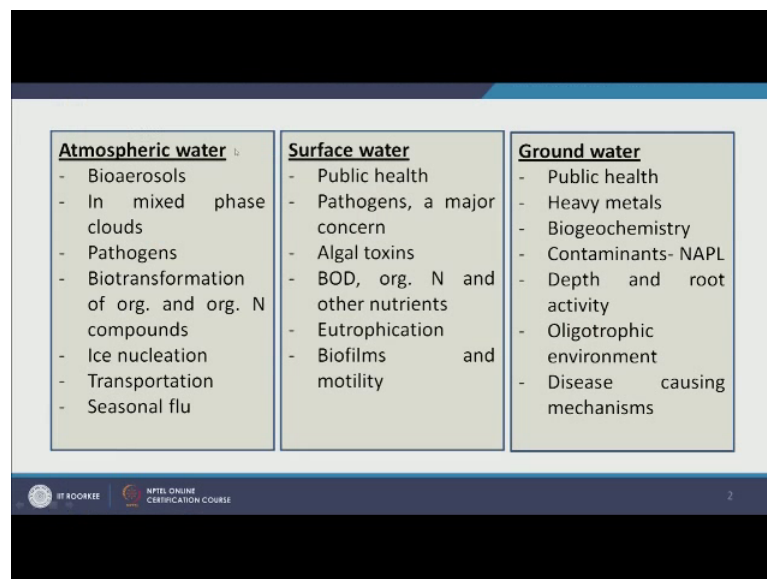


Applied Environmental Microbiology
Dr. Gargi Singh
Department of Civil Engineering
Indian Institute of Technology, Roorkee

Lecture – 18
Microbial Ecosystem III

Dear students. So, in the last lecture we talked about the microbial ecology in terrestrial systems and today we are going to talk about microbial ecology in aquatic systems, usually it is assumed when we hear the word aquatic we are talking about surface water so we might be differing to ponds to lakes to rivers and other surface water bodies; however, it is interesting and important to note that aquatic ecosystems are not limited to surface water systems. In fact, the water can exist in air, land like on the surface of land and below the surface of land and thus today in this class we will be talking about microbes and the microbial ecology in context of how do they come there? How do they survive in these ecosystems in air on surface and below the surface? So, let us get started.

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<u>Atmospheric water</u>	<u>Surface water</u>	<u>Ground water</u>
<ul style="list-style-type: none">- Bioaerosols- In mixed phase clouds- Pathogens- Biotransformation of org. and org. N compounds- Ice nucleation- Transportation- Seasonal flu	<ul style="list-style-type: none">- Public health- Pathogens, a major concern- Algal toxins- BOD, org. N and other nutrients- Eutrophication- Biofilms and motility	<ul style="list-style-type: none">- Public health- Heavy metals- Biogeochemistry- Contaminants- NAPL- Depth and root activity- Oligotrophic environment- Disease causing mechanisms

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So here we have the three different ecosystems is a one for atmospheric water surface water and ground water which is below the surface water. So, let us go through them one by one and then I will show you some specific pictures and schematics for each of these.

Atmospheric water; in atmosphere if I mean water is present, whether it is cloud whether it is the water vapor there absolute or relative humidity around us this water might at times serve as a small habitat for microorganisms and this is the reason why we have airborne illnesses such as tuberculosis and flu many flu viruses that can be transmitted through these air particles and if the air particles have a right condition the right humidity the right temperature then they can persist in this aerosols for long time. Now the aerosols so they are not dissolved in the air definitely they are suspended in the air these particulate matter these aerosols might be the good habitat for microbes to sustain and in that case we refer to them as bio aerosols definitely bio means life in the aerosols, the next very important example of bio aerosol is the mixed phased clouds.

So, in clouds where we have good amount of humidity where we generally tend to have particulate matter and definitely we have air so we have a mixed phase system the bacteria or any other microbe might find it a very suitable place to live in the cloud and then rain down upon wherever they rain down in case they do or just dispersed back in atmosphere it is very interesting that; this, atmosphere transportation of pollutants including microbiological pollutants is recently discovered phenomena in sense that earlier we did not give much importance to it, but now we are realizing the very important relevance of airborne transportation of pathogens and microbes a very clear example perhaps would be the example of flu.

