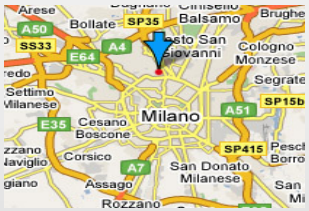
Research Center POLARIS
Particulate Matter and Health Risk

Fine PM biological effects – VESPA project



Marina Camatini
University Milano-Bicocca

Milan, November 30, 2009

Technical workshop for air quality researchers

Project development

I. Planning of the sites and set up of the experiments

II. PM2.5 and PM1 collections during the different seasons and in three sites of Lombardy Region with a different pollution impact: a urban site (Milan), a rural site (Oasis Bine Mantova) , a mountain site (Alpe S. Colombano, Sondrio)

III. Chemical and physical PM characterization

IV. Toxicity evaluation on in vitro systems (human alveolar cell line A549)

V. Evaluation of a possible correlation between the PM chemical composion and its biological impact (PCA)

VI. Proposal of a model suitable to define the PM health risk

I- Planning of the sites

Sites		Where	Description
urban	MI	Sarca Tower is a city place representative of the Milan area with heavy traffic	The sampler was placed above 50m from a traffic light
rural	OB	Oasi Bine WWF (Mantova Province)	This is a natural area with an extension of 100 hectare, is a WWF site, which lasts above 20 km from the cities of Mantova and Cremona
mountain	ASC	Alpe San Colombano (m. 2280 usl) (Sondrio Province).	It is a remote site, placed at the top of the Alpes chain ,over Bormio. It represents the climatic conditions of the Alpes , which are related with the pollutants atmospheric transport from the Po plain

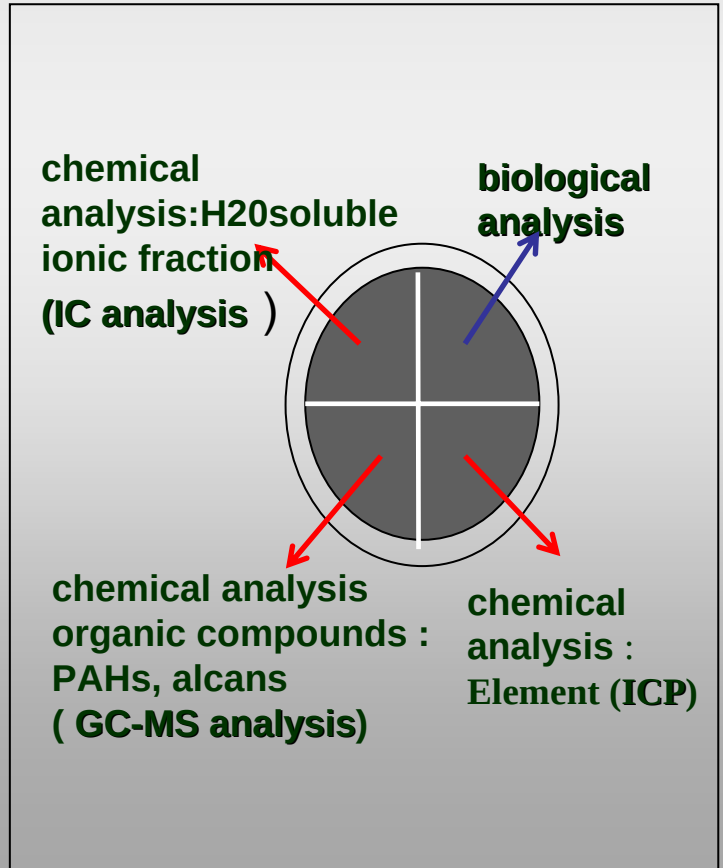
II - Samples (collected April 2007 - February 2008

Season	Period
spring	April 1 – 31 May 31
summer	Jun 1 – September 10
autumn	October 15- Novembre 20
winter	Dicembre 1 –February 28

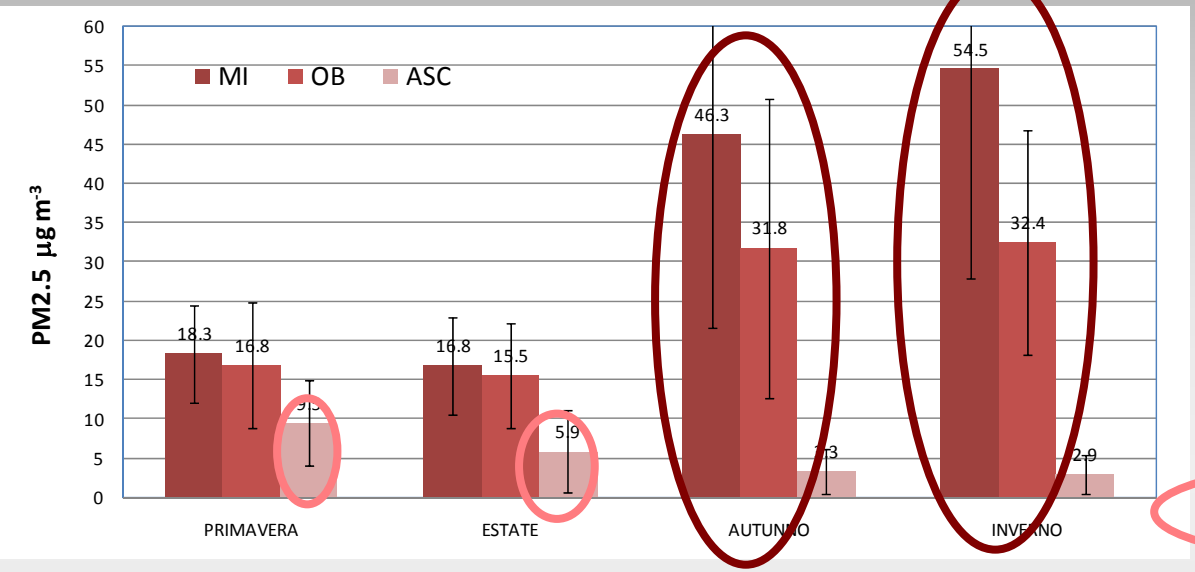
A low volume sampler (38,33l/min), with two lines for collection (PM2.5 e PM1) was used (Hydra model, FAI Instruments).
Samples were collected (24h/day)

An optic particle counter instrument was used, with 31 dimensional classes (0.25 a 32 mm-OPC 1.107 Grimm) and measure of particle concentration (n°/l)

Filters were PTFE, 47 mm diameter (PALL Gelman).
Gravimetric measure for mass concentration (mg m-3)



II- Mean daily concentration of PM2.5 e PM1 in MI, OB , ASC, during the different seasons



MI - OB

MI/OB= 1.8 - 2.0 autumn/winter

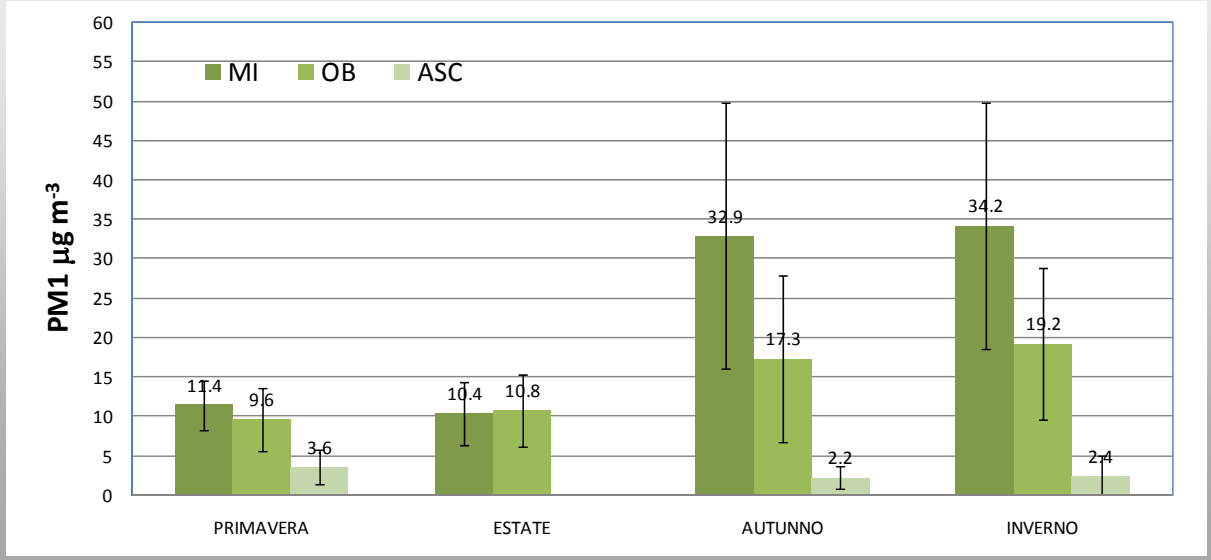
MI/OB= 1.0 -1.2 in spring/summer

ASC:

High concentration in spring/summer

PM2.5=9.5 (±5.4)

PM1=3.6 (± 2.2)



III- PM chemical characterization

	chemicals	origin
Inorganic compounds	**Water soluble ions**	
	$SO_4^{=}$, NO_3^{-}, NH_4^{+}	PM secondary PM semivolatile
	Ca^{++}, Mg^{++},	Mineral dust
	K^{+}, Cl^{-},	Combustion K^{+}, biomass oprigin
	Na^{+}, PO_4^{3}	
	Elements	
	Al,Fe,Zn As,Ba,Cd,Cr,Cu,Mn, Mo,Ni, Pb,V	Mineral dust (es. Al); Antropic origin (traffic, industries)
Organic compounds	**Carbossilic acids**	
	monocarbossilic acidi: C1, C2, C3; acidi dicarbossilic ac : C2, C3, C4, C5	PM seconday origin ; PM biogenic
	PAH	
	10 IPA (BaA, CPcdP, CHR, BbF, BkF + BjF, BeP, BaP, IcdP, BghiP); 1oxiIPA: (BaA7,12D); 1nitroIPA (1NP)	Combustion
	Linear alcans	
	C20- C32	Combustion PM biogenic (es. index CPI)

III- PM Chemical characterization

SO_4, NO_3^-, NH_4^+, OM*§ EC* are the principal chemical species

$SO_4^=$: 22-29% summer, 5-9% winter

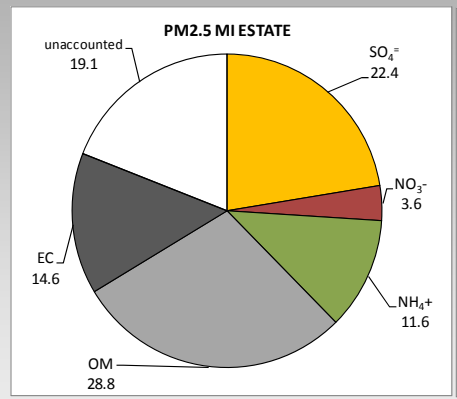
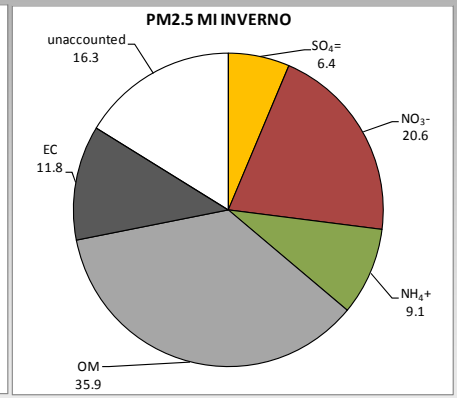
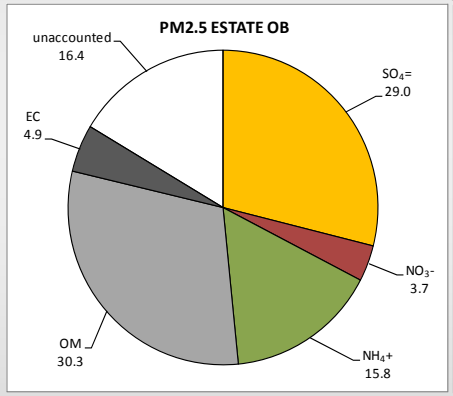
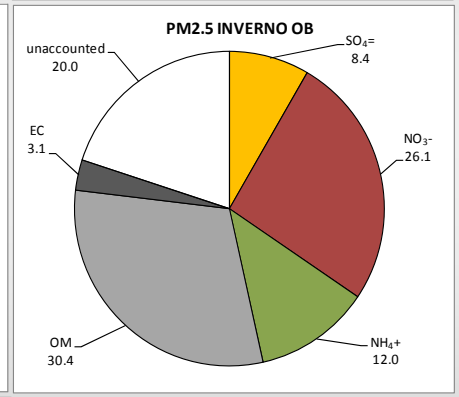
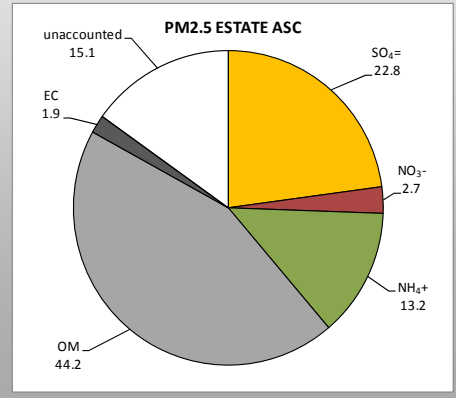
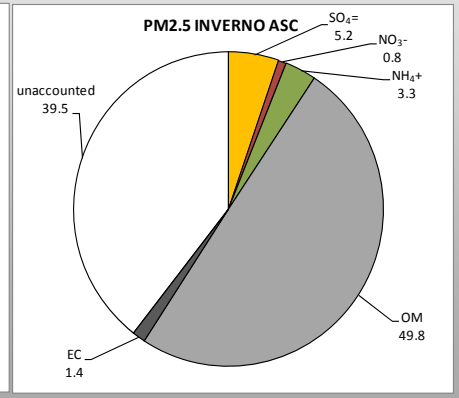
NO_3^- : 3-4% summer, 21-26% winter

MI, OB,ASC

MI: EC= 12-15%
OB: EC= 3-5 %
ASC: EC=1-2 %

ASC winter PM in free trposheric zone

•From the project QUITSAT (summer 07;winter 07-08)
§ OM=1.6*OC (MI, OB); OM=2.1*OC (ASC) (Turipin B.J., 2001)

	PM 2.5 summer	PM 2.5 winter
MI		
OB		
ASC		

CONCLUSIONS

Spring/Summer (MI, OB, ASC):

- High contribution from secondary PM, originated by reactive oxidative processes ($SO_4^=$, carbossilic acids), more evident in OB and ASC
- Contribution from soil dust and of biogenic origin

Autumn/Winter(MI, OB):

