Advance Analytical Course

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Lecture No. # 39

We must take a very serious look at the one very important part of our life and that is the drinking water, how this drinking water is getting contaminant contaminated, and the analysis and removal of the emerging contaminants in wastewater.

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And drinking water has become a prime importance in today's world, because we have found all over the globe that people are disposing wastewater without treatment or sometimes with ineffective treatment.

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Now, as a result, the not only the wastewater, but the wastewater when it leaches down to the ground water, even contaminates the ground water and most of the time the ground water which is used for drinking purpose also gets contaminated. So, what are the contaminants in water? The occurrence of trace organic contaminants in wastewater, their behavior during wastewater treatment and production of drinking water are key issues in the re-use of water resources.

When we try to look at water resources, we have to recycle. And in process of recycling, we see that the wastewater needs an elaborate treatment and if the elaborate treatment is not given to the wastewater, it cannot be used as or it has no re-usability as drinking water. Elimination of different classes of emerging contaminants, such as surfactant degradates, pharmaceuticals and polar pesticides in wastewater treatment plants was found to be rather low. So, sewage effluents are one of the main sources of these compounds and their treatment-resistant metabolites.

Analysis of several groups of emerging contaminants, acidic pharmaceuticals, antibacterial agents, acidic pesticides and surfactant metabolites in wastewater, applying conventional activated sludge treatment that is the AST and advanced treatment processes such as membrane bioreactors MBRs and advanced oxidation processes - the AOPs, as well as during production of the drinking water.

So, all these have been applied; the method suggests membrane bioreactors and the advanced oxidation processes the have been applied to make the wastewater suitable for drinking purpose, but the contaminants are of huge variety and of are of different chemical classes. They could be from pharmaceuticals, from surfactant factories, from various other pesticide industries, and therefore, to be able to treat all of them effectively is a big challenge.

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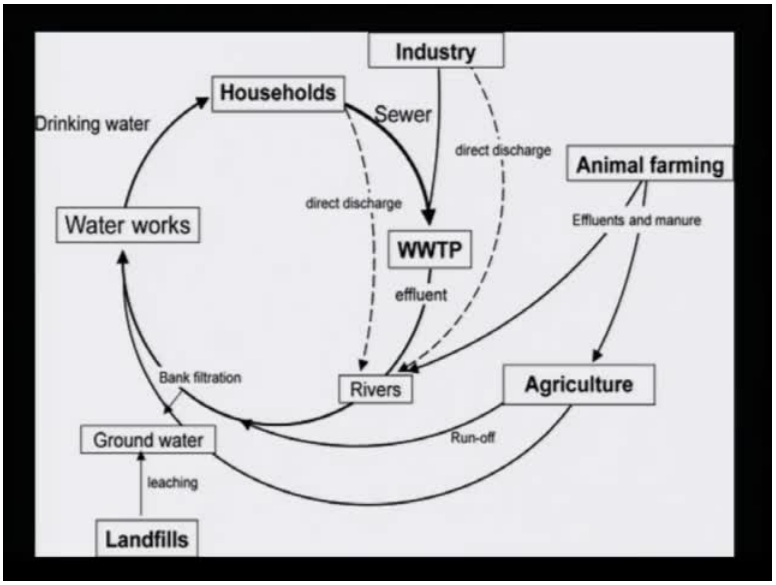

Now, if we try to look at this particular slide, we will see that, how the household water through the sewer, goes to the wastewater treatment plant and then it is then run into the rivers. The water works are supposed to be purifying and supplying the drinking water. So, that is what the water cycle looks like. Then, there are industries which make direct discharge, untreated discharge in the rivers and we have seen this example in river Ganga.

I will give you a small example. A friend of mine went to Haridwar, for a holy dip in Ganga. And when she took the holy dip in the evening, the water was fairly okay, but she still felt that there was some smell in the water; however, she just collected that water and brought it to her room. Next morning, they wanted to have an early morning water bath, water dip in the Ganges and they went back again, and this time, they found that the Ganges was absolutely having a very fowling smell.

Now, they still collected the water; they took the dip and they came back and had a bath even in a in their room, but to tell you this story, I just want to highlight the fact that all the industries in and around Haridwar were actually discharging their effluent directly, just the way the graph shows, that industries, they directly discharge their effluent or wastewater into the rivers without even sometimes giving them even the elementary kind of treatment. And that is how, you know, these river bodies, they get contaminated and then, of course, there are animal farmings and effluents from their agricultural runoff. That is also adding one to the pollutants in the rivers and then it is then going to the ground water, with and the landfills are also causing leaching into the ground water.

So, you see the whole, you know, situation is very grim. From every source there is contamination: from the household contamination is coming; from the industries, the contamination is coming; from the animal farming, the contamination is coming; from agricultural runoff, the contamination is coming; it is all going into the river or going into the ground water and therefore, the ground water that is supplied for drinking is also needs very serious kind of treatment, before it can be actually supplied for drinking purpose.

