Applied Environmental Microbiology
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Lecture – 48
Solid Waste Microbiology III

Dear students, in this last lecture for solid waste microbiology, we will be looking at what kind of microbial community we can find in a landfill or wherever we are disposing our solid waste and we will be looking at what are the factors that affect the microbial community structure and function in our landfill, apart from that we will, at the end also talk about three token research, that have happened on the microbiological structure of landfill and how the research was conducted and what their results were.

Alright, let us get started. So, in the last lecture, I talked about different kinds of landfill and these are the pictures from Ghaziabad landfill. Ghaziabad landfill and all of them are Indian landfills and the important things that I wanted you to notice, that many of our landfills, they resemble more like a dump yard, for example, this is more of a dump yard.

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Then a scientifically managed landfill; I also talked about the dangers of human beings and specifically children and at times, adults being exposed to toxic waste that we dump on a purely managed landfill. The another incident, that I shared was a recent landslide; recent at the time of recording this lecture, that happened in Ghaziabad, where due to

heavy rains, the waste from the top of the landfill slighted down and it killed two people and it slid into a nearby canal, for this is the dump yard and it slipped into the canal. So, now, the entire canal is dirty, it has toxins in it alright.

Then, I told you about the composition of the leach ate and the important thing that you need to note is that as time progresses, the chemistry of the landfill also progresses. Initially, we have a different kind of reaction, this is aerobic. So, the DO is, the oxygen levels are substantial. Oxygen is being consumed and then, comes the acidic phase, where the Ph drops rapidly and we have lot of different acids in the oxygen phase. We have hydrolysis of our waste.

Now, the hydrolyzed sugars are acidified in the second stage and in the third stage, methanogenesis shift sets in and this is where the solids, we start having solid loss and then, we move to fourth phase and fourth phase is where the solid lo loss is finally, substantial and the methane to carbon ratio becomes nearly one. Ratio of one and then, as we progress, methane might even be more than carbon dioxide and over the end.

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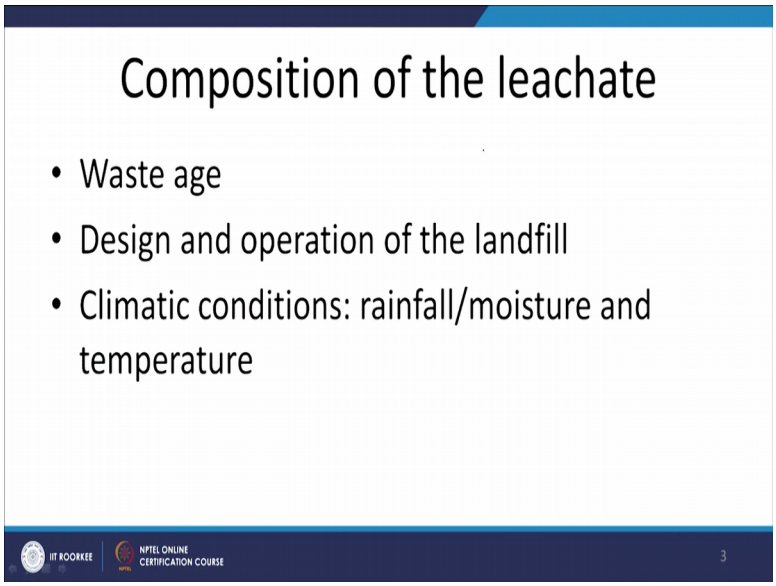

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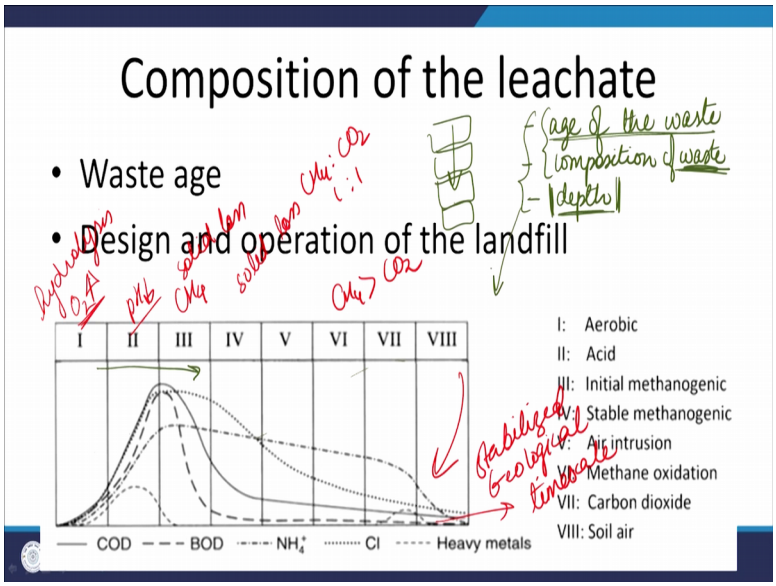

Finally, even if we infiltrate oxygen, we noticed that our landfill has stabilized and this happens or we hope it happens and in geological timescale. Now, this is very important for you to note, because you can see here, that the microbial community that function that microbial community in a landfill, that it is doing depends on multiple factors. First, it depends on the age of the waste, because as time progresses, the waste material that has been dumped into your landfill, will move from aerobic to acidic and to methanogenic phases.

