**Natural Dyes**

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**Lecture No. # 16**

Now, we have been talking so much about dyeing dyeing dyeing. What happens or how do we ascertain that a dyeid fabric or dye per say is actually nontoxic. Because we said, that the main criteria for rejection of syntactic dyes had been the reason for its toxicity. Now, because of being toxic, because of been carcinogenic, because of being allergenic, because of being mutagenic - these syntactic dyes were pushed out of the market or are being pushed out of them commercial market. And in place of the that there is a resurgence revival of natural dye. And we saw that, the method of the dying is also very safe, except for the role of mordents where also the it is recommend that copper and chromium should be avoided as far as possible, but if a shade is desired one can use this mordents.

So, how do we ascertain, that the naturally dyeid fabric is non toxic or has no chemical which are known to create health hazards or skin allergy and so on. So therefore, there was an importance for testing eco-friendliness of dyed fabrics or dyed textile. And in this lecture, we will try to understand the various methods of testing of these fabrics, and dyes in order to see theirs eco-friendliness and to understand their safety.

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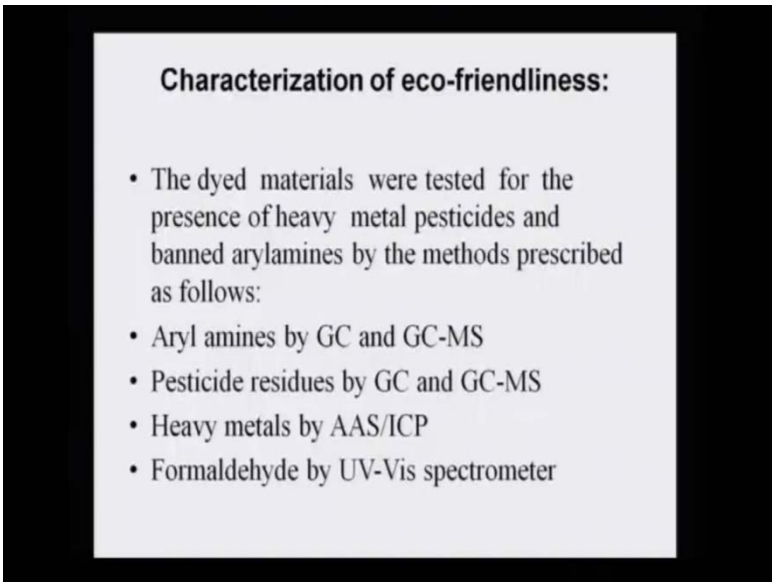


Characterization of eco-friendliness: the died materials were tested for the presence of heavy metals, pesticides, and banned arylamines by the methods prescribed as follows. Aryl amines were determine by GC and GC-MS, pesticide residues by GC and GC-MS, heavy metals by AAS that is atomic absorption spectrometer or inductively coupled plasma spectrometer ICP. And formaldehyde was evaluated by UV visible spectrometer. Now, I would like to draw your attention that this ban came after the German ban on Azo dyes. And it this emphasis for eco-friendliness or finding the whether the fabric - dyed fabric is eco friendly or not to human was emphasize mainly after that German ban.

Twenty two laboratories alone in India was set up by the textiles ministry, so that, the export that is the done from India to other countries must bare this certification that all the article that are exported are safe, and are eco friendly. And for that report, it was necessary to find out, whether there are Azo dyes by the means of analysis of the 22 banned arilameans. And this was done by gas chromatographie or gas chromatographie, mass spectrometry, then I told you that pesticide residues are there on cotton, because while cotton is being formed or due to agricultural practices, because pesticide is used for cotton crop. It is important to see, whether any pesticide residue remains on the cotton itself, and then into the yarn, and then into the fabric and so on.

And since, the you know some of the synthetic dyes are also are mordant dyes. Apart from that natural dyes are using mordent, is there any possibility of these mordant that is

the chromium, and the copper copper, and 9 such heavy metals. That is chromium copper, arsenic, led, cadmium, cobalt, nickels, zinc. These were the nine notorious elements, and mercury which had to be tested by the means of atomic absorption or inductively coupled plasma. Formaldehyde was another processing chemical which needed to be analyzed, and that was analyzed by UV visual spectrometer.

