There are many initial studies centred on the qualitative identification of polymeric organic fraction of the particulate matter, on the modifications of the matrix undergoing with the effect of temperature, light, time and enrichment in transport, polymerization's kinetics and thermodynamics of primary and secondary aerosol [9-10, 81-84]. All these studies employ the investigation of different compounds with a singular analytical method and a singular point of view, i.e. a treatment of samples, pertaining the research of interest, followed by a traditional or innovative extraction and a final analysis with a gas or liquid mass spectrometer.

In addition, to improve the detection of the polymeric organic fraction that comes from particulate matter, a method of study of the lignin oligomers and polymers arising from the biogenic fraction of two different samples of particulate matter by different extractive methods and final analysis has been developed.

Following, the general pattern of the analysis in use to perform the samples:

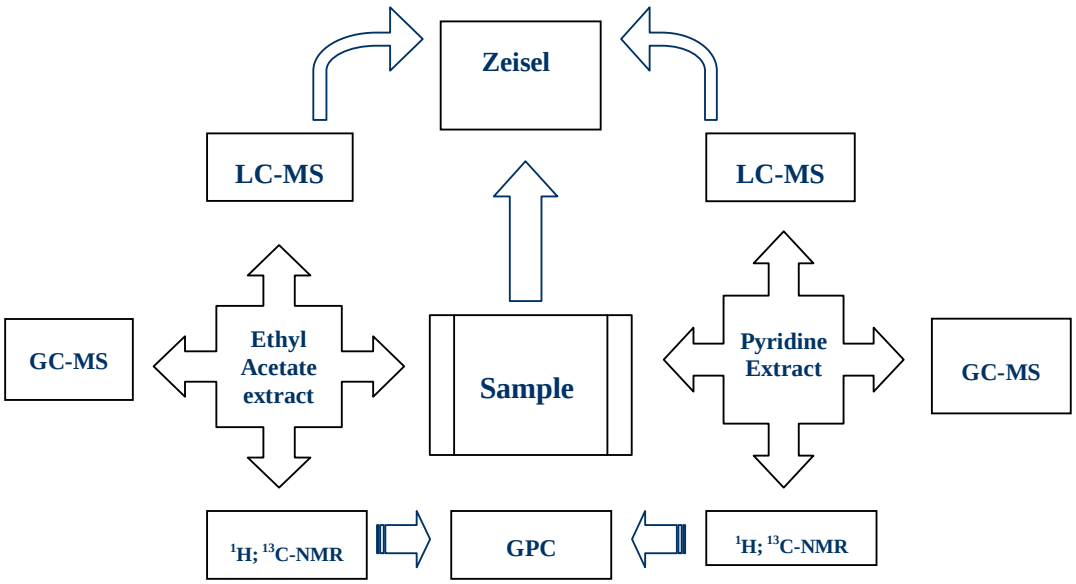


Fig.29 – Diagram of Particulate Matter characterization.

The diagram (fig.29), shows that an aliquot of particulate matter is only directly analysed with Zeisel analysis; the solvent extractable compounds (with ethyl acetate and/or pyridine) are analysed with LC and GC-MS, Zeisel technique, NMR (non destructive technique) and at least with a GPC.

Each analysis gives us different information about the semi quantitative, quantitative or qualitative presence of lignin oligomers or polymers. All methods able to extract pure lignin are adapted for analysis on particulate matter, at the concentration of interest. The data concerning the extractable compounds in an apolar organic solvent (ethyl acetate; acetonitryle), lead to the investigation of the low molecular fraction. Even in the exposed case, with Zeisel method, the methoxyphenols units like a model compound distinctive only of monolignols and oligolignols of lignin itself (thermodynamic and kinetic break) are principally found; with traditional GC-MS the monomers products of thermal degradation of lignin monomers and oligomers are principally monitored; with LC-MS the presence of the characteristic ions that the bibliography presents is investigated; with GPC the medium and ponderal molecular weight

is tracked. At last, with NMR analysis the results are confirmed from all developed methods. The same is conduced for the polar organic extract (water and pyridine). The PM (particulate matter) is only investigated with Zeisel method: this is an important data because of the impossibility to find a solvent or a combination of sequential solubilization that can extract all the organic mass of this complex matrix.
In the end, we can present a work model for the polymeric characterization of a complex matrix that could be confirmed with other analysis and investigations.

2.1 Materials and Methods

The following chapters focus on the techniques, the standard and sample treatments and the calibrations, set for every implemented type of analysis.

2.1.1 Gel permeation chromatography (GPC), [85-88].

Gel Permeation Chromatography (GPC), uses porous particles to separate molecules with different sizes. It is generally used to separate biological molecules and to determine molecular weights and molecular weight distributions of polymers. Molecules that are smaller than the pore size can enter into the porous particles and therefore have a longer path and a longer transit time than larger molecules that cannot enter into the gel particles. All molecules larger than the pore size are unretained and eluted together. Molecules that can enter into the pores will have an average residence time in the gel particles that depend on the molecules size and shape. Different molecules therefore have different total transit times through the column. The name gel permeation chromatography is used when an organic solvent is used as a mobile phase.
The collected fractions are often examined by spectroscopic technique to determine the number average molecular weight; weight average molecular weight and the concentration of the eluted particles. The **average molecular weight number** is a way to determine the molecular weight of a polymer. The average molecular weight number is the common, mean, average of the molecular weights of the individual polymers. Measuring the molecular weight of n polymer molecules, summing the weights, and dividing by n determinates it.

$$\bar{M}_n = \frac{\sum_i N_i M_i}{\sum_i N_i}$$

An alternative measure of the molecular weight of a polymer is the **weight average molecular weight**.
For the weight average molecular weight, the weigh polymer's dispersion is calculated by:

$$\bar{M}_w = \frac{\sum_i N_i M_i^2}{\sum_i N_i M_i}$$

Where N_i is the number of molecules of molecular weight M_i.
The ratio of the weight average to the number average is called the polydispercity index. The sample's solution (at the concentration of 1mg/ml) is injected on a column (with THF as solvent and eluent) packed with polystyrene and

divynilbenzene. For our purpose of study, the samples collected and analyzed with GPC technique are different extracted and acetylated particulates matters. The lignin polymers contain many hydroxylic functional groups (main – OH) situated on the aromatic rings or on the lateral chains, that is the reason why the samples had to be derivatized: an hydrophobic column makes a covalent bonding with the hydroxylic functional group. The HPLC system in use is an Agilent 1100, with a UV-VIS Detector set at 280 nm (in same case at 220 nm). Two different columns are used: a PL MIXED GEL E 3 μm (i.e. with a stationary phase of polystyrene with different reticulations of divynilbenzene) and a simple PL GEL 5 μm (i.e. with a stationary phase of polystyrene with the same reticulation). The eluent flow is set at 1 ml/min, with a loop of 20 μl. The calibration of the system allows finding the Mn and Mw grounded on the elution time as the correlation between the retention times and the molecular weight.

The calibration is performed by the injection of well-known compounds' weights: they are PL Polymer Standards (polystyrene resins) of Polymer Laboratories.

The following table shows the Mn and retention times of every single standard:

Mn	**t(r) 1**	**t(r) 2**	**mean t(r)**	**log Mp**
162	8,824	8,823	8,8235	2,209515015
580	7,778	7,743	7,7605	2,763427994
1060	7,463	7,432	7,4475	3,025305865
1310	7,26	7,252	7,256	3,117271296
1990	6,948	6,94	6,944	3,298853076
3370	6,559	6,557	6,558	3,527629901
4920	6,284	6,297	6,2905	3,691965103
8450	5,922	5,915	5,9185	3,926856709
13880	5,594	5,585	5,5895	4,142389466
19880	5,381	5,377	5,379	4,298416380

Tab. 12 – Mn retention time of every single processed standard.

Graph of retention times and the known molecular masses of the standard it's found a logarithmic correlation, fig.30.

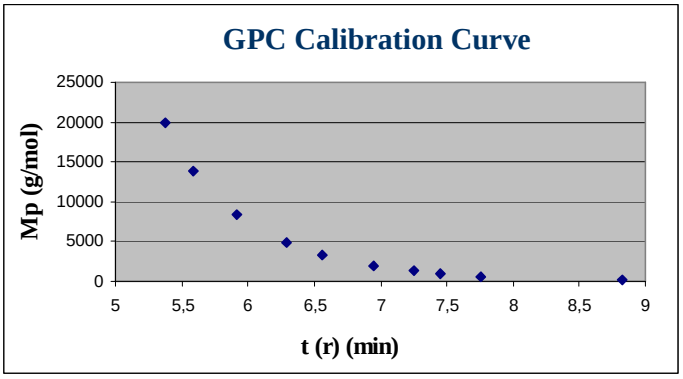


Fig. 30 – Logarithmic correlation in GPC analysis.

The correlation could be linearized converting the molecular weights in their logarithm, fig.31.

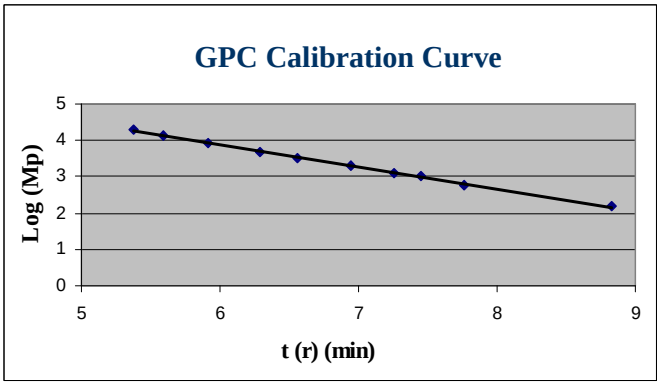


Fig. 31 – Linear calibration curve in GPC analysis.

The linear least square fitting gives the following equation:

$y = - 0.6077x + 7.5306$ $(R^2=0.9982)$.

It is also known that a polystyrene molecule has a different hydrodynamic volume from an oligolignol and that an oligolignol has a different hydrodynamic volume from a dilignol, and the like. So there is an approximation on the determination of polymer's molecular weights set with GPC technique.

See chapter 2.1.2: the set procedure is the same but the dry sample is dissolved in THF at the concentration of 1 mg/ml.

2.1.2 Zeisel Technique by Gas chromatography – Electron Capture Detector (GC-ECD) [87-90].

The gas chromatography is a separation technique in which a volatile solute is transported through a mobile gaseous phase (typically helium). The stationary phase is commonly a liquid covalent bonding to an inert support. A sample in a liquid phase (an organic solvent) is introduced into an injector positioned at high temperature to promote the passage

between the liquid and the volatile phase. The carrier gas with the solute carry through the column that separates the analytes. The Electron Capture Detector uses a radioactive Beta particle emitter: a metal of Nickel 63 exits by high temperature (more than 300 degrees). The electrons emitted from ^{63}Ni cause the nitrogen ionization and a steady current of electrons between the anode collector and a cathode and the ionization of nitrogen gas (make up gas). As the sample is carried into the detector by the stream of nitrogen analyzed molecules capture the electrons and reduce the current. The analyte concentration is proportional to the degree of electron capture. This detector is particularly sensitive to halogenous compounds.

The quantitative determination of metoxy groups is particularly important to the characterization of the lignin content in a particulate matter sample. Knowing the μmol of $–OCH_3$, it is possible to compare another quantitative or qualitative data coming from other technique like ^{13}C-NMR, GC-MS. The method consists in the transformation of –OCH3 groups in methyl iodide through a reaction between the sample and boiling hydrogen iodide:

$$R–O–CH_3 + HI \rightarrow R–OH + CH_3–I$$

In particular, the reaction device is suggested below, fig.32:

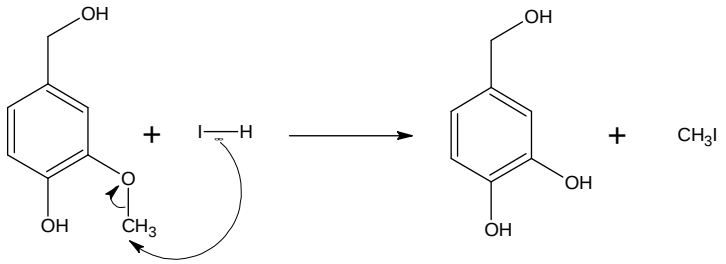


Fig. 32 – Mechanism of reaction in Zeisel technique.

The formed methyl iodide is performed by gas chromatography with an electron capture detector.

Because of a mole of CH_3I corresponds to a mole of $–OCH_3$, it is easy to calculate the sample's methoxy groups as a lignin monomer precursor.

Since the methyl iodide is a volatile compound it could be necessary to make immediately a liquid – liquid extraction with an organic solvent like benzene.

To promote the quality assurance, it's very important the use of an internal standard: it could be chemically and physically like the analyte and could have a different retention time: the trichloromethane satisfies these features.