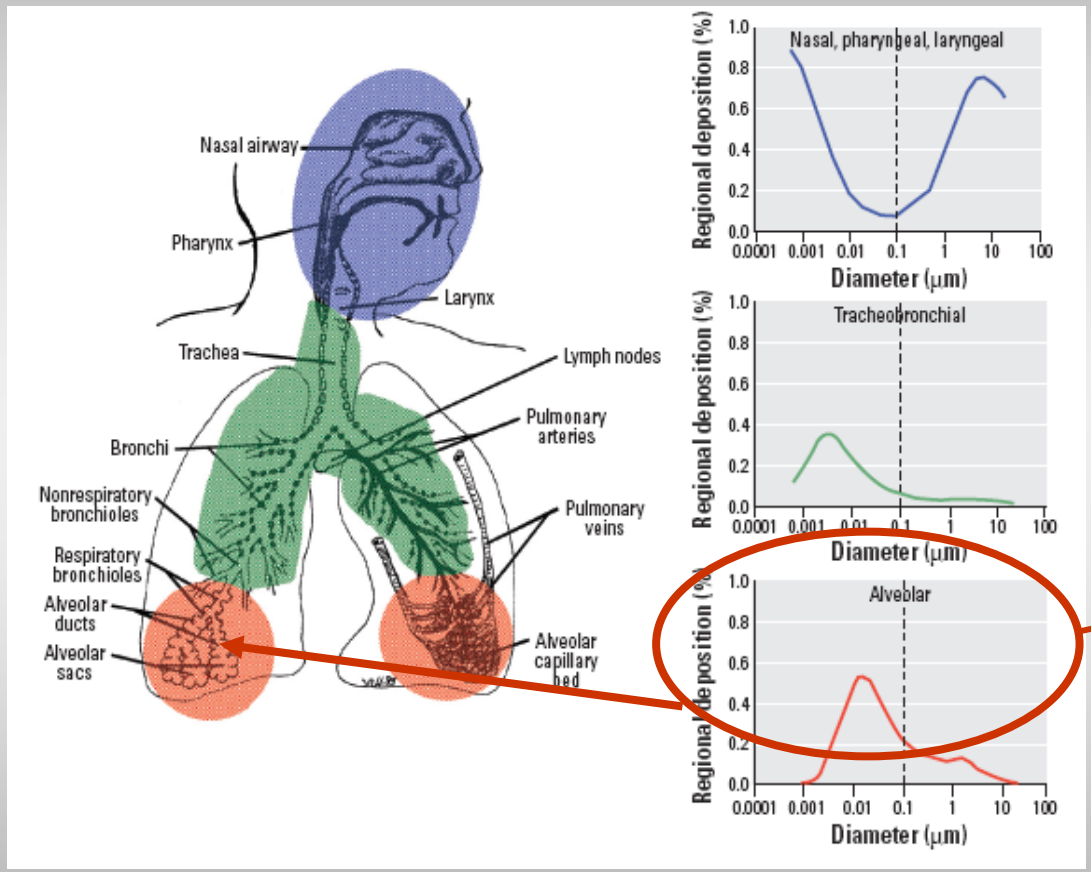
- Contribution from combustion origin (PAH), and biomass too (K^+..)
- High contribution from NOx

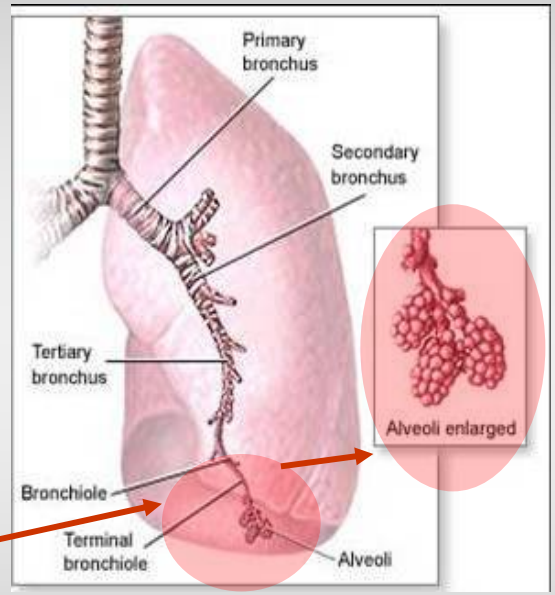
Autumn/ Winter (ASC) :

- PM from free tropospheric area. High level of elements (Fe, Al, Cr, Cu, Mn, Ni….)

IV-Toxicity evaluation

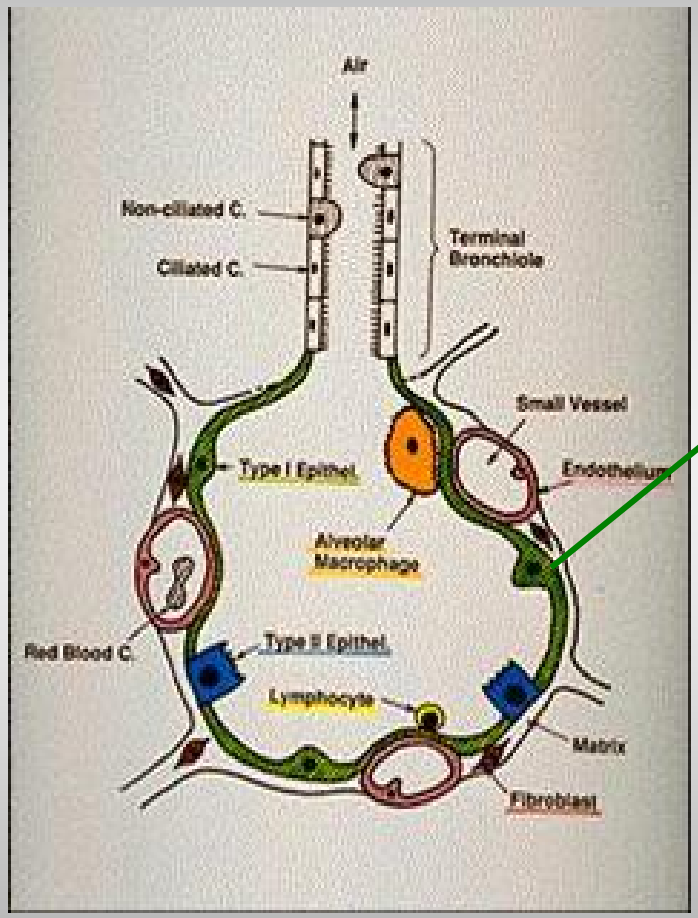
PM deposition in the respiratory apparatus



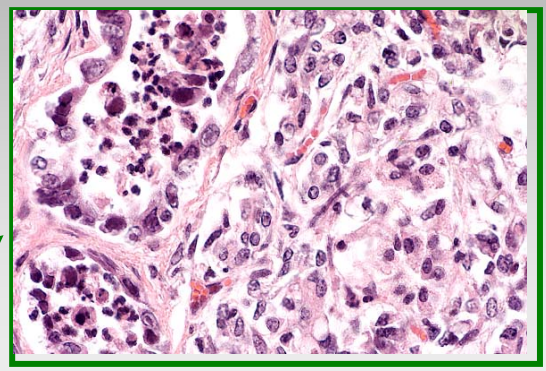


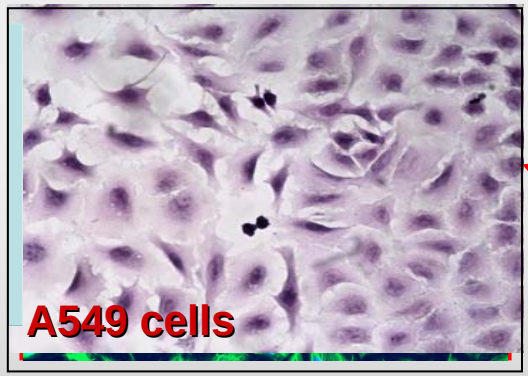
The different size fractions (0.01-100 um) enetr the three regions of the respiratory apparatus (Oberdörster, 2005)

IV-Toxicity evaluation on in vitro systems (human alveolar cell line A549)