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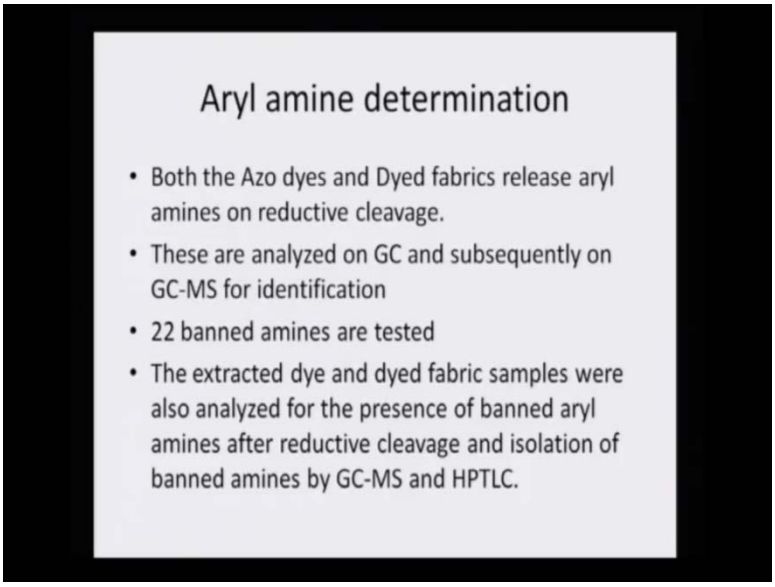


Aryl amine determination: Both the Azo dyes, and the Dyed fabrics release aryl amines on reductive cleavage. We have learnt this while ago when we are doing toxicity of dyes stuff, and we saw that how the n double bound n actually breaks, and gives one amino 2 naphthol. As one of the basic products of these Azo dyes. And of course, their substituted groups, but these amino amino compounds are extremely harmful. And that is why 22 such amino compounds were banned. And they were derived from the subsequent Azo dyes. These were analyzed on GC, and subsequently on GC-MS for identification.

I also told you that GC two compounds can have the same retention time. GC only recognizes the retention time of a compound, but that is not enough, in order to identify the compound it is also important to see the mass fragmentation pattern, which can only be obtain from GC-MS. So, two method for the validation or a cross checking that yes this is the, you know culprit compound must be carried out. And it is what the recommend procedure and the protocol. 22 banned amines have to be tested, the

extracted dye and dyed fabric samples were also analyzed for the presence of banned aryl amines after reductive cleavage, and isolation of the banned amines by GC-MS and even HPTLC.

So, one can use GC, GC-MS combination or GC-MS HPTLC combination. HPTLC is high pressure, a thin layer chromatography. Once it is identified and validated, then it is finally, identified you know, cross verified with help of amines. So, amines - mass spectrometry is the full and final identification; you know alma. Otherwise one will not be able to say, as I told you 2 compounds on TLC, can show same retardation factor value. They can can come as similar sport, but that does not go to prove that this is the same compound. Only when this compound is put into the GC-MS machine; the fragmentation pattern of every molecule is very specific. So, that becomes a main criteria for identification.

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Banned amines - The GC-MS analysis of 20 carcinogenic amines banned in textile and consumed product, and of interest in environmental monitoring of colorization process waste. For the analysis of amines from a range of consumer products a GC-MS system can be configured, and operated in a manner that is most suited suitable to achieve necessary detection level. World-class class performance with respect to reliability, continues to lead commercially available GC-MS instrumentation. An important consideration for laboratories doing routine GC-MS work; data handling tools previously

available for every specific environmental analyses have been expanded for more general use, providing greater easy to use in data analysis, and customized reporting.

So, you see that it the same machine which is actually present in the laboratory, can be used for the analysis of GC-MS analysis of the banned amines. Now, the only thing that one needs to do is to have standers of these 22 amines, make a program of in which all these 22 amines come as separate piece. And that was possible, because these are different compounds; they will elute differently, and each one then will be identified, and when such a possibility is there. Then one can identify all the 22 compounds in just one go, it is not necessary that one has to, if suppose one banned amines are there, 2 times analysis are has to be done no, on the same machine, because one Azo dye will release 2 amines at a time, but we can take the whole spectrum of, because any combination can be present in the Azo dye.

So, we do not know the fragment A, we do not know the fragments B. But the all the fragments, and enlisting on the same GC can be done. And when it is done, it is easy for us to identify which of the banned amine is present in that particular Azo dye, because the banned amines are coming from the reductive cleavage of that die. So, one dye will release 2 banned amines, and those can be identified from the list of 22.

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Banned amines: Because the concentration of amine in the product may vary, scan or SIM mode methods have been developed. With split injection of one micrometer

aliquots, mass detection in in scan mode allows easy detection of 0.1 ppm, and ultimate detection down to 0.02 to 0.04 ppm. The highest sensitivity is obtained by pulsed, split less injection combined with SIM mode, yielding a limit of detection of 5 ppb for injection volumes of one micro liter. For samples with concentration greater than 10 ppm, split injections are recommended. So, you see one can modify the process according to the need, and according to the availability of the dye on the fabric or as dye powder.