The system used is an Agilent 6890 with an ECD. It is equipped with a split-splitless injector and an Agilent HP-5 column of 30 m of length; 0.32 μm of stationary phase's film constituted by a covalent bonded fused silica gel with polyphenylsiloxanes (5%) and polymethylsiloxanes (95%).

For all analysis 1 μl of the sample extract is injected (a solution of chloroform in benzene) for three times to promote the quality assurance.

Following all the most important data methods.

Method Information

Injection Source: Manual

6890 GC METHOD

OVEN

Initial temp: 45 'C (On)

Initial time: 10.00 min

Final temp: 45'C (On)

Run time: 10.00 min

INLET

Mode: Split

Initial temp: 200 'C (Off)

Pressure: 10.42 psi (Off)

Split ratio: 19.609:1

Split flow: 45.0 mL/min

Total flow: 50.0 mL/min

Gas saver: Off

Gas type: Helium

COLUMN

Model Number: Agilent 19091J-413

HP-5 5% Phenyl Methyl Siloxane

Max temperature: 325 'C

Nominal length: 30.0 m

Nominal diameter: 320.00 um

Nominal film thickness: 0.25 um

Mode: constant flow

Initial flow: 2.3 mL/min

Nominal unit pressure: 10.43 psi

Average velocity: 37 cm/sec

Inlet: Front Inlet

Outlet: Back Detector

Outlet pressure: ambient

DETECTOR (µECD)

Temperature: 300 'C (On)

Mode: Constant makeup flow

Makeup flow: 60.0 mL/min (On)

Makeup Gas Type: Nitrogen

Electrometer: On

Makeup Gas Type: Nitrogen

SIGNAL
Data rate: 20 Hz

For the construction of calibration fit function six measurement standards of vanillic alcohol are injected (external standard) processed as shown in chapter 2.2 and extracted with in a 10 mg/l solution of chloroform (internal standard) diluted in benzene.
In a row the preparation description of all used chemicals is shown:

- **Vanillic Alcohol (high concentration solution)**

PM = 154.17 g/mol
Weigh = 23.4 mg
Solubilized 100 ml (0.1 l) ethyl acetate.
23.4 mg/ 0.1 l = 234 mg/l Vanillic Alcohol
PM CH_3I = 141.94 g/mol
234 mg/l *141.94/154.17 = 215.43 mg/l CH_3I

- **Vanillic Alcohol (low concentration solution)**

White. 0 ul (a processed sample as other solution without the addition of Vanillic Alcohol)
Solution **A**. 215.43 mg/l * **25 ul**/10000ul = **0.54** mg/l **CH_3I**
Solution **B**. 215.43 mg/l * **50 ul**/10000ul = **1.08** mg/l **CH_3I**
Solution **C**. 215.43 mg/l * **100 ul**/10000ul = **2.15** mg/l **CH_3I**
Solution **D**. 215.43 mg/l * **250 ul**/10000ul = **5.39** mg/l **CH_3I**
Solution **E**. 215.43 mg/l * **500 ul**/10000ul = **10.77** mg/l **CH_3I**

The dosed microliters are lead to dryness and then we proceed to the reaction and the final extraction; the extractive solution is set as shown:

- **Chloroform $CHCl_3$ (high concentration solution)**

d = 1,48 g/ml; PM = 119,38 g/mol; TITLE = 99 %; C = 5000 mg/l
1,48 g/ml = 1,48 10^6 mg/ml
10 ml : x ml = 1,48 10^6 : 5000 0,0338 ml = 33,8 ul
33,8 ul *100/99 = 34.13 ul
34 ul really prelevated of standard in 10 ml of benzene
C_{real}: 10 ml : 0.034 ml = 1.48 10^6 : x mg/l **5032 mg/l**

- **Chloroform $CHCl_3$ (low concentration solution) 200 ul in 100 ml of Benzene:**

100 ml : 0.2 ml = 5032 mg/l : x mg/l
x = 0.2 * 5032 / 100 = **10.064** mg/l

Firstly a linear square fit for a set of standards of CH_3I (the final analyte) is developed to compare the function's slope with the real final calibration curve ones, tab.13:

Standard Samples	CH3I Area	CHCl3 Area	CH3I/ CHCl3 Area/Area	mg/l real	mg/l Found	R% mg/l found/real	Average R%
A1	7565,9	17609,0	0,4297	0,5400	0,5230	96,8	99,9
A2	7800,4	17870,4	0,4365	0,5400	0,5376	99,6	
A3	8200,1	18404,1	0,4456	0,5400	0,5570	103,2	
B1	14679,0	19612,7	0,7484	1,0800	1,2063	111,7	108,2
B2	14620,0	20015,1	0,7304	1,0800	1,1677	108,1	
B3	13156,8	18447,5	0,7132	1,0800	1,1308	104,7	
C1	22360,9	19986,7	1,1188	2,1500	2,0002	93,0	93,5
C2	19715,8	17405,3	1,1327	2,1500	2,0301	94,4	
C3	23282,3	20803,6	1,1191	2,1500	2,0010	93,1	
D1	53923,0	19653,7	2,7437	5,3900	5,4833	101,7	101,2
D2	49129,5	18057,2	2,7208	5,3900	5,4342	100,8	
D3	57518,2	21100,6	2,7259	5,3900	5,4452	101,0	
E1	106611,2	19189,0	5,5558	11,3368	11,5116	101,5	99,9
E2	118368,3	21737,1	5,4455	11,3368	11,2749	99,5	
E3	110030,4	20361,6	5,4038	11,3368	11,1857	98,7	

Tab. 13 – results of the CH_3I processed standards.

The correlated function is shown in the following graph, fig.:

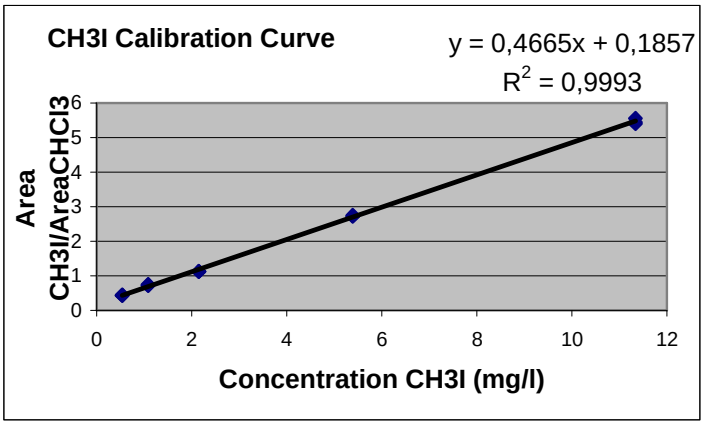


Fig. 33 – CH_3I Calibration Curve.

Then the final linear square fit for the set of standards of vanillic alcohol prepared as previously shown, i.e. in reaction, is developed. We can call them recoveries: in fact every single standard is processed like an unknown sample. Following the obtained data is presented, tab.14:

Standard Samples	CH3I Area	CHCl3 Area	Area CH3I/CHCl3	mg/l real	mg/l found	R% mg/l found/real	Average R%
O1	2377,4	20465,9	0,12	0,00	-0,03		
O2	2147,6	20928,9	0,10	0,00	-0,06		
O3	2059,6	19154,4	0,11	0,00	-0,05		
A1	8398,5	20330,1	0,41	0,59	0,59	100,7	96,9
A2	7750,2	19922,0	0,39	0,59	0,54	92,1	
A3	8137,0	20086,5	0,41	0,59	0,58	97,9	
B1	12933,4	17970,2	0,72	1,21	1,24	102,7	105,0
B2	13745,7	18772,0	0,73	1,21	1,27	104,9	
B3	12456,1	16709,5	0,75	1,21	1,30	107,2	
C1	22360,9	19986,7	1,12	2,15	2,09	97,1	97,6
C2	19715,8	17405,3	1,13	2,15	2,12	98,5	
C3	23282,3	20803,6	1,12	2,15	2,09	97,1	
D1	53923,0	19653,7	2,74	5,39	5,53	102,5	102,5
D2	49129,5	18057,2	2,72	5,39	5,48	101,6	
D3	57518,2	21100,6	2,73	5,39	5,49	101,8	
E1	106611,2	19189,0	5,56	11,34	11,48	101,2	101,2
E2	118368,3	21737,1	5,45	11,34	11,24	99,2	
E3	110030,4	20361,6	5,40	11,34	11,15	98,4	

Tab. 14 – Recoveries results for the final plotted calibration curve.

We can use the data found as recovery plotting the areas with the previous curve of CH3I, in the following table:

Standard Samples	mg/l fit with Y= 0,4665x + 0,1857	R% mg/l found/real	Average R%	Δ Average R%
O1	-0,15			
O2	-0,18			
O3	-0,17			
A1	0,49	82,6		
A2	0,44	73,9	78,7	18,2
A3	0,47	79,7		
B1	1,14	94,6		
B2	1,17	96,8	96,9	8,1
B3	1,20	99,2		
C1	2,00	93,0		
C2	2,03	94,4	93,5	4,1
C3	2,00	93,1		
D1	5,48	101,7		
D2	5,43	100,8	101,2	1,3
D3	5,45	101,0		
E1	11,51	101,5		
E2	11,27	99,5	99,9	1,3
E3	11,19	98,7		

Tab. 15 – Quality assurance of the obtained results.

It can be seen that there is a little loss of analyte in the reaction process and in the LLE (liquid extraction) that increases with the decrease of concentration. In every analytical system the error associated at low concentration is more than the high concentration one. The correlated function is shown in the following graph:

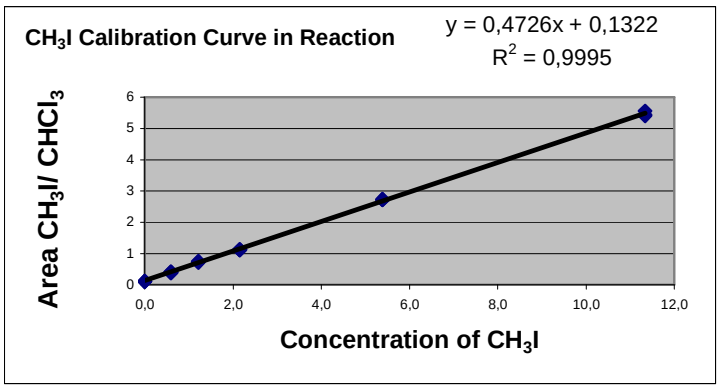


Fig. 34 – Calibration curve in use for Zeisel technique.

We can see how the two equations are similar both as slope and as y-intercept: this is another evidence of the goodness of the improved method.

Because of a sample's final result as concentration of vanillic alcohol is presented, every data plotted with previous linear square fit had to be converted in Vanillic Alcohol concentration.

The weighed talis qualis or extractable sample lead to dryness, is proceeded to the reaction with 5 ml of HI at 57% in the oven set at 150°C for 30 minutes. After the refrigeration 5 ml of water and 10 ml of benzene are added. Then a liquid-liquid extraction is made: the organic fraction is directly injected in the GC-ECD system.

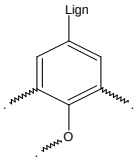
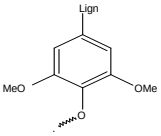
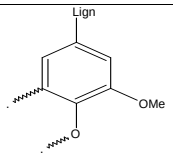
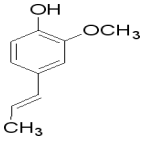
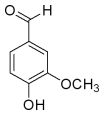
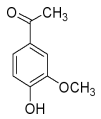
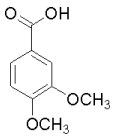
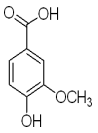
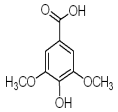
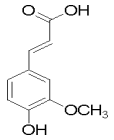
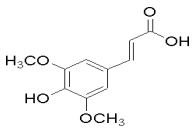
2.1.3 Analysis of Monolignols by Gas Chromatography – Mass Spectrometry (GC-MS) [10, 37, 84].

GC-MS is a technique that matches a gas chromatographic system of separation with a mass spectrometry analyser. In our study we have to use both acquisition modalities: SCAN to monitor the particulate matter's unknown low molecular weight compounds and SIM to quantify the lignin monomers markers as later described. For both types of analysis the sample must be derivatized by different modalities explained in the chapter 2.2.3.

For SIM analysis we must proceed to the construction of calibration fit function. Some characteristic 4-substituted methoxylated phenolic compounds (methoxyphenols) that still retain structural characteristics of the lignans precursors (molecular markers) are chosen.

The quantification of every single compound gives an indication of the original polymeric lignin precursors; the grade's presence of the low molecular weight fraction in the proceeded sample and the stage of the fragmentation of original polymers if the sample is fully characterised.

