Applied Environmental Microbiology
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Lecture – 40
Exposomes II

Dear students. Welcome back to Applied Environmental Microbiology. In the previous lecture we talked about exposomes, and we learnt about how different research groups are doing research, and better trying to understand the personal exposure versus the exposure that we would estimate from ambient air quality. Now similar research is being done on water pollution and on basically environmental microbiology. In this lecture we are going to briefly go through them. So, let us get started.

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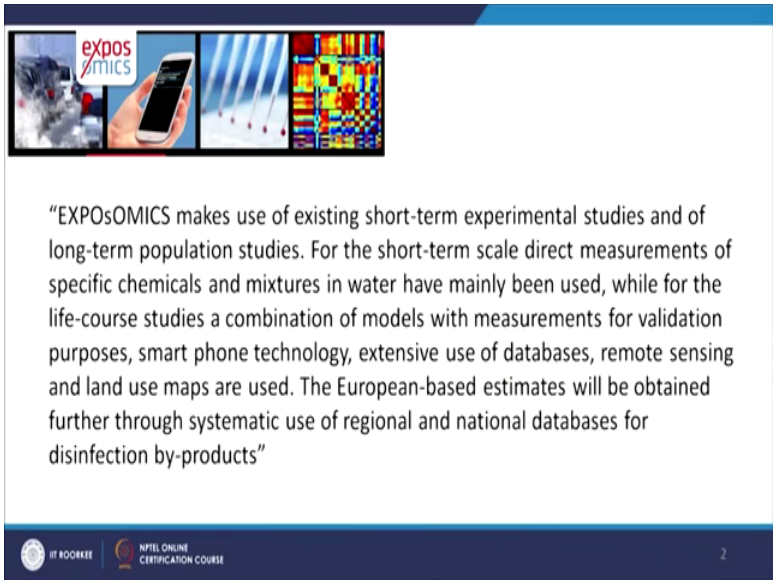

So, coming back to the facility Exposomics, that is doing with that last lecture we talked about the research and air pollution. So let us look at what work they are doing on water. Here is what they have to say; exposomics that their company institute makes use of existing short term experimental studies. So we have short-term experimental studies and long-term population studies. And then they have different kind of information that they get from short term experimental studies. For example, they find out what is specific chemicals and mixtures are present in water, and life course studies they understand what are the public health effects and long term exposure to different kinds of contamination.

They also use informations from other data bases such as remote sensing and land use maps to find out what the personal exposome is; and should we and validate the need for understanding the personal exposomes, studying exposomes instead of just what is there in my glass of water already.

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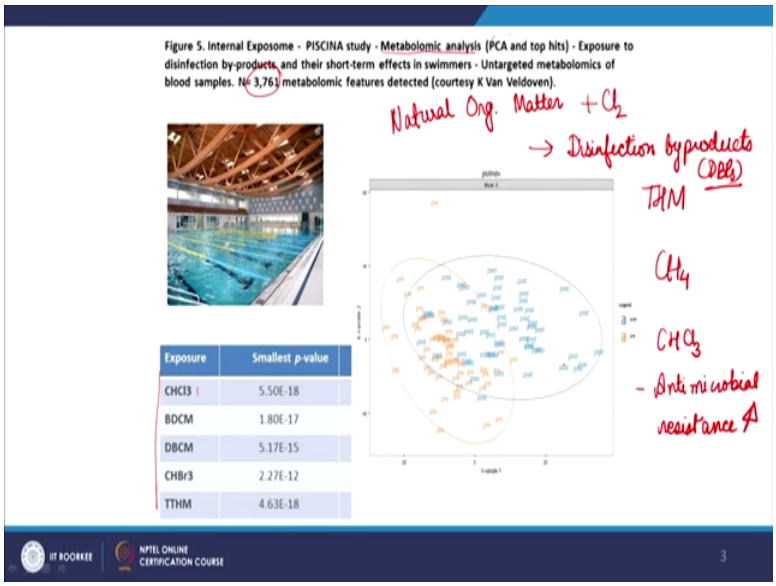

And so, this is one of the studies that they did in swimming pool. So, in swimming pool every time the water is filled or water is the swimming pool is cleaned the water is chlorinated, to disinfect the water and make sure that the people (Refer Time: 02:03) do not fall sick. Now, we know this thing that if there are natural organic matter; so this natural organic matter present in the water it will react with your halogen, the chlorine and it will create disinfection by products.