Next thing, it depends upon is composition of waste now; obviously, depending upon the composition of the waste, the progress with time from one phase to another would vary and a recent report has noticed that in India, nearly fifty percent of our waste is biodegradable. So at least, we can expect that the hydrolysis part, the acidification part they will happen pretty quickly and will result in a lot of loss in volume and production of leach ate over time.

The next thing, it depends upon, because it depends upon age and usually the dumping happens bottom top. So, we fill the bottom cells and then, the top cells in our trenches, it also depends on depth, also with depth, the conditions change. We have noted in previous lecture, that at 17.5 meter depth, the temperatures and the conditions in the landfill were extreme hemophilic and temperatures were very high.

So, basically depending on the time that has passed, since the vase was dumped, what kind of waste it is and what its environmental conditions are now as waste gets old, certain daughter products of degradation will get accumulated, the salinity would change, the Ph would change, pitch initially drops here and then, it normalizes back to 7 and it maybe even more depending on the composition. Overall what it brings us to is, two factors. The two factors that it brings us back to here are: - first, the foods.

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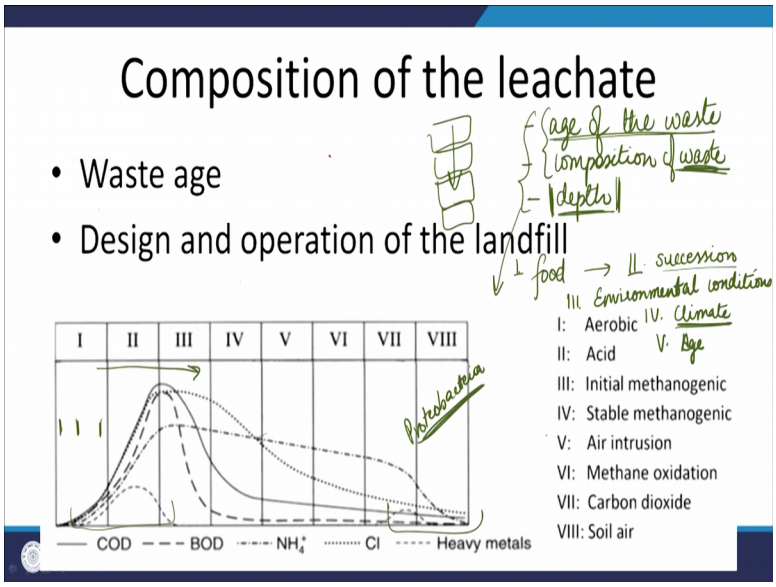

The kind of food we are giving to microbes. Now, depending on the kind of food that is available for microbial community, a certain population or communities that are better suited at consuming that food and its daughter products will flourish by, others will die out.

So this brings us to the second point, which is succession of microbial communities. Here, the microbial activity would be different and other kinds of microbes. The ones that love high microbial activity would thrive over here, Proteobacteria kind of microbes. I do not like lot of microbial activity; they will thrive because by now the food has been degraded to quite some extent and microbe belongings, many kinds of Proteobacteria would be happy.

So, we notice that from time to time, the microbial community undergoes succession. It is also quite possible that at the beginning of phase one and middle of phase one and the

end of phase, when the microbial community would look different, because the chemical conditions of the landfill would be different.

Which brings us to the third point: - the environmental conditions. Now, a question that this particular point brings up, ‘environmental conditions’ is that are we talking about local environmental conditions or does climate also play a role? Obviously, the climate too would play a role. So, that is now the fourth point. Now, this should highlight that even in cities that have similar climate, if the local environmental conditions are different and the kind of waste that is present is different, the age of the microbe, age of the landfill is different.

The succession of that microbial communities, have undergone is different than the microbial community is found at any given depth, at any given time in the landfill would be distinct. So, these are some of the factors that affect the microbial community structure. In the previous lecture, I also told you about how organic substances, chiefly cellulose and hemicellulose, the lignin, they degrade and either result in carbon dioxide or methane.