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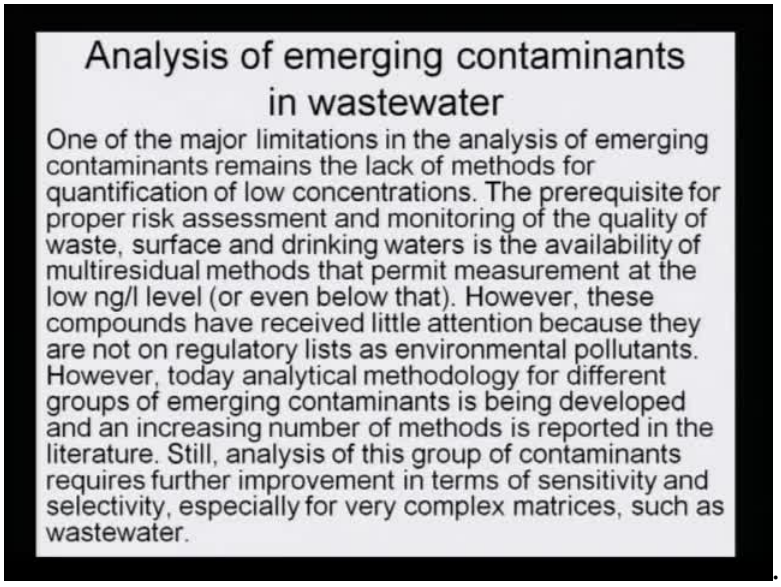

Analysis of emerging contaminants in wastewater - one of the major limitations in the analysis of emerging contaminants remains the lack of method for quantification of low concentrations. Now, some of these contaminants are in trace quantity or micro quantity,

and therefore, it becomes a big challenge to analyze them. The prerequisite for proper risk assessment and monitoring of the quality of waste surface in drinking waters is the availability of multiresidual methods that permit measurements at the low nanogram per litre level or even below that.

So, it is possible to analyze these emerging contaminants in wastewater, surface water, and drinking water, only because there is a very good method called multiresidual method, which permits the measurements even at nanogram per litre level or below that. However, these compounds have received little attention because they are not as regulatory list as environmental pollutants.

However, today, analytical methodology for different groups of emerging contaminants is being developed and an increasing number of methods is reported in the literature. Still, analysis of this group of contaminants requires further improvement in terms of sensitivity and selectivity, especially for very complex matrices such as wastewater. Why is wastewater very complex matrix? Because the wastewater from any industry, let us take the example of a textile industry. We will have all the, you know, chemicals that have been used in process 1, 2, 3, 4 and as many processes the textile has gone through. So, it is a collective, you know, contaminants, a list of contaminants will be present and therefore, the matrix becomes very complicated or complex, and to be able to pull out the nanogram present contaminant will be a big challenge. And there are no regulatory bodies to say that these are list of the contaminants that need to be analyzed. From every industry, it will vary.

So, textile industry will have a different kind of a wastewater chemical composition; the labor industry will have a different kind of a chemical composition in their wastewater, and so on and so forth. So, you look at any industry and if they are generating wastewater, the chemical profile will be entirely different. And to be able to, you know, selectively, you know, analyze one by one, all these contaminants, is a big challenge for the analysts.

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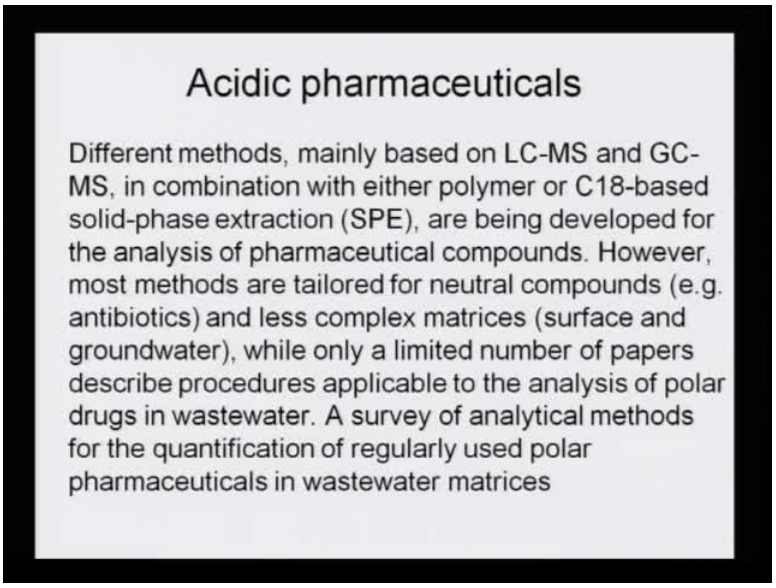

Acidic pharmaceuticals - some of the examples that we have taken here are for very specific compounds. If the contaminants are rich in acidic pharmaceuticals, what are the methods that can be usually applied for the analysis? Different methods, mainly based on Liquid Chromatography-Mass Spectrometry and GC-MS that is Gas Chromatography-Mass Spectrometry, in combination with either polymer or C18-based solid-phase extraction SPE, are being developed for the analysis of pharmaceutical compounds.

However, most methods are tailored for neutral compounds, that is antibiotics, and less complex matrices such as surface and ground water, while only a limited number of papers on research has describes procedures applicable to the analysis of polar drugs in wastewater. A survey of analytical methods for the quantification of regularly used polar pharmaceuticals in wastewater matrices has also been observed.

See, you see that the methods have been developed for non-polar compounds such as antibiotics particularly from less complex matrices like surface and ground water, but then wastewaters are very complex matrices. From there, how to analyze the polar drugs is a big challenge for the analyst; however, there are methods which make use of LC-MS that is Liquid Chromatography with Mass Spectrometry or GC-MS Gas Chromatography with Mass Spectrometry in combination with the C18 solid-phase extraction because the most important part is the extraction. The more beautifully the extraction or the more effectively the extraction can be done, the analysis will be facilitated accordingly.