A typical disinfection by product is Trihalomethane. So basically we know methane is CH 4, three of its carbon get replaced and we are left with CH Cl 3. Now these trihalomethanes or disinfection by products are not good for public health: many of them are carcinogenic, many of them are irritant, mutagenic, teratogenic and they are verse for ecology. So, if ones this water is released in the environment it is really bad for our aquatic systems, and micro definitely microbes that are affected by disinfection by product.

Now we also know that disinfection of product beside all these problems they also increase amount of antimicrobial resistance. The levels of an antimicrobial resistance in

water and wherever DBPs are exposed rises when DBP rises. So, definitely we do not want disinfection by products that is summarised as DBPs.

Now in swimming pools when the water is chlorinated over and over again we develop lot of DBPs, in fact you can smell them smell chlorine and then smell DBPs in the water. Now the peoples remain in the swimming pool are exposed to the DBPs. Now this is a part of their exposome. Now we want to know is it affecting their health, and if it is how much and how.

So, this was the study the piscina study and they looked at the internal exposome. So they are not looking at the external they are looking at internals, so you can understand they are trying to do metagenomic study, proteomic study, metabolomics study to and transcriptomic or transcriptomic study to understand how the internal environment, internal microbial community is being affected when they are exposed to DBPs. And for this particular study they did metabolomic and this is and then they did PCA which is principle component and severe statistical technique they did which is presented here.

So, basically you have the exposure to disinfection by products. These are disinfection by products that they noticed and the short term effect in swimmers, because they looked only in short term. And this is untargeted metabolomics of blood samples so they did not target, but they just did metabolomics. They did it for 3761 people which is a quite a lot number. So, if they have conclusive evidence for anything it should be statistically significant.

And they notice at they found these different disinfection by products in good quantities, and when they did the metabolomics untargeted metabolomics. So, they were not looking for acetate of glucose any particular metabolite, but they did the UPLC MS on the entire metabolites they found in the blood and serum. And on the orange what you notice are the pre samples, so pre swimming samples. So, this is the people who came for swimming and before they started swimming what a metabolites were, the profile of the metabolites and here you have post swimming in the blue; post swimming metabolites samples. So, their blood and serum were drawn prior to swimming and then after swimming.

Now we noticed that there is quite a shift. The metabolites move from this region to this region and some for some of these candidates this is people they shifted metabolites

quite significant. Now here is a thing, this might be because of the exposure to DBPs. It also might be because of exercise ones we exercise a metabolites chain. So, that is one issue that this study over came by looking at the physical exertion of people and whether they were swimming or just playing around the water dif and by basically noticing the physical exertion, and they did tried to delineate the effect of physical exercise versus exposure to DBPs.

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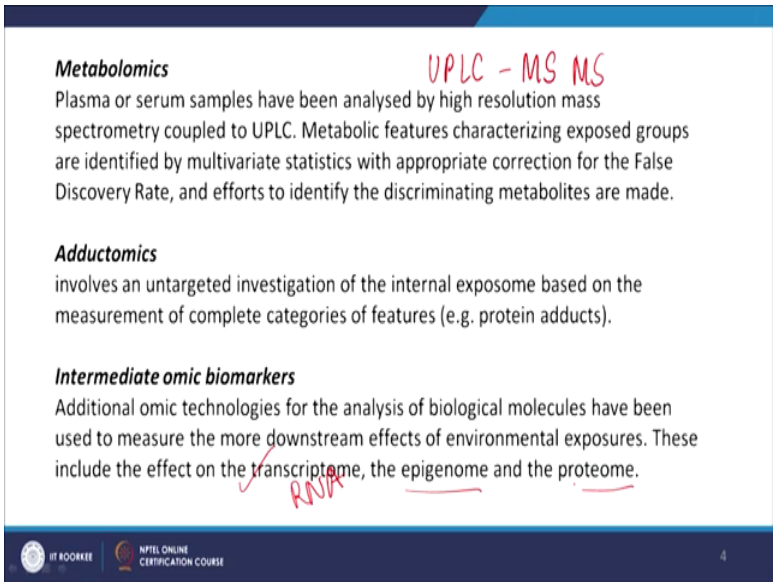

Now what are the different techniques that we use when we are trying to understand the effect on internal exposome of the internal microbiome or internal community and internal environment when we are exposed to different things? We can do metabolomics, which is what we just studied. We can do adductomics, we can do intermediate omic biomarkers. So, metabolomics is when we take their plasma or serum samples and we analyse them through high resolution mass spectroscopy, spectrometric coupled with UPLC.