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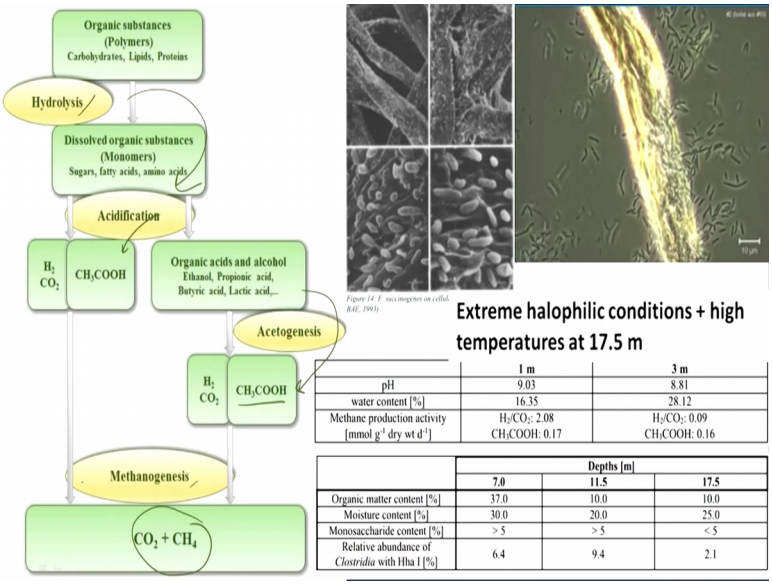

	1 m	3 m
pH	9.03	8.81
water content [%]	16.35	28.12
Methane production activity [mmol g^{-1} dry wt d^{-1}]	H_2/CO_2: 2.08 CH_3COOH: 0.17	H_2/CO_2: 0.09 CH_3COOH: 0.16

	Depths [m]		
	7.0	11.5	17.5
Organic matter content [%]	37.0	10.0	10.0
Moisture content [%]	30.0	20.0	25.0
Monosaccharide content [%]	> 5	> 5	< 5
Relative abundance of *Clostridia* with Hha I [%]	6.4	9.4	2.1

Now, the eventual fate is definitely methanogenesis, because we are not editing our landfills. So remember, first is hydrolysis where big polymeric substances like lignin, cellulose, hemicellulose; they break down into similar simple sugars, such as cellobiose and then, these are converted into assets such as prop ionic acid, butyric acid, lactic acid

which can undergo acetogenesis and they will all convert it to acetic acid, or it will directly from acetic acid, which will undergo methanogenesis and then, this is a brief.

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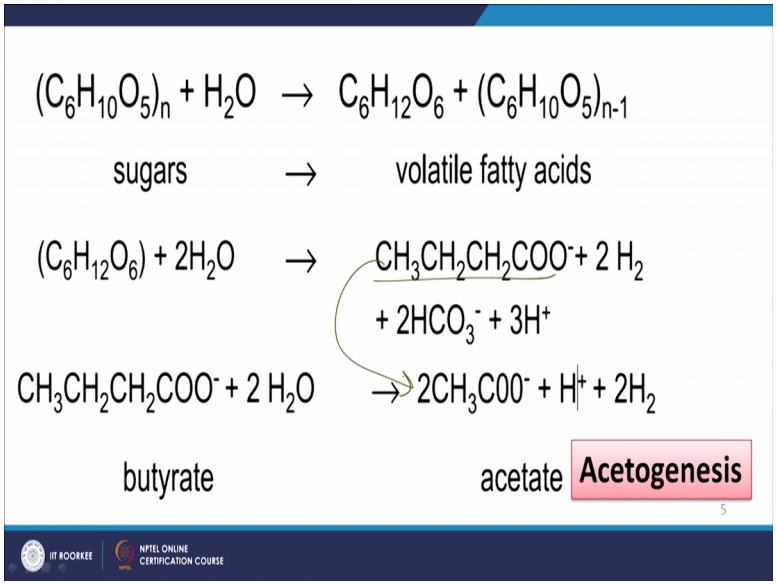

Review, first as hydrolysis then, there is fermentation where we get our acid. So, this is our acid Ph drops down and then, here is acetogenesis when all acid is converted into acetic acid and then, there is methanogenesis where methane is formed all righty.

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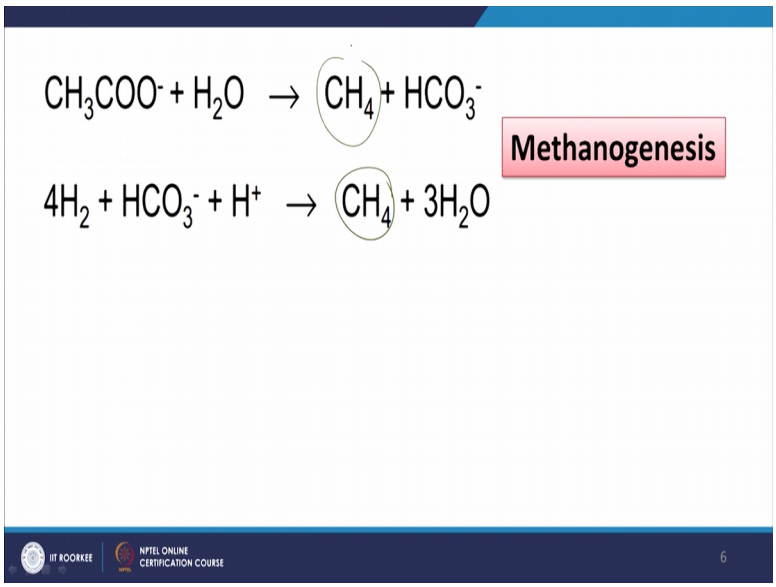