So, both scan method or SIM mode method can be used spilt or spilt less injection can be used depending on the quantity that is available for analysis. And it can go down to 0.02 to 0.04 ppm level also, and sometimes even up to ppb level presence or absences can be detected. Now these are the 22 banned amines, the list are according to commission of Europeans communities directives 2002.

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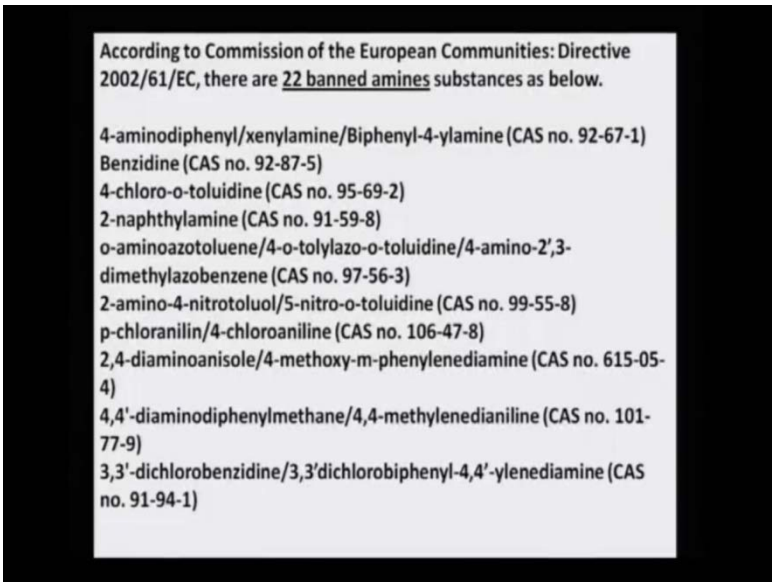


There are 22 banned amines substances as shown below. 4 aminodiphenyl, xenyamine, biphenyl-4-ylamine, Benzidine, 4-choloro-ortho-toluidine, 2-naphthylamine, ortho-aminoazotoluene, dye-aminoazobenzene, 2-aminio-4-notrotouol, 5-nitro-ortho-toludine, para-chloranilin or 2-4-diaminoanisole, 4-4-prime-diamino-diphynayl-methane, 3-3-prime-dichlorobenzidine, 3-3-prime-dimethoxibenzede, 3-3-prime-dimethylbenzedene, 3-3-prime-dimethyl- 4-4-prime-diaminodiphenylmethane, ortho-toluidin, Pera-cresidin, 4-4-prime-methyl–bis-2-chloroaniline, 4-4-prime-methylinedianiline, 4-4-prime-

oxidianiline, 4-4-prime-thiodianiline, ortho-toluidine, 2-aminotoluine, 2-4-toluylenediamine, 2-4-5-trimethylaniline, 4-aminoazobenzene, ortho-anisidine.

So, you see these are the various 22 banned amines that are released from Azo dyes, and these are the one's which are actually identified after the reductive clearage of the Azo dye.

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Similarly, toxic heavy metals can be analyzed. Toxic heavy metals contents in the dye or the dyed fabric were determined by using 2 machines. I said either one can use atomic absorption, spectrometer or inductively coupled plasma, optical emission spectrometer that is ICP. For analysis 1000 ppm solution, that is 0.1 gram digested in concentrated hydrochloric acid, and made up to 100 m l by adding distilled water was used. Metals - the heavy metal content in the extracted dyes were determined by using inductively coupled plasma spectrometer, and it was found that their concentrations are much below the stipulated limits.

The dye extract if it shows presence of zinc or mercury in less than 0.005 percent. This will be considered safe, because use of red listed heavy metals based mordents have been avoided. Thus dyeing process is eco friendly. So, as I told you, that by using 1 to 2 percent of copper or chromium in the list of the 9 heavy metals, see these heavy metals can come from the processing water. This heavy metal can come actually from the mordanting process. So, one has to take up serious check on the fabric by analyzing the

fabric, whether it contains heavy metals or not. And the 9 heavy metals that we use must remember are arsenic, cadmium, copper, cobalt, nickel, mercury, zinc and led. So, these heavy metals are the one's which need to be analyzed, because these are the toxic identified heavy metals which can (( )) create non ecofriendliness on the textile.