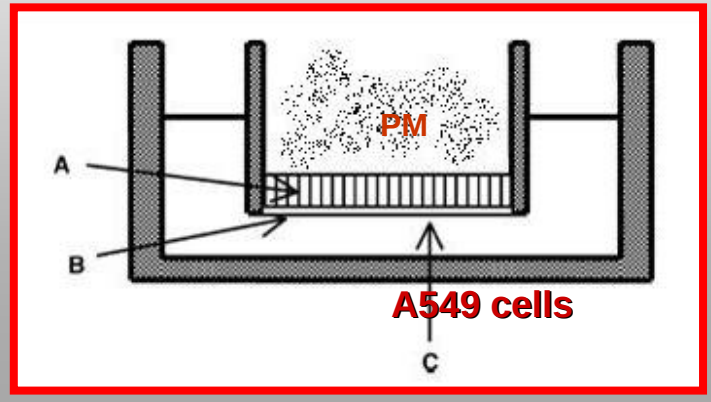


In vivo

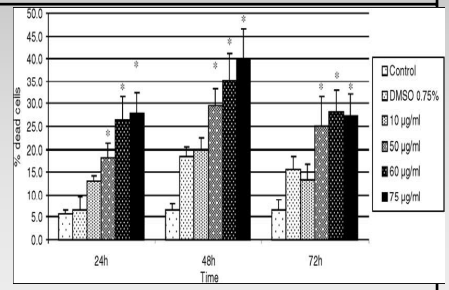
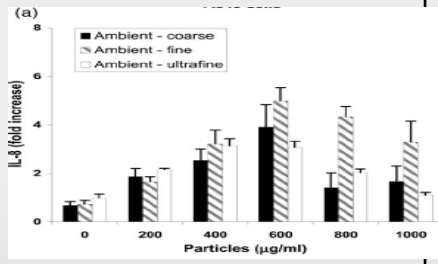
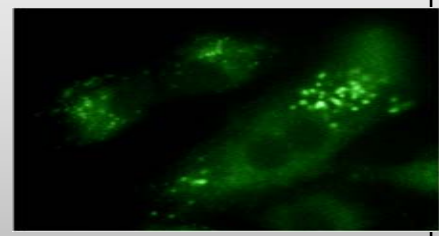
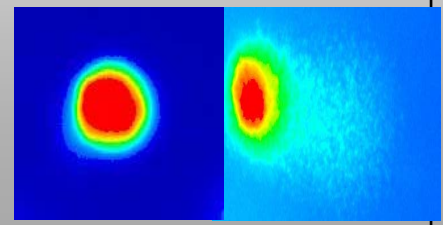




In vitro



IV-Toxicity evaluation on in vitro systems

Cell line evaluation	Parameters	Biomarker	Results
Cell toxicity	Cell viability	MTT, LDH	
Inflammatory evaluation	Protein expression	Interleukin IL-8	
Oxidative stress	Reactive Oxygen species (ROS)	Fluorescent microscopy, Cytofluorimeter	
Genotoxicity	DNA damage	Comet Assay	

IV Toxicity evaluation on in vitro systems

Particle extraction for cell toxicity assay: Teflon filters were extracted with the Sonica® ultrasound bath (four times ,each sonication for 20 min adding 2ml of sterilized water . Detached particles were dried in a desiccator and suspended in sterilized water to obtain aliquots at a final concentrationof 2ug/ul, and stored at −20 ◦C until use.

Cell colture:. A549 cells (American Type Culture Collection) were routinely maintained in OptiMEMmedium atpH7.2, supplemented with 10% inactivated foetal bovine serum and 1% penicillin/streptomycin and grown at 37 ◦C, with 5% CO2. Cells were seeded at a concentration of 1.5×10^5 in 12-well plate. After seeding, cells were treated

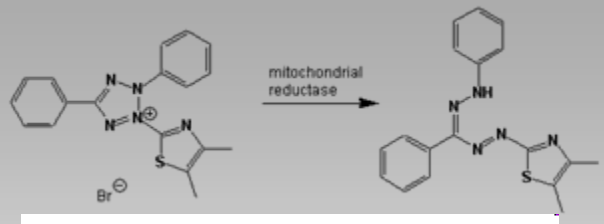
Doses used: were calculated considering that the mean fine quantity present in Milan during the winter was 50ug/m3. Daily ventilation is evaluated to be 20m3 of inhaled air, which contained up to 50ug/m3 of PM2.5. The exposition time for humans may be 6 h/day and the respirable quantity may be 65% and 30% the quantity reaching the alveolar epithelium. Thus the highest dose used was 25µg/cm2 corresponding to this calculation..

***Cell exposure*:** after 24 h from seeding, cells were treated with PM at the concentrations of 1,6 ,12 , 25 ug/cm2, in 1% FBS supplemented medium. 24 h was the exposure time Three independent experiments were carried out following the same experimental conditions. And ROS were evaluated at 2h

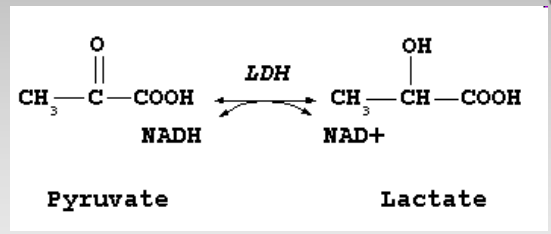
The comparison between the effects produced by PM2.5 and PM1 sampled in the three sites and at the different seasons have been performed on the results obtained by cells exposed to 6µg/cm^2.

IV Toxicity evaluation on in vitro systems

MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was used to measure cell viability.

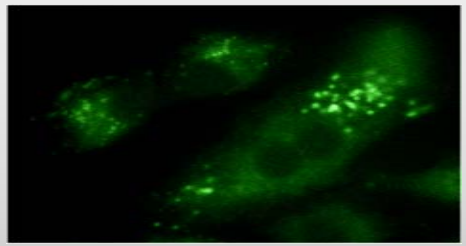


LDH. This enzyme is released from p.m. when it is damaged and it is cell viability parameter

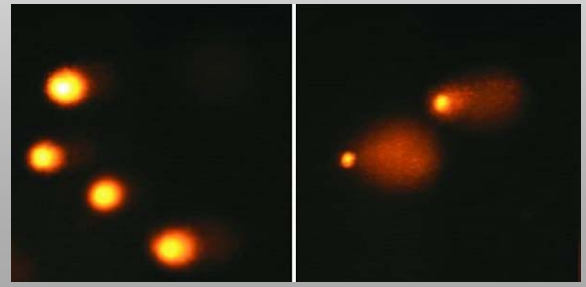


IL8.protein release in the colture media is a marker of inflammation process and the levels were determined by the immunochemical ELISA technique

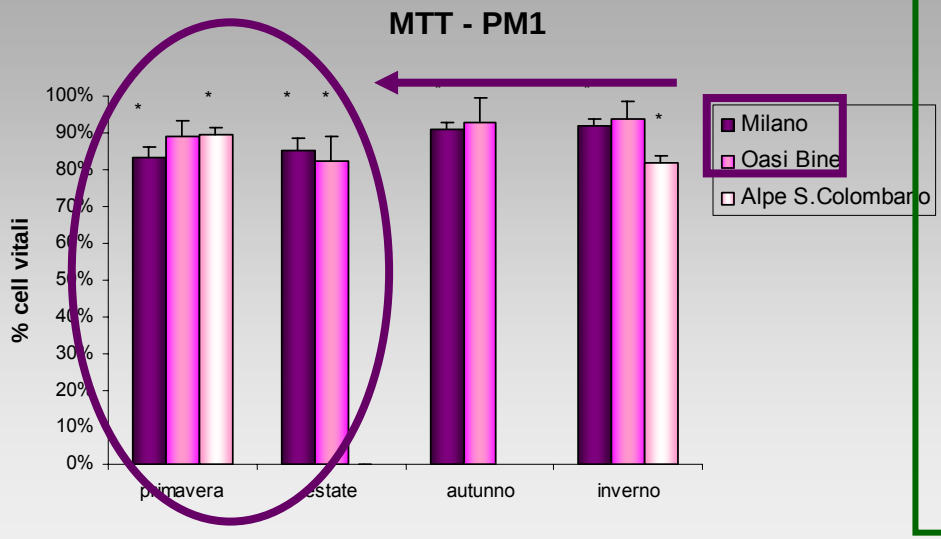
ROS production after treatments is detectable by the oxidation of DCFH to dichlorofluorescin (DCF), which is fluorescent (530 nm)