So, basically this is ultra high pressure liquid chromatography coupled with mass spectrometry or maybe we might have MS MS for better resolution. And then we get metabolic features characterising expose groups are identified by multi various statistics. So, remember as telling you about the exercise versus DBPs. So we can do multi component analysis and other statistical test to find out if the shift is because of DBP exposure of it is because of exercise.

And then in this way we can also do, we can identify which metabolic features. So this is untargeted metabolomic, we are not targeting a particular metabolite we can find out which of these metabolites are significant for study that we are doing which of them are not.

Then next we have adductomics. In this we do untargeted investigation of internal exposome based on measurement of complete categories of features such as protein adducts. In intermediate omic biomarkers we can look at biological molecules to understand a downstream effect of environmental exposure, these might include transcriptomics, epigenomics and proteomics.

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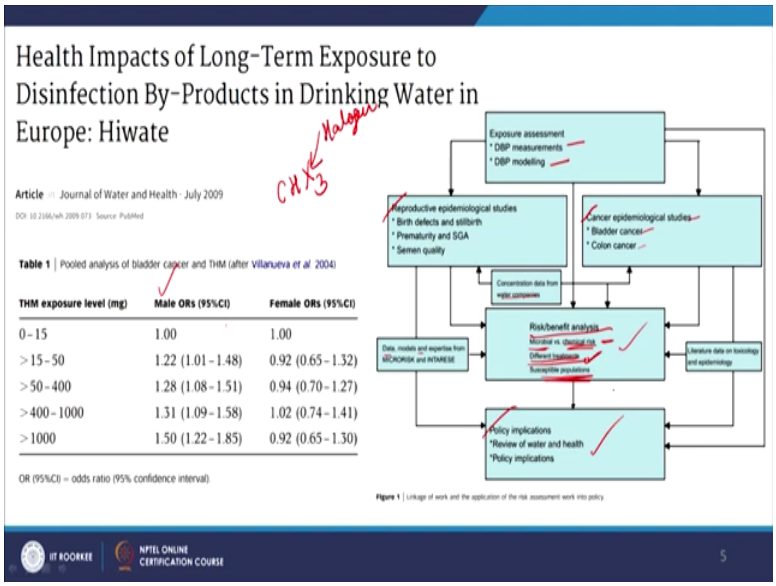

Already, now let us look at this study

published in 2009 health impact of long term exposure to disinfection by-products in drinking water in Europe. Now this is a drinking water and swimming water. So, they found out that to trihalomethane if you remember which is basically CH X 3, where X is your halogen. Now if you remember your periodic table the seventh column is your halogen column which includes fluorine, chlorine, bromine, iodine; and chlorine, bromine they are the typical halogen that we find in DBPs.

So, they look at the trihalomethane exposure what amount of trihalomethane disinfection by product was present in the drinking water. Now why would they be interested in that?

Because the presence of disinfection by products in drinking water which is definitely an environmental problem is very strongly correlated with bladder cancer. So, we want to understand how exposure to drinking water with high DBPs, high THM will result in bladder cancer. Now if the THM exposure vary from 0 to 15 upto 1000 milligram and OR is a odds ratio. In the odds ratio for males and females were detected separately and in bracket what you are noticing is the confidence interval. So, what is the odd ratio of developing bladder cancer when you are exposed to this amount of DBPs?

So, we notice that it increased for both male and female as the exposure of THM increase. With males it went all the way upto 1.5 odds ratio. Now how did this study happen, this is very important and how this kind of study where we know now that DBPs and drinking water are bad for health specially for men more so for men than for women and they might result in development of bladder cancer. Now this was their exposure study, they measured DBP measurement, they measure the model DBP consideration and if had cancer epidemiological, study on bladder cancer and colon cancer. They did both cancers and they found out that bladder cancer make more sense.