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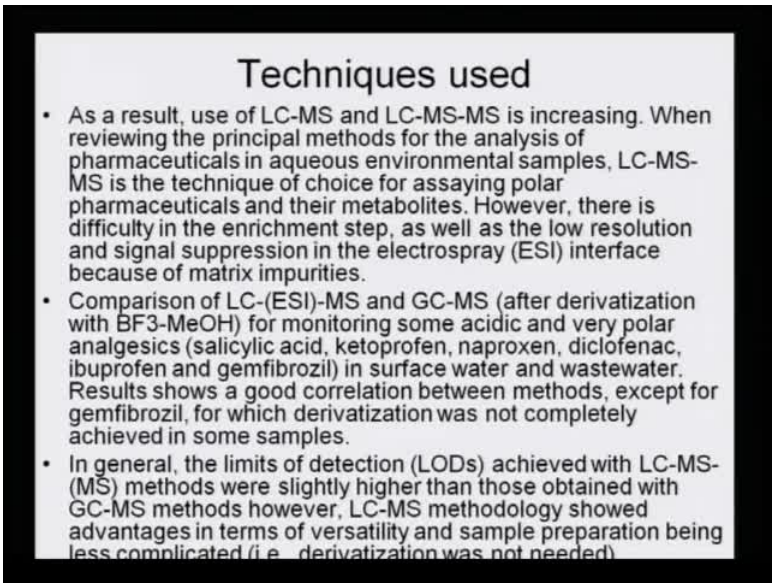

Most of the popular techniques that are used for analyzing the contaminants in water, wastewater, surface water, ground water are the following - as a result, use of LC-MS and sometimes LC-MS and MS, you see this is further, you know, hyphenated. It has two MS, is increasing. When reviewing the principal methods for the analysis of pharmaceuticals in aqueous environmental samples, LC-MS-MS is the technique of choice for assaying polar pharmaceuticals and their metabolites. However, there is a difficulty in enrichment step as well as the low resolution and signal suppression of the electro spray ESI interface because of the matrix impurities. So, one LC-MS is not sufficient for the analysis of pharmaceuticals. It is made to understand that LC-MS-MS that means two MS's are required. Why because the electro spray interface of the matrix impurities requires another MS to do actual fragmentation.

Comparison of LC-ESI-MS and GC-MS after derivatization with BF3 Methanol for monitoring some acidic and very polar analgesics like salicylic acid, ketoprofen, naproxen and all ibuprofen - these medicines, when they are present in surface water or wastewater, how they are analyzed with the help of this LC-ESI-MS and the comparison is made with the GC-MS. Results show a good correlation between methods, except for gemfibrozil, for which derivatization was not completely achieving some samples.

So, here, it is important that these drugs have to be derivatized first and then analyzed; otherwise, they cannot be analyzed like: salicylic acid which is nothing but you know the

simple aspirin that we use or ketoprofen, naproxen, diclofenac and ibuprofen; these are very typical analgesic drugs which need to be derivatized before analysis. In general, the limits of detection that is the LOD achieved with LC-MS-MS methods were slightly higher than those obtained with GC-MS. Obviously, when there is a further more MS hyphenated to LC-MS, it has to have greater sensitivity, whereas GC-MS has only one MS; so, the sensitivity also is lower and which is rightly reflected when the two methods are compared.

LC-MS methodology showed advantages in terms of versatility, and sample preparation being less complicated, that is derivatization was not required. Another advantage is that if one is using LC-MS-MS, then derivatization may or may not be required, but when one is using GC-MS, derivatization is a must because it has to be converted into a volatile derivative to be able to pass through the GC column.

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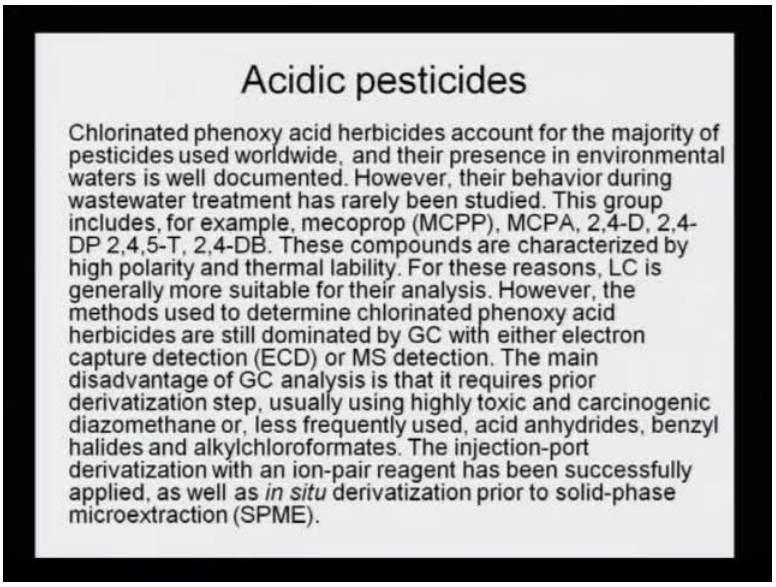

Similarly, there are very specific methods for the analysis of this water contaminant called acidic pesticide. Chlorinated phenoxy acid herbicides account for the major majority of pesticides used worldwide, and their presence in environmental water is well documented; however, their behavior during wastewater treatment has rarely been studied. This group includes, for example, the mecoprop that is MCPP, MCPA, 2, 4-D, 2, 4-DP, 2 4 5-T and 2, 4-DB.

These compounds are characterized by high polarity and thermal lability. For these reasons, the Liquid Chromatography is generally more suited for their analysis; however the method used to determine Chlorinated phenoxy acid herbicides are still dominated by GC. So, one can make use of GC, but LC that is Liquid Chromatography are also popularly used for these kind of herbicides such as MCPP, MCPA, 2, 4-D, 2, 4-DP, 2, 4 5-T and 2, 4-DB.

