification of copper from the plants. The fungus *Candida* sp. is tolerant of the copper toxicity. With the metallo activation mechanism the peptides are easily formed in the yeast cells (Danesh et al., 2013). Melanin is regarded as the dark brown coloured pigment produced by some specific fungal species and having very higher molecular mass and is hydrophobic in nature as well as are negatively charged. The melanin compound is found in the outer layer of the cell wall of fungi aligned with the chitin (Kumar. V, Dwivedi S. K., 2019b). For the formation of melanin compound, various mechanisms are used like the oxidative polymerization in which catechol plays a very important role. Melanins have a very complex structure and many functional groups are involved in this like the carbonyl group, phenols, hydroxyl and methoxy group etc. (Sheron et al., 2009). All these functional groups have a chelating ability. The fungus *Cladosporium* sp. has very high absorption capacity than another fungus-like *Penicillium* sp. and this can easily absorb the heavy metals like Lead, Nickel, Zinc, Cadmium, Chromium, Arsenic and Copper etc. (Kumari, P., & Kumar. P., 2019). Melanin compound also helps the DNA stand from rupturing. Like melanin, the siderophores are also the negatively charged atoms and have a very strong interaction with the metal ions. The siderophores are made from the negatively charged oxygen atoms like alcoholates, carboxylates and hydroxymates etc. (Ruley et al., 2006). Iron atoms like ferrous, ferric more interact with the siderophores. The siderophores can easily remove the arsenic from the contaminated soil with the efficiency of about 90%. In the bioremediation of metals, for siderophore formation bacteria is very important (Hamba, Y., & Tamiru, M., 2016). Bioreduction is also one type of chelating process in which the toxicity of metals is less because of the decrease in the oxidation state. The heavy metal Chromium is mostly found in a very stable form that is Cr III and CrVI among these two the form Cr VI is very harmful and is toxic in nature than another one. The transformation of Cr VI to the other form that is Cr III is important for their reduction to mitigate the toxicity (Khan et al., 2019). This reduction needs much energy and the whole process depends upon the glucose. The fungus *Aspergillus* sp. produces the enzyme glucose oxidase which helps in the reduction mechanism. This is also used for the functioning of glycolytic pathways (Fu, F., & Wang, Q., 2011). If there is any reduction in the transformation of Cr VI then there is an ultimate increase in the production of reactive oxygen species (ROS). This whole process is multistage and requires a transfer of various electrons (Kumar, V., & Dwivedi, S. K., 2020). Myco precipitation or bio precipitation is the other technique which also helps in the removal of heavy metals mainly from the waste water. This process is of two types that is intracellular precipitation and extracellular precipitation (Chanley et al., 2007). In the process of extracellular precipitation and metal ion, the fungus donates the anionic species and the cell wall of fungus provides a medium to complete the whole reaction. While in the intracellular precipitation the metal ions emerge from the inner side of the cell wall of the fungus and the anionic species present in it precipitates (Antonovics et al., 1971). The last type of precipitation is the intracellular precipitation in which the precipitated products can join with the surface of the fungal cell wall and can diffuse this with the help of precipitation (Colpaert, J.V., Van Assche, J. A., 1987). For the removal of lead the anionic oxides and phosphate, sulphates, massicot, anglesite and pyromorphite are required. The fungus *Paecilomyces javanicus* helps in the precipitation of lead in the form of lead oxalate, Plumbonacrite and Cerussite etc. (Kumar. V., Dwivedi, S. K., 2021). Along with this, the phosphatic anions are also helping

in the precipitation and bio-remediation of uranium. The precipitation of the heavy metals like uranium also forms a complex compound of such heavy metals like bassetite, ankoleite, uramphite and chemikovite etc. (Macnair, M. R., 1987). The fungus *Aspergillus niger* forms the mineral compounds by using the low grade ores of uranium like uranium acetate hydrates. The fungus segregated from the mines of zinc also produces the oxides of zinc. The precipitation of Strontium and Calcium by the fungus produces the different forms of Strontianite, Calcium chloride, Vaterite and Calcite etc. (Prasad, R., 2017). The biological volatilization of metals is called as biovolatilization. The process of bio volatilization is very important for the removal of heavy metals from the soil, water and environment. Mainly this process is used for the removal of heavy metals such as Mercury and Arsenic present in the plants and soil micro-organisms like fungi, bacteria and algae (Anahid et al., 2011). In Arsenic this process is used mainly to convert the non-volatile species of Arsenic to the volatile species of the heavy metal Arsenic. In the methylation process, the methyl group is used for the reduction of Arsenic pentaoxide to the Arsenic trioxide with the help of fungus *Scopulariopsis* sp. Firstly the Arsenic pentaoxide is converted with the help of Arsenious acid into the other form of Arsenic that is Arsenic trioxide with the reduction process (Kumari, P., & Kumar, P., 2020). Various chemical compounds are used in this conversion process like Arsenobetaine, Dimethylarsinic acid and Arsenoribosides. All these compounds are also present in the fungus-like *Rhizophagus* sp., *Penicillium* sp. and the fungus *Aspergillus* sp. The heavy metal Mercury is one of the most volatile element and is very toxic among all the heavy metals (Romheld, V., & Marschner, M., 1983). But this heavy metal Mercury can be easily volatilised with the help of micro-organisms like fungus and bacteria. The contaminated sites of the affected areas of mercury can be easily remediated with the help of these organisms. These bacteria and fungi remediate the contaminated site by the enzymatic reduction (Dekock, P.C., & Mitchell, R. L., 1957). For the reduction of Mercury, the enzyme mercury reductase is used. The fungal species like *Scopulariopsis* sp., *Candida* sp., *Aspergillus* sp., *Saccharomyces* sp., and *Cladosporium* sp etc. are used for the volatilization of heavy metals like Thallium, Selenium, Bismuth and Antimony etc. (Awad, F & Romheld, V., 2000). The methyl mercury is specified as a very toxic species of Mercury and sometimes it also inhibits the growth rate of micro-organisms like bacteria, fungi and algae etc. Along with this sometimes the fungi also changes into the less toxic form of metal into the highly toxic form of heavy metal. Some of the fungal species like *Saccharomyces* sp., and *Candida* sp. can easily tolerate the mercury stress but at some limit, because they are not able to grow under the concentration above 0.70ug/ml (Dietz, A. C., & Schnoor, J. L., 2001).