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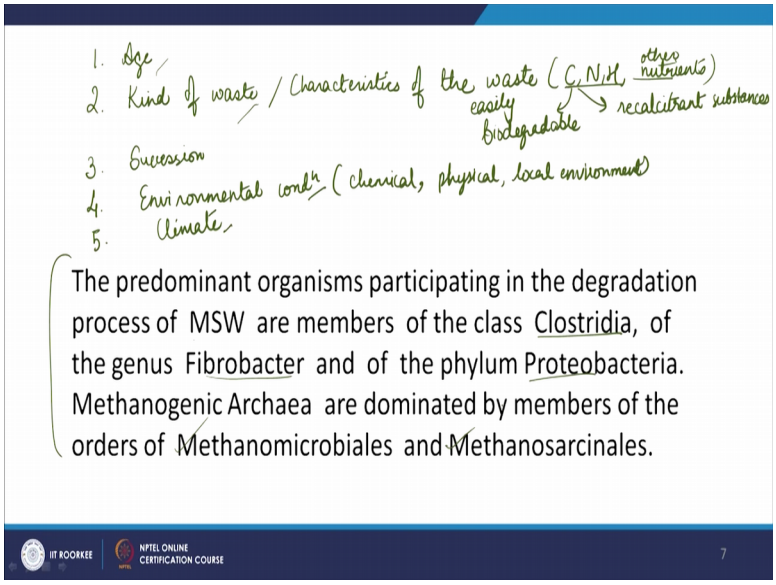

So now, let us look at a microbial community structure. Now, to review all that we have learned, we know that the age of the landfill may affect some microbial community structure. The second thing, we know that affects a microbial community stretch structure is the kind of waste, we can also call it the characteristics of the waste and this, among many other things will include definitely the C, N, H ratio and ratio of other nutrients - micro and macro nutrients and again, when we talk about C, N, H or carbon in itself, we want to talk about easily biodegradable substances and recalcitrant substances.

The other thing, the third thing that it depends upon is the microbial community succession we have talked about it, briefly in one of the previous lectures, where I mentioned that we might start with five community members and without any abrupt change in the environment, it is quite possible that some of these populations might disappear over time with microbial succession.

So, this succession factor is important. Next, we know the environmental conditions are important and this, in here we are talking about the chemical conditions, we are talking about physical conditions and local environment, for example, it is quite possible that our landfill is right next to an agriculture field. Now, because it is an agriculture field, there is regular irrigation, perhaps, not very low level of ground water table and lot of NNP being used N, P and K being used as fertilizers.

So, we will have a very different input of if any infiltration of the local runoff into landfill and that will affect your environmental conditions. The next thing, we talked

about was the climate. Now, a question that this begs is, if there are two landfills in two different cities, which have similar climates, similar environmental conditions and similar kind of waste and similar age built in microbial communities, be exactly the same. Now, that is subject to research and we are going to talk about one of the research that we will talk about it.

Now, this is a summary of another brief study, which showed that in municipal solid waste, most of the microbes were members of clostridia, fibrobacter, Proteobacteria and then, the methanogenesis I carried out by Achaea, were dominated by the methanomicrobiales and methanosarcinales, they have already talked about them earlier. Now, let us look at a study that has been recently presented and this study is in frontiers of microbiology and in this study, what they did was, they looked at landfills from different states of us.

Now, it is notable that ours is a vast country with diverse geography and this and each of them have distinct landscape and local climate and thus, the landfills have very different climatic conditions and also because, the demography of USA is very diverse, in local environmental conditions are also very different for each of the landfills.