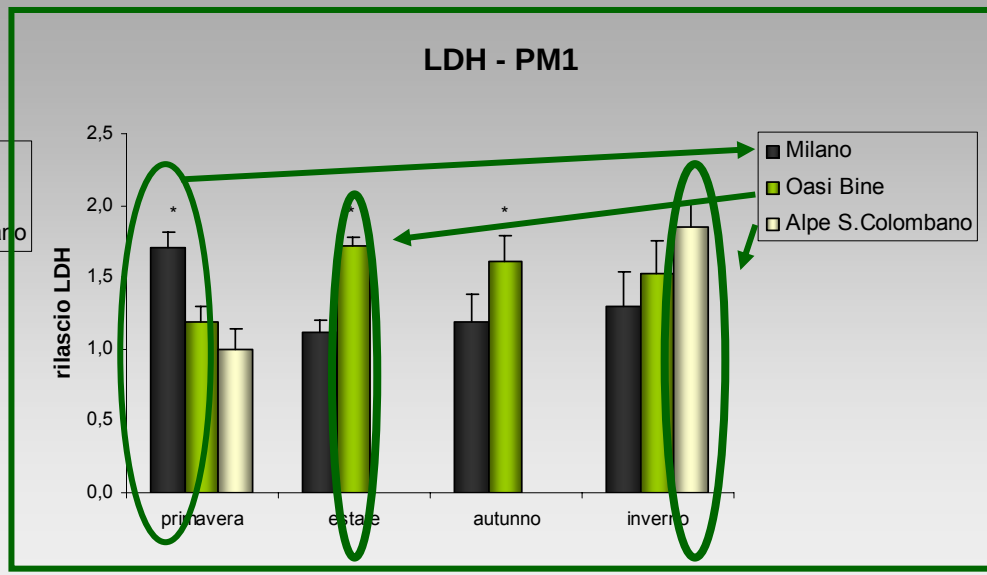


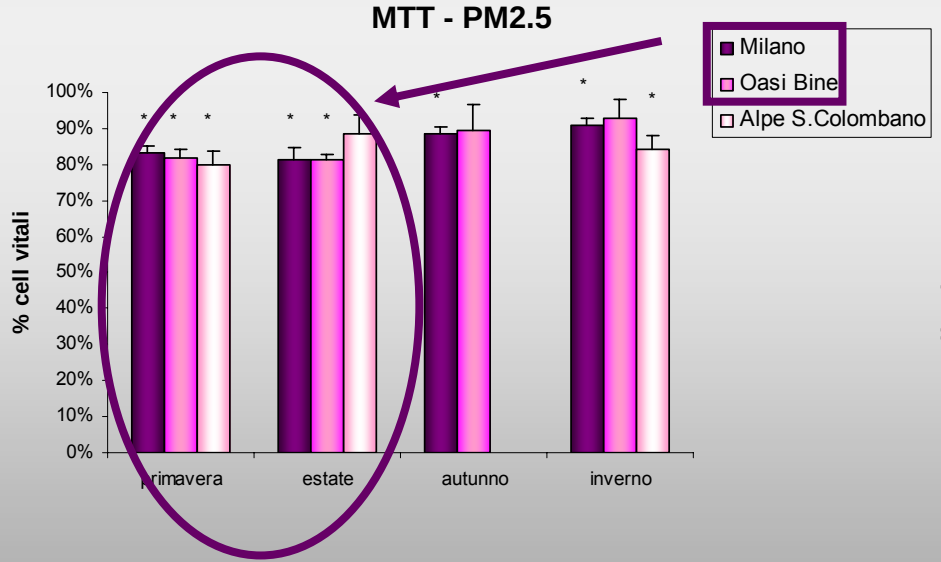
Comet assay is a staining of DNA and fluorescence determine the extent of DNA damage.