FACTORS RESPONSIBLE FOR THE REMOVAL OF HEAVY METALS: For the removal of heavy metals from the contaminated sites various factors may affect like the temperature of that site, pH, and adsorbent used, time and concentration of the pollutant present there. The pH of the solution is the most important factor which determines the removal of heavy metals from the contaminated site (Bhattacharya et al., 2014). In this, the mycoremediation process is used by growing the desirable fungus in the contaminated area. The removal capacity of the metal or the pollutant depends upon the adsorbent used in the solution and the interaction between the adsorbent solution and the number of metal ions present in it (Kumar, V., 2006). The fungus can tolerate the toxic effects of the metal they can increase their biomass by destructing the level of heavy metal. The main factors involved in the remediation of heavy metals are shown in fig. 3.

Figure 3:- FACTORS INVOLVED IN HEAVY METALS REMEDIATION PROCESS**Reference:- Prepared by Authors**

This fungus can make their energy from the heavy metals and decrease the level of that heavy metal from the contaminated site (Bellion et al., 2006). With the increase and decrease in the pH of the fungi, the growth of the fungi is affected which leads to the decrease in the detoxification of the heavy metals. The fungus *Aspergillus niger* provides very good results for the removal of heavy metals like nickel, arsenic and copper etc. (Robinson et al., 1999). In high pH, the removal rate of the metal is less because at high pH there is the formation of metal hydroxide. The surface of the fungus at high pH has a negative charge on it and this will make some strong bonds with the metal ions. Due to the dissociation of a functional group the detoxification process affected (Babel, S., & del Mundo Dacera, D., 2006). Temperature also plays a very vital role in the removal of heavy metals and it depends upon the adsorption rate. With the increase in temperature the endothermic process occurs and the adsorption rate of the pollutant or heavy metal increases (Pilon-Smits, E., 2005). In the removal of Zinc and Nickel the fungus *Penicillium sp.*, combines with the bentonite and thus endothermic reaction occurs but in some cases if the reaction is exothermic the adsorption rate decreases but the temperature at that time increases. With the decrease in the adsorption rate the two attraction forces get destructed which are present in between the metal ions and the functional groups (Baker, A., & Saleh, T. A., 2020). Due to the re-orientation of the components of the cell wall of fungus and the ionization capability of the cell wall, there is a strong affinity occurs in between the metal ions and their active site. So, in this way the temperature is very important to be getting moderate and stabilizes during the removal of the heavy metals (Montarges-Pelletier et al., 2008).

CONCLUSION

We have already discussed about the major consequences of heavy metals and their bioremediation as we all know these heavy metals are toxic in nature if accumulated in large amounts. Bioremediation is the most simple and sustainable way to remove HMs as compare to other conventional methods because they are more costly and time consuming. It has been prove that using fungi and microbes are the easiest way to treat contaminated water. in many studies they have focussed in only single metal and their remediation but in this study we have discussed about the removal of multi metals removal fungi which can act on the multiple heavy metals at a time, and as a result we have seen using fungi in bioremediation gave very good results. Introducing fungi which are metal tolerant in multiple metal contaminated sites are best technique to reduce the concentration of heavy metals. In the site of contamination various pollutants of heavy metals are present including, hydrocarbons, sulphates, fluoride, nitrate, pesticides, weedicides and others and species of fungus have the ability to remove out all these pollutants even if it is present in multiple amount. Not only in wastewater but also in plants fungi have the capability to reduce the heavy metal accumulation

in the grain of the plant, as we can apply this fungi which forms bond with plants in wetlands for the treatment of wastewater but till now this technique is not in limelight. The fungi is also present in biofilter and bioreactor as in the viable or dead form which have the capability to remove all the wastewater contaminated with heavy metals. In this paper we have shown the table where we have indicated the fungi and in which group it belongs to, usually this fungus belongs to ascomycetes group which have high tendency to tolerate heavy metals. Fungi not only reduce these heavy metals but also degrade or convert it into toxic to non-toxic nature as use it as their own nutrients, in this way nothing goes waste. Along with fungi and microbes genes are also involved in the technique of bioremediation.

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