Environmental Quality:
Monitoring and Analysis
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Lecture No. 33
Monitoring and Measurement of Microorganisms

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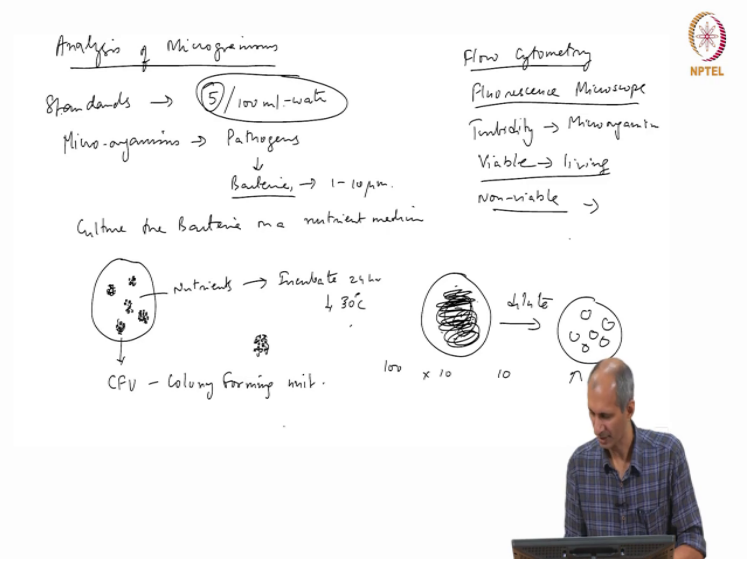

So, here if we look at the standards that people use for analysis of microorganisms, for example CPCB standards for microorganisms will be like say 5 per 100 ml or 5 microorganisms for 100 ml. So, the microorganisms we are talking about are predominantly pathogens. Many of the pathogens are bacteria, there are a few viruses and all that but mainly bacteria and for water quality, people count the number of bacteria in it. Here we focus on water only. There are bacteria in air also, fungal spores and all but there are no standards for it yet. It is still going on people are trying to develop it but it will take some time for it to happen.

So, how do you count say 5 per 100 ml? This bacteria size is around 1 to 10 microns, it means if I take 100 ml sample, I have to see it, it is difficult to count, so you need a microscope. So how do you do this? You take 100 ml of sample you filter it put it on a filter paper and observe the filter paper if somewhere in the filter paper there are 5-micron spots. Filter papers are big 2.5 centimeters in size, you have to search for 5 microns somewhere, so, it is not very easy.

So, this is a big challenge, counting microbial populations is a big challenge. One of the old standard methods is that people use what is called as a culturing method. A lot of people work on these various ways of doing it, but one of the simplest methods is to take a water sample and you culture the bacteria on a nutrient medium. Typically, what you do is, you take a plate filled with some nutrients. There are some standard nutrients. Nutrient is something in which the bacteria will grow, uses that as a substrate and it will multiply. So, what people do is they take say 1 ml of water sample and put it on a nutrient medium. And in the sample say there are 5 bacteria and you cannot see it because it is micron size. And then you incubate it for a day or 24 hours at some temperatures 30 degrees centigrade or 25 degrees centigrade. Then what happens is, they allow the bacteria to multiply. So, the bacteria multiply and it becomes small cluster, multiply and grow around here. So, when it becomes big you can see it. So, you have formation of a colony, one bacterial cell will multiply 2, 4, 6, 8 it multiplies in some fashion and whatever was this one single dot, you cannot see now has become a colony.

So, this is called as CFU or a colony forming unit. So, this takes around 24 hours. So therefore, you cannot get an immediate value of this thing, you have to take our sample put it on a nutrient medium, wait for 24 hours then you can see either directly or you can look at it under a microscope you can see this, you can see it and you can see directly, this is one way, there are other ways of doing it where you can look at it under a microscope. So, if you are looking for small numbers such as 5, you need to do this kind of thing because it will increase the size and you are able to identify the colonies and therefore the number of microorganisms. So, what if it has 100 already? If you have 100, you have no problem because there are other methods of doing it. But if you do culturing when you have very high concentration is you already have a lot of dots and this at the end of one day, you may get the big jumble, a big mass and you cannot differentiate how many originally were there. So, usually the analysis prefers that, if you have a very large number, you dilute it so that you can get distinct masses of colony forms. So that you back calculate using the dilution. For example, if you have 100 you dilute it 10 times so you have 10 so you find 10 colonies hear you multiplied by 10 to say 100 colonies or 100 colony forming units that is count.

So, 100 colonies unit in 1 ml so if 10 ml is you cannot take 10 ml on an agar plate or any other nutrient, you take more than small amounts, so you have to multiply it by that volume. The assumption here is that it is uniform again. So, you have to do multiple samples, same rules of analysis apply here you are taking 1 ml for analysis from a big tank of water I am not sure if this is uniform, so you have to take multiple samples, same rules apply.

So, microbes are treated like particles, so you can also look at it like a particle and look at it in a microscope and there are a lot of instruments now available, which use microscopy in order to count bacterial cells and these are not standard methods, but people use what is called as flow cytometry. So, flow cytometry is used in diagnostic analysis in blood. When you do blood analysis, you will see them laying there using flow cytometry will count the number of red blood cells, white blood cells, and all the cells which are about the same order of magnitude.

So, there are similar kind of techniques used, where they will take a sample of water and send it through a small channel. And one bacterium will go one after the other. So, they will count but it is not the standard method yet to be used in this thing. There are issues about sample representativeness and all that in this kind of things. And this is a big challenge, microbial analysis is a very big challenge. And this is a very simple way of doing it.

So, people use other ways of detecting bacteria also which includes putting a dye something called a staining. They put a dye, this dye will go and absorb on different organisms in order to distinguish between which bacteria which fungus it is and then you can use what is called as a fluorescence microscope in order to detect and count. The more sophisticated method if you want to know exactly what bacteria is there and all that, you can look at the bacteria look at morphology you can look at DNA analysis and get what is the bacteria present there and all that. So, in the analysis of our scheme of things, that is a very sophisticated thing. So, you really want to find out very specifically if there is a disease that is being caused you want to know what it is, but for general water quality, people use a simple method to see if something is growing or not.

In general, if the concentration of microorganisms is very high, it will show up as turbidity. So water is not clear, which means it could be because of bacteria a lot of bacteria is there so it is a

suspended particulate matter and that's why there is turbidity. The turbidity is one measure of microorganisms but not always, you cannot be sure. So the only way to make sure that it is a microorganism is to put it on and see if it is growing.

So that is a surefire thing we of saying because there is viable. What we mean by viable, viable is living is a term called viable and non-viable means it is a dead cell which will not grow. So, the dead cell will not grow you like organic matter organic carbon it constitutes in that fraction it will not grow and it will not cause probably much harm as much harm as a pathogen will because when generally people are worried about microorganisms.

Because they are pathogens and they will grow the cause an infection is happening that means something is growing bacteria is growing in the body so, that is a non-viable organism which does not grow does not that much of a danger then viable arguments. So, I think I will stop the analysis part here we have a lot of ground to cover. So, I will start the next section.