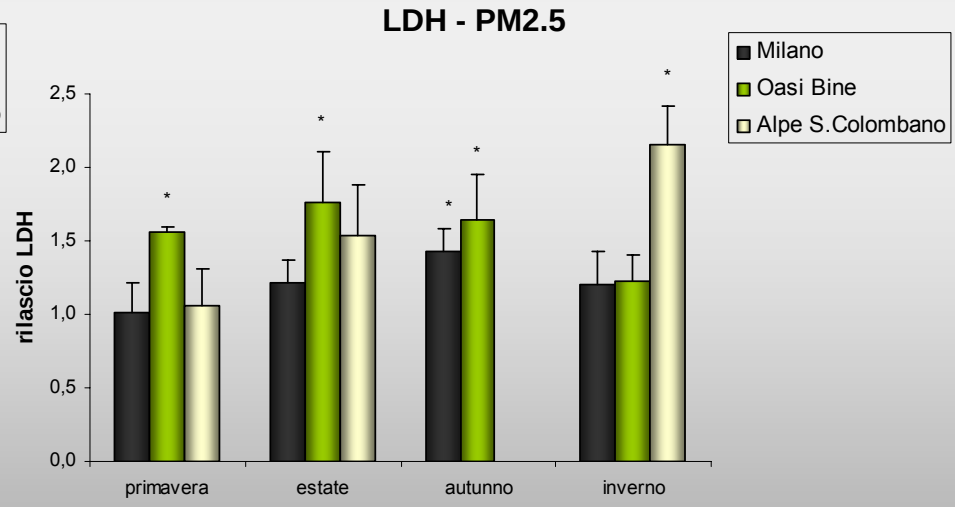


Citotoxicity







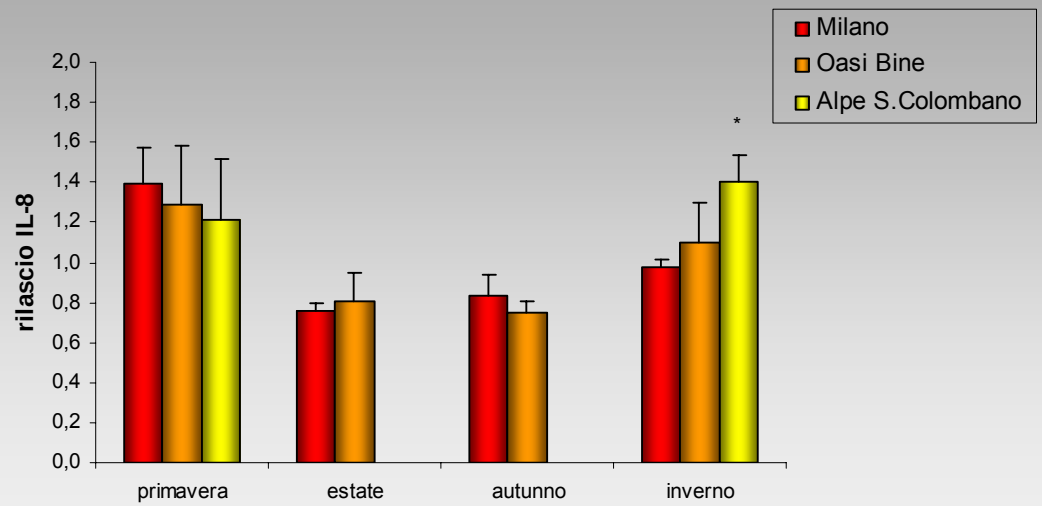


Cell viability is affected in spring and summer at Mi and OB

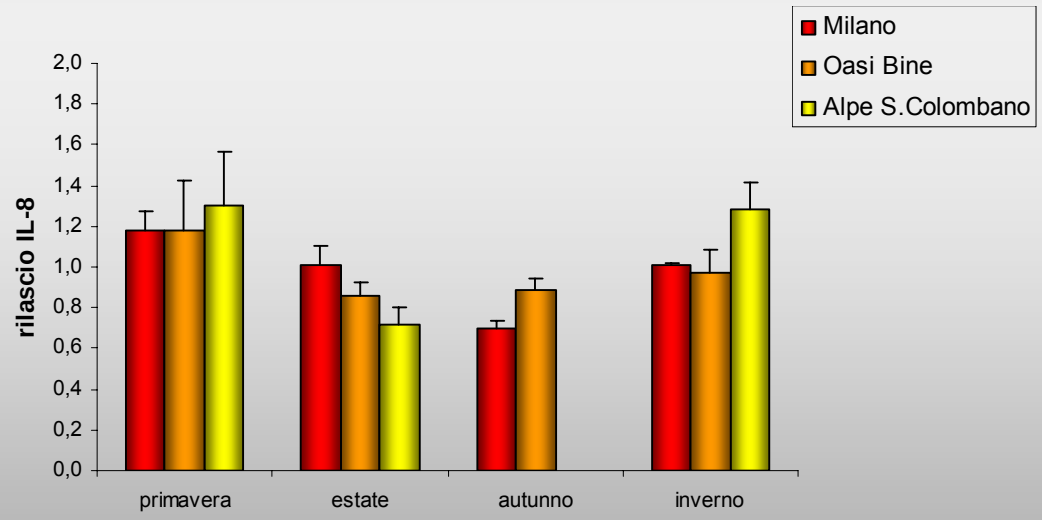
p.m integrity is affected in spring at MI , in summer at OB and in winter at ASC.

IL-8 Cytochin expression

PM1

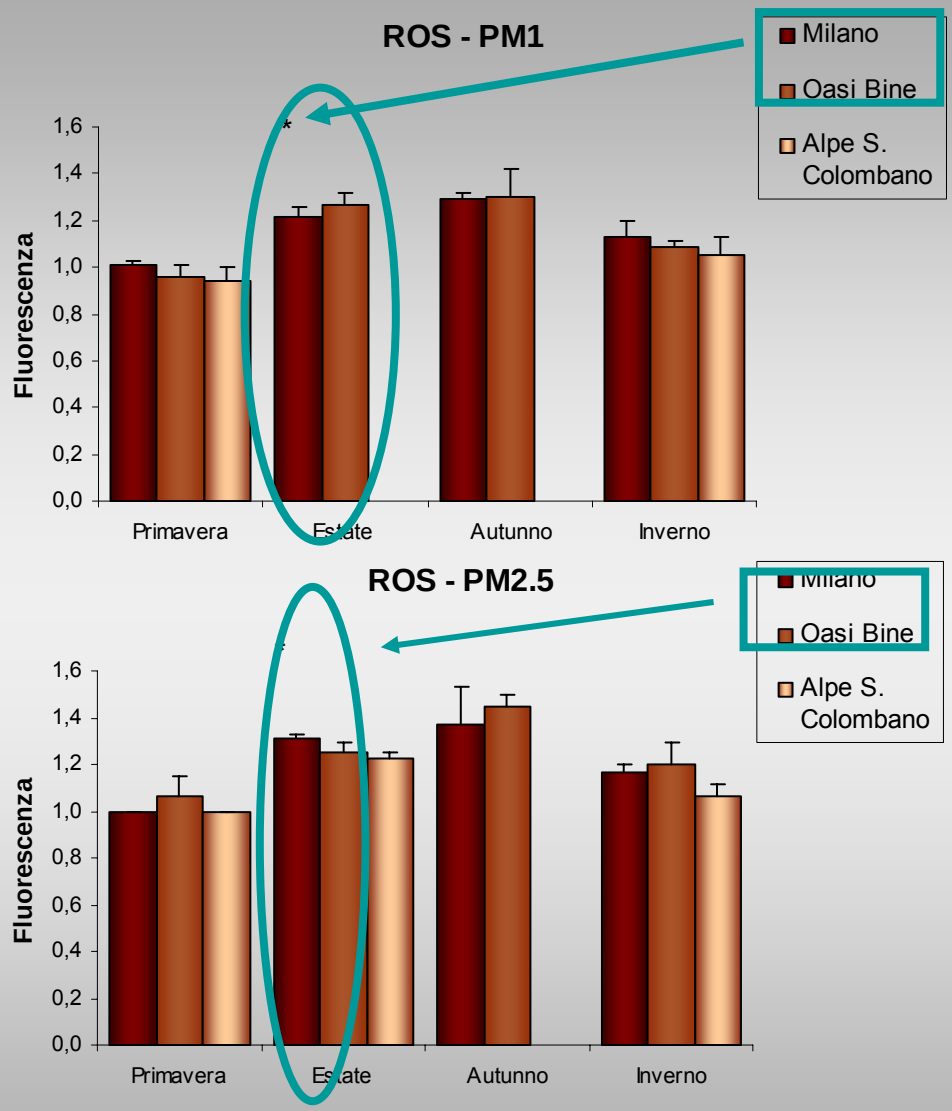


PM 2.5



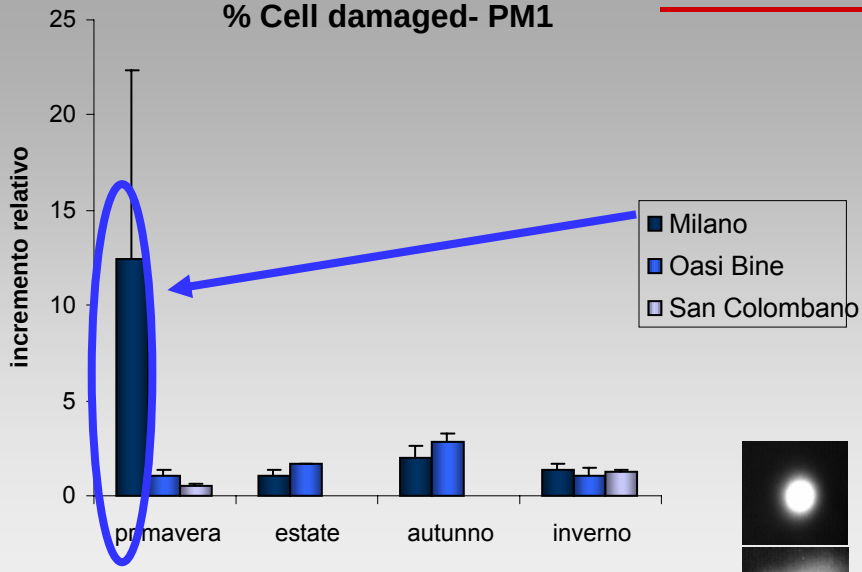
PM1 and PM2.5 do not have a significant inflammatory potential, even IL-8 is augmented in MI summer and at ASC during winter

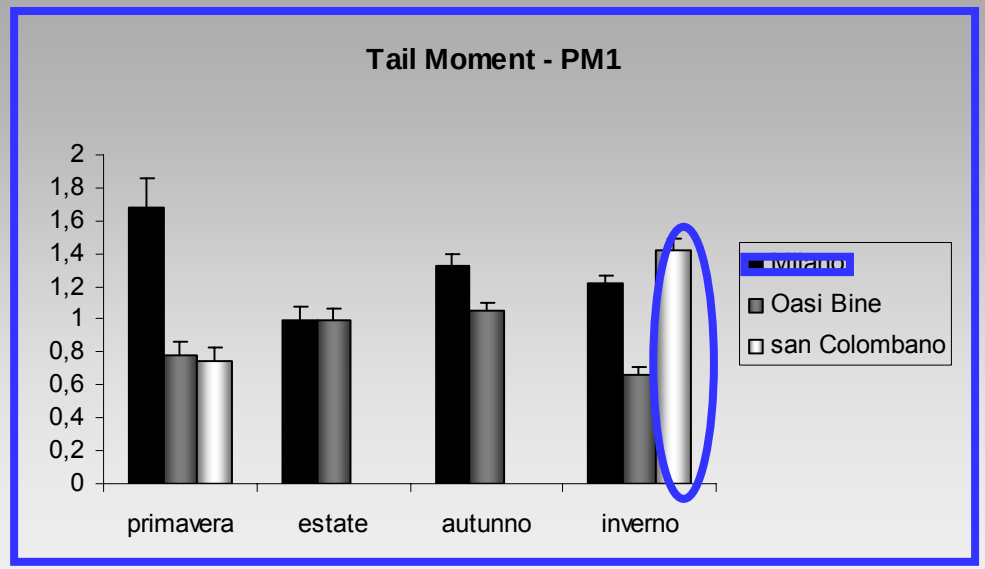
Reactive Oxygen Species (ROS)