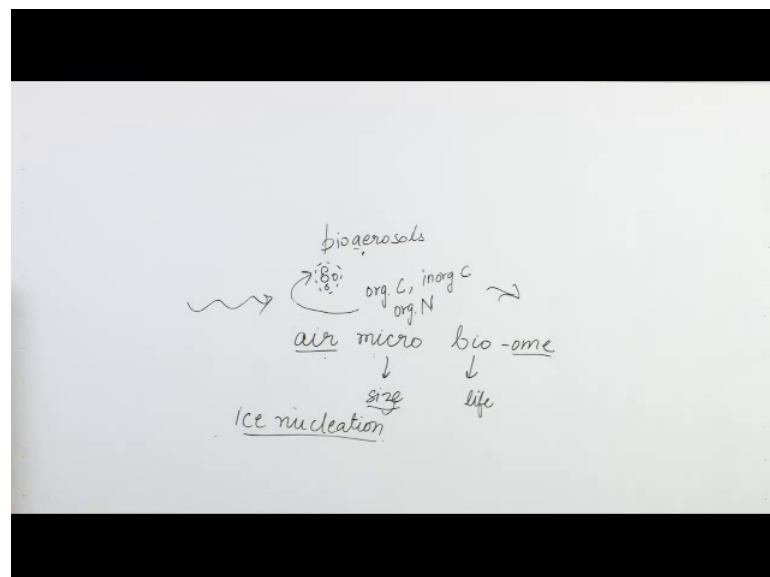
So, every year we have a flu season in our side of the world which is Indian subcontinent and Chinese continent subcontinent and are nearly after 6 months we have the flu season in the north American and south American continent and it is interesting how the flu virus that is spreading the annual flu in our part of the world shares genetic similarity with the flu virus that will spread of would have spread flu 6 months later in the north America and south American continent, implying that there is a high probability and likelihood of the flu virus getting transported through some airborne mechanism from the Indian and Chinese subcontinent all over to Americas and the other the other way is also true that the flu virus from north and south America travels to India and china and actually across the globe, but we happen to be diametrically opposed it.

So it is a good example suggesting that the air micro biome is very important and in fact I might say this I want to take a Segway and talk about how relevant this air micro biome is nowadays? Is by the immense amount of research that is being that is focusing itself

on the air micro biome; we are very interested to see how the microbes are spread through the air? How diseases are spread through air? And how our indoor air is not only different in terms of its physical and chemical characteristics compared to the outdoor ambient air, but also in its microbiological perspective. So, the one way to think about is about this is the moment we enter a room; we shed off microbes from our body, the moment a dog enters the room let us say I have a pet dog any enters the room he shedding off microbes all over the room, now these microbes will travel to different parts of the room undergo transformations and will die somewhere probably free and thus will have a very different air micro biome inside compared to outside.

So, now coming back to the atmospheric water why would it be very important for us? Why we as human beings would be very interested in atmospheric water and the microbes that live in it well; obviously, they are interested about public health and it so happens that there are certain pathogens that do very well in these bio aerosols given the right humidity and air condition and we will talk about them, the other thing is very important because there are microbes present in the air we call it air micro biome I will try like to write this down actually.

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So, we can split it into different parts this particular word can be split. So, definitely air is you know; air, micro on a small scale so this is giving you an idea of the size we are

talking about, bio means life and when we talk about ome like omecs we are talking about a technique or a strategy that involves all the forms of biology present.

So, if you remember in one of the previous lectures I talked about how less than or nearly 1 percent of microbes we know how to culture them when we can culture them in lab, but majority of them are not yet culturable in our laboratories and thus we go for high throughput amplicon sequencing based techniques which allow us to look at the entire microbial structure, now this word ome is a general is a general suffix to many words implying that everything is included. So, air micro biome means all that life that exists in micro scale in the air; obviously, it is not possible to have a clump of microbes roaming floating in the air because due to their weight they will come down due to gravity.

So, we have definitely a size limitation now because the microbes are present in the air so let us think of it this way let us say this is a bacteria and this is a clump of few bacteria and there is some moisture around them and they are floating happily in the air and let us say the air has some organic contaminants or organic carbon and they have some organic forms of nitrogen and maybe not even necessarily organic it can be inorganic form of carbon as well. So, these electron donor potential electron donor electron acceptors nutrients and carbon source can very well be transformed in the bio aerosols. So, if you remember the; we talked about the microbial energetics and we talked about how microbes harness the energetic exchanges in their environment to use it as energy source to use it as carbon in nutrition source the same thing happens in the air. So, this is very important because this might be actually catalyzed in conversion of many primary pollutants into secondary pollutants.

