Environmental Biotechnology
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Lecture - 42
Microbial Interactions with Heavy metals and Metalloids

Welcome to the next lecture of our course on environmental biotechnology and in this particular lecture we are going to discuss about the microbial interactions with heavy metals and metalloids and this will of course, include the radionuclides also and we will learn how bio remediation of these toxic compounds or toxic metals can be done through the microbial action.

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So, now, the basic concepts those will be covered in this particular lecture will be the bioremediation of metals. So, we will get ourselves introduced to the basic concepts that will we will highlight the importance of the microbial biocatalysis in or catalytic mechanisms in bioremediation of metals, a basic concept of metal microbe interaction will be discussed and finally, the metal bioremediation ways to achieve the remedial goals and some of the aspects of metal microbe interaction also will be discussed in detail.

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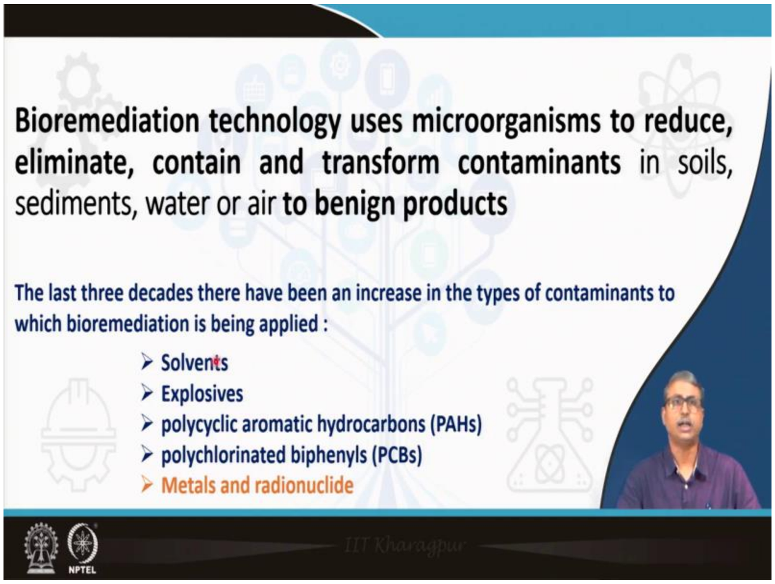

So, bioremediation technology, as we have already discussed, uses the microorganisms to reduce eliminate, contain and transform contaminants in soil sediment, water or air to benign products. So, this is a broad technology and as we already have learned, that it includes not necessarily only the kind of detoxification or disappearance of the molecule or the complete removal of the molecule or the contaminant, but also it includes the reduction elimination containment and transformation of the contaminants.

And in the last 3 decades, there have been an increase in the types of contaminants to which the by remediation is being applied, almost all kinds of environmental pollutants are being subjected to bioremediation particularly using the microbial systems so called microbial bioremediation and these include the solvents, different explosives, polycyclic aromatic hydrocarbons, polychlorinated biphenyls and metals and radionuclides. So, with respect to metal and radionuclides, we are going to have this lecture because the other organic solvent discussion we have already concluded.

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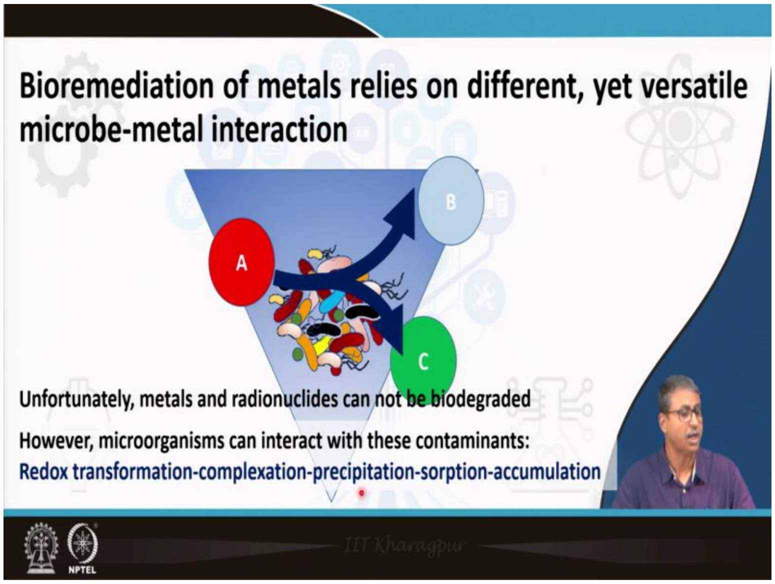

Now bioremediation works by either transforming or degrading the contaminants to non-hazardous or less hazardous chemical or less hazardous compounds that we already know. Now, when in a contaminated environment, we have the microbial communities or microbial cells, which are capable of acting on the pollutant molecules, the polluted molecules like A is expected to be converted into the compound B or maybe A will be converted to compound C

Now, there are actually 2 distinct types of biological transformation reactions catalyzed by the microorganisms which are naturally present in the contaminated environment and by virtue of the natural catabolic abilities, which are basically due to the enzymes and their intrinsic potential to deal with the toxic pollutants, the pollutants might undergo the bio transformation reaction or they might undergo the biodegradation type of reactions.

During bio transformation type of reactions are is kind of an alteration of the molecule or atomic structure of a compound occurs. So, during bio transformation reaction, the compound may not undergo any kind of breakdown or the oxidation in terms of the complete or mineralization kind of things, which we perhaps will see in case of organic pollutant biodegradation. But the particular pollutant molecule like pollutant A may be converted to some form.

Which is possibly having less toxicity or having an altered chemical structure chemical behavior and that could be useful for environmental control environmental remediation purposes. So, these are the 2 different types of biological transformation rather I will say which are expected from the microorganisms. One is basically the degradation of the compound which is more often is seen in case of the organic pollutants where the organic substances are broken down.

During the course of the microbial interaction and as we have learned earlier that these are basically manifested by the oxidative metabolism in most cases in some of the cases like with chlorinated solvent etcetera, reductive dechlorination or reductive dechlorination, dehalogenation type of reactions might be also there. So, essentially these are biodegradation type of reactions, but everything is not subjected or cannot be bio degraded because for example, the heavy metals cannot be degraded.

But the heavy metals and similarly different other pollutant molecules can be converted into in terms of their chemical structure atomic structure. So, that they are environmental fate environmental toxicity, they are mobility etcetera are altered. So, often in in microbial bio remediation process, we need to rely on such kind of processes as well. So, these are the 2 basic types of processes which are basically deployed by microorganisms and these are found to be more useful for developing the bio remediation processes.