However, methods used to determine Chlorinated phenoxy acid herbicides are still dominated by GC, with either Electron Capture Detection or that is the ease, one can use GC with ECD detector or GC-MS the main disadvantage of GC analysis is that it requires prior that derivatization step, usually using highly toxic and carcinogenic diazomethane, or less frequently used acid anhydride, benzyl halides, and alkylchloroformates.

The injection port derivatization at the injector port with an ion pair reagent has been successfully applied as well as in situ derivatization prior to solid phase micro extraction is possible. So, if we are using LC-MS, then there is no need for any kind of derivatization for acidic pesticides or herbicides, but if we are making use of GC with ECD detector or GC-MS, it is necessary to derivatize, and sometimes this derivatization requires very hazardous chemical like diazomethane and also sometimes use of acidic anhydride, acid anhydride, benzyl halide and alkylchloroformate. Now, this kind of injective port derivatization at the injective port with the help of this ion pair reagent can be successfully carried out and it has been done with the help of the solid-phase micro extraction in situ; on that also, it can be derivatized.

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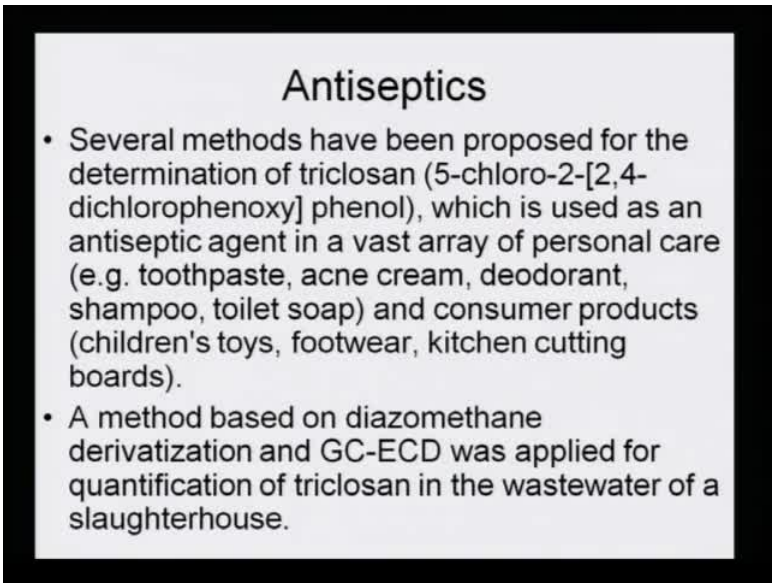

Antiseptic compounds - several methods have been proposed for the determination of trichlosan that is 5-chloro-2-2, 4-dichlorophenoxy phenol, which is used as an antiseptic agent in vast array of personal care such as tooth paste, acne cream, deodorant, shampoo, toilet soap, and consumer products such as children's toys, footwear, kitchen cutting boards, etcetera.

Now, to be able to analyze these and this particular antiseptic 5-chloro-2-2, 4-dichlorophenoxy phenol, which is so popularly used in so many substances of daily use, it is a big challenging method. The method based on diazomethane derivatization and analysis on GC with ECD detector was applied for the quantification of triclosan in the wastewater of slaughterhouse as well as other places. So, you see that diazomethane derivatization is a prerequisite in order to be able to analyze these antiseptics.

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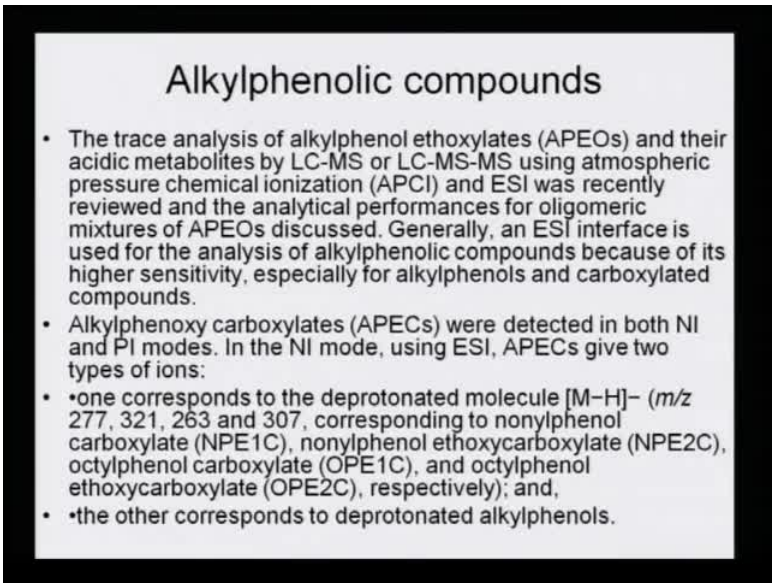

Alkylphenolic compounds - the trace analysis of alkylphenolic ethoxylates and their acidic metabolites by LC-MS and LC-MS-MS using atmospheric pressure chemical ionization method or ESI method was recently reviewed, and the analytical performance for oligomeric mixtures of APEO, that is the alkylphenolic compounds that phenol ethoxylates, would be carried out very effectively.

Generally, an ESI interface is used for the analysis of alkylphenolic compounds because of its higher sensitivity, especially for alkylphenol and carboxylated compounds. So, you see that these compounds are very typical surfactants and they need to be analyzed because and the method should be very soft, electrospray method for the ionization in the MS; otherwise, it is not possible to analyze this alkylphenol so easily. And because the ESI interface is well suited because of its high sensitivity, it is matching with the requirement of these compounds, alkylphenolic compounds. The alkylphenoxy carboxylates were detected in both, NI and PI modes. In the NI mode, by using ESI gives two types of ion; one corresponding to the deprotonated M minus H that is m by z you will find at 277, 321, 263 and 307 corresponding to nonylphenol carboxylate, nonylphenol ethoxycarboxylate, octylphenol carboxylate and octylphenol ethoxycarbolate respectively.