So, again a short revision from your basic environmental science goals it is taught to all undergraduates' now, primary pollutant is a pollutant that is originally released from the source, so if I have a chimney it is releasing some smoke from the industry that is the primary pollutant all the pollutants in the smoke, now when it goes out in the air it comes in contact with light; comes in contact with other phenomena, including bio aerosols and then it undergoes a transformation and makes secondary pollutants already many a times the secondary pollutants are more harmful for public health than the primary pollutants, so we are very concerned about it and we are particularly interested in knowing how bio aerosols play a role and we are finding out increasingly the play a good amount of role.

The other thing is bio aerosols and my air my program in itself is very important because remember how we how we teachers taught you for many years how clouds are formed? We told you well the nucleation happens in such a way there is a particle and then the water condenses around it and there grows and then low and behold it is a mini droplet which then becomes a droplet and then next thing we have is raindrops right.

So, it does not always have to be a particle particulate matter and in that particulate matter in fact, it can be a bacteria or a group of bacteria that are sticking to each other. So, bacteria can also serve the role of particulate for nucleation of rain and ice then this is very important let us say we talk about ice nucleation. So, when we talk about ice nucleation not only are we talking about how microbes will help or continuing to help formation of clouds formation of rain, but also if the temperatures are low enough then we might have preservation of these microbes so very long time, let us say there is a bacteria or there is a virus or there is any other kind of microbe that is pathogenic and we are not interested in making ensuring the persistence of that microbe in the environment, but it undergoes ice nucleation and now it has a protective layer around it yeah which maintains it is temperature to below and this can be transported over long distances.

So, apart from affecting the weather the local weather conditions and the local chemical scenario, so what kind of pollutants you have? What kind of chemistry you have? And what kind of pathogens you have? What does to air microbe microbes do? As I mentioned about seasonal flu; this is very important, the particulate matter travels all across the globe, so pollutants from let us say released in china will cause acid rain in took you and will cause los Angeles to suffer excessive deposition of dust. So, it is not just particulate matter that is getting transported, but it is also the micro biome so we are increasingly interconnected we always have been interconnected in terms of bacteria alright. So, now let us move to a surface water, so this was atmospheric water and we will briefly go over it again in surface water wells one of the biggest thing about surface water is that we are very concerned about public health and why would that be so? Because from large part of our world yet in definitely our country it also surface water is still the primary source of raw drinking water, so we take surface water like a river water lake water be treated and then we use it as drinking water.

Now if the quality of our surface water is not good; then we are in troubles, so when we talk about surface water and surface water microbiology ecosystems we are very

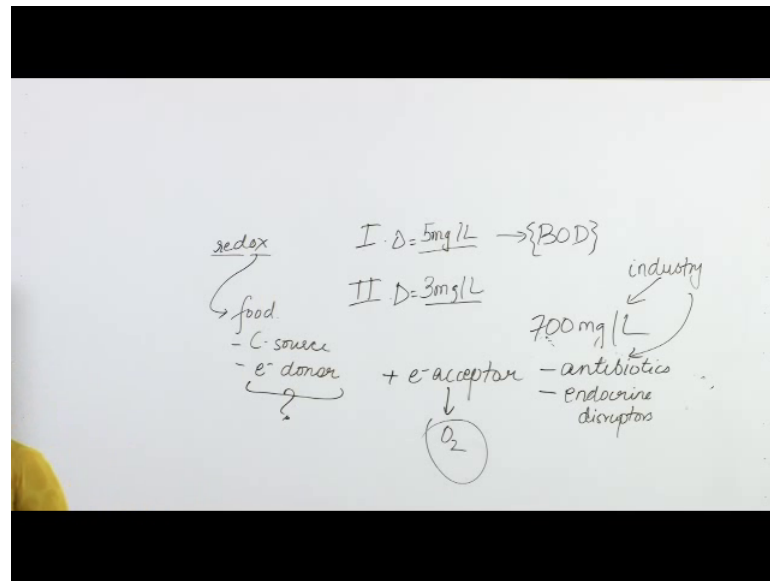
interested in what is going what it is re percussion would be for public health. So, we interested in pathogens and continuing with our interest in public health and next thing is algal toxins, so most of you must have heard about eutrophication where we have excess of nutrients and low and behold things grow and early dies about a body suffers and at times we also have algal blooms and some algae not all some algae produce algal toxins which are not good for us we briefly go over it again and then coming back to the basic parameters which affect the persistence of pathogens which affect and can drive the algal growth of biofilms are B O D organic nitrogen and other nutrients.