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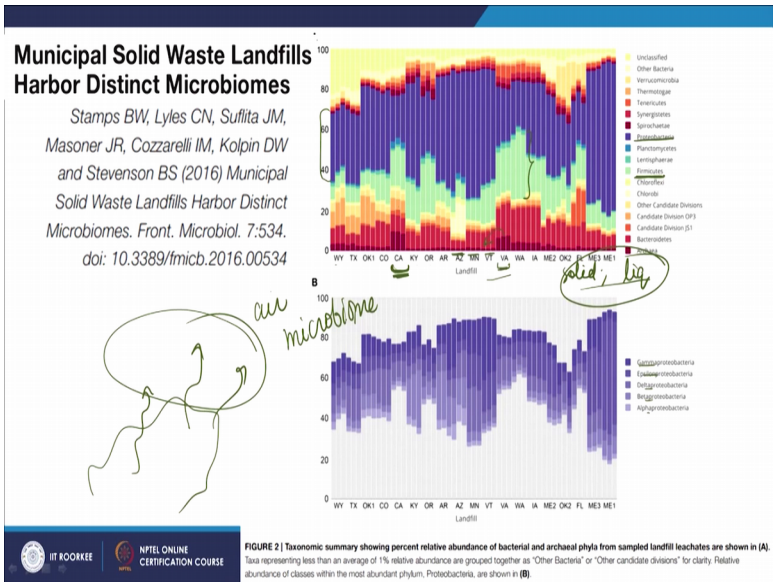

FIGURE 2 | Taxonomic summary showing percent relative abundance of bacterial and archaeal phyla from sampled landfill leachates are shown in (A). Taxa representing less than an average of 1% relative abundance are grouped together as "Other Bacteria" or "Other candidate divisions" for clarity. Relative abundance of classes within the most abundant phylum, Proteobacteria, are shown in (B).

Now, what they did was, now, these are the abbreviations of states such as Texas, Oklahoma, Colorado, California, Kentucky, Oregon and so and. So, forth and these on Y axis, you have the microbial communities so the ones in dark red are Achaea. So, in

north Californian landfills, had higher levels of Achaea, just like the ones in Virginia. Now, notably California and Virginia have very different climates. Virginia typically constitutes of low lying hills and valleys. California on the other hand is very diverse includes deserts, includes mountains and includes very pleasant weather areas, with very pleasant weather also. In fact, parts of California are coastal and thus, they have very coastal along with having high mountains right away right nearby.

So, the environmental conditions of California are very - very different within California. So, we are talking about interstate climate and intrastate climate comparison, notice; however, all the landfills in California had higher amount of Achaea.

Now, why would this be? So, it is quite possible that the segregation policy of California is different from rest of the country and thus, the quality of waste and the characteristics of the waste received in the landfills, in California are different. Now, in the red, you have bacteria IDs and then, we have different unknown candidates' - chlorobi, chloroflexi and firmicutes. So, these are firmicutes, the one in green.

They are important because, the first cellulose degraders which carry out hydrolysis or beta one for glucose fluidized bond, were found to be members of firmicutes and then, we have other kinds of microbes and the blue ones are Proteobacteria. So, this is your Proteobacteria. Now, if you look at the Proteobacteria and we divide them into different kinds of Proteobacteria, the same thing here, we notice that within this, we have different levels or a different amount of different gamma, epsilon, delta, beta and alpha Proteobacteria.

So, there are two messages here. First, from one state to another the microbial community, it is different right? The second is that, within a particular state, there is more similarity than there is between two different kinds of state.

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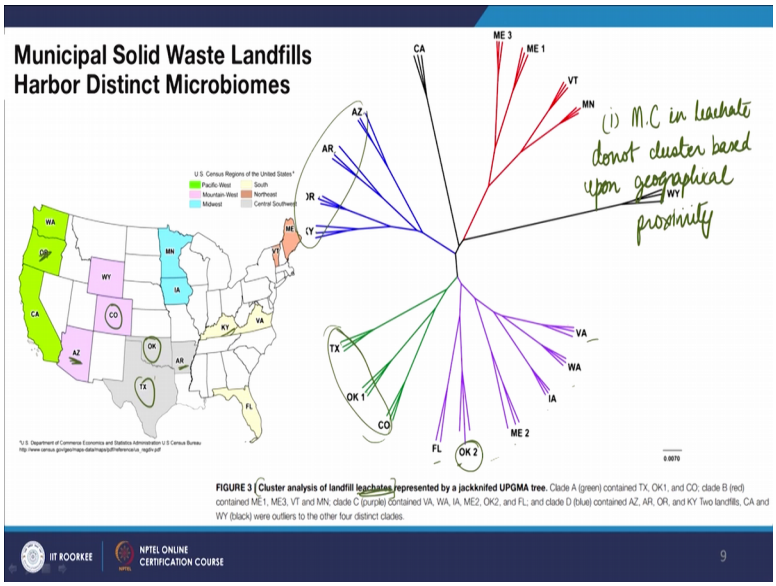

Now, these are the states that were sampled and this is the cluster analysis of landfill leach ates. Now, this is landfill leach ate, it is not the landfill sample. So, they did not take out the waste and then do microbial community analysis of the waste. Instead, they collected the leach ate. Now remember, an ideal landfill and which is true for most developed countries and economically developed countries and many developing countries as well the landfills, are very scientifically managed and as such, they have a very nice land leach ate collection system, which drains down the leach ate and then, pumps it out and removes it.