In the following table the analytes correlated with their main originated lignin:

Compound	**Structure**	 **H**	 **G**	 **S**	**MW**
4-Allyl-2-methoxyphenol (Isoeugenol cis; trans)				√	164.21
4-hydroxy-3-methoxybenzaldehyde (Vanillin)				√	152.15
4-Hydroxy-3-methoxyacetophenone (Acetovanillone)				√	166.17
3,4-Dimethoxybenzoic acid (Veratric Acid)				√	182.17
4-Hydroxy-3-methoxybenzoic acid (Vanillic Acid)				√	168.15
4-Hydroxy-3,5-dimethoxybenzoic Acid (Syringic Acid)			√		198.28
Trans-4-Hydroxy-3-methoxycinnamic Acid (Ferulic Acid)				√	194.19
4-Hydroxy-3,5-dimethoxycinnamic acid (Sinapic Acid)			√		224.21

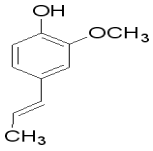
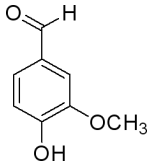
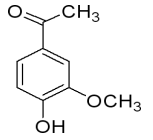
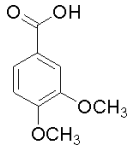
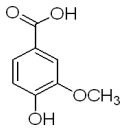
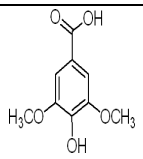
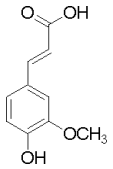
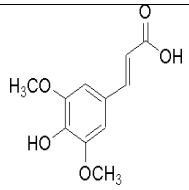
Tab. 16 – GC-MS monitored compounds.

Preliminarily a high concentration standard is made with the referred solid compound in ethyl acetate as dilution solvent to set a 10 mg/l mix solution. Then it is set the dilute standards at 500 μg/l and 50 ug/l. A rate of the standard solutions (1 ml) is carry on the derivatization by N,O-Bis (trimethyl silyl) trifluoroacetamide with 1% of trimethylchlorosilane (BSTFA + TMCS); ($CF_3C[=Nsi(CH_3)_3]Osi(CH_3)_3$) (20 ul) and positioned on a hotplate set at 200°C for 30 minutes. Silanization reactions are used to enhance GC analysis for polar non-volatile analysed compound. The sylation reaction replaces the active hydrogen on protic functional group, that reduces dipole-dipole interactions and subsequently increases the volatility of the derivating parent compounds. The general reaction is:

$(CH_3)_3SiX + R'\text{-}H \quad \rightarrow \quad (CH_3)_3Si\text{-}R' + HX$

There are many available trimethylsylation reagents: BSTFA can be used to derivatize all protic functional groups, including non-sterically hindered alcohols; carboxylic acids, amino acids; amides; amines and enols.

Each compound weighs 73,191 amu (a $(CH_3)_3Si$- unit) in addition to its molecular weight for each derivatizable functional group with a contemporaneous subtraction of a hydrogen ion. So the products molecular weight and their molecular ion are explained in tab. 17.

Compound	Structure	MW	Molecular ion monitored	First Fragment ion monitored	Second Fragment ion monitored	Third Fragment Ion monitored
4-Allyl-2-methoxyphenol (Isoeugenol cis; trans)		164.21	236	221	206	
4-hydroxy-3-methoxybenzaldehyde (Vanillin)		152.15	224	209	194	
4-Hydroxy-3-methoxyacetophenone (Acetovanillone)		166.17	238	223	208	193
3,4-Dimethoxybenzoic acid (Veratric Acid)		182.17	254	239	195	165
4-Hydroxy-3-methoxybenzoic acid (Vanillic Acid)		168.15	312	297	267	223
4-Hydroxy-3,5-dimethoxybenzoic Acid (Syringic Acid)		198.28	342	327	312	297
Trans-4-Hydroxy-3-methoxycinnamic Acid (Ferulic Acid)		194.19	338	323	308	249
4-Hydroxy-3,5-dimethoxycinnamic acid (Sinapic Acid)		224.21	368	353	338	323

Tab. 17 – SIM monitored for GC-MS compounds processed.

In general these compounds, in a non-derivatization status, lead to a fragmentation with a loss of a piece or the totality of the lateral chain or with a loss of -CO from the hydroxylic group. Because in this case the hydroxylic group is protected by the Siloxane group, only the other view of fragmentation occurred. But, as we can see in the previous table a group of original compounds reacts to a fragmentation, the new derivatized compound losses the $–CH_3$ groups of Siloxane unit: for example in the case of Acetovanillone 223 is the fragment ion with a loss of a $–CH_3$ group (-15); 208 the fragment ion with a loss of two $–CH_3$ groups (-30) and 193 the fragment ion with a loss of three $–CH_3$ groups (-45). The same is for Sinapic Acid (353, 338, 323) and other compounds in the list.

Sometimes the compound loses all the Siloxane group (Me_3Si-) with its oxygen bonded: (M-73-16 = M-89). This occurs for example for Veratric, vanillic and Ferulic Acids (fragment ions 165; 223; 249 respectively).

Subsequentially the full GC-MS method in use is reported:

Inlet

250°C

17,32 psi

34,4 ml/min total flow

0,8 min 30,0 ml/min purge flow to split vent

2,0 min 20,0 ml/min gas saver

Splitless modality

Column

Constant flow

17,32 psi

1,00 flow

26 average velocity

Oven

°C/min	°C	Time (min)	Tot. time (min)
	55	1,00	1,00
10	76	2,00	5,10
10	280	2,17	27,67
30	290	2,00	30,00

Detector

280°C

SIM Program

Range time (min)	Monitored ions
0-20,35	194-206-209-221-224-236
20,35-22,50	165-193-195-208-223-238-239-254-267-297-312
22,50-30,00	249-297-312-323-327-342-353-338

The calibration curve for each compound monitored is evaluated for each sample processed: it is important because the standards have to be analysed in the same batch of the samples, i.e. each sample will have its calibration curve as shown in the experimental chapter.

2.1.4 Structural Investigation by Liquid Chromatography – Mass Spectrometry (LC-MS) [91, 92].

LC-MS is a technique that matches a high performance liquid chromatographic system (HPLC) of separation with a mass spectrometry analyser. This kind of Detector consists of three basic parts: an atmospheric pressure ion source; a mass analyzer and a detector system. First in the source chamber the ions from the sample are produced; then the mass analyzer splits ions of different masses; finally the data is collected to generate the mass spectrum.

The ion source in use is an Electrospray Ionization (ESI) chamber: the ions are formed by a room temperature capillary with an applied electric charge: the molecules (come from eluated sample and solvent) are spruced by a nitrogen gas flow; nebulized charged spruce drops are formed that under a Colombian force explode in ions; the positive or negative ions formed are directioned to the analyzer under applied acceleration potentials.

A quadrupole filter allows the transit of few m/z ions; the other ones could be gone earth or moved away as neutral ions. The current potential applied to the four metallic bars that set up the electrodes determines transmitted and collected m/z ions. At last an electron photomultiplier amplifies the signal.

When the data is collected in the full scan mode, a target range of mass fragments is determined and inputed into the instrument's method. A typical broad range of mass ions to monitor with a LC quadrupole analyzer would be from m/z 50 to m/z 2000. Both Full Scan and SIM technique are possibly set. The differences between GC-MS performance and LC-MS ones are located in the ionization mode: in the atmospheric chamber the molecules acquire less energy, so the resulted ions are the molecular ions (M^+; M^-; $(M+1)^+$; $(M+1)^-$; $(M-1)^+$; $(M-1)^-$) or the adduct ions formed by weak bonds with an ion or a molecule solvent: $(M+Na)^+$ for example.

The importance of this technique is both, the capacity maintenance of the molecule sample tale qualis in general deriving from the particular ionization technique, and the possibility of monitoring monomeric and polymeric organic compounds containing polar functional group: these compounds in fact are volatilize with difficulty and are thermally degradable, so it’s impossible to monitor them by GC-MS without a previous derivatization.

In our study we used SCAN acquisition modes with a direct injection of the sample. We have obtained a spectrum of the different samples, in which we have compared the low molecular weight standards spectrum and the ions found in bibliography for the oligomers and polymers coming from lignins. At last we have crossed the found data with the GC-MS and the GPC one. In the first case, we could have or not have a confirmation of the presence of the found monomers; in the second case, we could have or not have a confirmation of the presence of high molecular weight compounds. The limit of the technique is that, in a total spectrum only a relative presence of an ion could be seen, but a particular ion couldn’t be seen with a direct injection but with a previous chromatographic separation in SCAN or SIM mode. So these results have to be considered preliminary data. In the next chapter the chosen ions, with their bibliographic reference, and the data’s method, could be seen.

For the selection of the researched ions it was necessary to carry out a focused bibliographic study of many articles regarding the lignin structure and the environmental researches on wood markers. Crossing this data, we can set the following table:

m/z	MW	Ion. Mode	Name	Formula	Bibliographic Reference 1	Bibliographic Reference 2
122	168,15	nd	Fragment of **Vanillic Acid**	C7H8O4	D.R. Oros et al., 2006	
123,5	124	ESI-	Fragment of dimeric lignin model compounds: β-O-4 (M=320.4); β-β (M=350.4); **Guaiacol**	C7H8O2	D.V. Evtuguin, F.M.L. Amado, 2003	D.R. Oros et al., 2006
136		ESI-	Fragment of 2-Hydroxyphenilacetic Acid		Cappiello et al., 2002	
137	138	ESI-	4-Hydroxybenzoic Acid	C8H10O2	Cappiello et al., 2003	
139		ESI-	Coumaric acid	C7H10O1	Cappiello et al., 2003	
	141		3-HYDROXYGUAIACOL	C7H9O3	D.R. Oros et al., 2006	
149		ESI-	fragment of Sinapic acid and Acetovanillone		Cappiello et al., 2003	
150	150	ESI-	**4-VINYLGUAIACOL**; Fragment of Acetovanillone	C9H10O2	D.R. Oros et al., 2006	Cappiello et al., 2003
151	152	ESI-	Anisic acid; 2-hydroxyphenilacetic acid; **Vanillin**; 4-PropylLcathecol	C8H8O3	Cappiello et al., 2003	D.R. Oros et al., 2006
151,5		ESI-	FRAGMENT OF dimeric lignin model compounds: β-β (M=358.4); β-5 (M=358.4)		D.V. Evtuguin, F.M.L. Amado, 2003	
154	154		**SYRINGOL**; 3.5-DIMETHOXYPHENOL; VANILLYL ALCOHOL	C8H10O3	D.R. Oros et al., 2006	
157		ESI-	trans-1.2-cyclopentadicarboxylic acid ; fragment of tricarballylic acid		Cappiello et al., 2003	
nd	164		ISOEUGENOL; p-COUMARIC ACID	C10H12O2; C9H8O3	D.R. Oros et al., 2006	
165	166	ESI-	ACETOVANILLONE	C9H10O3	Cappiello et al., 2003	
165,5		ESI-	FRAGMENT OF β-O-4 (M=320.4)		D.V. Evtuguin, F.M.L. Amado, 2003	
	166		**3-GUAIACYLPROPANE**; p-HYDROCOUMARIC ACID	C10H14O2	D.R. Oros et al., 2006	
167	168	ESI-	VANILLIC ACID	C8H8O4	Cappiello et al., 2003	D.R. Oros et al., 2006
	178		**CONIFERYL ALDEHYDE**; 4-METHOXYCINNAMIC ACID; METHYL p-COUMARATE	C10H10O3	D.R. Oros et al., 2006	
179		ESI-	Phenilmalonic acid		Cappiello et al., 2003	
	180,2		**CONIFERYL ALCOHOL**; GUAIACYLACETONE; 3-GUAIACYLPROPANAL; METHYL p-HYDROCOUMARATE	C10H12O3	D.R. Oros et al., 2006	
181	182	ESI-	**SYRINGALDEHYDE; Veratric Acid**	C9H10O4	Cappiello et al., 2003	
	181,5	ESI-	FRAGMENT OF β-β (M=418.4)		D.V. Evtuguin, F.M.L. Amado,. 2003	
	182		METHYL VANILLATE; VANILLYL ETHANOL	C9H10O4	D.R. Oros et al., 2006	
193	194	ESI-	Ferrulic acid	C10H10O4	Cappiello et al., 2003	
	194		SYRINGYLPROP-2-ENE	C11H14O3	D.R. Oros et al., 2006	
195,5		ESI-	FRAGMENT OF β-O-4 (M=320.4; 350.4)		D.V. Evtuguin, F.M.L. Amado, M 2003	
	196		**ACETOSYRINGONE**; **HOMOSYRINGALDEHYDE**; 3-GUAIACYLPROPANOIC ACID; METHYL HOMOVANILLATE	C10H12O4	D.R. Oros et al., 2006	
	198		HOMOSYRINGIL ALCOHOL; SYRINGIC ACID	C10H14O4; C9H10O5	D.R. Oros et al., 2006	
	210		**SINAPYL ALCOHOL; SYRINGYLACETONE**; 3-SYRINGYLPROPANAL	C11H14O4	D.R. Oros et al., 2006	
	212		HOMOSYRINGIC ACID; METHYL SYRINGATE	C10H12O5	D.R. Oros et al., 2006	
223	224		Synaptic acid	C11H12O5	Cappiello et al., 2003	
225,5		ESI-	FRAGMENT OF β-β (M=350.4)		D.V. Evtuguin, F.M.L. Amado, 2003	
271,4		ESI-	FRAGMENT OF β-O-4 (M=320.4)		D.V. Evtuguin, F.M.L. Amado, 2003	
301,4		ESI-	FRAGMENT OF β-β (M=350.4)		D.V. Evtuguin, F.M.L. Amado, 2003	
309,5		ESI-	FRAGMENT OF β-5 (M=358.4)		D.V. Evtuguin, F.M.L. Amado, 2003	
311,5		ESI-	FRAGMENT OF β-β (M=358.4)		D.V. Evtuguin, F.M.L. Amado, 2003	
319,4	320,4	ESI-	β-O-4		D.V. Evtuguin, F.M.L. Amado, 2003	
327,4		ESI-	FRAGMENT OF β-β (M=358.4);β-5 (M= 358.4)		D.V. Evtuguin, F.M.L. Amado, 2003	
339,4		ESI-	FRAGMENT OF β-5 (M= 358.4)		D.V. Evtuguin, F.M.L. Amado, 2003	
342,4		ESI-	FRAGMENT OF β-β (M=358.4)		D.V. Evtuguin, F.M.L. Amado, 2003	
349,4	350,4	ESI-	β-O-4		D.V. Evtuguin, F.M.L. Amado, 2003	
357,4	358,4	ESI-	β-β; β-5		D.V. Evtuguin, F.M.L. Amado, 2003	