Now, bioremediation of metals and metalloids and nucleotides relies on different yet versatile micro metal interaction. Because one of the fundamental reasons that the microbes the need to deploy a separate and very specialized set of reactions in terms of dealing with the heavy metals or radionuclides or metalloids. The reason is that metals cannot be biodegrade it you cannot degrade cadmium or chromium or lead or arsenic or uranium, what microbe can do microbe can to some extent transform it in many times.

We see we will learn about these things that microbes many times they enzymatically they carry out the chemical transformation of the toxic heavy metal compounds. So basically the metals and radionuclides cannot be biodegrade. So, you cannot consider or compare the degradation of organic pollutants with heavy metal of bioremediation process because metals and radionuclides cannot be degraded.

However, microorganisms can interact with these contaminants like the heavy metal radionuclides contaminants and they can facilitate an array of processes and these processes include a redox transformation that is the change in the oxidation state of the pollutant molecule maybe arsenic or chromium or uranium or any other metals or metalloids or any nuclides whose the oxidation states are of important factor in in regulating their environmental toxicity, environmental significance.

Their ability to bind to different mineral phases, our ability to dissolve and remain in the aqueous phase for example complexation, precipitation, absorption and accumulation are the other processes which are responsible for controlling the behaviour of the other toxicity, the mobility of metals and radionuclides in environment and these are carried out by the microorganisms present in the environment. So, these are the reactions or the possible processes which are sub which are available with the microorganisms to deal with the toxic heavy metals or similar metallic compounds.

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So since heavy metal are not subjected to biodegradation per se, as compared to the organic pollutants, so the remedial goals when we have a contamination by chromium or uranium or arsenic or something like that, these are heavy metals we cannot biodegrade or cannot oxidize them straight away, so that they become more decomposed into carbon dioxide or water. However, the remedial goals can still be achieved using microorganisms which will facilitate.

The precipitation and does immobilization of the inorganic contaminants like the heavy metals or the radionuclides. The precipitation means they will no more be available in the aqueous phase and they will remain immobilized on the solid surfaces. So just imagine the groundwater. Where are the aquifers where the pollutant molecules like chromium or uranium might be there due to some anthropogenic activities?

And by some means, if you are able to precipitate this uranium, for example, or convert the arsenic, transform the arsenic in a form where the modified form of arsenic precipitates or the uranium precipitates and gets immobilized on the solid surfaces like the minerals then the water which is flowing will remain free of those contaminated or contaminants. The second option could be concentration and does the reduction in the volume of the contaminated matrices.

Sometimes particularly with respect to the toxic radioactive metals and radionuclides the volume of the waste becomes a very serious concern in the front of the environmental technologies and environmental biotechnologist. So, what we have learned that the volume of the waste can be significantly reduced if we are able to use certain specific microorganisms who can actually bind to these radioactive or the otherwise toxic metals and sequester them, accumulate them absorb them onto their cell surface.

And thereby, we can store that bio accumulated or bio dissolved materials in a much smaller volume compared to the total volume of the waste. So, from the waste management point of view, that gives us ample opportunity to deal with the large volume of waste particularly where the radioactivity or the hazard of the waste are of major concern because storing few lakhs of gallons a few lakhs of liters of water or contaminated waste liquid is a very difficult task rather than few hundreds of liters.

So few hundreds of liters may be stored or can be managed much more efficiently compared to the few a few maybe million liters of the waste volume. The third one is the compartmentalization of the metals to a part of the environment in which their harm is reduced. That is another aspect where in the natural in the insitu condition, we can actually deploy certain specific microbial processes which carry out transformation reactions and those transformation reactions are enabling the metals to compartmentalize in certain part of the environment and thereby they are environmental harm, environmental mobility or bio availability is significantly reduced.

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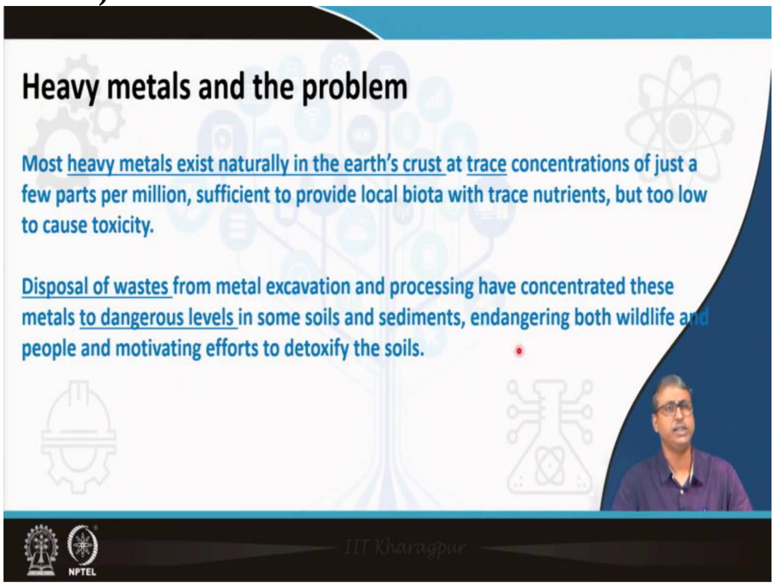

Now, in this section, we are going to learn something about the heavy metals particularly in terms of the problems which are posed by these heavy metals when the microbes are taken into consideration in particular. Now, most of the heavy metals exist naturally. So, heavy metals are part of the earth crust and from the early evolution, we see that the local microorganisms local biota, all kinds of animals and plants are habituated they are equipped to handle with the heavy metals.

Because and most of the heavy metals and many of the maybe heavy metals are required as a trace nutrients like zinc, copper, cobalt etcetera. But at low concentrations, these are low concentration means that the parts per million are below that. So, at very low concentrations biological systems are equipped to utilize the metals as a part of their essential nutrients trace elements that we consider as our micronutrients that we consider.

But they may pose toxicity or they may cause toxicity if the concentration thresholds have the same metals like the copper, zinc, nickel etcetera, which are otherwise very useful as trace nutrients are present in excess concentration. Now how the excess concentration appears because the naturally the earth is not made up of that excess concentration unless and until we are dealing with some ores of mineral deposits.

It is basically the anthropogenic activities particularly the disposal of waste from metal excavation, metal processing and other industrial activities that actually have led to a huge level of rise in the level of these heavy metal concentration and which we call a dangerous level because the concentration of heavy metals are now more than what the biota or the microbes the plants are naturally equipped to handle with and in some of these soils and sediments which are we call them contaminated soils and sediments.