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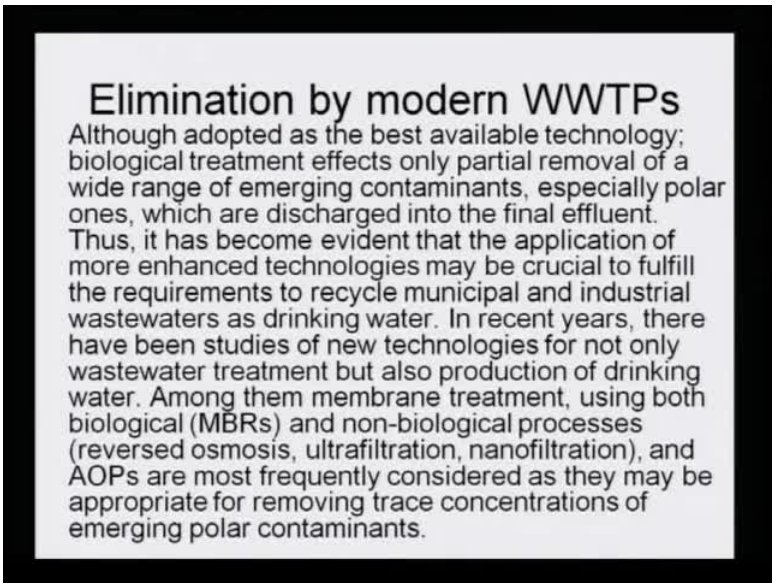

So, these are various types of surfactants that need to be typically analyzed by LC-MS-MS machine and cannot be escaped, because they make serious contamination in water. Elimination by modern wastewater treatment plants, although adopted as the best available technology, biological treatment effects only partial removal of wide range of emerging contaminants, especially polar ones, which are discharged into the final effluent.

Thus, it was it has become evident that the application of more enhanced technologies may be crucial to fulfill the requirements to recycle municipal and industrial wastewater as drinking water. In recent years, there have been studies of new technologies for not only wastewater treatment, but also production of drinking water. Among them the membrane treatment using both biological membranes and non-biological processes - reversed osmosis, ultrafiltration, nanofiltration and so on, have been applied, and the oxidation processes are most frequently considered, as they may be appropriate for removing trace concentrations of emerging polar contaminants.

So, now, what has been done? That in big countries, in more advanced countries, in the developed countries, what they are trying to do? They are trying to eliminate various processes at the wastewater treatment plant itself, and therefore, by the use of modern technology that is for treatment, they are using membrane for both biological as well as non-biological processes. Membranes are used, and therefore, a lot of contaminants are

removed by these membranes. And of course, the oxidative processes, the biological processes of treatment of wastewater are other means.

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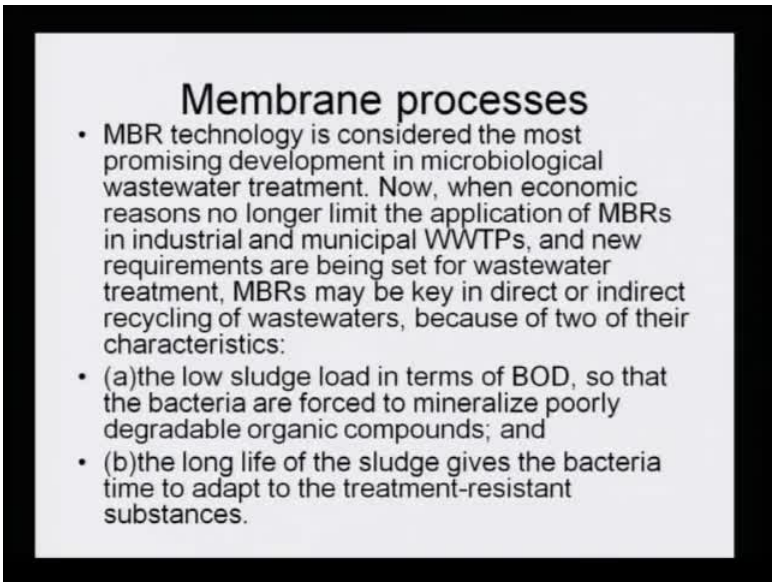

Membrane technology or membrane processes - MBR technology is considered the most promising development in microbiological wastewater treatment. Now, when economic reasons no longer limit the application of the MBRs in industrial and municipal wastewater treatment plants, and new requirements are being set for wastewater treatment, MBRs may be key in direct or indirect recycling of wastewater. So, it is of course, analysis is important, but what is being done at the root level? Only the membrane technology or other kind of processes are being applied on the wastewater treatment plant so that the contaminant level in the waste treated water becomes very low; the low sludge load in terms of BOD, so that the bacteria are forced to mineralize poorly degrade organic compounds and the long life of the sludge gives the bacteria time to adapt to the treatment-resistant substances. So, there are advantages, very important advantages that the membrane technology is actually very useful because these bacteria can face very little low sludge, and therefore, they can thrive on it and they can mineralize on it, and because they can have a long life on the sludge, therefore, the treatment is most effective.