358,9		ESI	CONIFERYL ALCOHOL'S DIMERS		De Angelis et al., 1999	
371,5		ESI-	FRAGMENT OF β-β (M=418.4)		D.V. Evtuguin, F.M.L. Amado, 2003	
377,2		MALDI	CONIFERYL ALCOHOL'S DIMERS		De Angelis, Fregonese, & Verì (1996)	
387,4		ESI-	FRAGMENT OF β-β (M=418.4)		D.V. Evtuguin, F.M.L. Amado, 2003	
393,4		MALDI	CONIFERYL ALCOHOL'S TRIMERS		De Angelis, Fregonese, & Verì (1996)	
402,4		ESI-	FRAGMENT OF β-β (M=418.4)		D.V. Evtuguin, F.M.L. Amado,. 2003	
415,4		MALDI	CONIFERYL ALCOHOL'S TETRAMERS		De Angelis, Fregonese, & Verì (1996)	
417,4	418,4	ESI-	β-β		D.V. Evtuguin, F.M.L. Amado, 2003	
509		ESI-	Spruce dioxane lignin		Evtuguin et al. (1999)	
537,1		MALDI	CONIFERYL ALCOHOL'S TRIMERS		De Angelis, Fregonese, & Verì (1996)	
551,0		MALDI ESI-	Spruce dioxane lignin; CONIFERYL ALCOHOL'S TRIMERS		De Angelis, Fregonese, & Verì (1996)	Evtuguin et al. (1999)
556,1		ESI	CONIFERYL ALCOHOL'S TRIMERS		De Angelis et al., 1999	
581		ESI-	Spruce dioxane lignin		Evtuguin et al. (1999)	
613		ESI-	TRIMERS OF Eucalyptus globulus dioxane lignin		D.V. Evtuguin, F.M.L. Amado, 2003	
643		ESI-	TRIMERS OF Eucaliptus globulus dioxane lignin		D.V. Evtuguin, F.M.L. Amado, 2003	
675		ESI-	Spruce dioxane lignin		Evtuguin et al. (1999)	
734,2		ESI	CONIFERYL ALCOHOL'S TETRAMERS		De Angelis et al., 1999	
737,2		MALDI	CONIFERYL ALCOHOL'S TETRAMERS		De Angelis, Fregonese, & Verì (1996)	
747		ESI-	Spruce dioxane lignin		Evtuguin et al. (1999)	
839		ESI-	tetramers of Eucaliptus globulus dioxane lignin		D.V. Evtuguin, F.M.L. Amado, 2003	
853		ESI-	Spruce dioxane lignin		Evtuguin et al. (1999)	
868		ESI-	tetramers OF Eucaliptus globulus dioxane lignin		D.V. Evtuguin, F.M.L. Amado, 2003	
914,9		ESI-	Spruce dioxane lignin		De Angelis, Fregonese, & Verì (1996)	
926		ESI-	Spruce dioxane lignin		Evtuguin et al. (1999)	
1060		ESI-	Spruce dioxane lignin		Evtuguin et al. (1999)	
1065		ESI-	pentamers OF Eucaliptus globulus dioxane lignin		D.V. Evtuguin, F.M.L. Amado, 2003	
1090		ESI-	Spruce dioxane lignin		Evtuguin et al. (1999)	
1092,5		ESI	CONIFERYL ALCOHOL'S PENTAMERS		De Angelis et al., 1999	
1094,7		MALDI	CONIFERYL ALCOHOL'S PENTAMERS		De Angelis, Fregonese, & Verì (1996)	
1095		ESI-	Pentamers OF Eucaliptus globulus dioxane lignin		D.V. Evtuguin, F.M.L. Amado, 2003	
1110,8		MALDI	CONIFERYL ALCOHOL'S ESAMERS		De Angelis, Fregonese, & Verì (1996)	
1193			Spruce dioxane lignin		Evtuguin et al. (1999)	
1269		ESI-	Spruce dioxane lignin		Evtuguin et al. (1999)	
1273,1 1272,6			CONIFERYL ALCOHOL'S EPTAMERS		De Angelis, Fregonese, & Verì (1996)	De Angelis et al., 1999
1291		ESI-	hexamers of Eucaliptus globulus dioxane lignin		D.V. Evtuguin, F.M.L. Amado, 2003	
1321		ESI-	hexamers of Eucaliptus globulus dioxane lignin		D.V. Evtuguin, F.M.L. Amado, 2003	
1451,0		ESI	CONIFERYL ALCOHOL'S OTTAMERS		De Angelis et al., 1999	
1452,8		MALDI	CONIFERYL ALCOHOL'S OTTAMERS		De Angelis, Fregonese, & Verì (1996)	
1466		ESI-	Spruce dioxane lignin		Evtuguin et al. (1999)	
1632,2			CONIFERYL ALCOHOL'S NONAMERS		De Angelis, Fregonese, & Verì (1996)	
1809,6		MALDI	CONIFERYL ALCOHOL'S DECAMERS		De Angelis, Fregonese, & Verì (1996)	

Tab. 18 – LC-MS indagated ions, [10, 83, 92-96].

When, in the table, the compounds are more than one, the structure's formula can be referred to the highlighted ones. The bibliographic reference presents the molecular weight (MW) or the molecular ion (m/z) monitored with the ionization technique. In our samples we investigated that the same ion if the ionization technique is the same, the MW if there is only indicated the molecular weigh or if the ionization mode is different, in both cases with the trueness of +/-1 amu. Therefore we consider only the ions with a relative intensity of 2%: so we can abort the interferences coming from the samples and the instruments. The samples treated with ultrasounds in acetonitrile or ethyl acetate for 5 minutes are ever at the concentration of 5 mg/ml. Other samples, previously extracted with different solvent, could be analysed by LC-MS taking them to dryness and then dissolving them again in CH_3CN. Below, the instrumental conditions for the implemented method.

LC condition
0,5 ml/min total flow;
50% Acetonitrile; 50% Milliq Water
Ionization Mode: ESI-
ESI conditions : as Tune file
Mass range : 100-2000 amu
Acquisition time : 0,8 interval/sec
Quadrupole Analyzer : as Tune file.

2.1.5 Structural Investigation by Nuclear magnetic resonance Spectroscopy (NMR) [85, 86, 89, 97, 98].

The structural elucidations of unknown organic matrix analysis are accomplished by non-destructive spectral method. NMR, UV and IR analysis gave more information about structural unit content but needed high concentrated samples. So it is important to make more than one analysis with the same sample aliquot.
In NMR technique the radio frequency electromagnetic radiation interacts with the magnetically active nuclei of the samples, subjected to a strong magnetic field (B_0). Each nucleus in a molecule's sample experiences a magnetic field slightly from the external ones B_0: the difference from the resonance frequency is called the chemical shift, δ, usually given in parts per million (ppm). How a nucleus senses the spin states of neighbouring nuclei through chemical bonds (scalar coupling) is characterized by the coupling constant J, i.e. the splitting of lines in Hz. Therefore it could be given a different longitudinal and transverse relaxing time (T_1 and T_2 respectively), to change the behaviour of chemical shift and to obtain more structural information.
NMR allows the inspection of the whole structure i.e. the whole range of structural units present in the sample but since a complex matrix like the organic extract of particulate matter is a mixture of different concentrated compounds subsequently separated with difficulty, signals are commonly overlapping or covering. The most informative nuclei in NMR spectroscopy of lignins are the proton and carbon isotopes ^1H and ^{13}C. ^1H nucleus (natural abundance 99,98%) is the most sensitive nucleous for NMR spectroscopy. The larger chemical shift dispersion in ^{13}C NMR (over 220 ppm vs. 12 ppm in proton NMR) and the more distinct chemical shifts and narrower line widths to ^1H spectra make ^{13}C NMR a highly attractive tool for structural studies of lignins but it is also less sensitive (1,11% of natural abundance).

In our work, before the analysis, the samples, i.e. a solvent extract, can be or not be acetylated, in relation to the data we would obtain.

The pyridine sample extract (more than 25 mg/ml) is added Ac_2O in a 1:1 volume rate. The sample is positioned in an oven at 50°C for a night. Then the pyridine and the sub products reaction are eliminated taken to dryness three aliquots of 20 ml of EtOH; three aliquots of 20 ml of toluene and three aliquots of 20 ml of $CHCl_3$.

The solid residual is solubilized in a deuterate solvent ($CDCl_3$) and transferred in a NMR tube at the concentration of 25 mg/ml.

2.1.6 Structural Investigation by Ultraviolet –Visible Spectrometry (UV-VIS) and Infrared Spectrometry (IR) [87].

The optical spectrometer uses both the near ultraviolet and visible portions of the spectrum. In our study we have used the latest UV-VIS detector is a Diode Array containing a number of photosensitive diodes in the form of a multi-layer sandwich.

The Infrared instrument in use is a Nicolet FT-IR AVATAR 360. The residual particulate matter powder samples coming from extractable different solution system were analyzed: water; ethyl acetate, pyridine, respectively; in order to confirm the absence of residual organic matter non extractable.

For the extractable organic compounds, different confrontable solvents are used. The extraction is made with an ultrasonication bath for 5 minutes. Structural characterisation requires the analytes speciation with sub sequential solvent extraction, the extract is centrifuged at 3500 RPM for 5 minutes, and then the extract is collected and the residual particulate matter is ready for another solvent extraction.

2.1.7 Samples collecting system

The atmospheric particulate phase has been investigated by collecting it through gravimetric samples between PM 2.5 fraction and course fraction. In a gravimetric sampler, the atmospheric particle phase is collected on the head of the sampler, then it is weighted for the analysis. The use of *high volume sampler* is necessary to collect enough quantities of sample: the atmospheric particle phase is chemically analysed for trace compounds and for structural characterization analysis that require high quantities of sample. High volume sampling system (*High Volume PM 2.5, Tisch Environmental*) has been used during summer campaign from *May to July 2007, in the urban area of Milan*. Low Volume sampling system (*Low Volume PM 2.5, FAI Instruments – Hydra Dual Sampler*) has been used during spring campaign, from *March to April 2005, in the same urban area*. The urban area of Milan is characterized by very high pollution levels for atmospheric particles, but during spring and summer few alkanes corresponding to biogenic source are also found. The main annual chemical composition of PM 10 for the urban area of Milan is reported below: 8% of NH_4^+; 14% NO_3^-; 11% SO_4^{2-}; 9% Mineral Dust ("crustal elements": Al; Si; K; Ca; Ti); 1% Trace Element (V; Cr; Mn; Fe; Ni; Cu; Zn; Br; Pb) ,(43% of total Inorganic fraction) and a 43% of Total Carbon (i.e. EC – Elementary Carbon + OC – Organic Carbon) [99]. The OC concentration in an urban area is about 20% of the total sample, the EC

fraction depends more on local emission [81]. Only a small fraction of the OC is extractable. The unresolved non-extractable fraction is attributable to natural plant wax, composed in polymers, probably partly coming from lignin and cellulose units. Therefore it is probable that these polymers are highly concentrated in the course fraction because of their molecular weight and diameter size.

The samples collected are weighed by an analytical balance characterized by four numbers after the gram.