Now they had this study of information on the kinds of cancer being detected from their epidemiological study for the region and they knew that amount of THM because it is measuring it regularly. Now the exposure assessment dis-assessment; how much DBP you people are being exposed to, and then modelling the exposure gives us we can link it with the cancer studies and we can also link it with the reproductive epidemiological study, because you also know that DBPs affect the simon quality, the rematurity, birth defects, stillbirth and other problems. We can also gather data from water company that supply water; what are the amount of NOM natural organic matter in your water, what are amount of chlorination you are adding or what kind of disinfectant you are adding, how much DBPs people and were observing in the consumer end.

So, all these information we can link with cancer epidemiological study and reproductive epidemiological study. And what we gain out of this linking is we get data, we get models and we get expertise from different people and we can do this benefit analysis and we can revise policies. So, risk analysis will tell us is it better to allow some microbes to grow in the water so that we can dues the level of disinfectants and thus reduce the level of trihalomethane or DBPs. Or is it better to allow people to get higher

risk of getting bladder cancer and other diseases that are because of higher exposure to or exposure of higher amount of DBPs trihalomethane.

So we can do, this kind of we can answer these questions from this kind of studies. That should I increase the clock level of disinfectant enough so that people are safe from acute infectious diseases or should I reduce its so that they inchronic level they do not get exposed to, they do not get higher chances of bladder cancer.

We can also understand different treatments, for which the water is being is given to different kind of population you can decide what is more important here and we can also identify susceptible populations. For example, very clearly the male participants of the study showed a higher chances of developing bladder cancer than the female participants. So once we know the susceptible population we can do differential treatment. For example, the elderly might more susceptible to getting infectious diseases soo you might say boost up their disinfectant level so they do not get infectious diseases and the bladder cancer is be ahead in the future, we would not care it much we can addict that.

Now what this tells us? It helps us decide the policy, it helps us decide the what are the health and the implications of policy. And these studies also develop literature and toxicology in epidemiology which overall help us understand risk benefit, analysis and also helps us do understand how our policies effecting health and how we should change our policy.

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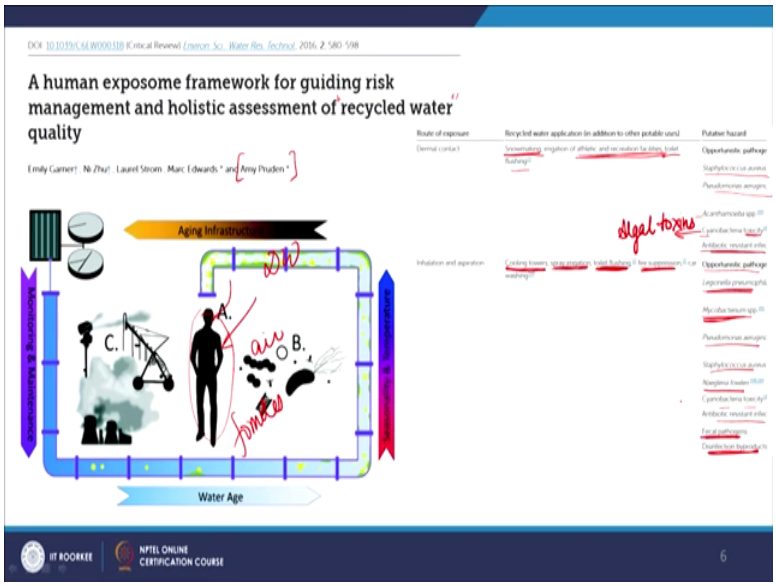

Alright, now this is a study by Dr. Emily Garner it (Refer Time: 13:02) and she worked on human exposome framework for reclaimed water. So, now across the world water is a very fresh water portable water is a scarce resource. So, whether I want to use water for irrigation or for drinking or for washing or other purposes it is rare, a do not rare pardon me it is scarce. Now because it is scarce we have the concept of reclaimed water. So, reclaimed water is basically I have wastewater from my city we treated to high now standards that we can now use it as raw drinking water or drinking water. So, we are reclaiming the used water.

Now this can happen in two ways: you can do it intentionally which is called recycling of water, recycled water reclaimed water or it can be de facto, where the waste of one city are upstream, the other cities raw drinking source and automatically the affluent treated affluent or untreated affluent from the upstream city becomes a raw drinking water for the second city, so unintentional reclaiming of water.