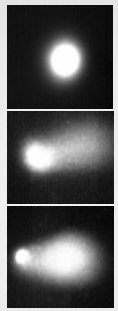


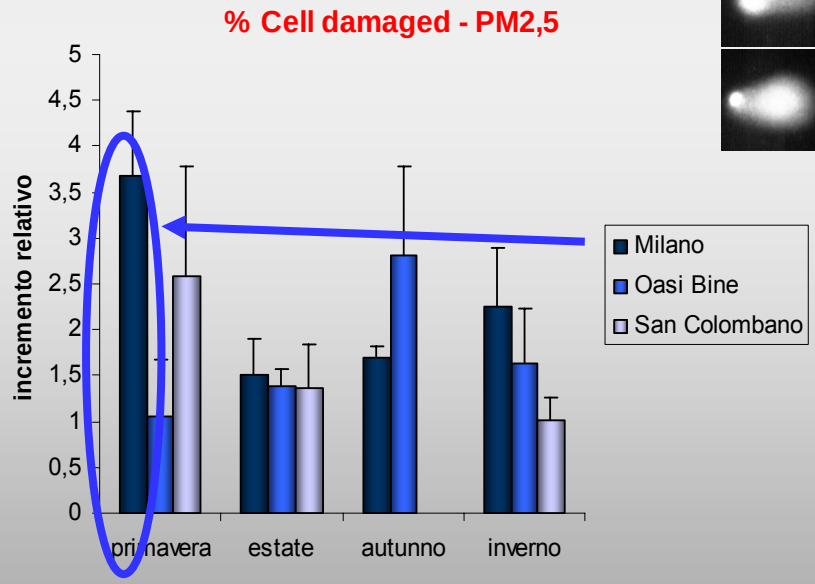
ROS production has a significant value at MI and OB in summer

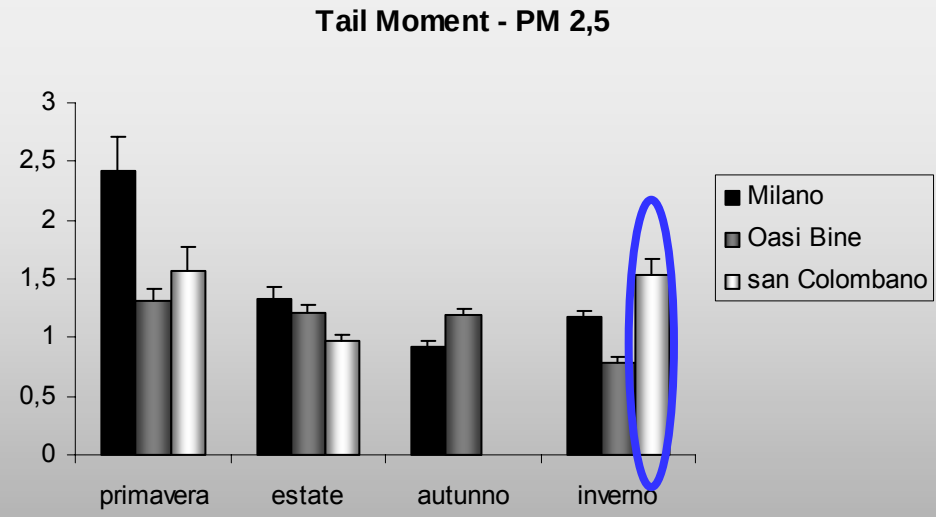
DNA damage









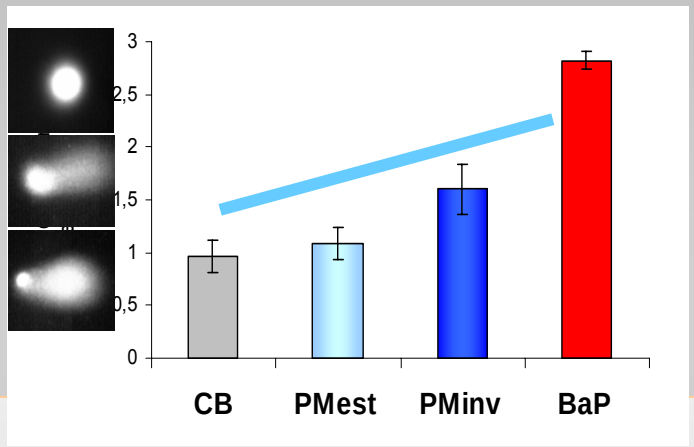


The % of damaged cells is significant in spring .

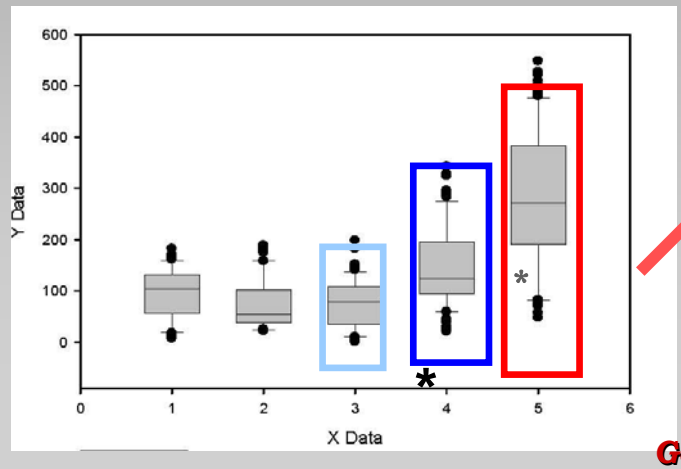
The damage is significant at MI in spring and at ASC in winter

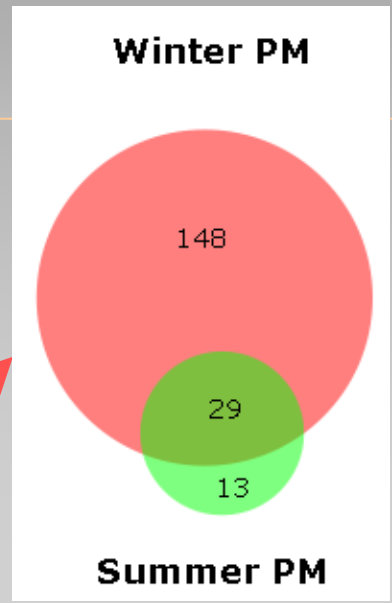
Comet assay

% of comet cells



Tail moment





Gene expression profiling in A549 esposte a PM2.5

DNA damage has been quantified , utilising the data of "tail moment", which evidences also the quantity of DNA fragments produced by exposition

The gene number . activated by winter PM is higher than that of summer . Many of these genes are involved in detoxification **mechanism**

In preparation with C.Battaglia (LITA Segrate)

winter PM2.5 produce significant DNA damage

CONCLUSIONS

- **Citotoxicity: cell viability is diminished in spring and summer at MI and OB; p.m is damaged in spring at MI, in summer at OB, in winter at ASC**