So, let us look at what B O D is? I have briefly mentioned about B O D in past I think it is a very good time for me to talk about B O D. So, B O D stands for biologically biological oxygen demand some people also call it biochemical oxygen demand, basically it is amount of oxygen that will be consumed in 5 days or in any tiny amount of time depending on what with my time duration for B O D is and why would this oxygen consumption happen? Because they we assume and we believe that there are organics present in the water and there are microbes that want to eat it. So, if organics are present in water and I provide microbes that love to eat it I want to know how much oxygen would dip in T amount of time; T could be 3 days, T could be 5 days, T could be infinity depending on what I am interested in. So, the more the B O D in other words the more oxygen is consumed we know that they were more based to begin with, so I like to think of this in terms of let us take a favorite food; let us say, your favorite food is noodle you love noodle let us say maybe you do not, but let us imagine or pick any other food you are like idly is one of my favorites, so we can pick idly or anything really pizza.

So, you take let us say you have 1 kilo of noodles you can finish them you need you can finish them if you are a bacteria you can finish them let us say in 1 day, but now I increase a load of the food and make it 100 kilos, know the 100 kilos will take longer time for you to finish alone, but let us say you cannot afford longer time we have limited the time, so this from 0 to 3 days or 0 to 5 days or 0 to infinity whatever we have limited the time and we make enough humans we bring in humans in to finish the 100 kilos of food.

Now the oxygen that would be consumed by the humans or by the bacteria to consume that the hundred times more amount of food will be directly proportional to number of amount of food, so if there is less food for bacteria to digest they will need less oxygen.

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So, let us remember for redox reaction; there two components to it, one is the reduction and the other is oxidation, so if we have lot of food and food here is both carbon source and food is also the electron donor, typically food when we talk about food for bacteria it is biology it is organic waste and they are all electron donors. So, if I want to consume electron donor, so if I want electron donor to undergo oxidation so that it can release energy for microbes there has something has to be reduced. So, what will be reduced? Electron acceptor and in case of biological oxygen demand or biochemical oxygen demand; however, we put it that electron acceptor of choice is oxygen that is why it is oxygen demand, so we do not know how much waste is present we do not know how much electron donor is present, but what we can and we cannot measure it we want to find out how to measure it. So, one way we can measure electron donor is we can find out how much oxygen would be consumed to finish this food or in other words we can find out how much oxygen would be consumed by microbes if there are enough microbes to eat they will as much electron donors as they can within time feet time period.

So, let us say in first 5 days; let us say in, so I take water sample two water samples sample 1 and sample 2 and in sample 1 I make sure that both samples I make sure that there is enough oxygen and after 5 days and I had bacteria by the way. So, I take sample 1 and sample 2 both have enough oxygen, both have some amount of waste material I do not know how much biodegradable waste material or how much electron donors they

have, so I will add lot of bacteria to them or the bacteria that I know will eat of these in case they are not already present we eat of the electron donor, so now bacteria will try to drive this reaction. So, let us say in 5 after 5 days of incubation I noticed that the dissolved oxygen in sample one has dropped by 5 milligram per liter and in the other it has dropped by 3 milligram per liter, so this is the delta and duo this is the consumption of dissolved oxygen, so I know that more oxygen was consumed in samples 1, so if more oxygen was consumed in sample 1 I can say more electron donor was there to be digested with this oxygen more of it was present.