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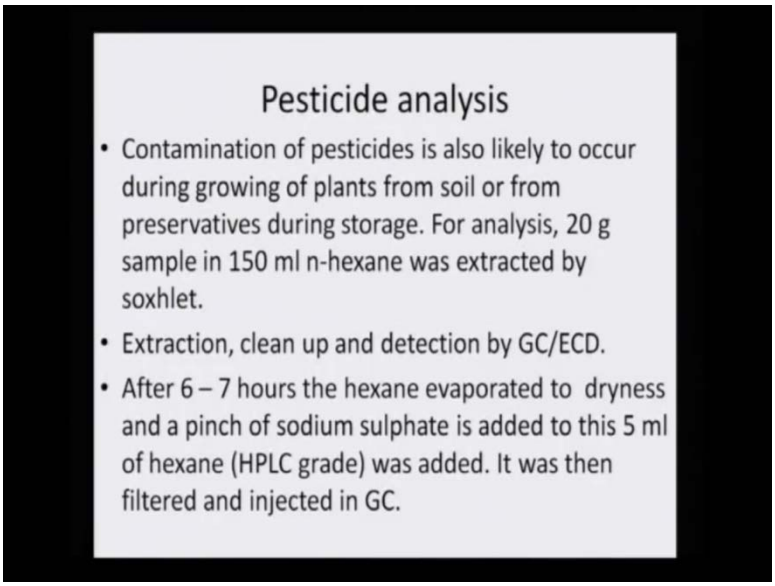


Pesticide residue analysis - As I have been telling that even reminisce or residue of pesticide, on the cotton that was grown in the farms can be carried over to the final product or even up to the dyed product. So, contamination of pesticide pesticide is also likely to occur, during growing of plants from soil or from preservatives during storage. For analysis 20 gram samples in 150 m l hexane was extracted in soxhlet. So, these pesticides are very nicely, they dissolved in hexane. So, a soxhlet extraction of the fabric with the hexane itself can give the pesticide residue extraction, cleanup, and detection by GC/ECD. That is electron capture detector with GC can identify the retention time.

But again for validation, we need GCMS. After 4 to 7 sorry after 6 to 7 hours, the hexane evaporated to dryness, and a pinch of sodium sulfate is added to this 0.5 m l of hexane which should be HPLC grade, and then the it is filtered and injected in GC. First the GC analysis is carried out, and then subsequently the analysis must be repeated on GC-MS to cross verify. Now this extraction process is very typical for only pesticides, this is not the procedure that followed for the aryl amines. There reductive cleavage is to be carried

out, because Azo dye per say is not analyzed on the GC machine or GCMS machine; it is the banned amines which are I read out the names to you the 22 banned amine.

So, similarly pesticide analysis is quite different. Although the same GC, and the GCMS machine is being used, but in the case the aryl amine, it was GC with FID detector, where as in the case of pesticides, because most of them are you know, these pesticides are chlorinated or even if they are prosperous containing or amino containing these banned amines. They have they are analyzed or on the GC, but the detector is different. So, the pesticides are different, they are analyzed on GC with ECD detector whereas the banned amines are detected by GC with FID detector, but both of them are validated on the GC MS.

So, this should be made clear, because the procedure of banned amines and pesticide may appear to be similar. But they are not truly similar. I will repeat that the in the banned amines, there is a reductive cleavage method involved, before the sample can be injected into the GCE where as in the case of pesticide, it is direct extraction from the fabric; so, in the solvent hexane, because hexane is one of the best solvent for these particular pesticide desolation.

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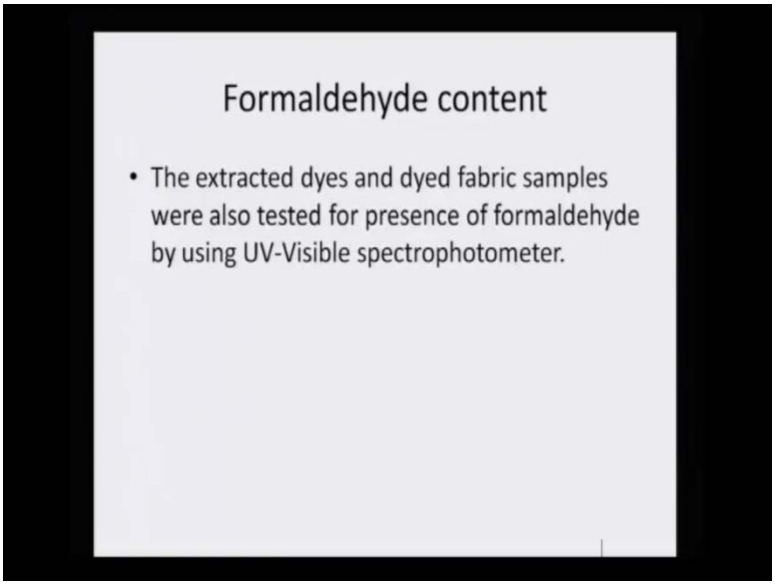


Formaldehyde analysis - The extracted dye, and dyed fabric samples were also tested for presence of formaldehyde by using UV visible spectrophotometer. Now, formaldehyde does not come from any you know source, but it is a part of that textile processing, where

formaldehyde is being used. Now, any excess amount of formaldehyde on the fabric can create skin allergies. So therefore, it should be tested, before it is exported and for its eco-friendliness.