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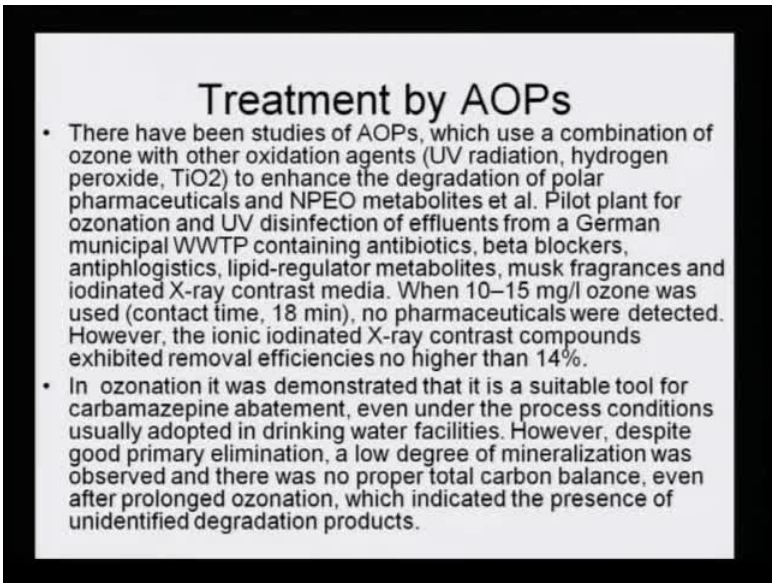

Treatment by AOPs - there have been studies of AOPs, which use a combination of ozone and other oxidative reagents UV radiation, hydrogen peroxide, titanium dioxide to enhance the degradation of polar pharmaceuticals and NEO metabolites.

It is found to be that you know these needs to be treated with very good oxidizing agent. Pilot plant for ozonation and UV disinfection of the effluents from a German municipal wastewater treatment plant containing antibiotic beta blockers antiphlogistics lipid regulator metabolites musk fragrances and iodinated X-ray contrast media; so, when 10 to 15 milligram ozone was used, contact time required was 18 minutes; no pharmaceutical were detected; however, the ionic iodinated X-ray contrast compounds exhibited removal efficiency no higher than 14 percent. So, when a combination of such matrix is present, it is possible that only few can get oxygenated and the others may not even get even affected.

In ozonation, it was demonstrated that it is suitable tool for carbamazepine abatement, even under the process conditions, usually adopted in drinking water facilities; however, despite good primary elimination, a low degree of mineralization was observed and there was no proper total Carbon balance even after prolonged ozonation, which indicated the presence of unidentified degradation products.

So, sometimes, what happens is that even when ozone is passed to for a longer period of time, it is not effective because of some unidentified degraded product which hampers

the effectivity of ozone. And therefore, no one method is full proved, but these are all corrective measures so that some of the compounds can be broken down, can be oxidized, can be mineralized and removed from the wastewater, and the effective wastewater treatment can be brought about.

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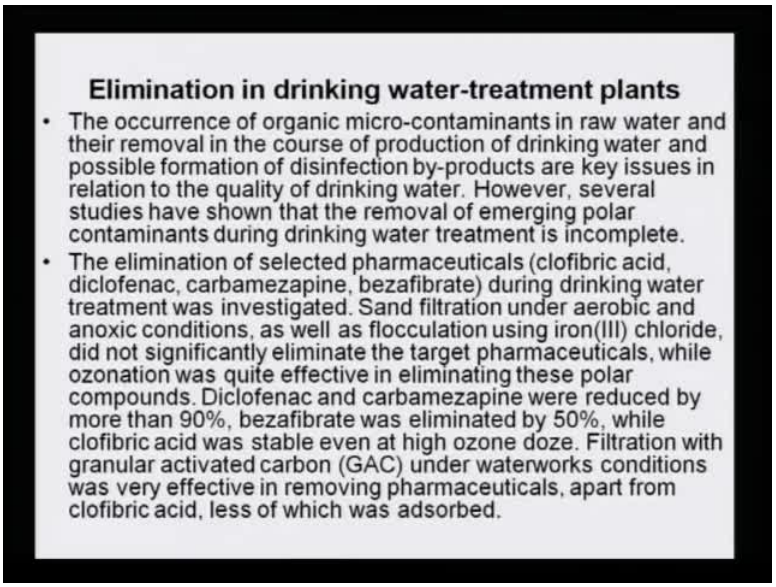

Elimination in drinking water treatment plant - now, this is of at most important to human beings because it is to be understood that drinking water is of prime importance to all; one and all. The occurrence of organic micro-contaminants in raw water and their removal in the course of production of drinking water and possible formation of disinfection by-products are key issues in relation to the quality of drinking water. However, several studies have shown that the removal of emerging polar contaminants during drinking water treatment is incomplete.

Elimination of selected pharmaceuticals that is the clofibric acid and some of these medicines during drinking water treatment was investigated. Sand filtration under aerobic and anoxic conditions as well as flocculation with Iron III chloride did not significantly eliminate the target pharmaceuticals, while ozonation was quite effective in eliminating these polar compounds. The diclofenac and the carbamezapine was reduced by more than 90 percent, the bezafibrate was eliminated by 50 percent, while the clofibric acid was stable even at high ozone doze. Filtration with granular activated

carbon that is the GAC under water works condition was very effective in removing pharmaceuticals, apart from the clofibric acid, less of which was adsorbed.

See, you see, a combination of method is most often required when we are talking about drinking water; no one method you will see. I told you so many examples: the acidic pharmaceuticals, the surfactants, and then the antiseptics, the medicines, they all are present in the matrices, and to be able to remove that with just one method, it is impossible.

So, combinations of sand filtration, ozonation, use of granular activity charcoal - these are some of the standard methods. Elimination of neutral and acidic nonylphenols also can be brought about with very effectively by using LC-MS-MS method. We have already described the method LC-MS-MS. So, first it is allowed to set and flocculation is done by rapid sand filtration it takes care of 7 percent purification.