Other case reclaiming water is a reality in India, it is rampant in India and because of high population density and the closeness and are also the closest of our cities and towns. And also because of our poor waste management or poor wastewater treatment and collection; collection in the first place reclaimed water is often a de factor in our country. However, much research is needed in that aspect.

So when we talk about recycled water then it is not de facto, when it is intentional then there are certain guidelines about: what its quality should be and even when we are talking when it is de facto there is certain guidelines for wastewater treatment affluent. So, it should meet certain qualities and (Refer Time: 14:51) the wastewater should be treated in wastewater. But that is not often in the case, so let us look here.

So, this is your wastewater treatment plant and you monitor and maintain it regularly. And then when the water has been treated it goes to drinking water treatment plant, it gets cleaned. And as it passes through your water distribution network, the microbes infiltrate the system or they them they re grow, they form biofilms, they colonise that microbial communities increase and get richer and richer. And this is also your infrastructure aging the water age is increasing, your disinfectant is decaying, and at times when disinfectant decades we get nutrients depending on disinfectant, some microbial community will grow. And by the time this human being consumes on the consumer end, not only the person is exposed to more microbes, some of them can be pathogens, but also the chlorine level has decreased and it is quite possible that the DBPs are significant.

Also this person A is being exposed to environmental microbes and environmental genomes and viruses and insects and pollution that is present in the environment. All of this together including drinking water, air, fomites, so fomites are surfaces which have pathogens attached to them and if we touch them then we get exposed to it, and they all of them contribute to exposome. So, now let us look at how recycled water when it comes to recycled water how that is potentially exposing us to different kinds of diseases. So now the person might be exposed by a touch certain diseases we just need to be in touch with the water contaminated water and you fall sick, some might be through injection route, some might be through inhalation route. So let us look at different routes of exposure and what are the diseases that we can get through them.

Through dermal contacts, so through touch: so this in US particularly where this study was just snow making reclaimed water is used for snow making, where people like to ski when this snow has not fallen. So, people might, will touch the snow and that that is how they might get infected, also athletic and recreation facilities. So, if I am irrigating the grass in an athletic field then the players and people on that field they will get exposed to the microbes that are present in reclaimed water. And also if, because it is in athletic or

recreation facility the chances of injury are very high; so not only dermal contact, but the blood contact can also happen and also toilet flushing.

Now in this case in dermal contact we are mostly concerned the operationistic pathogens such as Staphylococcus aureus, Pseudomonas aeruginosa, Acanthamoeba cyanobacteria. Though cyanobacteria that produce toxins, we can summarise this as Algal toxins. And then we are also interested antibiotic resistant infection. In fact in Amy Pruden who is the corresponding author of this paper has done lot of research on the presence of antibiotic resistant genes in reclaimed water. And she has shown that the reclaimed water has elevated levels of ARGs - antibiotic resistance genes present in them. So we are also interested in; we are it is also probability that through dermal contact the people would be exposed to antibiotic resistance microbes.

Next is inhalation and aspiration. So, let us say I am standing next to waterfall and I inhale the water. Now, this is an issue in cooling towers for example, many for thermal power stations, pre irrigation, drip irrigation where we are trying to minimise the use of water toilet flushing. Now it is a very interesting study in fact at the same institute (Refer Time: 18:24) this one and very established air pollution expert and Dr. Marc and she is working now, she worked few years ago and how the way we are flushing the toilet not the way pardon me; the toilet flushing might be brought for exposure to Ebola virus.

Fire suppressions, so you have fire hydrants and when you are trying to throw the fire you are aerosolising water, car washing also shower. In this case you are concerned about opportunistic pathogens such as (Refer Time: 18:54) which causes mild lung infection, micro bacterial species which also includes the tuberculosis and micro bacteria. Pseudomonas Aeruginosa is really bad skin infections Dr. (Refer Time: 19:07) also Naegleria Fowleri from previous class is the brain eating amoeba and then Algar toxins, antibiotic resistance infections Fecal pathogens also and disinfection by products.