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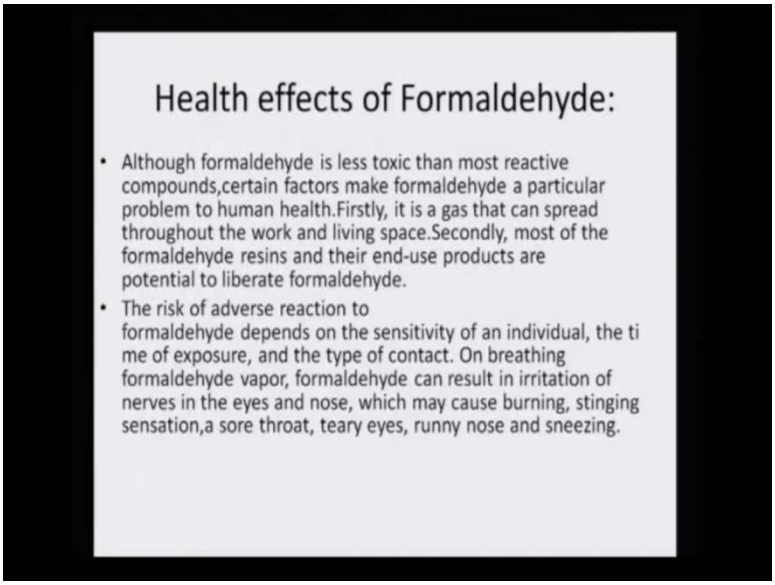


Health effects of formaldehyde: Although formaldehyde is less toxic than most reactive compound certain factors make formaldehyde, a particular problem to human health. Firstly, it is a gas that can spread throughout the work and living space. Secondly, most of the formaldehyde reasons, and their end use products are potential to liberate formaldehyde. The risk of adverse reaction to formaldehyde depends on the sensitivity of an individual, and the time of exposure, and type of contact. On breathing formaldehyde vapor - formaldehyde can result in irritation of nerves of the eyes and nose, which may cause burning, stinging, sensation and a sore throat teary eyes, running nose and sneezing.

So, these are various effects that are caused not only the skin allergies, but it can also create immediate effects on throat, ears, eyes etcetera. Skin contact with formaldehyde can cause skin rashes, and allergic skin reactions.

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Formaldehyde is well known sensitizer for dermatitis. And instances of dermatitis resulting from wearing clothing, containing high levels of formaldehyde have been documented. It was only after that, that it was felt formaldehyde formaldehyde also must be tested. Because after the processing of the fabric; the remnant of the formaldehyde can create this problem. So, therefore, it should not be present. So, that the user is not put to a difficult situation. The international agency for research, and cancer a world health organization panel of 26 scientists have concluded, that the formaldehyde is a human carcinogen.

So, it is as serious as that, and that is why just the way toxic nine heavy metals were analyzed. Similarly, the formaldehyde residues or remnants must be analyzed on the finished product. What causes the release of formaldehyde of from where does the formaldehyde actually come from the textile.

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The formaldehyde containing chemicals are most effective, cross linking agents for wrinkle resistance, dimensional stability, and flame retardant properties of textiles. So, whenever textile is you know prepared, specially for being flame retardant or for having some kind of you know wrinkle free a resistance material or if they have to have some kind of stressed look or so, some kind of coatings are done. And these coating actually release formaldehyde. During the finishing process the N-methylol compounds in formaldehyde containing reagent can react with the hydroxyl groups of the cellulose. Resulting in preferable cross linking reaction; they may also react with themselves or with NH group which occupy the action places of cross linking agents.

So, that is how they first attached to it, and then subsequently the formaldehyde is released from the cross linking agent.