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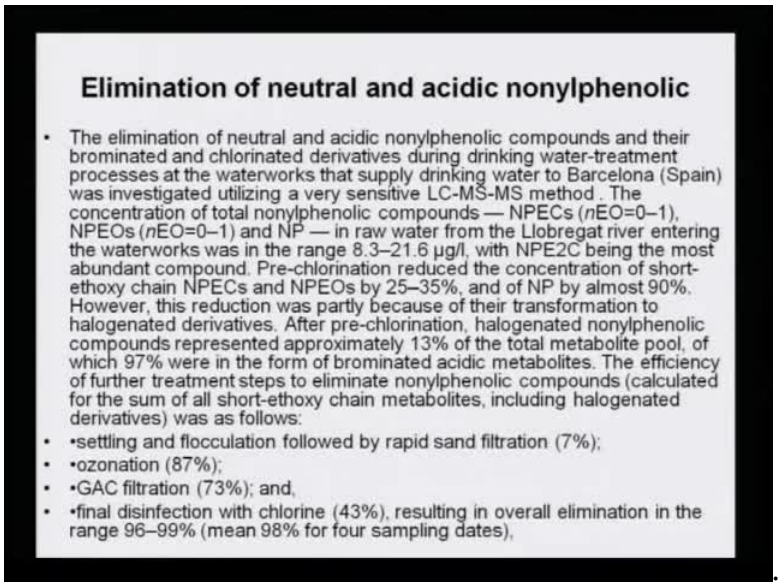

Then ozonation takes place and 87 percent purification is possible. Then, with the use of Granular Activity Charcoal, another 73 percent you know of such metabolites can be removed and final disinfection with chlorine resulting in overall elimination in the range of 96 to 99 percent can be achieved for the drinking water.

So, you see it is not such an easy process to remove these neutral and acidic nonylphenol ethoxylates because they are very complicated structures and they need to be separated

through various steps by using various reagents, by sand filtration, ozonation, granular Granulated Activated Charcoal; only then, the it becomes purified to be able to be like fit for drinking water.

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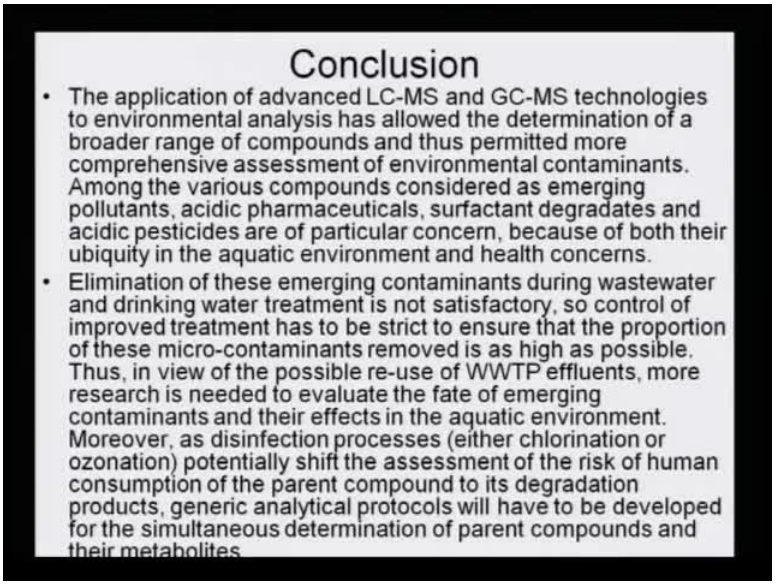

So, to conclude this, I would say that application of advanced LC-MS and GC-MS technologies for environmental analysis is allowed to determine a broad range of compound, thus permitted more comprehensive assessment of the environmental contaminants, particularly in water. Among the various compounds considered as emerging pollutants, acidic pharmaceutical, surfactant degradates and the acidic pesticides are of particular concern, because of both their ubiquity in the aquatic environment and health concerns because they are so harmful to our health; that is why it is important to eliminate and thus entire reuse and reviewing of the wastewater treatment plant effluent must be taken into consideration. A disinfection process must be included that is clorination and ozonation, and therefore, one can expect that the levels of contaminant would be reduced.

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So, with this, we come to the last part of our lecture which is then related to analytical method validity and quality assurance. Having learnt all this while, the various processes that can be carried out, the analytical method, one must understand the importance of its validity and quality assurance, and its correlation with quality assurance.

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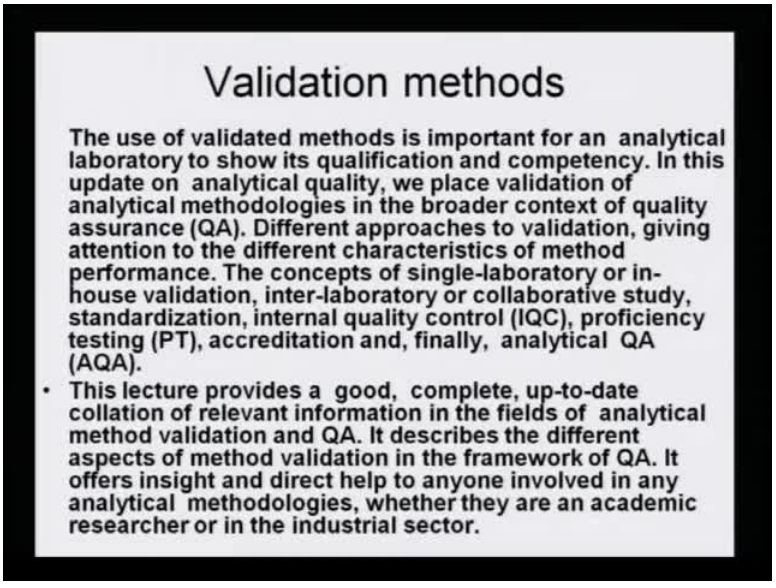

Validation method - we have said that it is important to see whether the machine is validated or not. The use of validated methods is important for an analytical laboratory to show its qualification and competency. In this update on analytical quality, we place

validation of analytical methodologies in the broader context of quality assurance; so, quality assurance can only be correlated if there is the method is validated.