And these higher level of heavy metals, toxic heavy metals, toxic radionuclides metalloids etcetera. They are endangering both the wildlife and people and motivating the effort to detoxify the soils. So we are trying to look into the aspects that how natural microorganisms, even plants and fungi etcetera. They how they behave when they are exposed to the higher concentration of these toxic metals and how we can learn from them and then develop technologies which could possibly help us to have bio remediation processes.

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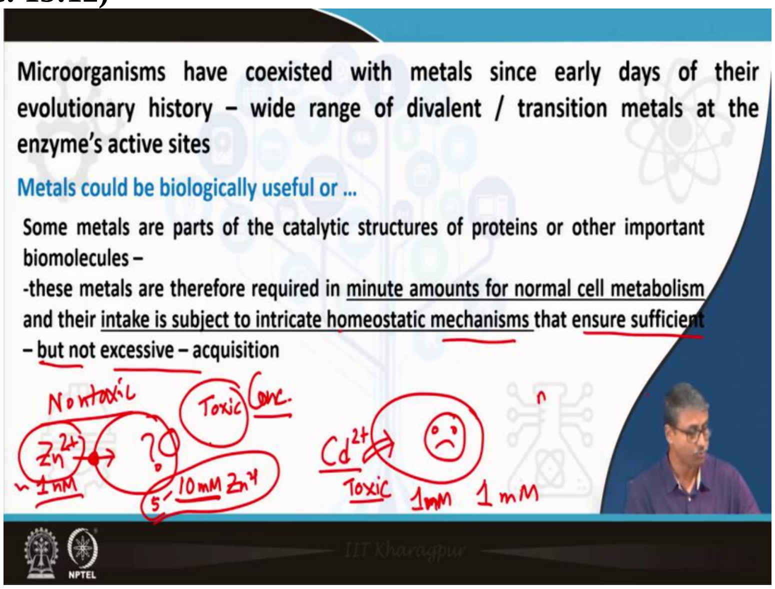

Now, microorganisms as I mentioned have coexisted with metal since the early days of their evolution. So, from the day 1 of their journey in this planet they are living with the metals because metals are the integral part of the earth crust and a wide range of divalent and transition metals are present are involved in the enzymes active sites. So, many enzymes we see the content this trace metals like zinc, nickel, cobalt, iron etcetera which are required to traces condition and many times.

Only grow the cells in the laboratory we may not add these heavy metals as such as the nutrients because they are supposed to be coming from the other materials that we that we supply as a growth medium but in a natural condition in a natural environment microorganisms they just acquire these divalent and transition metals which are required as a trace ions for them. Now metals, many metals as I mentioned, zinc, cobalt, cadmium zinc, cobalt, copper etcetera, iron, magnesium.

For example, could be biologically very useful because they are the part of the integral part of the enzymes which require many metal enzymes for example, we call these enzymes the required the metals for their activity, but they may be toxic, they may be creating a lot of stress to the organisms if the concentrations are beyond the desirable range even for the required essential lines.

For example, copper or zinc copper will be the trace element required by the cell zinc, nickel are required by the cells for different enzymes, but if the concentration of the copper or zinc or nickel is elevated beyond certain threshold limits, then possibly the microorganisms and all other organisms will experience toxicity toxic effect, because of this higher concentration. So, at that point of time, they are biological relevance will be different because then they are stressor they are toxic compounds.

Now, some metals that the like we are referring to these metals, which are considered to be the trace elements and they are the part of the catalytic structure of the proteins or other important biomolecules. These metals are therefore required in minute amount for the normal cell metabolisms and as a result, the intakes of these metals are highly controlled within the cell. So, as we have learned over the years, that within a cell is naturally growing in an environment, if we have.

For example, zinc, so, these cells would like to transport zinc inside the cell. So, how this will be done for that there will be some transporter, so this is called metal transporter. So, these metal transporters are of different types, these metal transporters can be of general type, general type means they are capable of transporting any divalent cation of comparable ionic radius and comparable chemical behavior or they may be highly specific type.

Now, these transporters are produced by the cell so, the cell has the DNA and from the DNA they encode the proteins they encode the mRNA and from the mRNA following translation the proteins are produced and the proteins are these transport proteins. So, basically the transport proteins are part of the genes which are present in the organism itself. So, these transport essentially that means is under the control of the cellular process.

So, cell essentially can control this how because these are manifested these are carried out the transport is carried out by this carrier complex or the transporters. So, these transporters which may be general transporter or maybe divalent transporters or maybe very specific transporter for a particular iron like zinc or nickel or cobalt or copper, there may be specific types of so, in either of the cases the cell can control the expression and the production.

And the formation of these transporter molecules and hence they can actually they can control the transport of the essential trace elements and therefore, that is considered as that the intake is subjected to intricate homeostatic mechanism, it depends on how much is the requirement by the cell. Like for example, the cell is requiring alkaline phosphate which is zinc containing enzyme. So, if a cell for instance is growing in an environment where a lot of organic phosphate is there.

So, if the cell is growing on an environment where a lot of organic p is there, and the organic p needs to be metabolized, so that the p phosphate is available to the cell, so, that cell can transport the p. Now, for this process, the cell will require the alkaline phosphatase and other phosphatases. Now, the alkaline phosphatase particularly is a periplasmic enzyme these alkaline phosphatase will require zinc divalent zinc iron will be required for the alkaline phosphatase.

Now, if that this particular cell is growing under the organic phosphate in presence of organic phosphate then possibly requirement of zinc will be slightly higher compared to the environment where the cell is not growing under organic phosphate rather the cell is growing with inorganic phosphate. That means, the cellular requirements determine how much will be the transport of the zinc into the cell.

So, the behavior of the cell with respect to the divalent cation zinc is entirely in the hands of the cell itself that what the cell wants, so, that the sufficient quantity is supplied, but this supply must not be excessive. So, under this condition, the cell will maintain the homeostasis that means the equilibrium so, that the level of the zinc which is accumulated inside the cell will be always linked to the requirements.

How much the cell requires that will be the major criterion for determining how long this transportation will occur. So, under certain other environmental conditions, some other heavy metals we will come back to the zinc maybe very soon, but maybe some other metals they seem to have no biological relevant function for example cadmium. So, for example, cadmium if there along with zinc, then cadmium is not required because cadmium to Cd 2 plus ions are not required by the cells.

But by some means, Cd cadmium 2 plus ion is capable of entering into cell then that cadmium is undesirable for the cell and that is going to cause harm or damage to the cell. Why because these heavy metals either they are non-essential, they are not required at all. So, they are fundamentally identified as non-essential. So, they are not required for the cell or they are required, but at much lower concentration.