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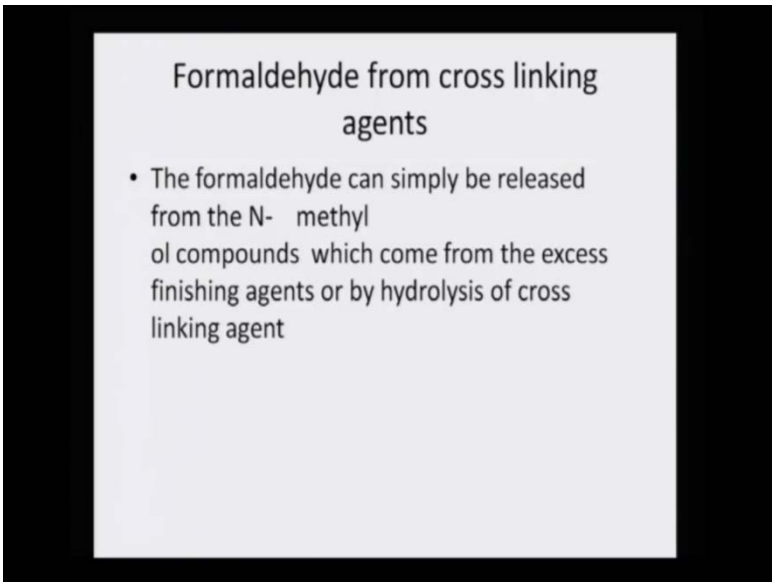


So, the formaldehyde can be simply released from the N-methylol compounds which come from the excess finishing agents or by the hydrolysis of this cross linking agent. So, all those which are causing the cross linking on the fabric, eventually slowly release the formaldehyde, and that is what needs to be analyzed and cross checked.

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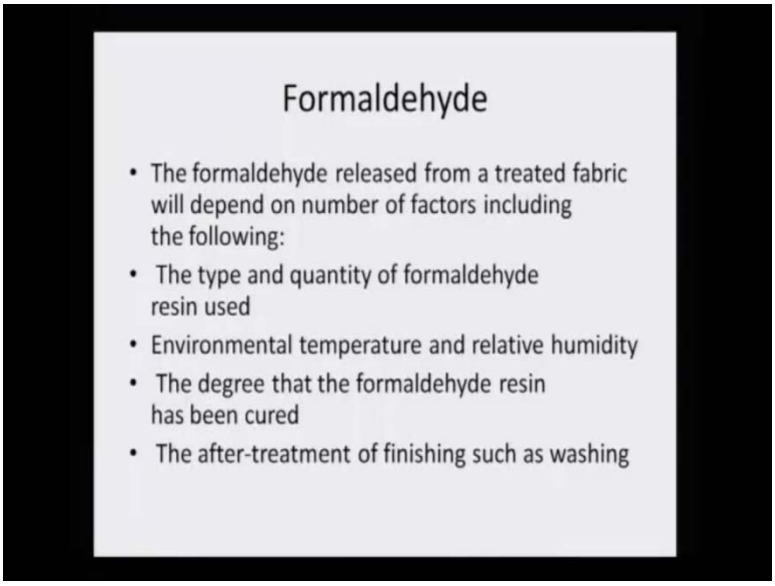


Formaldehyde released from a treated fabric will depend on number of factors including the following. The type and quantity of formaldehyde that has been used or formaldehyde resin that has being used. And environmental temperature, and relative

humidity, because all this will actually cause, formaldehyde is a gas. So, anything that can facilitate the gaseous substance to evolve from the material, from the resins material will facilitate. So therefore, environmental temperature, and relative humidity can also release or will facilitate or make facile release of formaldehyde.

The degree that the formaldehyde resin has been cured, so if the curing is improper; then also the release of formaldehyde from the formaldehyde resin is possible. The after treatment of finishing such a washing, will also cause formaldehyde to relief. So therefore, all this all the various reasons, why formaldehyde releases in the finished product? And because it is already established human carcinogen, one needs to do this kind of testing, in order to prove that this all though this processing, like if a fabric has to be made wrinkle free, use of formaldehyde resin is a must is mandatory cannot be avoided.

So, if it cannot be avoided, it is better to test what is the level of formaldehyde whether it needs any more treatment. So that all the formaldehyde is first removed after the treatment process is done

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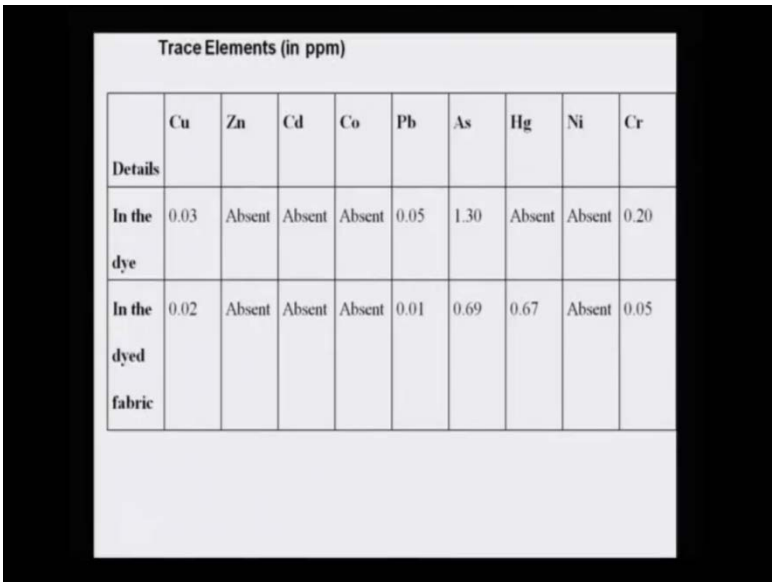