2.2 Experimental Part: Result and Discussion.

In order to improve the initial studies centred on the qualitative identification of the polymeric organic fraction coming from particulate matter, [9, 10, 81-84], a lignin olygomers and polymers study method of two different samples has been developed, with the general pattern of analysis shown below:

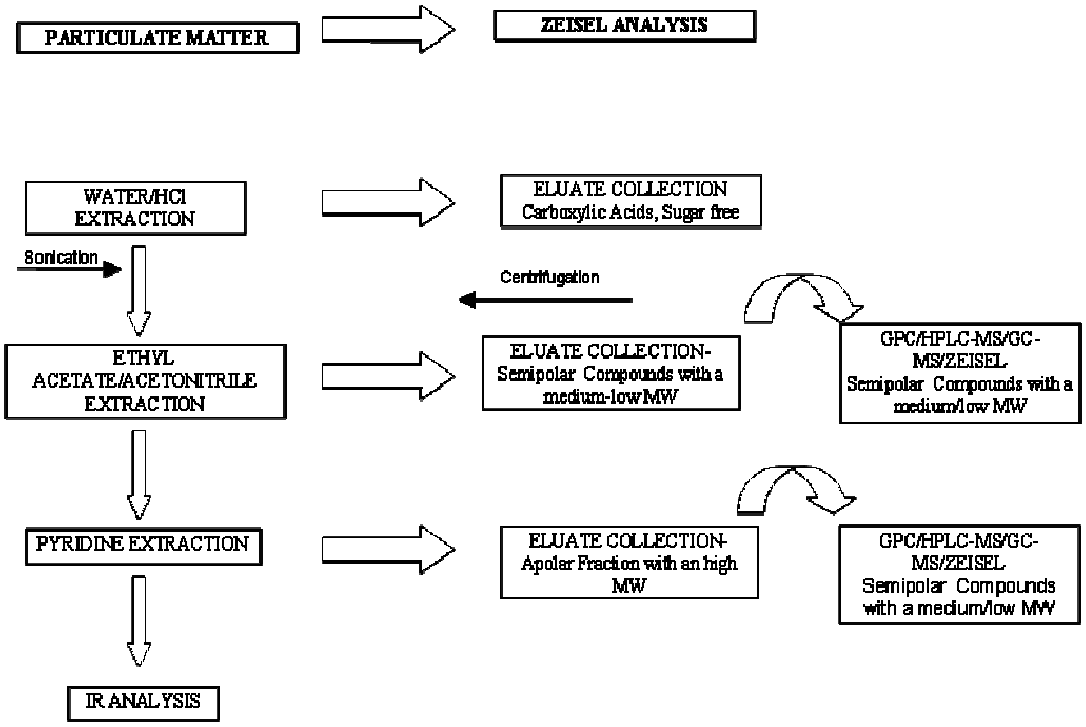


Fig. 35 – General pattern analysis for particulate matter lignin like polymers.

The results have be compared to improve the structural and quantitative useful information.

2.2.1 Gel permeation chromatography (GPC) Results.

The GPC chromatograms show that in both all 2005 and 2007 extracts samples there isn't any signal before the retention time of six minutes for the presented calibration (PL MIXED BED Column), so we don't have Mn over 5000 Dalton.

It could be true if it is considered that it is not probable that lignin polymers are present as such in the particulate matter, but in the form of fraction (degradated or not) of the original polymers.

Fig.36 shows the chromatogram of an ethyl acetate extract and fig.31 the pyridine one (it must be considered that the pyridine extract comes from a sub sequential analysis of a ethyl acetate extract).

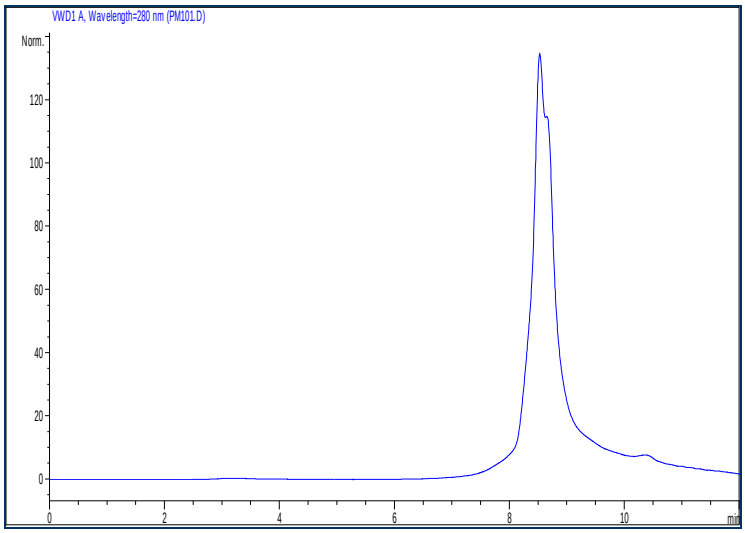


Fig 36. Chromatogram of an Ethyl Acetate extract of the 2007 PM sample, acquired at 280 nm with the PL Mixed Bed Column.

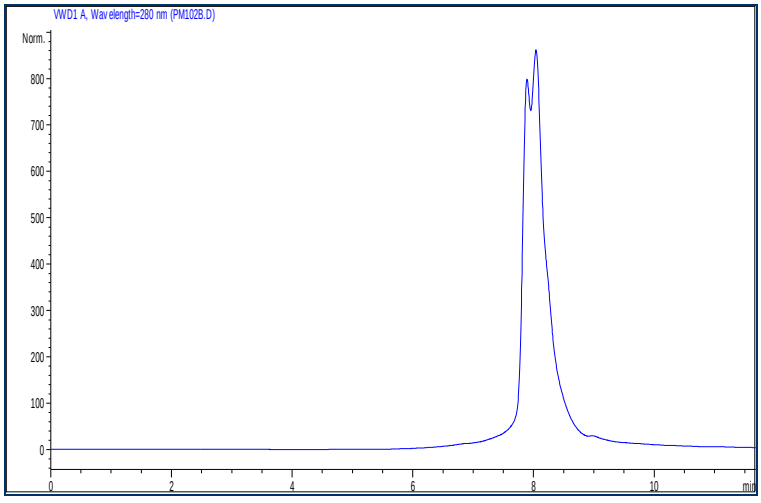


Fig 37. Chromatogram of a Pyridine Residual extract of the 2007 PM sample, acquired at 280 nm with the PL Mixed Bed Column.

We can see that the main molecular fraction is set at retention time of eight minutes, for the pyridine extract (fig. 37) and of nine minutes for the ethyl acetate extract: the pyridine extract contains an higher Mn, as shown in the table below (tab. 19):

Dates	Extract Type	Nm	Column	Name	Mn	Mw	Mw/Mn
PM2005	Ethyl Acetate Extract	280	PL Mixed Bed	AE	215.5	349.4	1.4
PM2005	Pyridine Residual Extract	280	PL Mixed Bed	P	520.2	1337.4	2.6
PM2007	Ethyl Acetate Extract	280	PL Mixed Bed	AE	204.2	279.7	1.4
PM2007	Pyridine Residual Extract	280	PL Mixed Bed	P	678.0	1428.2	2.1
PM2007	Pyridine - Water Residual Extract	280	PL Mixed Bed	AP	497.7	1075.3	2.2
PM2007	Pyridine - Water Residual Extract	210	PL GEL	AP	432.7	18608.5	43.0
PM2007	Pyridine - HCl Residual Extract	280	PL Mixed Bed	HClP	521.5	972.5	1.9
PM2007	Pyridine - HCl Residual Extract	280	PL GEL	HClP	253.8	1084.0	4.3
PM2007	Pyridine - HCl Residual Extract	210	PL GEL	HClP	246.3	6641.6	27.0

Table 19 – Results of GPC's analysis

Legend: *Pyridine Residual Extract:* fraction collected after an ethyl acetate extraction and a pyridine extraction; *Pyridine - Water Residual Extract:* fraction collected after water, ethyl acetate extraction and pyridine extraction; *Pyridine - HCl Residual Extract:* fraction collected after an HCl 0.1 M solution, ethyl acetate and pyridine extraction.

The analysis at 210 nm presents a higher average molecular weight (Mw) considering wavelength of absorption: the lignin oligomers and polymers absorb more at 280 nm while the cellulose absorbes at 210 nm (the main samples absorption as we could be seen later in UV chapter). In general the 2005 samples have a minor content of oligomers and a major content of monomers because of seasonal sampling. Therefore the HCl pyridine residual extract has a minor Mn considering the major disgregative powder of an acid solvent on the cellulose polymeric units.

2.2.2 Zeisel Results

The quantitative determination of methoxy groups is performed both on extractable sample and on the original particulate matter talis qualis. The sugar content doesn't interfer with the final results: the processed standards of glucose and cellobiose give a signal comparable with the white ones.
It is important to evaluate the unsolubilized or partly solubilized solid fraction that otherwise could be obtained only with complex methods of extraction.
This is the reason why in tab.20 the Zeisel analysis results compared with the weighed samples processed are shown.

Sample	mg		TOTAL pg/mg		pg/mg	pg		% TOTAL		%
	PM	Extract	PM	Extract	Extract	PM	Extract	PM	Extract	Extract
	22,1		1804,3			39875,9		0,18		
2005	65,7	10,6		478,3	2964,6		31424,3		0,05	0,30
	5,3	0,9								
	20,3		5644,9			114591,5		0,56		
2007	9,3	4,2		1237,7	2740,6		11510,6		0,12	0,27
	6,4	1,1								
2005/2007			0,32	0,39	1,08					

Tab. 20 – Results of Zeisel analysis: it is shown that the concentrations refer to the PM weighed (column 3 – Total PM) when the analysis is conducted on the particulate matter talis qualis; the Total Extract concentration (column 3) and the Extract one (column 4) if the sample is before extracted and then reacted is presented. In column 5 and 6 the absolute picograms are presented; in column 7 and 8 the percentages on the total concentration and in the column 9 the extracts one. In the last line the 2005 and 2007 samples data is compared.

The extract concentration samples for 2005 (2964.6 pg/mg) and 2007 (2740.6 pg/mg) particulate matter are the same (rate 1.08; 0.30%; 0.27%): it could mean that the monomeric lignin fraction has the same percentage distribution in comparison to the other compounds, in the organic solvent extractable sample for the spring and summer campaign. The total concentration is ever greater in the PM talis qualis (1804.3 to 478.3 pg/mg for the 2005 sample; 5644.9 to 1237.7 ppm for the 2007 sample) than in the organic solvent fraction: it could mean that there are not only monomeric compounds but even polymeric lignin's units. At last the spring campaign shows a major concentration of both extractable and talis qualis fractions. Total composition percentage depends on the samples: about 0.2% for 2005 sample and about 0.6% for 2007 sample: the summer campaign has about three times the lignin content.

2.2.3 Gas Chromatography – Mass Spectrometry (GC-MS) Results

The total ion chromatograms for the SIM analysis of a standard are shown in fig.38 below.

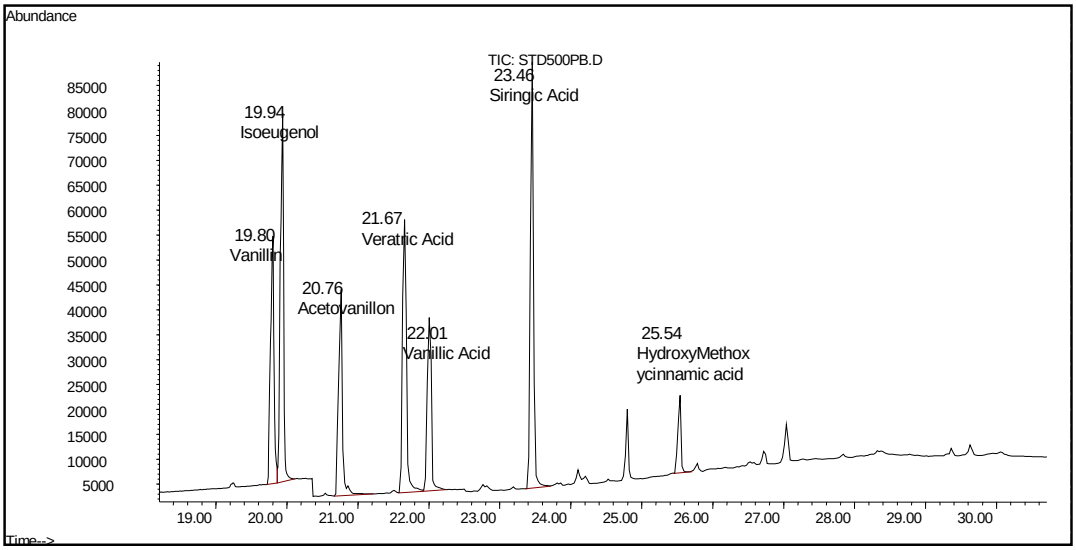


Fig.38 - Standard 500 ug/l of the monomer mixture selected on the chromatogram at their retention time (see chapter 2.1.4).