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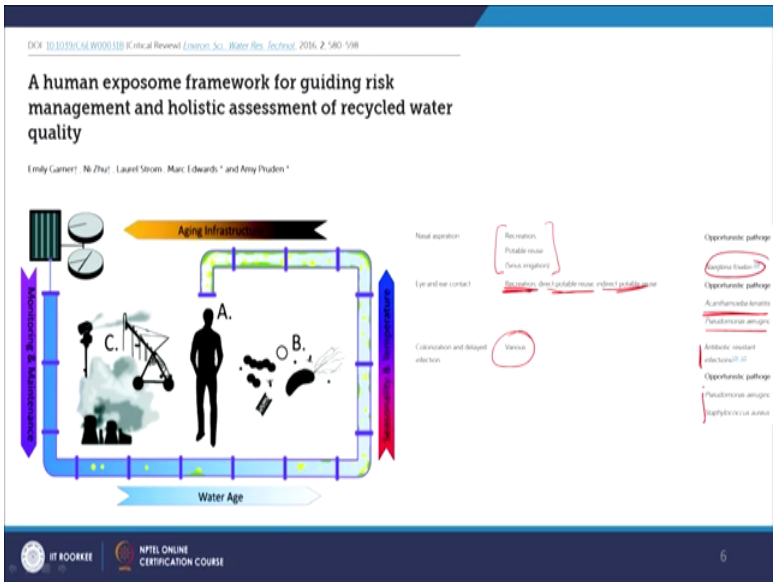

Now if you look at nasal aspiration. Now if we look at nasal aspiration, now this is more concern, in recreation, importable use when I am drinking the water. So, if I am drinking I by mistake aspirated through nasal route or I am doing sinus irrigation which many people in India do actually is called Neti Pot Technique for cleaning the sinus, so in this case the major dangerous of Naegleria Fowleri.

Now eye and ear contact if I am washing my face or the water enters my ear. This might happen during recreation like let say like I am swimming or just playing with the water or direct portable use, I am drinking water I am splashing it on my face and then in this case I am interested in (Refer Time: 20:00) ok. It is causes horrible infection in the eye and Pseudomonas Aeruginosa. Again then we might have colonisation in delayed infection and there various ways of getting them. Here again I am interested in antibiotic resistant genes infections Pseudomonas Aeruginosa and (Refer Time: 20:18).

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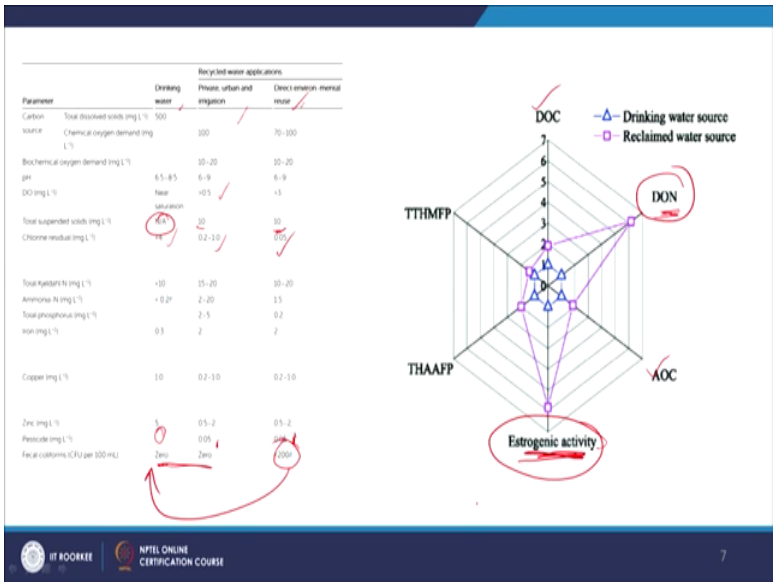

Now, if you look at our drinking water and a reclaimed water in this study they highlighted how the recycled water that in that is allow in US for different purposes whether it is private and urban irrigation, indirect environmental reuse, differs in the quality from the drinking water. For example the cod of drinking water has to be very low compared to the recycled water. Also the pH is similar but the dissolved oxygen is very high in drinking water versus the recycled water that is used for private irrigation and direct environmental reuse.