Now, if we try to look at any fabric, and see a results like this. That you see that copper, zinc, cadmium, cobalt, led, arsenic, mercury, nickel and chromium. These are the various metals that have been tested from the dye, as well as from the dyed fabric. And the result obtained is that from the dye there is this 0.03 percent of the trace metal of copper, zinc,

cadmium, cobalt are the absent; led is 0.05 in the dye and it is 0.01 in the fabric. In the arsenic in the dye arsenic is present as 1.3 ppm. And in the dyeid fabric it is only 0.69, mercury is absent, but it appears in the dyed fabric. So, it may be coming from the water or some other source. Nickel is absent in both the cases, and chromium is present as 0.2 in the dye, and is present on the fabric at as 0.05.

So, what does this show, that normally the even if the these toxic nine heavy metals, one or more than one are present in the dye; by the time they are put on to the fabric. The amount is generally reduced, which means that the processing process takes care, and on the fabric, even if we are using mordant. The concentration of copper and chromium are much much less, and they are in I would say in the trace quantity.

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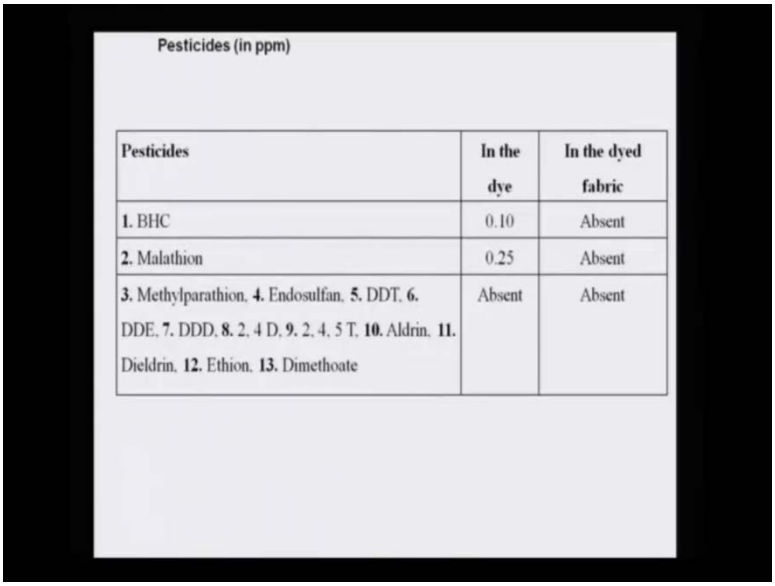


Similarly, if we try to look at a result obtained from the pesticides analysis. It shows that BHC that is benzenehexachloride is 0.1 in the case of dye, it is absent in the fabric. Melathion is present as 0.25 in the dye, but its absent in the fabric. And all others like methyl, parathion, eldosolfan, DDT, DDE, DDT and 2 4 D, 2 4 5 T, aldrin, dieldrin, ethion, dimethoate all these rest of them are absent. So, even when the pesticide is present on the fabric or in the dye is still is absent on the dyeid fabric. So, it is not getting you know, it is not even present in the trace quantities, but this was possible to know, because the analysis was carried out both the dye, as well as the dyed fabric. If such an excise is not done, one would not be able to know what is the source of the pesticides?

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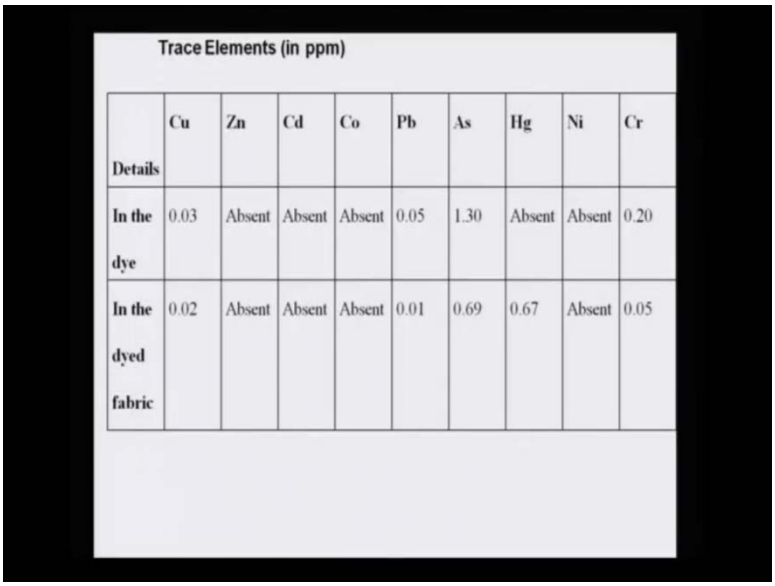


And similarly, what is the source of the heavy metal one will not be able to know unless until, this kind of eco friendly testing excise is carried out.