So, now, you have 2 situations in one situation so, in one of the situations you have only the iron which is required by you like zinc 2 plus you will lead zinc 2 plus. So, you have a transporter and the gene concentration is controlled, but that zinc should be at the Nano molar or micro molar level so, maybe it is close to or around some Nano molar level around 1 Nano molar. So, that is sufficient for the cell but in the environment you might have a millimolar level or maybe 5 to 10 millimolar level zinc.

So, in that case the concentration is so high compared to what is required in this condition the zinc is toxic, but in this condition zinc is non-toxic. So, the cellular response of cellular homeostasis will be adjusted in such a way that when the concentration threshold of the desired heavy metal is made that means the constitution threshold is below the toxic level, the cell will deploy a type of response to the heavy metal.

Because the zinc is already identified as the essential but and its level is also in the nontoxic level. So, that time the cellular response will be different, but when you have much elevated zinc, no matter whether the zinc is trace element required by these bacteria not only because the concentration is so high, then the zinc become toxic and then the cellular response will be something different that we will study that what happens when the cell are exposed to a particular element which is already a trace element required by the cell.

But in a given environment is present in a very high concentration that means at this point of time, the concentration of these particular elements of the heavy metal is a point of concern. Now there is another situation where you have cadmium which has no biological function. So, what will happen? So, cadmium will obviously be not desired inside the cell has no desire to take cadmium inside the cell because it has no biological role, but the cadmium might try to enter inside the cell.

This is the property of the cadmium because it might resemble the transporters which are general type divalent cation transporters, and those divalent cation transporters can be utilized by cadmium to enter inside the cell and then the cell will response differently. So, cellular response will be different cell will not be happy at all because the cadmium at any level it is toxic. Now cell will have its own mechanisms to combat this situation. If the concentration of the cadmium is low like in a Nano molar level, then the response will be something if it is millimolar level, then the response will be different that we will see later.

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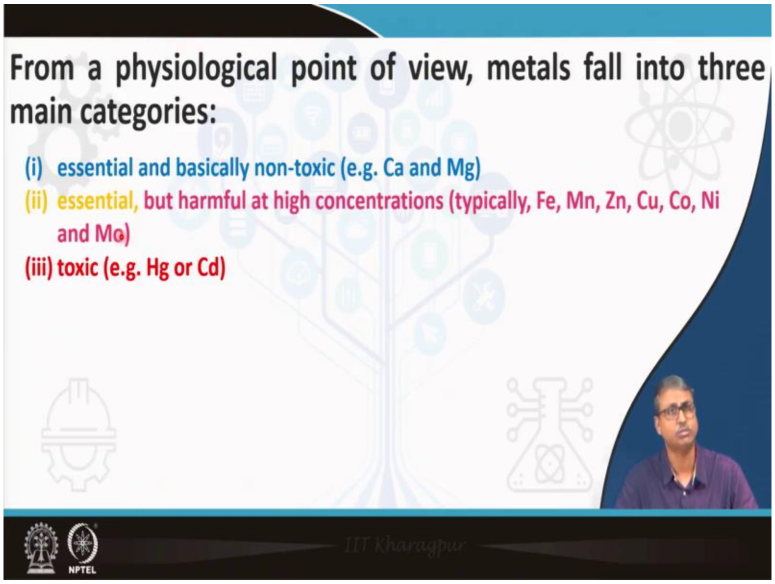

So, now, from the physiological point of view, we are able to then categorize 3 types of the metals 1 is essential like zinc, cobalt, all these things will come later, but the some are even more general type like calcium, magnesium and these are called essential but basically nontoxic. So, even if the concentration goes slight higher, the cell would not mind, because these are not toxic, they do not have the similar type of effect that otherwise the heavy metals like zinc, copper, cobalt might have.

The second category is the essential but harmful at higher concentration like iron, manganese, zinc, copper, cobalt, nickel and molybdenum, they are required but at low very low concentration, if the concentration level crosses that threshold, which varies of course from cell to cell different environment also they become toxic and the third one is obviously toxic, they are always toxic, like mercury and cadmium.

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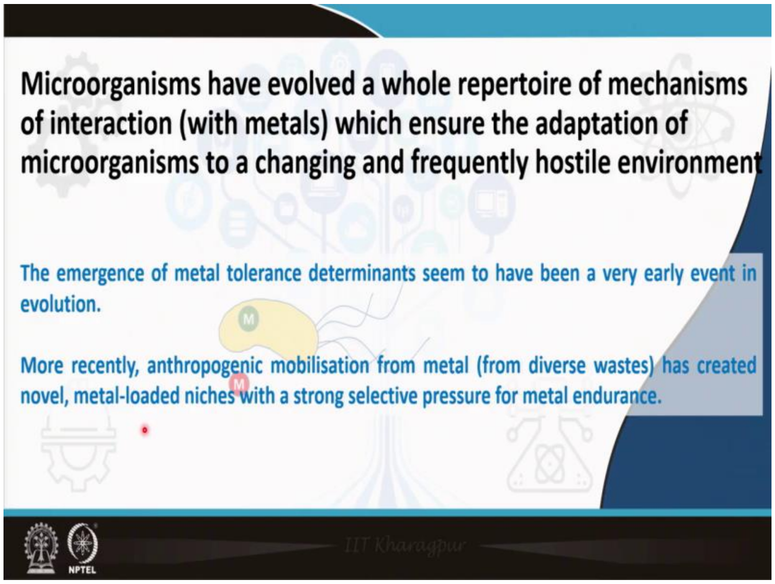

Now, microorganisms have evolved a whole range of mechanisms of interactions with these metals to ensure the adaptation of microorganism to changing and frequently hostile environment because the environment is not static. So, environment is changing always depending upon different physical chemical conditions, the level of heavy metals are also going to be changed. So, microorganisms are equipped to deal with such situations. So, for example, a bacterial cell is shown here and you can see one M is a metal ion.

So, in one case, the metal may be required by the cell to say take up this metal and utilize it. In other case, the cell will not take up the metal and we will just discard this cell will not take it into inside the cell. So, that is the same cell has different mechanisms of interacting with the metal ions. Now, the emergence of the metal tolerance determinant that is the genetic determinants seem to have been very early event in the evolution.

Ever since the microbial cells are experiencing the different concentration thresholds or concentration levels of different heavy metals. They are learning that how to deal with them. So, they have initially adopted maybe some general strategies and then they have started evolving some specific methods of dealing with the heavy metals that we see today and in more recent time, particularly with the industrial revolution and all those things, the anthropogenic mobilisation from the metals of metals or different metals from diverse waste materials has created novel metal loaded niches with strong selective pressure for metal endurance.