The total ion chromatograms for the SIM analysis of a sample are shown in the fig.39 below.

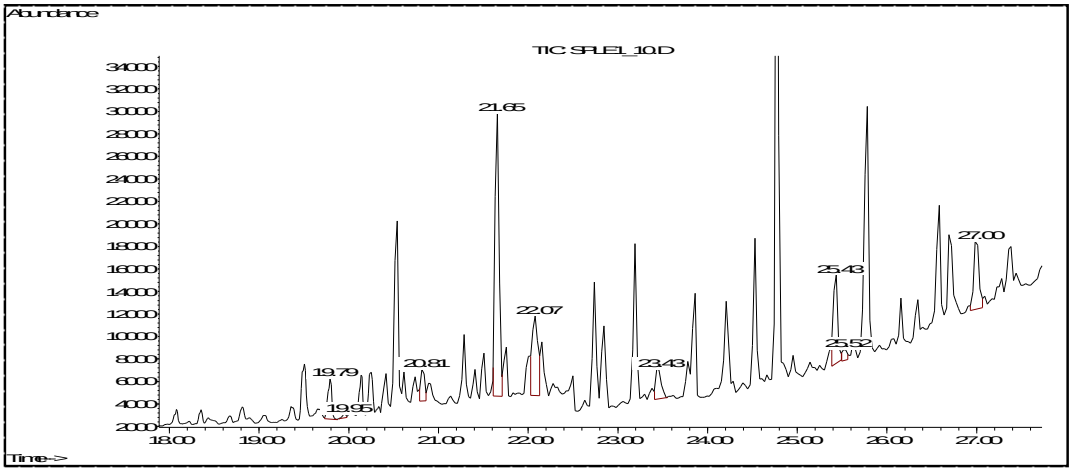


Fig. 39 – A chromatogram of the PM 2005 sample.

As we have reported in chapter 2.1.4, the calibration curve for the monitored compounds is evaluated for each processed sample. In succession examples of calibration curve for the same analyte for both 2005 and 2007 samples are shown:

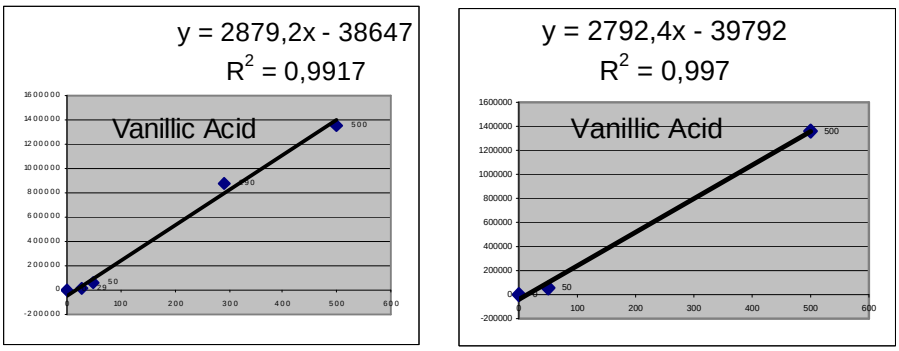


Fig.40 – Calibration curve of Vanillic acid for the 2007 sample and for the 2005 sample respectively: the intercept and the slope are very similar.

In table 21, in succession a significative difference of composition (about the compounds found and their concentrations) of the processed samples is shown.

Compounds Name	PM 2005 pg/mg Extract with Ethyl Acetate	PM 2007 pg/mg Extract with Ethyl Acetate
Vanillin	0,6	0,4
Isoeugenol	<0,9	<7,8
4-hydroxi-3-methoxy acetophenone	3,1	56,4
veratric acid	10,1	5,5
vanillic acid	7,7	10,7
siringic acid	0,6	4,7
trans-4-hydroxy-3-methoxy cinnamic acid	<0,9	<3,3
sinapic acid	9,7	28,6
Tot. Markers (pg/mg)	31,8	106,3

Tab.21 – Concentration Results of the samplesprocessed twice expressed in pg/mg.

In table 22, it can be seen that the concentration in 2007 sample is more than the 2005 one. The total percentage shows that these compounds are a low presence for the summer and spring campaigns.

Sample	mg		Total	Extract	pg	%	%
	PM	Extract	pg/mg	pg/mg		Total	Extract
2005	5,3	0,9	31,8	187,4	168,7	0,003	0,02
2007	6,4	1,1	106,3	618,4	680,2	0,011	0,06
2005/2007			0,30	0,30			

Tab. 22 – GC-MS analysis results for 2005 and 2007 samples extract.

2.2.4 Liquid Chromatography – Mass Spectrometry (LC-MS) Results.

For the selection of the researched ions it was necessary to carry out a focused bibliographic study of many articles regarding the lignin structure and the environmental researches on wood markers. Crossing this data we can set the following table (tab. 23):

SAMPLES	*PM 2005*	*PM 2007*				
	A	*A*	*WAP*	*HClAP*	*W*	*HCl*
Fragment of vanillic acid	***122***	Nd	nd	nd	nd	nd
Fragment of dimeric lignin model compounds:β-O-4 (M=320.4); β-β (M=350.4); Guaiacol	***124***	Nd	***124***	nd	nd	nd
4-Hydroxybenzoic acid	nd	Nd	137	nd	nd	nd
Coumaric acid	***139***	Nd	***139***	nd	nd	nd
4-Vinylguaiacol; fragment of Acetovanillone	***150***	Nd	***150***	nd	nd	nd
Anisic acid; 2-hydroxyphenilacetic acid; Vanillin; fragment of dimeric lignin model compounds: β-β (M=358.4); β-5 (M=358.4)	nd	Nd	***151***	nd	nd	nd
Isoeugenol; p-Coumaric Acid	***163***	Nd	***163***	nd	***163***	nd
Acetovanillone; Fragment of β-O-4 (M=320.4)	nd	Nd	***165***	nd	nd	nd
Vanillic Acid	***167***	Nd	***167***	nd	nd	***167***
Coniferyl Aldehyde; 4-MethoxyCinnamic Acid; Methyl p-Coumarate	nd	Nd	***178***	nd	nd	nd
Phenilmalonic Acid	nd	Nd	***179***	***179***	nd	nd
Syringaldehyde; Veratric Acid; Fragment of β-β (M=418.4)	nd	Nd	***181***	nd	nd	nd
Methyl Vanillate; Vanillyl ethanol	nd	Nd	***182***	nd	***182***	nd
Syringylprop-2-ene	nd	Nd	nd	***194***	***194***	***194***
Fragment of β-O-4 (M=320.4; 350.4);Acetosyringone; Homosyringaldehyde; 3-Guaiacylpropanoic Acid; Methyl Homovanillate	nd	196	***196***	***196***	***196***	***196***
Homosyringil Alcohol; Syringic Acid	nd	***198***	***198***	***198***	***198***	***198***
Sinapyl Alcohol; SyringylAcetone; 3-Syringylpropanal	***209***	***209***	***209***	nd	***209***	nd
Sinapic acid	nd	***223***	nd	nd	nd	nd
Fragment of β-β (M=350.4)	***226***	***226***	nd	***226***	***226***	nd
Fragment of β-O-4 (M=320.4)	nd	nd	***271***	***271***	nd	nd
Fragment of β-β (M=350.4)	nd	nd	***301***	***301***	nd	nd
Fragment of β-5 (M=358.4)	nd	nd	***309,5***	nd	nd	nd
Fragment of β-β (M=358.4)	nd	***311,5***	***311,5***	***311,5***	nd	nd
β-O-4 (M=320.4)	nd	nd	***319***	nd	nd	nd
Fragment of β-β (M=358.4);β-5 (M= 358.4)	***327***	nd	nd	nd	nd	nd
Fragment of β-5 (M= 358.4)	nd	nd	nd	***339***	nd	***339***
Fragment of β-β (M=358.4)	nd	nd	nd	***342***	nd	nd
Conyferyl Alcohol's Dimers	***359***	nd	***359***	nd	nd	nd
Fragment of β-β (M=418.4)	nd	nd	***371,5***	***371,5***	nd	nd
Conyferyl Alcohol's Dimers	nd	nd	***377***	nd	nd	nd
Fragment of β-β (M=418.4)	nd	nd	***387***	nd	nd	nd
Conyferyl Alcohol's Trimers	nd	nd	***393***	nd	nd	nd
Conyferyl Alcohol's Tetramers	***415***	nd	***415***	nd	nd	***415***
β-β (M=418.4)	***417***	nd	nd	nd	nd	***417***
Conyferyl Alcohol's Trimers	nd	nd	***537***	nd	nd	nd
Spruce dioxane lignin; Conyferyl Alcohol's Trimers	***551***	nd	nd	nd	nd	nd
Conyferyl Alcohol's Trimers	nd	nd	***556***	nd	nd	nd
Spruce dioxane lignin	nd	nd	***581***	nd	nd	nd
Trimers of Eucaliptus globulus dioxane lignin	nd	nd	***613***	nd	nd	***613***
Spruce dioxane lignin	nd	nd	***675***	nd	nd	nd

LEGEND	
A	EXTRACT WITH ETHYL ACETATE
WAP	EXTRACT (RESIDUAL WITH WATER; ETHYL ACETATE) IN PYRIDINE
HClAP	EXTRACT (RESIDAL WITH HCl 0.1 M; ETHYL ACETATE) IN PYRIDINE
PM	PARTICOLAR MATTER
W	EXTRACT WITH WATER
HCl	EXTRACT WITH HCl 0.1 M

Tab.23 – LC-MS results for the processed sample extracts.

It can be seen, that the 2007 samples contain a higher presence of oligomers. These results agree with the GPC's data analysis.

The sample's composition of low molecular weigh compounds agrees for both particulate matter extracts. As we could expect, some high molecular weight compounds are found in the HCl extraction: these compounds could come from cellulose oligomers that don't solubilize in water but need a mild acid solution. It must be considered that, for example, the α-D-Glucopyranoside ($C_{18}H_{32}O_{16}$) – a trisaccharide - has a molecular weigh of 504 g/mol; the Sucrose (cellobiose – $C_{12}H_{22}O_{11}$) has a molecular weigh of 342 g/mol; the d (+)-Mannose and the L-Glucose ($C_6H_{12}O_6$) have a MW of 180 g/mol.

In figure 41 below, a sample's LC-MS spectra (PM 2005 – Extract with Ethyl Acetate).

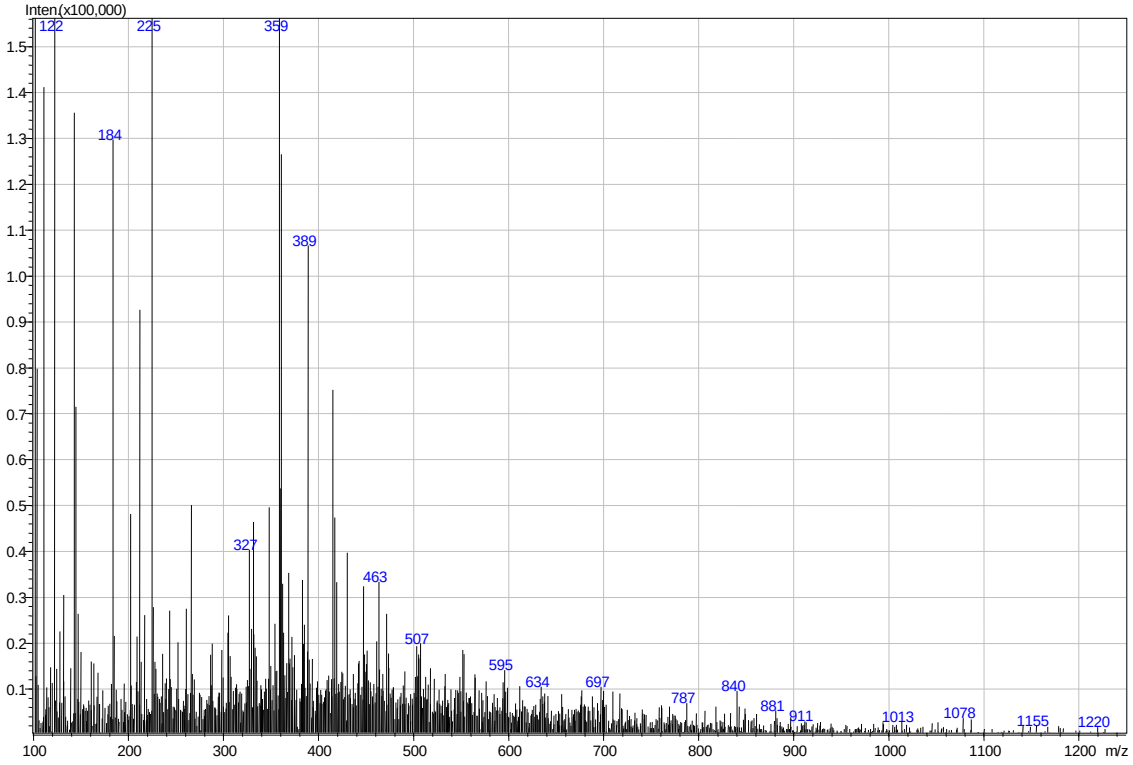


Fig.41 – The ESI-LC-MS Spectrum fro the Ethyl Acetate 2005 extract.