So, the leach ate is a collection of everything, that could dissolve in the water produced by metabolism or received through rain and definitely involves a lot of recalcitrant organics and water products of degradation of the waste. So, and the leach ate it. So, happens also, has lot of microbes present in it and therefore, it makes sense to do cluster analysis of leach ate, now; obviously, the microbial community who made the leach ate, some of their members would fall into the leach ate and be collected in the leach ate collection system and that is how, we assume that the leach ate microbial community composition is representative of microbes present in the in the landfill.

So, look here, these are the states that were sampled and here, we have Colorado, Oklahoma and Texas grouping together. Now, Colorado is here, Oklahoma is here and Texas is here. They are neighbors. So, just maybe it makes sense that, there is something

very similar in this, that they are leach ate microbial community, has clustered together. Now, let us take another example. Here, we have Kentucky, Oregon, and Arizona. Arizona is here. So, these states have clustered together as well. Now, let us locate them on the map. We have Arizona here, we have AR here, we have Oregon here and we have Kentucky here.

Now, unlike this particular cluster, this cluster is geographically separated. In fact, none of the states that have last, whose leach ate composition, has clustered together our neighbors. So, Kentucky's here and Arizona is here and Oregon is here and Oklahoma is not here sorry. So, now, notice that now, this implies that geographical closeness is not necessarily the reason for microbial community.

Similarity and that is the first hypothesis, that maybe Texas, Oklahoma and Colorado have similar leach ate microbial community composition, because they are next to each other, cannot be accepted. Now, similarly here, we have Virginia, Wisconsin, Iowa, and Oklahoma. Another sample from Oklahoma and Florida and these states are also not geographically all clustered to each other. Now, let us move on.

So, here in the key home, the key message is that microbial communities in leach ate that do not cluster, based on geographical proximity. So, we can basically say that, there are other factors that decide which kind of leach ate would be similar to each other and which would not be. Now, another study that, this another part of this study was looking at different kinds of conditions.

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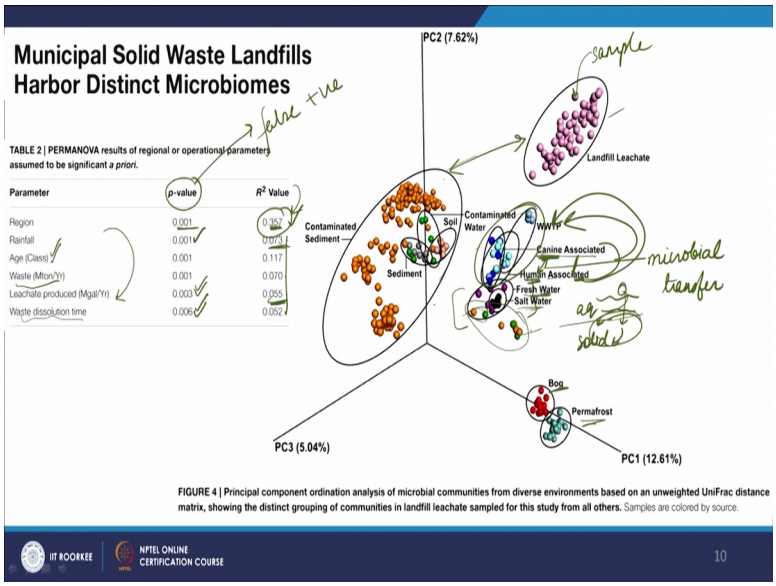

That affects the microbial community structure and their relevance to the microbial community structure. So, if it is not geographical proximity as represented, as seen here, then what is it? Is it the region and it notice that, yes it is region. The P value is significant and the relation is nearly 35.7 percent, which is higher than any of the factors they noticed.

So, perhaps two different landfills located in seeing in Delhi, are likely to have similar microbial community composition; however, landfill in Chennai, will probably have a very different microbial community composition than a landfill in Guwahati or in Kolkata. So, the region definitely contributes to up to 36 percent to thirty 35.7 percent of similarity. Now next thing is rainfall. Now, if a landfill receives a lot of rain compared to another landfill, that will affect the microbial community there and we noticed, yes the value is significant and let me briefly explain to you what P value is.

So, this P value tells you the probability, that whatever test you have done, whatever you comparison you are making, is actually true. It is not a false positive or a false negative, actually, this P value tells you about the probability, that what you are noticing is a false positive. So here, the probability is very - very low. It is less than 0.1 percent. So, this means, it is probably correct that rain crime region has up to 35.7 percent the R square contribution to the microbial community structure. Rainfall on the other hand, had relatively lower and if you go down the other thing that is very important is age, which

makes sense if there are two old landfills, they probably are in similar or close by phases and that is the microbial community structure, would be similar and then, we had rainfall and the waste.