So therefore, in such a niche where we have more heavy metal concentration, we can expect that the microorganisms living over there, they might be deploying more intelligent and more efficient mechanisms.

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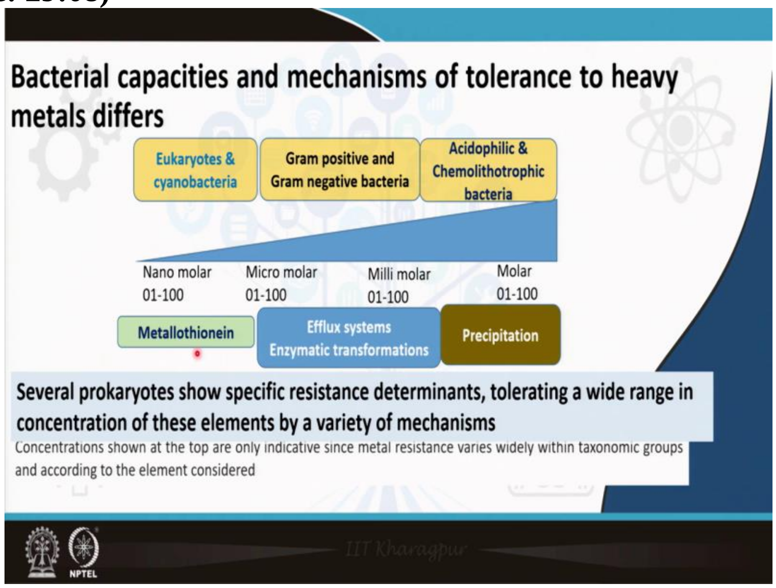

Now, if we look at the overall spectrum of the different microbial or bacterial abilities to deal with the heavy metal or different type of heavy metals, so, this is a kind of a general plan that the with the concentration varying from nano molar to the molar level. The organisms like eukaryotes of cyanobacteria, they have a very specific type of way of dealing with this like using the metallothionein or some kind of these proteins are utilized to deal with the toxic concentration of the heavy metals which are of course at very low level.

At moderately higher level the bacteria particularly talking about more on bacteria, the gram positive and gram negative different bacteria, including the E. coli, and desulfovibrio and many other members. They deploy large numbers of systems a variety of systems which include the efflux systems that will learn the enzymatic transformations which are very specific to specific toxic metals and metalloids.

And at very high concentration of metals which are at molar level, which are more frequently encountered in the mind sites mostly the metal mind sites, we see that very high level of metals metal concentrations are prevailing. So, acidophilic microbes and chemolithotrophic bacteria they deploy again another set of specific set of mechanisms which help them to deal with this very, very high concentration of the toxic metals and of course, that include the precipitation as one of the major means.

Now, several prokaryotic organisms bacteria and archaea, this whole specific resistant determining, so, these processes are often very specific. So, as I mentioned earlier some mechanisms might be general like production of metallothionein or similar Phyto implant system, we see the Phyto chelating something comparable to that, but this kind of sulfhydryl group containing small protein molecules have been found to be very efficient at low concentrations thresholds.

But at higher concentrations, the microbe's bacteria, they deploy a large number of systems. So, and the specific genetic determinants for that type of resistance systems or transformation systems are all very well studied.

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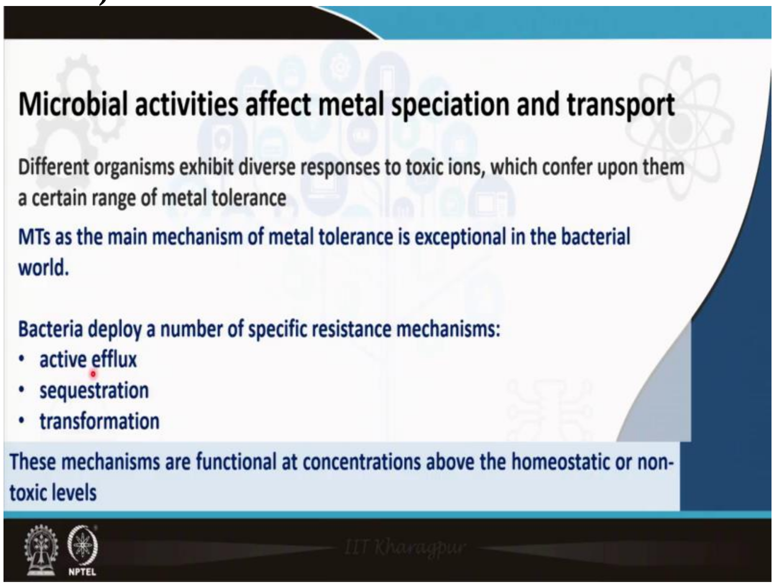

Now, microbial studies microbial activities that affect the metal speciation and transport will be discussed, the different organisms exhibit diverse response to toxic ions which confer upon them a certain range of metal tolerance. So, they know how to deal with this toxic metal concentration. So, one idea that I presented is a metallothionesis, which are basically a family of metal chelating proteins are mostly used by eukaryotic organisms and cyanobacteria we have found that they are also capable of producing these metallothionein.

So, it is mostly eukaryotic mechanism, but in some prokaryotes, they are also present, but they are more sensitive to metal toxicity particularly towards the lower concentration regime and what we have seen that eukaryotic organisms are more sensitive than the prokaryotes. So, prokaryotes they use a whole set of different mechanisms compared to the eukaryotic cells which are which are capable of producing these metallothioneins for combating the low concentration of heavy metals and that actually help the cells to regulate the intracellular metal.

Because these content this alpha hydrilla SH groups, these SH groups are capable of binding the divalent metal cations. So, forming the metal sulfur bonds and the metal sulfur bonds are used to to chill it due to precipitate the metal inside the cell. So, that the metal divalent metal ions are not available for interaction with any other cellular components. Now, MT's as our metallotheionin as the main mechanism of metal tolerance is exceptionally in the bacterial world.

Generally, we do not see much of bacterial interest in producing these metal at tiny. So, what bacteria do bacteria they deploy a number of specific more specific because metallothionein is not a very specific mechanism that is more towards a divalent broad divalent cation or divalent heavy metal homeostasis mechanism bacteria that deploy active efflux sequestration and redox mediated different transformation processes through which they try to withstand the toxicity and also often the detoxify actually the metals.

Now, these mechanisms are functional at concentration above the homeostatic or nontoxic level. So, like for zinc or copper or nickel which are actually required essential trace elements for them such this kind of like the efflux pump may be operating when the concentration goes beyond certain threshold limit or for cadmium even or mercury we can see that the transformation or the active efflux is actually operating even at much lower concentration. So, it depends upon what are the metals, what are the type of microorganisms and if which environment they are actually interacting.