So, I can say that the more electron donor was present in samples 1 compared to sample 2 in other words; more waste material was presented sample 1 compared to sample 2, so this is what B O D is, by looking at how much oxygen is demanded I get an idea of how much organic or biodegradable waste is present in the water, so this is what we call as biological or biochemical oxygen demand already. So, in surface water this is one of the most important parameter that we love to measure because just by looking at B O D we sort of know how fast microbes will grow because let us say in the beginning I do not have any micro present in the water, but I have a very high B O D loading; let us say, I have 700 milligram per liter which is pretty higher actually it is pretty high yeah. So, I have 700 milligram per liter of B O D and there are no microbes but eventually because there is food the microbes will find a way and once they find a way they will proliferate. So, I know the potential of how much the total microbial growth can happen in this, I know how much hydro tropic plate count I can expect from this sample very quickly if it has such high B O D loading.

So, B O D loading tells me an idea of how much microbes can grow? So, this is very important and the other thing is the question is; where did such high amount of organic waste or biodegradable waste come from? So probably it came from a sewage raw sewage or partially treated or incompletely treated sewage.

So, if it is coming from sewage then I can definitely expect fecal contamination and there is a very high chance of pathogens being present. So, high B O D also tells me that there is a high chances of pathogens being present, the other thing is if it came from industrial sources not sewage; let us say, there is some industry that has very high B O D load.

Let us say biscuit industry and they have lot of it will probably be higher than 700 milligram per liter, but they have a very high B O D load high amount of waste that they are releasing in their effluent and there are no pathogens present there are no micros, but eventually over time they very likely to consume that, the other thing is if it is industrial waste is a very high chance that they have other chemicals that they are also releasing. So, let us not take this kid because we assume that everything in biscuit is edible and healthy or not unhealthy, let us take pharmaceutical industry and they have very high amount of B O D right all let us a considerably high amount of B O D, so they are not just releasing biodegradable waste of food for bacteria which bacteria will eventually consume and bring it quite below to the nearly detection limit or they know below our regulatory standards, but they are also releasing other contaminants that are not good for us for example; they might be releasing a lot of antibiotics yeah, so they might be releasing a lot of antibiotics the pharma industry or they might be releasing lot of endocrine disruptors which are basically chemicals that hurt us hurt an endocrine system and thus upset our hormonal cycles. So, whenever there is a high B O D all red alarms should all alarm should go red and the red herring should tell us either there are a lot of pathogens or a lot of contaminants present or there is a huge potential for pathogens to grow alright.

So, again when it comes to surface water we are very interested in B O D, the other thing we are interested when it comes to surface water is how the electron gradient how the light gradient and energy eage changes with depth? Alright so let us take case of lake; now remember in the previous lecture when I talked about; population, communities and gins I drew a diagram of a lakes, so I encourage you to go back and revisit the previous lecture and note that; as we went below, from the surface of the lake and we increase the depth there far thereby the energy yield of any redox reaction reduced the electron acceptor gradient went from aerobic reactions to anoxic reactions which included; nitrate reduction, a ferric reduction, sulfate reduction, sulfur reduction all the way to methanogens and then to sorry all the way to fermentation and the methanogens and acid to genesis. So, we notice that the microbial communities in surface water will vary with depth. So, one kind of microbes might be very prevalent in the top surface may not be, so prevalent in the deep surface and this is where lot of research is going on and we will briefly go over ha how microbes change through that then; obviously, temperature is

important the lakes or stationary relatively stationary bodies of what surface water they usually have a temperature gradient and undergo twice a year or once a year mixing.

So, this is mixing what happens when the top layer and bottom layer have such temperature that the bottom layer wants to come up and top layer wants to go down, now we know this that water is most dense when it is at 4 degree Celsius anything less it is lighter anything more it is lighter. So, again as the temperature of the atmosphere changes temperature of the top surface changes the lake will undergo mixing cycle and you will study this in other courses environmental science courses or in general E V S course also. So, as the mixing happens or does not happen the microbial communities also change, the other thing the surface water that we need to be hmm careful about so apart first thing we need to be aware of is depth the other you need to be aware of is a electron acceptor gradient and then we need to know about definitely how deep the light goes because of turbidity the depth might change, then we need to know about the mixing how mixing is going on.