How much waste is being put here? So, if more waste is being put here, there is more food, it is perhaps a large landfill and has quite similar environmental conditions. Now, leach ate produced which will depend upon the rainfall, it will also depend upon the kind of waste, also had some definitely significant contribution to the kind of microbial community and then, the waste dissolution time. Now, this is very beautiful and I had not talked about this earlier.

So, let me talk about this. Some ways like petroleum, hydrocarbon, for example, are not very. So, not very soluble in water and thus, have higher dissolution time. So, they will degrade slowly and the microbes that degrade them will evolve, thrive, slowly. Some other contaminants degree dissolve really well and fast like certain aromatic compounds and thus, they will degrade fast and the microbes that eat them will show up earlier on the timescale and then, disappear over time.

So, the dissolution time, they again coming back to the characteristics of the waste effects significantly, affects the composition of microbial community. Now, the same study went ahead and looked at the microbial community in different parts of the environment and built environment and noticed the similarities between microbes from different parts of the environment. So, each dot here represents one sample and they are color coded.

So, you can look at the color and then, you know what kind of sample we are talking about and what it is saying, is that look these are the samples from different landfill leach ates and even though, they are quite dissimilar from each other, as we noticed here and here, when compared to other environments they all cluster together. So now, here we have soil sample, cerumen sample. So, the landfill leach ate microbial community would definitely be distinct and separated on a typical PCA diagram. Now, this is principal component ordination analyses. So, the more close two samples are placed in this particular diagram, the more similar the microbial community structure is.

So, landfill leach ate cluster together and sediments clustered together, the contaminated soil and water cluster together, wastewater treatment. First, you together canine

microbial community associated with canine and humans are mixed up with wastewater. So, we know now, what is going into our wastewater? So, basically it is saying, that human and canine microbiology is shared and from what I understand, this canine, we are talking about are the pets that live with humans. So, if you have a pet, you are probably sharing a lot of your micro biome with your dog, with your cat, with your pet and you are participating in its micro biome with you.

So, there is some very nice microbial transfer happening, now, the human waste like urine and poop go into your wastewater treatment plant and thus, they all cluster together. Here, we have fresh water in salt water, which being the aquatic media, they have some similarity, some closeness and then, this is bog and this is permafrost very different from everything else.

Notice here, that some contaminated sediments and sediments are same or similar to freshwater and saltwater microbial communities. Then, there are two other sediments which makes sense, if the sediment has aquatic system near it, then it is quite possible that freshwater and saltwater microbes, some of them fall and our example, when we are sampling the sediment right. So, this is basically telling you that different environments will have distinct microbial communities and looking at the chemical community structure.

We can actually get relationships between different environments, for example, now, we know that saltwater and freshwater or in two, there is closeness with these particular samples. We can know that these samples probably had two phases: - echoes phase and solid phase. So, these elements were here, the water was here, and certain microbes which thrive really well in fresh and saltwater settled here, or when they died, they drop the DNA here and then, this was sampled as sediment and also the human canine association and its contribution to wastewater treatment plant is clear by such an analysis.

Now, this kind of sequencing, you can do by many different techniques and fourth generation third generation sequencing techniques, as mentioned in previous lectures will be very helpful for you to get this information. Now, let us look at another study.

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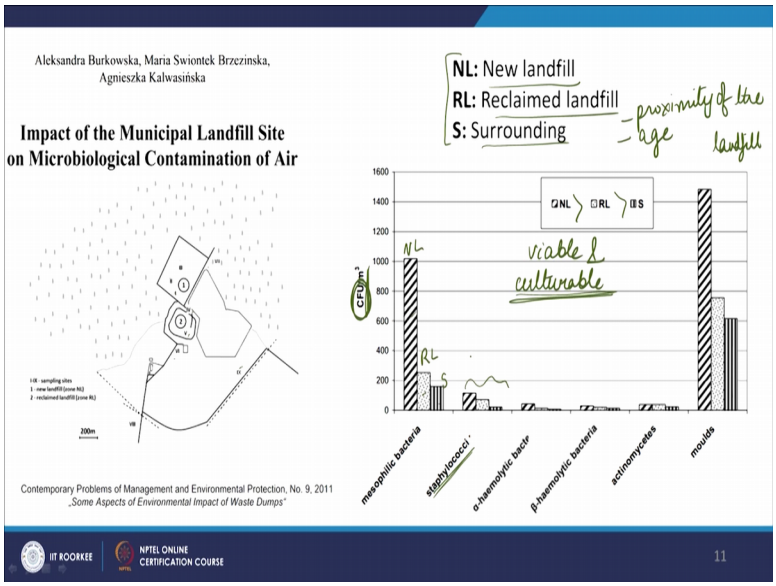

Now, this is again the recent study from 2011 and what they are looking at is, we have a landfill now, and it has a very complex microbiology, very rich microbial communities as is noticed here, very rich microbial communities. Now, these are in solid phase or in case of leach ate, they are in liquid phase. Now, some of it now, we know Henrys law right? So, the concentration in the air is related to concentration in the liquid and in the solid, some of it perhaps, some of the microbes perhaps move on and they become part of the air right.