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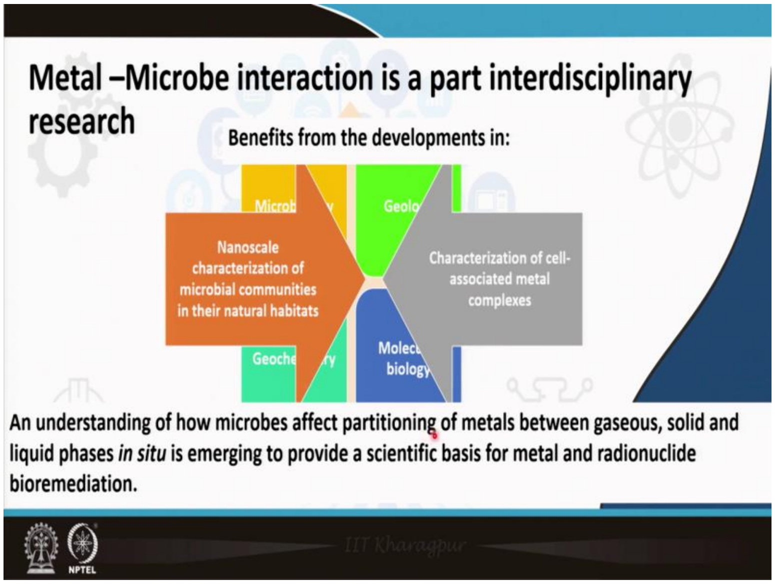

Now, in context to bioremediation, this metal microbe interaction is developed as a part of interdisciplinary research area where we see the assemblage of microbiology along with that geology, geochemistry and molecular biology, because essentially, these microbes the need to interact with these metals in a in a practical context, which will have a lot of geological parameters, geological factors within that.

And we have a number of benefits from these and this could be actually the Nano, micro to Nano scale characterization of the microbial communities in their natural habitats to understand what type of microbes are involved with different metals, what are their responses, how actually they are dealing with the toxic metals and nuclides. On the other hand, the characterization the cell associated metal complex is because often the metal ions are subjected to change.

In their oxidation states or changing their chemical other chemical properties like including their solubility in the aqueous medium. So, under such conditions, how and what are the properties of those deposited metals or sequestrated metals and their stability etcetera, those are also characterized and an understanding of how microbes affect the partitioning of the metals between the gaseous solid and the liquid phases in situ, because in practically the heavy metal pollutions will be the outside world.

How, exactly microbes will be capable of partitioning the metal. So, that they are not coming to in the aqueous phase and that is emerging to provide a scientific basis for the metal and radionuclide microbe interactions.

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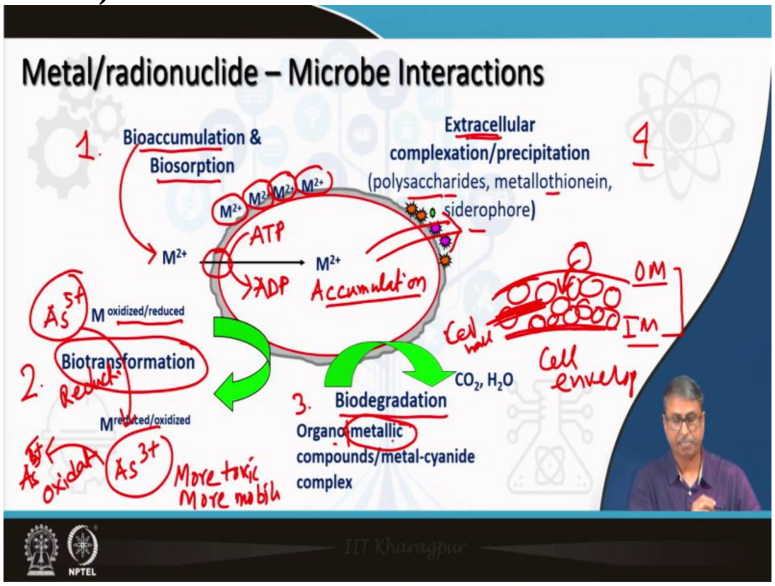

So, in this general way, if we look at this, so, microbes here the microbial cells, are one of the cells I have drawn and the M 2 plus is indicating the divalent metal ions. So, as you can see, there could be 4 basic mechanisms that I tried to put here. So, 4 mechanisms we will begin with first I brought the categorized into bioaccumulation and biosorption although these could be again splitted into 2 events like bioaccumulation and biosorption.

But the idea here is that in case of bioaccumulation, the divalent cation is transported inside the cell, either using some general transporter or some specific transporter by some means, the toxic metal ion will come inside the cell and we will begin accumulation. So, you will find that large number of divalent or the metal ions are deposited or accumulated within the cell. So, that is called the bio accumulation process.

That is a part of the introduction and of course, this is a property of a living cell because lot of transporters will be engaged and these transporters sometimes, they need energy also. So, we need to spend ATP for that or another transmembrane ingredient. So, we need to spend energy in some other form either ATP or transmembrane ingredient. The other one is biosorption, which is more like in a physical chemical process, where the whether the cells are living or cells or nonliving is not that important.

Biosorption is a physical chemical process where the divalent cations will be physically or chemically or both physical chemically, they will be dissolved. Now, the word dissolved means both adsorption and absorption are included in that and in this case, the bacterial or the cellular structure the cell in the lab plays a very important role because that is full of different negatively charged groups and those negatively charged groups they allow the divalent cations to resolved.

So, that includes both the adsorption and then absorption. So, absorption is on the surface and then gradually it moves in. So, now, if we imagine that the outer membrane and you have the periplasmic space or the cell wall part, so, you have the cell wall and any gram-negative bacteria, you have also the inner membrane. So, the heavy metals will gradually enter and reach up to the inner membrane.

Sometimes they have been found that they are completely loaded the cell envelope region which is covering entire piece of this outer membrane inner membrane and the cell wall part that is called the cell envelope. It is totally loaded with these metals, because it is both the adsorption first and then absorption this is a physical chemical process we will discuss about this later. The second and very important and interesting phase is the bio transformation.

Which is found to be mostly redox driven process oxidized and reduced either oxidized or reduced species are converted to some other form. For example, if we take an example, that arsenic 5 plus for example, can be reduced to produce arsenic 3 plus and vice versa so arsenic 3 can be oxidized to produce arsenic 5. Now, when the; microorganisms change the oxidation state or uranium for example, uranium one form to the other.