Suspended solids are not undetectable, whereas there is some suspended solid present in both. Again the chlorine is residual is very low for recycled water, but it is quite high for drinking water. Again the TKN is very high for the recycled water and the standard for recycled water compared to drinking water. Same is to for ammonia nitrogen and total phosphorous. It is iron and zinc. Well, zinc is comparable in all. What is interesting is that some pesticides are allowed in private urban irrigation, recycled water used for itic pubic purposes, irrigation purposes and direct environmental reuse, but none of them is allowed for drinking water. Also faecal coliforms are allowed when they are used for direct environmental reuse reclaimed water, but definitely not allowed in drinking water.

So, even if I take this direct environmental the water that is good enough for direct environmental reuse and try to use it as a drinking water it requires for the treatment. The other thing that this paper talked about this is study from this information from other

research, but what they talked about was in the blue here we have drinking water and in the purple we have the reclaimed water. So they notice that ok, the certain parameters of drinking water and reclaimed water are quite in agreement with each other for example AOC, DOC, TTHMFP, THAAFP. They are quite agreeable with each other they and we also measure them like disorder or organic carbon ascendible organic carbon.

But then there are other things like disorder organic nitrogen and estrogenic activity; so endocrine destruction that are present in the water and that are much higher in reclaimed water. So as we reclaimed the water, it continues recycling it over and over again they are likely to increase more and more, and thus they will effect microbial growth, algar growth when they are thrown in the environment and the health of the people. And this is again where the; this is another example where the exposomes study help us identify that. This is what people are getting exposed to if they are drinking reclaimed water, the amount DBPs, the amount of DOn, the amount of endocrine destruction.

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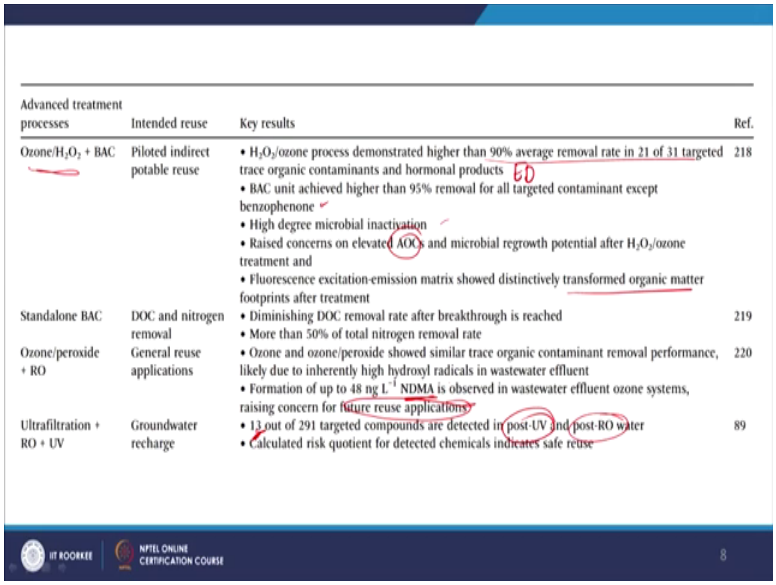

And now we can do an health study and understand the health risk assessment.

Now this study also looked at advance treatment process so that can be used for getting rid of the contaminants. They looked at ozone H 2 O 2, oxidation followed by BAC, which is biological activated charcoal reactor. And the internet used for piloted in directed portable reuse and then they notice that more than that this one have more than 90 percent average removal in 21 or 31 targeted trace or organic contaminants and

hormonal product which are endocrine destructors. Also the BAC achieved higher than 95 percent removal of all targeted contaminants except benzophenone. And then there was high degree of microbial inactivation. So, microbial present will not a problem, but it released a concern because AOC is increased which will increase the microbial growth potential ones the oxidants have died out, have been consumed.

Also that, they the florescence excitation emission matrix such as florescence microscopy showed that that transform from organic matter, the organic matter had been transformed. Then they did standalone BAC, so remove the advance oxidation part just stand alone biological activated charcoal. And wanted to do this for removing doc dissolve organic carbon and nitrogen and they notice that the removal rate of DOC reduces after the break through point is reached. Also 50 percent of total nitrogen was removed. And then they did ozone peroxide against same thing and RO which is reverse osmosis. And they notice that because of high hydroxide radicals in wastewater affluent, the removal was similar to an ozone ozone peroxide will be similar. They also notice that lot of NDMA was present was developed in water so that will grace concerns on when you are thinking of future reuse application.