And the next thing we need to know when it comes to surface water is how the nutrients are changing with depth? So, at nitrogen levels are changing in the phosphorus levels are changing so we will study through the about these things in subsequent slides. Now another important thing about surface water is that; in fact, whether it is atmospheric water surface water or ground water if they are clean if they have not has suffered heavy human impact or impact of human activities and they relatively clean they are likely to have less amount of nutrients compared to other environments for example; some parts of the lake might be oligotrophic, some parts may not be. For example; the sediment zone may not be lacking essential nutrients, but wherever the water is clearer and cleaner it might like the nutrients and in that case the oligotrophic microbes they devise certain techniques to survive one of them is motility they learn how to swim towards move food. So, the bacteria that you notice or the microbes you notice growing in surface water bodies like; even ocean or lake, pond, river would have beautiful whip like structures that help them flow that help them move it is a flagellum or any other estrogen.

So, one is motility that is how they develop; the other is that they make biofilms and this is our last point in this slide for surface water biofilms and motility, so in biofilms what these microbes do is that; they stick with each other and I think in one of the previous no in the upcoming lecture when we talk about drinking water micro biome I will be telling

more deeply about biofilms what biofilms are how they are helpful? Briefly for now it is important for you to know that when my probes stick together they are better able to capture the nutrients in the water so they act like a sea when they capture whatever nutrients go they also protect each other so it is almost the penguin effect you know how penguins clubbed together and when a storm comes and only the penguins are the periphery of their group of the heap will suffer and the young ones inside are saved from the storm.

So, this is exactly what the bacteria do and in biofilms they have an added advantage that they secrete extra polymeric substances this secrete them and they make matrix around them which gives them further protection alright, then we move on to groundwater again if not surface water then our next source of water is ground water, people have been thinking of getting water from atmosphere but the technology still needs some more development for mass utilization, but ground water definitely is a major source of raw drinking water.

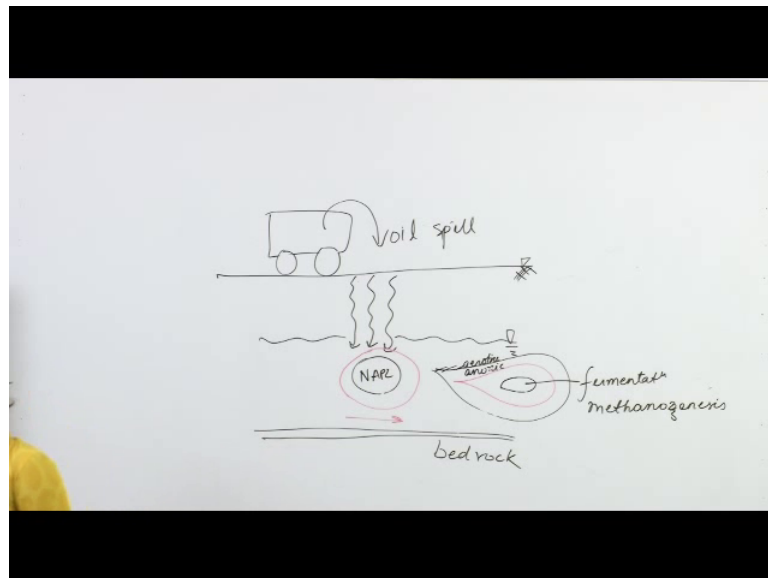
So, our first concern again is public health, now when we think of ground definitely in the first few feet up to the first few feet of the below the earth's surface we will see a lot of nutrition and we will see a lot of microbial communities; whether they are anoxic or facultative aerobes, facultative anaerobes or aerobic microbes in the reason is because this is very close to at the surface it gets a lot of nutrient deposits like; biomass that deposits on it and then human activities impacted and that is why it is not unexpected to see quite rich microbial communities and diverse microbial communities in the top surface of the soil, interesting thing happens when we go deep towards the deep aquifers.

So, initially it was an assumption that with ground water microbial growth is not an issue but now we are noticing it is not necessarily in terms of severe pathogenic presence there are two pathogens we will talk about them, but there are microbes in and around ground water who are very happy to be in those oligotrophic even all you go trophic sometimes not necessarily oligotrophic but definitely anoxic zones and in presence of water and they really not only affect the quality of water, but they also affect the minerals and the chemistry of the soil around them we will talk about this.