So, when we do these kinds of what we see the microorganisms are doing these kind of redox mediated transformations or maybe for chromium also or for iron or other redox active metals. So, that leads to some kind of change in the chemical behaviour because, if we compare arsenic 5 and arsenic 3, arsenic 3 is more toxic and more mobile compared to arsenic 5. So, arsenic 5 is relatively stable compared to that.

Now, possibly then the microbes will not try to do this reaction because if they are doing these then possibly, they are generating more toxic species, but then there could be some other 5 who can again transfer this arsenic 5 into arsenic or arsenic 3 into arsenic 5 through oxidative reactions. So, you can have both reactive reactions and oxidation reactions all kinds of redox reactions are possible in the environment depending upon the redox conditions and the type of microorganisms.

So, the idea is these bio transformation type of reactions they play a very important for example, the chromium for uranium we have seen that a lot of successful bio remediation process have been developed. The third one is the biodegradation. It is not the degradation that we have learned during organic pollutants, but this is the other the degradation where organometallic complexes because metal organic complexes are found to be more toxic more mobile.

They move very fast within the environment; they are accumulated in the living cells very fast. So, often microorganisms are capable of breaking down this organo metallic bond for example, retail marketing. So, if you break the retail market that bond between the marketing group and the retail group then the marketing becomes less mobile compared to the retail marketing molecule and then same the similarly for the arsenic also.

So, since microorganisms often they deploy some enzymes to break down the bond and then deal with the metallic element separately. The fourth one is the extracellular complexation or precipitation by different cellular ligands or cellular molecules produced by the microbes for example, polysaccharides metallothionein or siderophore like compounds, which are produced by the cells and they are released. So, once they are released these molecules when they are released or produced outside the cells, they are capable of extracellular complexation and precipitation of the metals.

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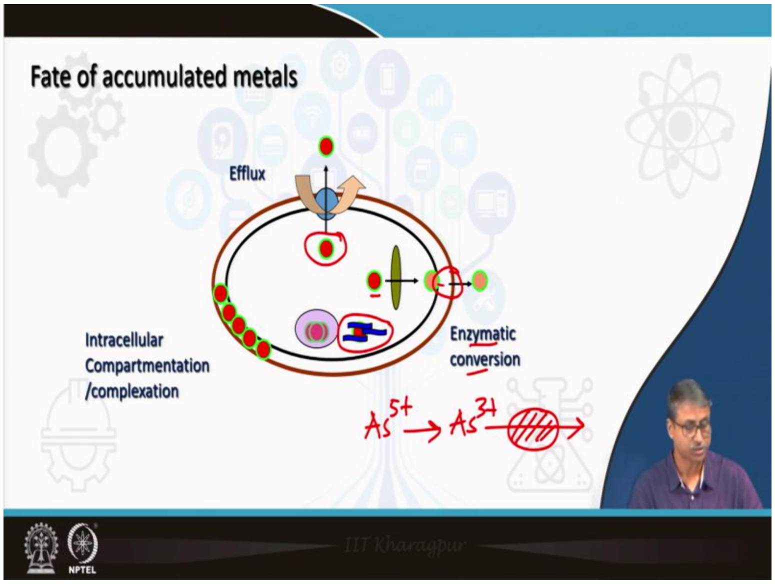

So, now, if the; metals are accumulated in the cells for the in the case of bioaccumulation that we have learned. So, what happens to the accumulated data for that also we see that there are a number of strategies developed by these microbial cells or bacteria in particular. So, one of the most prominent ones is the flux where the accumulated metal is thrown out or exported outside the cell by spending energy like either ADP or proton motive force or other source of energy.

So, that the cell is free from the metal. In other instance, we see the sale is capable of compartmentalizing the metal ions. For example, in case of copper, we have seen some bacterial strains of pseudomonas they are able to sequester the copper only in these periplasmic or the cell envelops space. So, that is a kind of their strategy they produce a specific type of copper binding proteins only in the cell envelope region.

And this cell envelope region copper binding protein they bind the copper and then allow the compartmentalization of the copper only in the periplasmic region. So, that the cell interior is free from the copper otherwise the copper will have its toxic effect and the cell may not be the feeling good with that the stress. So, these are kind of all the strategies that the cells are developed, but they execute and the other one is the enzymatic conversion.

For example, with chromium with arsenic, we see that there is different type of enzyme only the mostly the reductases type of enzymes, which converts these metal toxic metal like chromium. For example, to a form which can be if fluxed out. So, in that case there may be like mercury,

Mercury can be reduced and then the mercury can be reduced Hg 0 Hg 2 plus 2 Hg 0 and Hg can diffuse out of the cell or can be a flux pump can be they are that the flux pump will help the cells to actually transport out.

For example, in case of arsenic, we see that there are cytosolic arsenic reductases which actually reduce arsenic 5 to arsenic 3 and then arsenic 3 is fluxed out by a specific transporter pump. Alternatively, cells can also produce certain small protein molecules or a few amino acid chain molecules to sequester the metals inside the cell maybe the metallothionein is one of these examples. So, the again the metal is sequestered and unable to interact with anything else inside the cell.

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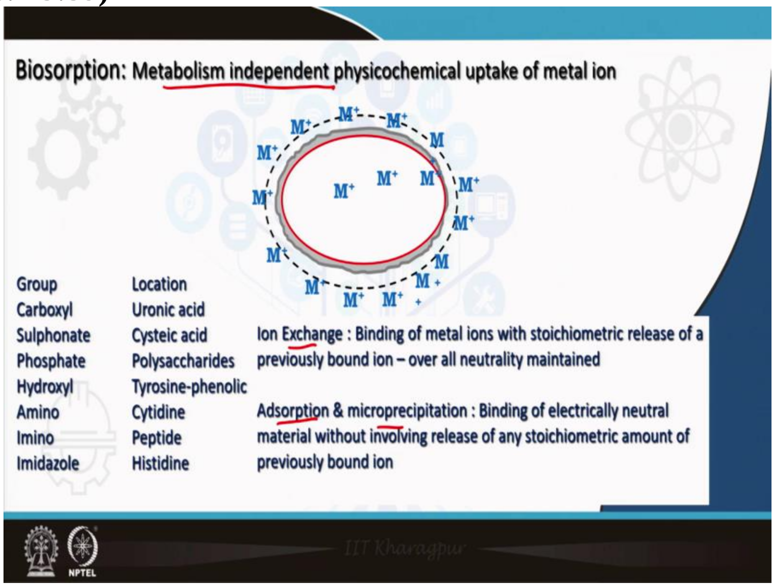

In case of biosorption, which we have already discussed, that is a metabolism independent physical chemical process where a large number of functional groups present within the cell envelop regions including the carboxyl, sulfonate, phosphate, hydroxyl etcetera, they are involved and it involves basically in large number of processes, but among them the ion exchange and absorption micro precipitation have been found to be most prominent in case of the bias option process.