2.2.5 Nuclear magnetic resonance Spectroscopy (NMR) Results

For NMR structural characterisation of our samples firstly we have started with a ^{1}H-NMR analysis of a non-derivatized Ethyl Acetate extract of PM 2005 (10 mg/ml in deuterate acetone solvent – $(CD_3)_2CO$).
In figure 42 the relative spectrum is shown: the presence of an intense alkane signal at 1.3 ppm is clear; the solvent signal at 2.1 ppm and many overlapped signals at 3.5 ppm and 7.5 ppm. The 7.5 ppm signal could be referred to phenols; the 3.5 ppm signal could be referred to the 1-O-4 sugar bonding: because of their high presence, these polymers cover the methoxy groups signals.

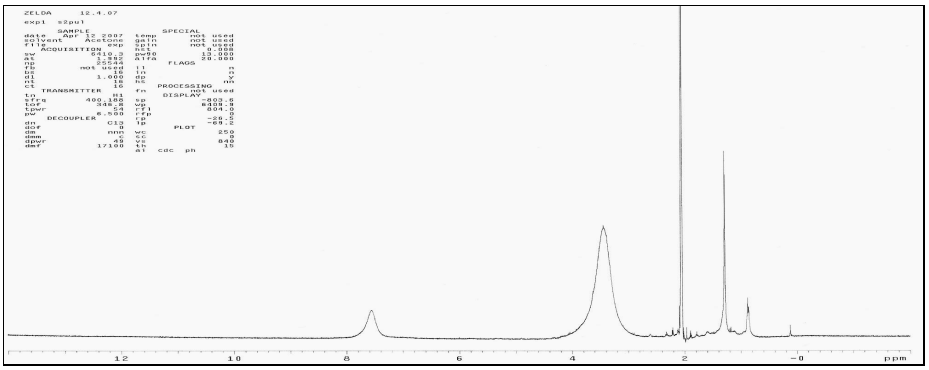


Fig. 42 – The ^{1}H-NMR Ethyl Acetate Extract spectrum.

Because the organic solvent extract contains a high concentration compounds interfering with the lignin fraction characterisation, it must be necessary to make sub sequential extractions in order to obtain more structural information.
The same aliquot sample (PM 2007), was firstly extracted with Ethyl Acetate, derivatised as explained in the 2.1.6 chapter, and then processed for a ^{13}C-NMR analysis in $CDCL_3$ solvent; the residual matter of ethyl acetate treatment was extracted with pyridine and then the derivatized sample processed for a ^{13}C-NMR. In the figure below the two NMR spectra are shown:

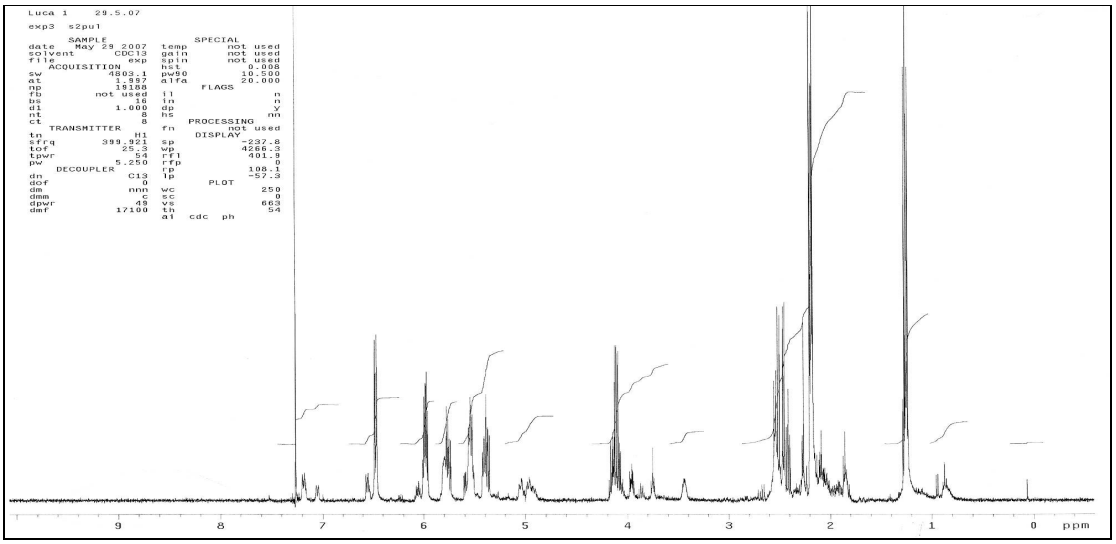


Fig.43 – ^{13}C-NMR Spectrum of the Ethyl Acetate Extract.

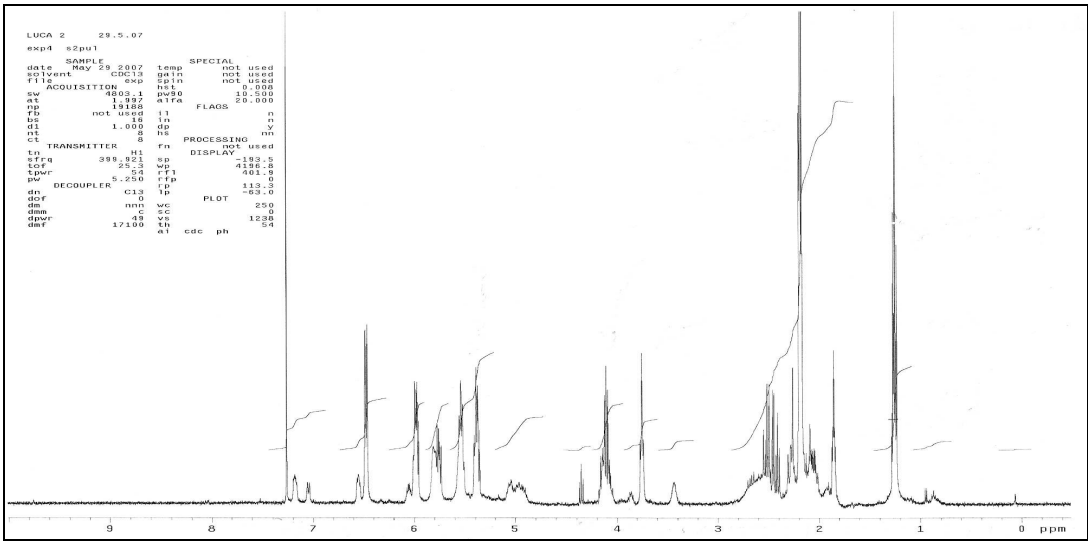


Fig.44 - ^{13}C-NMR Spectrum of the residual matter pyridine extract.

The two spectra present the same signal, i.e. the extracts have the same composition. The only significant difference is the 3.75 ppm pesk of the pyridine extract (fig.44): it is absent in the spectra of fig.44 and could represent the presence of lignin –OCH_3 group.

For both samples we can say that the signal at 1.3 ppm is characteristic of alkanes; the 2.2 ppm peak is referred to the derivatized –OH; the 4.1 ppm could represent the cellulose presence (1-O-4 interunits bonds); the 5.4 ppm the Alkenes signals.

2.2.6 Ultraviolet –Visible Spectrometry (UV-VIS) Results.

The entire processed sample by GPC analysis (Ethyle Acetate and Pyridine extract dissolved in THF) is processed though a UV spectrum: for both samples the maximum wavelength is 210 nm (this confirms the polysaccharide presence).

2.2.7 Infrared Spectrometry (IR) Results.

The same derivatized sample characterized for ^{13}C-NMR Spectrometry was processed by a FT-AT-IR Spectrometer: the two samples extracts spectra are shown in the figures below:

Fig. 45 – FT-AT-IR Spectrum for the Ethyle Acetate Extract.

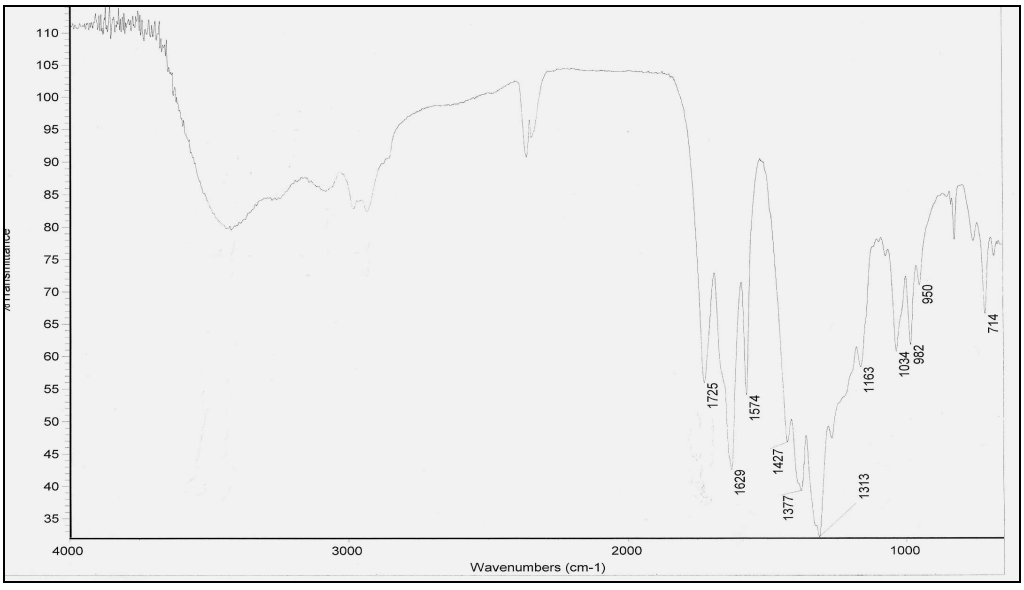


Fig. 46 – FT-AT-IR Spectrum for the residual matter pyridine extract.

Like the ^{13}C structural results, the IR spectra show the same signals profile:

3388 nm: the –COOH groups resistant to the derivatisation

3100 nm: $–OCH_3$ aromatic group;

2900 nm: alkanes; aromatics

1725 nm: -C=O derivatized carbonyl

1629 nm: aromatics;

1427 nm: alkenes;

1377 nm: alkynes;

982-950 nm: aromatic alkenes.

For the characterisation of the real sub sequential analysis extraction capacity (i.e. the organic fraction total extraction), it was necessary to analyse the last residual particulate matter powder samples coming from extractable Figure 47 shows the overlapped signal of two different residual powders coming from the 2007 sample processed separately: they present the same profile.

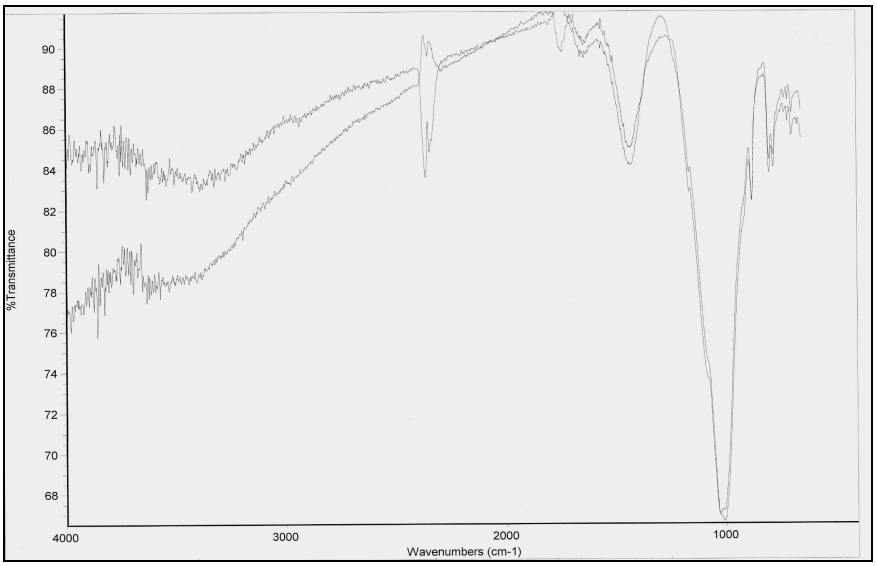


Fig.47 – Two 2007 samples residual powder overlapped, processed by FT-AT-IR Spectrometer.