So, what we can analyze this, as is here I have my garbage dump or let us in case of a well maintained landfill, I have a well maintained landfill. Now, it is there, rich microbial degradations that are happening here, some of the microbes will escape in the air and thus, they will affect the air microbial community. We have known this, that air also has substantial diversity in the kinds of microbes and thus, the air micro biome will be affected here.

Now, this is very - very important and very - very relevant in countries like India, where due to lack of space, the people live very close to landfill and interact with landfill directly. So, if you look at this particular slide, you can see that the human beings are playing in either, playing in under landfill or collecting waste from the landfill or interacting very closely with the landfill and thus, if there are airborne particles, thus if there are airborne microbes that leave the landfill and come here, it is quite possible that

these humans, if these microbes are pathogenic, then the humans will fall sick thus, it is very - very important to understand the air, the microbial community in the air, near and above landfills and this is exactly what the study did. So, the study is called impact of municipal landfill site on microbial contamination, microbiological contamination of air.

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So, what they took there, this is the landfill site, they have the numbers from 1 to 9, are their sampling locations so 1, 2, 3, 4, 5, 6, 7, 8 and 9. So, they sample this area and they are trying to understand, how the air in this area and they want to know what would be the microbiological quality of the air and what they noticed was that, they have three different regions, among these that they classified these nine sampling points into the new landfill area -the reclaimed land fill and the surrounding area.

So, notice that the new landfill area is now represented by dash. So, this is new landfill area, this is a reclaim landfill and this is your surroundings. Then, notice that mesophilic bacteria were most in the new landfill area, followed by reclaimed landfill and in surrounding.

Staphylococci were present and remembered staphylococcus, aurous and other staphylococci are bad for healthier pathogenic and they were also significantly present and we noticed that, in all of the cases, the new landfill had more microbial community members that were detected, then reclaim landfill followed by surrounding. So, basically right above and in the landfill and if the landfill is young so, age and proximity with

landfill that is what will affect the microbial quality of the air. Now, one thing to note here is the unit on Y axis a CFU per meter cube. Now, CFU is Colony Forming Unit. So, this has only captured microbes that are viable and culturable.

So, they might there are lot of microbes that will not be viable and they would not have been captured by this and also the microbes that are not culturable, which we now know, it is the majority of microbes in the environment than these figures might look very different.

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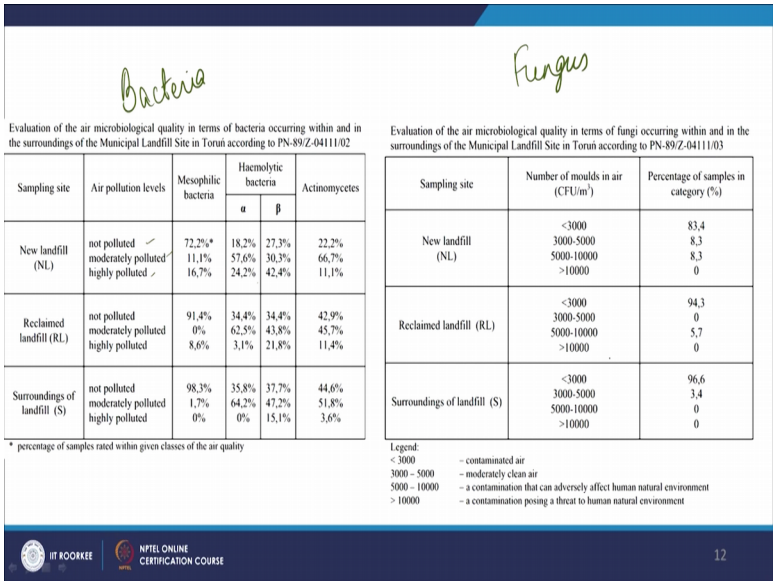

Now, this is the summary of the work that they have done in new landfill area. They divided air pollution levels into three different groups - not polluted, moderately polluted and highly polluted and this is what they observed, the percentage of mesophilic bacteria, hemolytic bacteria and actinomycetes. So, this is for bacteria and here, they have for fungus. So, not only a bacteria, but fungus and fungal spores were also present in the air. So, the air around landfill is pretty clear, is not necessarily safe and a study like this needs to be carried on side to side basis.

So, it is a site - specific study case to case basis study to determine the safety of air for workers, who work in the landfill and children and people who roam around the landfill, but this is very important. We have not done such kind of work in India, but it is very - very relevant here. So dear students, this is all for today's lecture. In the next lecture, we

will look at microbes that affect our built environment and then, we will proceed on to microbes that affect our public health.

Thank you so much.