It just remembers that this is including both the absorption and adsorption both type of processes is there and it is a metabolism independent process where the cells need not be leaving when these buyers options of product bioremediation point of view there have been a considerable research interest and process development have been done, because they can be performed using dead or metabolically inactivated cell also.

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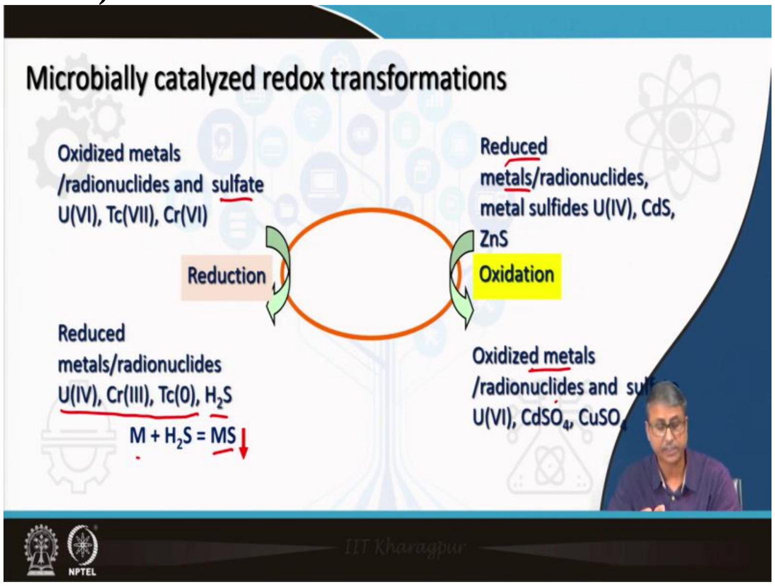

Now in case of the microbial immediate Redox transformation that is called bio transformation reactions, the oxidized forms of different metals already nuclides like uranium, technetium, chromium etcetera, can be reduced to some form like uranium 6 to uranium 4 chromium 6 to chromium 3 or technetium 7 to technetium 0 and all these will lead to altered mobility they are more in they are actually prone to precipitate they are prone to precipitate in the aqueous environment where the pH is around neutrality.

So, you will find that almost the aqueous phase is free from toxic uranium or toxic chromium because most of the uranium and chromium they have been precipitated in the solid phase. So, the water which is flowing will be free from these ions. Another aspect is the reduction of the sulfate, sulfate reduction leads to the production of sulfides and these sulfides can react with divalent metal ions and further produce the metal sulfides and metal sulfides.

The precipitate so that is another means by which microorganisms they interact with these toxic metals and radionuclides alternatively, these metals and radionuclides can be oxidized also. So, a large number of metals uranium, including uranium 4 or cadmium sulfide, zinc sulfide, arsenic etcetera we see that the reduced species are subjected to oxidation and then the oxidized forms are produced, but we need to remember that the again the oxidized forms might have different environmental behaviour.

But whether oxidation will proceed or reduction will proceed that depends on the environmental condition, particularly the redox condition of the environment itself and also the microorganisms which are present over there.

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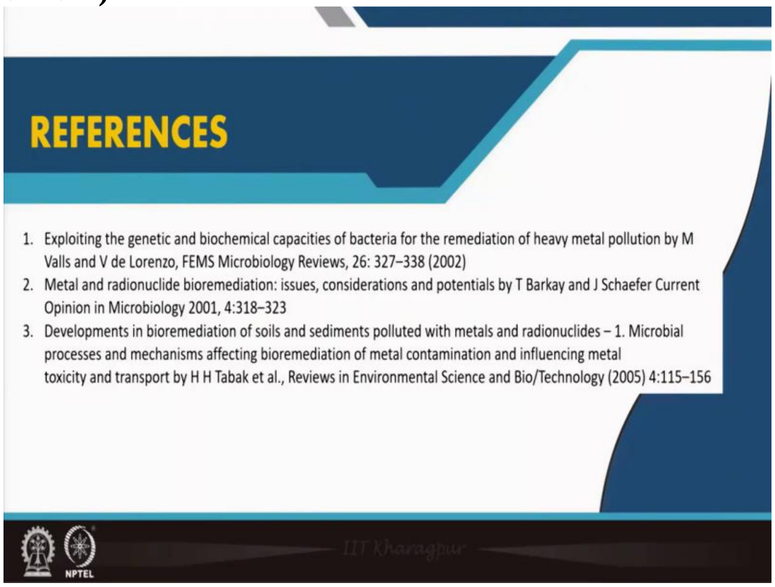

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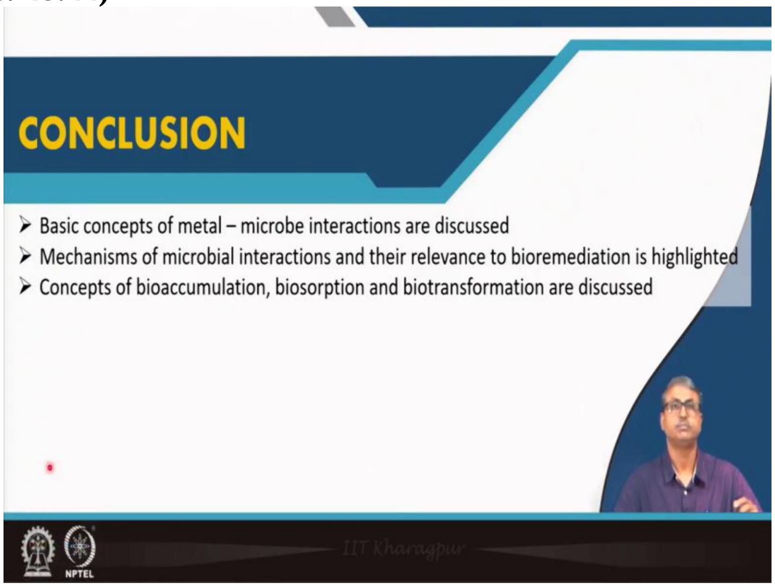

So, for this part of the lecture, the following references can be used and in conclusion, we have discussed the basic concept of metal microbe interaction, mechanisms of microbial interaction and their relevance to bioremediation is highlighted and the concepts of bioaccumulation biosorption and biotransformation are also discussed in today's class. Thank you so much.