And so let us see here, so this is biogeochemistry so bio life geo earth chemistry. So, the life in earth it changes earth and it changes earths chemistry the other thing about ground

water we are interested in is contaminants. So, often what happens as we spill things and then the spill percolates down and reaches the ground water in our ground water is contaminated here I have the word NAPL non aqueous phase liquids, so these are non-aqueous so non water phases liquid, so when they reach the ground water they have their own plume they have their own plume that they make they do not necessarily mix in the ground water these are one kind of contaminants, especially petrochemicals they will definitely make a plume a NAPL plume now depending on the physical and chemical characteristics of the plume it will undergo degradation, it will undergo mixing to some extent in transportation through the ground water and affect the microbiology. I have drawn this diagram before, but please go ahead and take another look at these.

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Alright students, so take a look at this diagram let us imagine that this is the surface of the earth and there was a tanker carrying oil and it spills the oil; the oil percolated down the down the earth through the layers of the terra and here we have ground water, now please note that this diagram is just a schematic just a cartoon of ground water from water does not flow in waves like this it just passes through the earths pole, so it looks very different but yeah it is quite a discernible and significant flow and let us say it is flowing in this direction.

So, from my right to left and this is when the oil has spilled and percolated down, because it is non aqueous it will not mix with the water as much and because of the

surface tension of water and the contaminant it is a petrochemical they will try to form a meniscus you know a blob of NAPL non aqueous phase liquid, alright over time and pretty instantly also actually the NAPL will undergo slight dissolution so some of it will have a slight equilibrium with the water so this is the red thing and as it moves ahead it will undergo a shape deformation; like this, so it's definitely this is an exaggeration or may not be an exaggeration.

So NAPL is here and the dissolution level looks like this and the reason for this is the resistance provided by the earth or the soil force to movement of NAPL and when this happens because before this happens also, when the dissolution happens the microbes start eating whatever they can because you know microbes bacteria and other microbes they love to live in the hydrophilic they love to live in water and that is how their cellular membrane is made, if they live in oily places in the cellular membrane will be disrupted remember a hydrophilic and hydrophobic ends, so the cell membrane makes use of the fact that inside the cell and outside the cell it is a water based medium and the integrity of the cell membrane is maintained.

So, they do not like to enter into NAPL if they do they would not persist very long most of them at least, but in the dissolution layer they will grow and as they grow they will start consuming the electron acceptor, they start consuming the NAPL which is electron donor and the more they consume the more of it would dissolve and after some time the volume or the amount of NAPL left would reduce yeah if the volume total of it increase, but C/V concentration into volume will definitely reduce where biotic reduction.

Now as remember this is electron donor so some electron acceptor is also getting consumed, so the first electron acceptor to get consumed to be oxygen energetically most favorable. So, at the periphery of the dissolve plume we still have some oxygen left, so this will be your aerobic zone and then you will have anoxic zone, now in anoxic zone you might see; nitrate reduction, you might see iron reduction or sulphur reduction, sulfate reduction and so and so forth and we notice that the closer we come to the plume will go to anaerobic metabolism which will look like fermentation or methanogenesis alright our acetogenesis and the reason is that the first dissolution there will happen here right very close to NAPL and the micros will consume it right away they are waiting here when the discussion start we start consuming. So, they will deplete the oxygen here first

the near the NAPL and then eventually even that is consumed and the NAPL has traveled further apart then here the oxygen will be consumed, further apart here the oxygen will be consumed for now it has reached here, so this is the aerobic way place the aerobic lay way of oxygen is turn left.

So, we notice that in case of ground water we might in the same local region we might get very different microbes, so here we might have aerobic microbial community here we have nitrate reducers sulfate reducers methanogens and thus ground water can be very diverse both in terms of function and in terms of phylogeny alright. So, in ground water the oligotrophic environment plus the contaminant create pretty interesting phenomena and we notice that we have very diverse microbes so notice all of them tend to have very diverse microbiology atmospheric water is very important for us because of bio aero source and how diseases spread geographically, surface water because still they are all drinking major source of raw drinking water and ground water because in many places we have contaminated it and it affects biogeochemistry's, so we are interested in understanding the microbial ecology of ground water and now other thing about ground water is that usually there bacteriologically safe there are, but there are two pathogens that are known to survive ground water conditions; one of them is eco lie and the other is cryptosporidium, now keep this in mind and remember it for when we are going to talk about drinking water micro biome.

Alright dear students, I think this is all for today and in the next lecture we will go ahead and we will talk about atmospheric water surface water and groundwater more in depth. So, I will show you some of the cycles that undergo in all these three ecosystems environment and how they impact public health and why they important for us.

Thank you.