The total absence of organic compounds signals: a spectrum of a silice standard to confirm the main inorganic residual presence of these crustal compounds in both samples (figure 48) is processed:

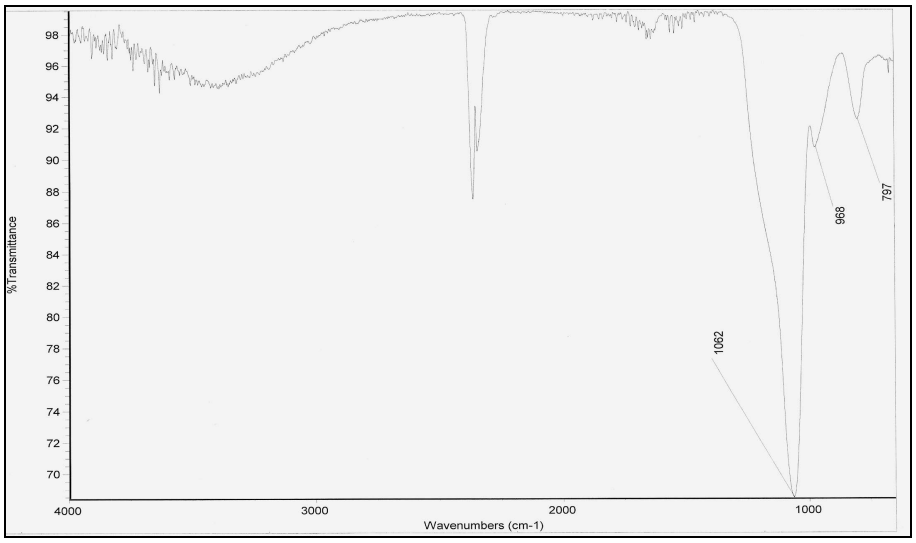


Fig.48 – Standard of Silice processed by FT-AT-IR.

2.2.8 Compared Analysis Results

For all required samples it was important to estimate the weighed extractable content. In the table below the obtained results are presented:

SAMPLE	Mg				%		
	PM	***CH_3CN***	***$CH_3COOC_2H_5$***	***C_5H_5N***	***CH_3CN/PM***	***$CH_3COOC_2H_5/PM$***	***C_5H_5N/PM***
2005	4,8	0,8			16,7		
2005	6,8	1,2			17,6		
2005	5,3	0,9			17,0		
2005	5,8	1,4			24,1		
2005	7,3	1,1			15,1		
2005	5,1		0,7			13,7	
2005	65,7	10,6			16,1		
2005	102,9		41,8	60,5		40,6	58,8
2007	30,6		4,0			13,1	
2007	10,2		1,5			14,7	
2007	15,6		7,1	5,0		45,5	32,1
2007	33,5		5,8	6,3		17,3	18,8
2007	6,4		1,1			17,2	
2007	9,3	4,2			45,2		
2007	5,3	0,9			17,0		

Tab. 24 – Results of the weighed extracts (PM = particulate matter).

In general, with a organic solvent like acetonitrile and ethyl acetate the percentage of weighed extract is near 15-20%, i.e., if the totality of the sample is considered, it constitutes an half of organic matter, and it leads to 30-40% of the organic fraction. The table shows that three aliquots present a major extracted fraction (40.6, 45.5 and 45.2 % respectively). In particular the aliquot of 2005 particulate matter (102.9 mg) is the processed sample for the ^{13}C-NMR

spectrometry. It could mean that the same composition found for both acetate and pyridine extracts could be an exception for this sample's aliquot. The NMR analysis must be confirmed with other processed aliquots. Therefore, in general the pyridine extraction capacity on the residual aliquot fraction is higher (from 18.8 to 58.8 %). In fact a greater fraction is solubilized with pyridine, but another one precipitates after the solubilization: this can be seen in the weighed data but not in the structural one. Comparing the Zeisel and the GC-MS data, we can set the following table:

DATA	Mg		TOTAL pg/mg			EXTRACT pg/mg		pg			% TOTAL			% EXTRACT	
	PM	Extract	Zeisel PM	Zeisel Extract	Tot Markers	Zeisel Extract	Tot Markers	Zeisel PM	Zeisel Extract	Tot Markers	Zeisel PM	Zeisel Extract	Tot Markers	Zeisel Extract	Tot Markers
2005	22,1		1804,3					39875,9			0,18				
	65,7	10,6		478,3		2964,6			31424,3			0,05		0,30	
	5,3	0,9			31,8		187,4			168,7			0,003		0,02
2007	20,3		5644,9					114591,5			0,56				
	9,3	4,2		1237,7		2740,6			11510,6			0,12		0,27	
	6,4	1,1			106,3		618,4			680,2			0,011		0,06
2005/2007			0,3	0,39	0,30	1,08	0,30								
Zeisel AE/PM; TOT Markers/ Zeisel PM2005			0,3		0,02										
Zeisel AE/PM; TOT Markers/ Zeisel PM2007			0,2		0,02										
TOT Markers/ Zeisel AE 2005					0,1		0,1								
TOT Markers/ Zeisel AE 2007					0,1		0,2								

Tab.25 – Compared results between Zeisel and GC-MS analysis.

Table 25 shows that the rate from acetate extract (AE) and the particulate matter Zeisel results to be about 1/3 (0.3) – line 5, for the 2005 sample. For the summer campaign the rate is 0.2: this represents a subsequent confirmation that the summer 2007 sample contains a higher percentage of oligomers and polymers. Therefore the markers rate is at 0.02 for both samples: the aliquot of monomers percentage doesn't change if compared with the totality of sample and even with the extracts. We can also monitor the situation in detail with table 6 shown below: the GPCs are reported; Zeisel; GC-MS analysis with the percentage comparison for both 2005 and 2007 samples and for each processed aliquot: most data presented only a confirmation of the exposed conclusion, but there is an interesting concordance between the percentage of different aliquot sample for the GPC's 2007 and 2005 analysis. Only the pyridine residual HCl and Acetate extract (A/HclAP – 80.4%) seem to have lost a high content of monomers and intermonomeric units.

In the last column it is underlined that if the GPC's data concords, the difference in the two samples is not the grade of polymerisation but their content amount and their monomeric composition. It is an important result to characterise two different seasonal and dating samples.

SAMPLES	PM 2005				PM 2007								% PM 2005 / PM 2007		
	A	**P**	**PM**	**%A/P ; A/PM**	**A**	**P**	**WAP**	**HClAP**	**PM**	**%A/P ; A/PM**	**%A/ WAP**	**% A/HClAP**	**A**	**P**	**PM**
Mn	215,5	528,4		***40,8***	204,2	678,0	497,7	253,8		**30,1**	**41,0**	***80,4***	105,6	77,9	
Mw	349,4	1177,5		***29,7***	279,7	1428,2	1075,3	1084,0		**19,6**	**26,0**	***25,8***			
Mn/Mw	1,4	2,2		***62,3***	1,4	2,1	2,2	4,3		**64,9**	**62,3**	***31,9***			
ZEISEL pg/mg eq. vanillic alcohol	478,3		1804,3	26,5	1237,7				5644,9	21,9			38,6		32,0
pg/mg vanillin	0,6				0,4								168,4		
pg/mg isoeugenol	<0,9				<7,8										
pg/mg 4-hydroxi-3-methoxy acetophenone	3,1				56,4								5,5		
pg/mg veratric acid	10,1				5,5								183,1		
pg/mg vanillic acid	7,7				10,7								71,7		
pg/mg siringic acid	0,6				4,7								13,0		
pg/mg trans-4-hydroxy-3-methoxy cinnamic acid	<0,9				<3,3										
pg/mg sinapic acid	9,7				28,6								34,0		
Tot. Markers (pg/mg)	31,8				106,3								29,9		

LEGEND

A ESTRACT WITH ETHYL ACETATE

P ESTRACT (RESIDUAL WITH ETHYL ACETATE) WITH PYRIDINE

WAP ESTRACT (RESIDUAL WITH WATER; ETHYL ACETATE) IN PYRIDINE

HClAP ESTRACT (RESIDAL WITH HCl 0.1 M; ETHYL ACETATE) IN PYRIDINE

PM PARTICOLARTE MATTER

% DATES REFER TO FRACTION IN ORDER OF INDICATION; PM 2005 / DATES OF PM 2007

Tab.26 – Summerising results for the lignin like polymers.

Reporting the main quantitative and structural data, we can summarize in general that, (Appendix):

- The maximum average molecular weight (Mn), is about 700 Dalton;
- The maximum weight average molecular weight (Mv), is about 15.000 Daton;
- The content of polymers is ignorable compared with the oligomers one (trimers and dimers) in the solubilized organic matter;
- The cellulose content has an important role in the grade of polymerization definition but not in the concentration data;
- The total lignin concentration is greater in the PM tal qualis than in the organic extractable fraction, fig.49;
- With the developed sample's treatment, all organic matter could be extracted, but not solubilized.
- The concentration of the lignin total fraction is higher in the PM tal qualis (5645 pg/mg) than the extractable organic fraction (2741 pg/mg), fig.49.

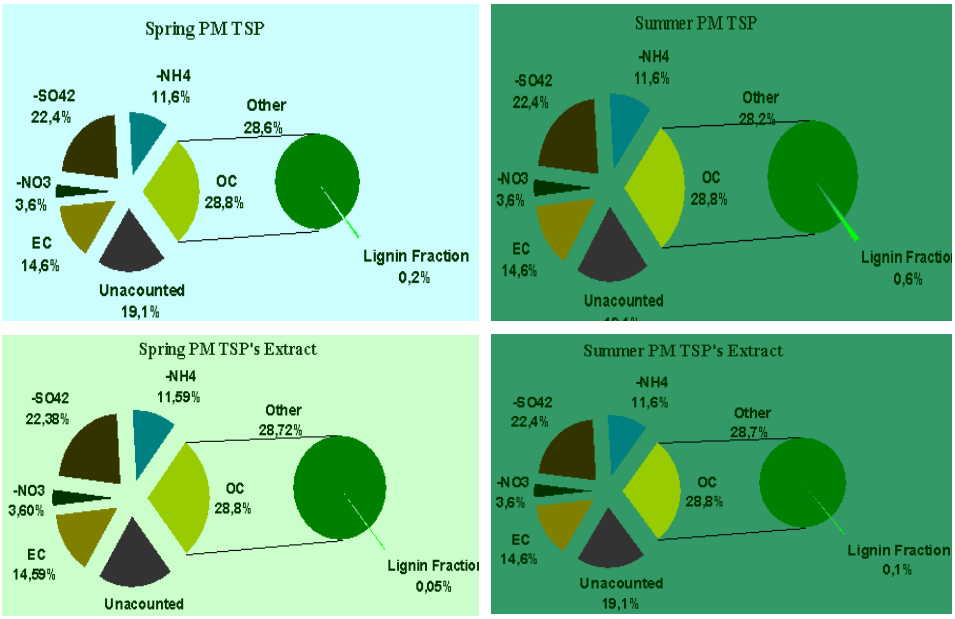


Fig.49 – The lignin fraction particulate matter content expressed in percentage respect the Organic Carbon (OC) data or the Urban City of Milan.

About the processed two samples we can explain that, fig.50 and Appendix 1 – tab.1:

- The Summer Campaign has a greater contribution of lignin content than the Spring Campaign – as we expected;
- The two samples' data agrees with their grade of polymerization.

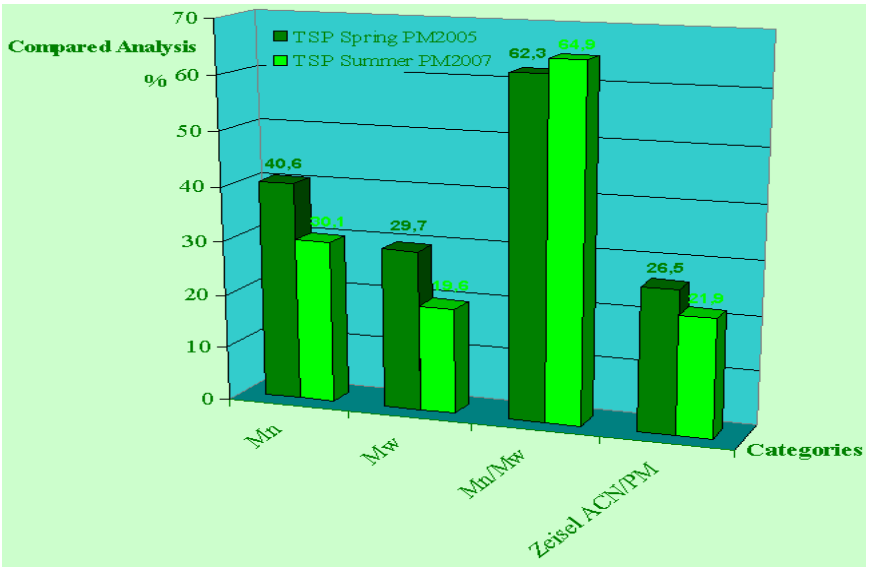


Fig.50 – Compared data for the Averaga Numeral Molecular Weight (Mn); Average Ponderal Molecular Weight (Mw); Grade of Polymerization (Mn/Mw) and Fraction of Zeisel concentration between Acetonitrile and Particulate Matter data obtained.