Also they did ultra filtration followed by RO followed by UV treatment. And they notice that 13 out of 291 targeted compounds were determined, were detected after UV and after RO. So, see we notice that none of our techniques even advance oxidation, advance treatment techniques of full proof. So, in most these are important, more studies are required to see when these compounds are still present in water how they affect health and how much reclaiming of the water is permissible and how much is not.

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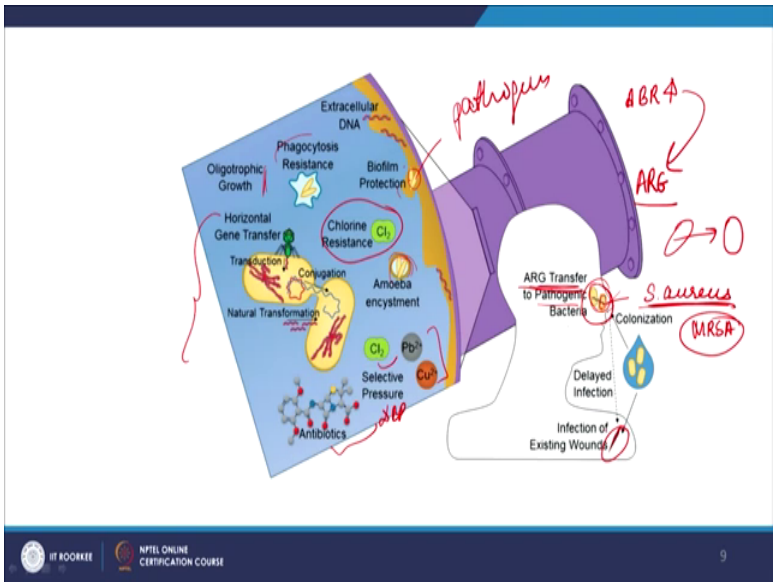

Now, this a very very beautiful diagram again from Dr. Garner's paper. And this is a cross section of a pipe and if you notice here in the pipe here biofilms growing there some extra cellular DNA, there is biofilms are protecting pathogens and we also have microbes that have developed phyto cyto resistance. So, no amoeba can eat them protozoa cant fetch it undergo (Refer Time: 25:54) for them. This oligotrophic growth, so microbes are growing in scarce nutrient environment, this horizontal gene transfer which is a nightmare antimicrobial resistance. There is also resistance through disinfection. The amoeba can form a cyst around the pathogens.

So, these are pathogens of concern. So, pathogens may find home in the biofilm, they may find home in the inside an amoeba and they refuse to die. The amoeba might assist them and then they might be selective pressure from heavy metals, from antibiotics, from disinfectant and DBPs disinfection by-products. All of it will enhance the antimicrobial resistance. So, antibiotic resistance, antibiotic resistance will increase.

And the way we measure antibiotic resistance because of this gene transfer, how important the genes are is by measuring the antibiotic resistance genes ARG; so the ARg genes. Now these bacteria are not necessarily pathogenic, they might be pathogens, if they are pathogens we do not want to get exposed to them, but let us say they are not pathogenic. Let us say in our nose or in our body we already have staphylococo aureus present.

Now if this staphylococo aureus over populated will have staph infection and this is susceptible to antibiotics. But now these bacteria have developed antibiotic resistance and then I am exposed to them, then the ARG can transfer to pathogenic bacteria. So, these benign microbes transfer them to; this benign microbe will transfer the antibiotic resistance to and pathogens. And now this staphylococo aureus could be resistance to microbes such as is in case of MERSA and MRSA.

Now other possibilities also delayed infection, for example, is knees and I have get wounded or just now MERSA that has resistance microbes and menus are exposed to other people and now the wounds can get infected very bad.

So dear students, this is all for today, the importance of exposome. So, we want to look at all what is happening not only what are you being exposed to, but we also want to look into what is happening in the environment from which you are exposed microbial or chemical contaminants are coming. And what kind of problems we are facing and we might face in near future.

And this is all for today. In next class, in next lecture we will go more into antimicrobial resistance which is quite a burning topic and it is very important in our country for us to brain antimicrobial resistance to put an end to it. So, this is all for today.

Thank you very much. See you later.