Different approaches to validation, giving attention to different characteristics of method performance. The concepts of single-laboratory or in-house validation, inter-laboratory or collaborative study, standardization, internal quality control, proficiency testing accreditation and finally, analytical quality assurance is what actually matters for a laboratory. Because if we say that we have generated an analysis, that does not mean it is an absolute value. Can it be repeated and if the machines are working properly, if the method is validated, only then there will be repeatability.

So, all other things, that is a single-laboratory concept and the inter-laboratory or collaborative study standardization is a must; otherwise, my result and result from another laboratory will differ. And if the analysis is differing, that means it is not being done or the method is not validated.

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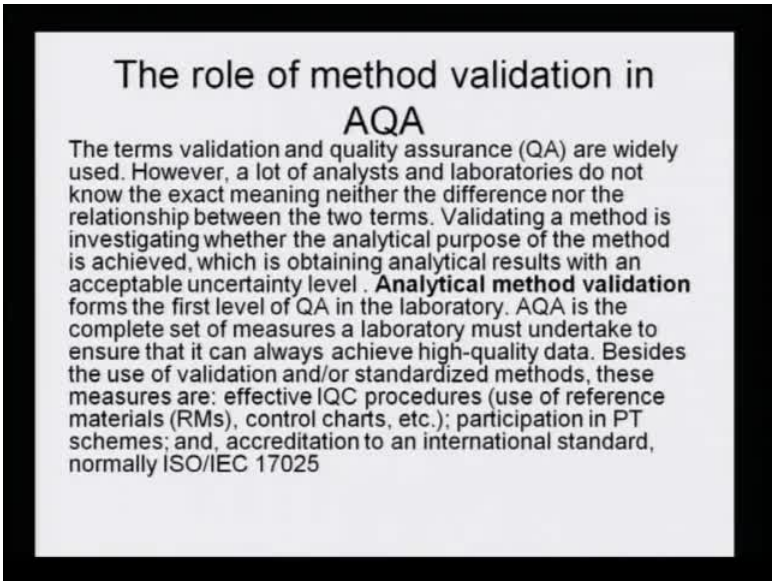

This lecture provides a good, complete, up to date collation of relevant information in the fields of analytical method validation and quality assurance. It describes the different aspects of method validation. So, if we try to look at the role of method validation, it is the term validation and quality assurance are widely used; however, a lot of analysts and laboratories, do not know the exact meaning, neither the difference nor the relationship between the two terms.

The validating method - when we say that, we are validating a method; it is investigating whether the analytical purpose of the method is achieved which is obtaining analytical results and with the acceptability of uncertainty level. That means it should not have too much of an error. Analytical method validation forms the first level of quality assurance; therefore, they go hand in hand.

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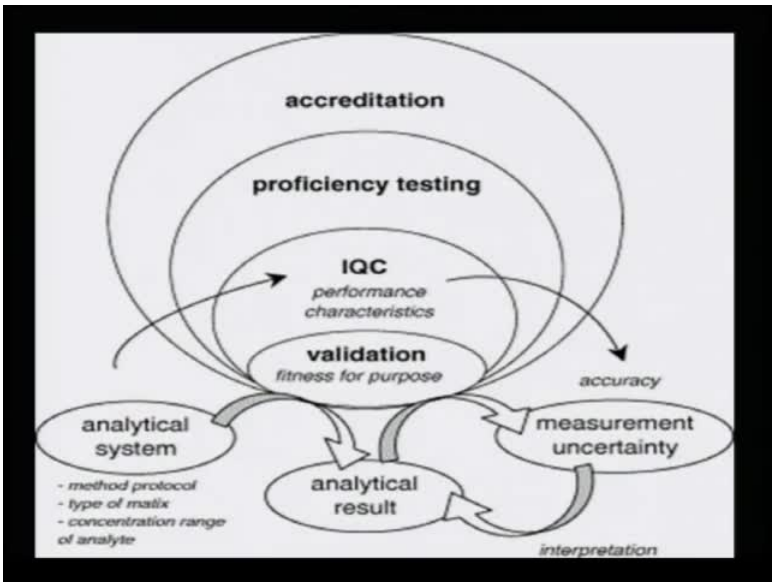

If the method is validated, if the machine is working properly, the it is assured that there is a quality assurance from the results that are derived from those machines. If you try to look at this particular slide which is the last slide of the lecture, I would like you to appreciate that any analytical system which generates analytical results, give measurements and that could fall in the range of an uncertainty or certainty.

The accuracy will only be established, if we have an inter-laboratory quality control and the method is validated, fit for the purpose, and therefore, proficiency test and accreditation are important factors in this line of quality assurance. So, if we try to now take an overview of the entire course that we have just gone through - the advanced level of analytical chemistry course, you will see that I tried to take you through the journey of various chromotagraphic and spectroscopic methods, including the fact that we must also understand that every analysis needs validation and that requires, that gives us the quality assurance for finding out whether the analysis has been done properly. So, with this we have come to an end of this course. Thank you.