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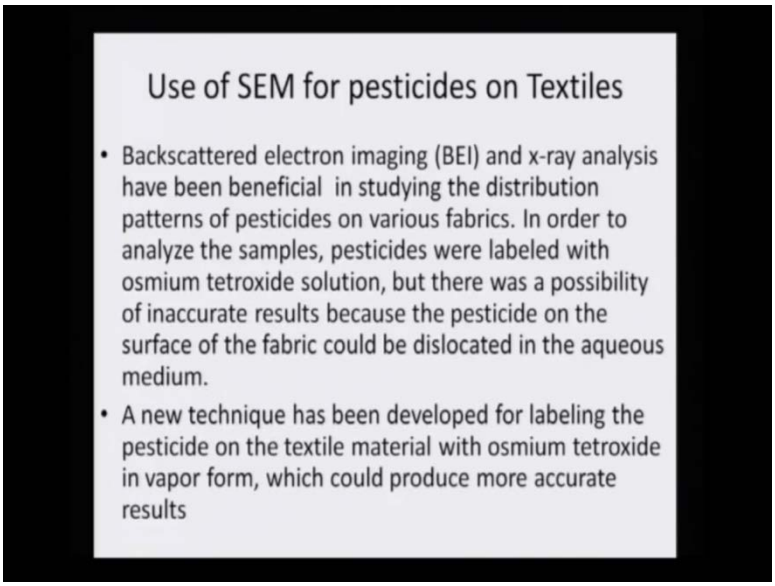


Now, there has been lot of research, to find out how one can even use some other tools to find out, whether pesticides pesticides are still adhering to the textiles or not. There was one research paper which I came across, and I thought I would like to discuss this with you people. Back scattered electron imaging that is BEI, and x ray analysis have been beneficial in studying the distribution pattern of the pesticides on various fabrics. In

order to analyze the samples - pesticides were labeled with osmium tetroxide solution, but there was a possibility of inaccurate results, because the pesticide on the surface of the fabric could be dislocated in the aqueous medium. A new technique has been developed for labeling the pesticide on the textile material with osmium tetroxide in vapor form, which could produce more accurate results.

So, you see that this particular imaging of the surface of the fabric was done by the scattering electron microscopy, and because of the effect of the backscattered electron imaging. The osmium tetroxide has a tendency to react with these pesticide molecules on the surface of the fabric, but what was happing that when solution of osmium tetroxide was used, these the pesticide would get delocated, dislocated. But an new technique was developed, where labeling of the pesticides on the textiles material could be done. And osmium tetroxide was used in the vapor phase. So therefore, more accurate poisoning, and presence of the pesticide could be ascertain.

Now, these are certain very new techniques, which are not actually the being practiced for eco-friendliness testing, the routing eco-friendliness testing. There pesticide is analyzed only by GC and GC MS. But in this is nevertheless an attempt which uses a new technique, and's you know scanning electron microscopy is one new technique which has been used to locate the pesticides on the surface of the a textile. Because some can only do a surface morphology, and it cannot get into the depth whereas the other analysis the GC, and the GC analysis is through the extraction of the pesticides.

So, there the fabric is completely destructed, and this is the non destructive analysis where one can do a surface study by using vapors phase osmium tetroxide on to the surface, and identifying the presence of the pesticides on the surface of the textile. So, with this, we have come to an end of the eco-friendliness testing of the textile. But at the same time, I would also like to emphasize, that these testing are valid for not only naturally dyed fabric, but also for the synthetically dyed fabric. The testingful eco-friendliness in the dye fabric holds good for syntactic as well as natural dyed fabric, why because under no chance the export material should have any of these banned chemicals.

So, what are these banned chemical? The Azo dyes which release 22 banned amines, the toxic heavy metals which are 9 in number, and the residues of pesticides and formaldehyde. So, all these 4 need to be analyzed by different techniques, and to a

certain that whether it is sourcing from the dye or whether it is coming from the processing of the dyed fabric, that can be only ascertain when the comparative analysis of the dye, and the dyed fabric is made for all these parameters for checking their eco-friendliness.