- **Genotoxicity: DNA is damaged in spring and summer at MI; in winter at MI and ASC**

- **Inflammation process: is significant in spring at MI and in winter at ASC**

- **ROS : are expressed in summer at MI and OB**

V- Evaluation of a possible correlation between the PM chemical composition and its biological impact (PCA)

PCA (Principal Component Analysis) is a multivariate analysis able to find correlations among variables. The variables are: the biological effects and rhe PM chemical composition.

n: PM1 and PM2.5 samples - MI, OB, ASC sites - seasons = 21

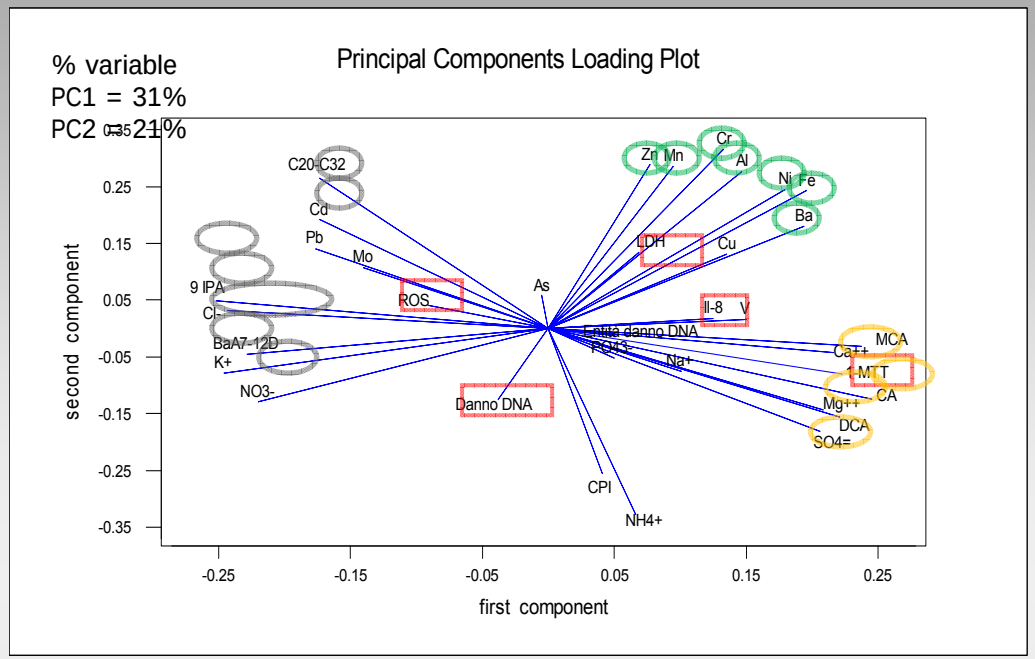
p: variables

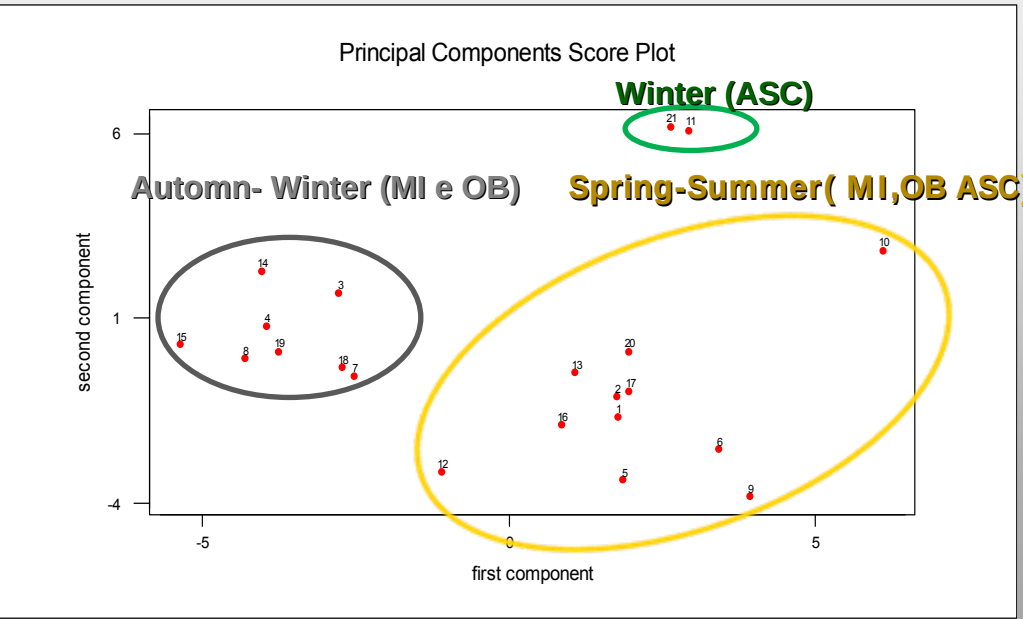
biological effects (6)
-MTT; LDH; IL-8; ROS; DNA damage.
dose 6μg cm^{-2}

=35

chemical composition (29):
9 inorganic ion ioni inorganici, 13 elements, MCA
(Σ monocarboxylic acids); DCA (Σ dicarboxylic acids); C20-C32 (Σ linear alkanes);
9IPA (Σ IPA); BaA7,12D.
RESULTS: % MASS COMPOSITION

PCA: different clusters in function of seasons





Spring/Summer PM (MI, OB and ASC)

BIOLOGICAL EFFECTS: cell mortality (1-**MTT**), Damage to m.p.(**LDH**), inflammatory responses (**IL-8**)
CHEMICAL COMPOSITION: secondary PM(CA, $SO_4^{=}$), dust from ground (Ca^{++}, Mg^{++})

Autumn/Winter PM (MI e OB)

BIOLOGICAL EFFECTS : ROS
CHEMICAL COMPOSITION : PM products from combustion (IPA, K+), elements (Cd, Pb)

Winter PM -ASC

BIOLOGICAL EFFECTS : citotoxicity **(LDH)**
CHEMICAL COMPOSITION : elements (Fe, Al, Cr, Ni..

The Sites in the Po plain (MI e OB) have the same characteristics

- PM1 AND PM2.5 ARE IN THE SAME CLUSTER

Seasonality prevails on both the geographical location and the PM distribution when a correlation is done between the biological effects and the chemical composition

VI -Development of a model suitable to define the PM health risk

A tentative has been performed to create a INDEX RISK (IR) which integrates the experimental data of:
- the mean concentrations (mg m-3) of PM1 and PM2.5 in the sites of MI, OB e ASC, for the seasons
- the biological effects produced on the pulmonary alveolar cell line A549
The calculation of the risk index considered in detail:
dose-response curves from exposure of A549 to 6ug/cm^{-2} of PM1 and PM2.5 in MI, OB, ASC and different seasons
average atmospheric concentration (C =µg m^{-3}) of PM1 and PM2.5 in different sites and seasons, converts to cell exposure dose (D =mg cm^{-2})

$$IR_{i,j} = \left|(a_{i,j} * D_j + b_{i,j}) - 1\right|$$

IRi,j = RISK INDEX calculated for the i-th biological response and the j-th sample of PM (PM2.5 and PM1) for each site of the Lombardy Region (MI, OB and ASC) and season.

•C*j*, is the j-th dose of PM (PM1 o PM2.5) calculated from environmental concentration measured in each site of the Lombardy Region (MI, OB, ASC) and season.

•$a_{i,j}$, $b_{i,j}$, are the parameters (intercept and slope) of the dose-response curves for each toxicological test performs on PM1 or PM2.5 for each site of the Lombardy Region (MI, OB, ASC) and season.

ALGORITHM for CALCULATING the TOTAL RISK INDEX

$$IRtot_j = (IRcit_j + IRgen_j)/\max(IRcit_j + IRgen_j)$$

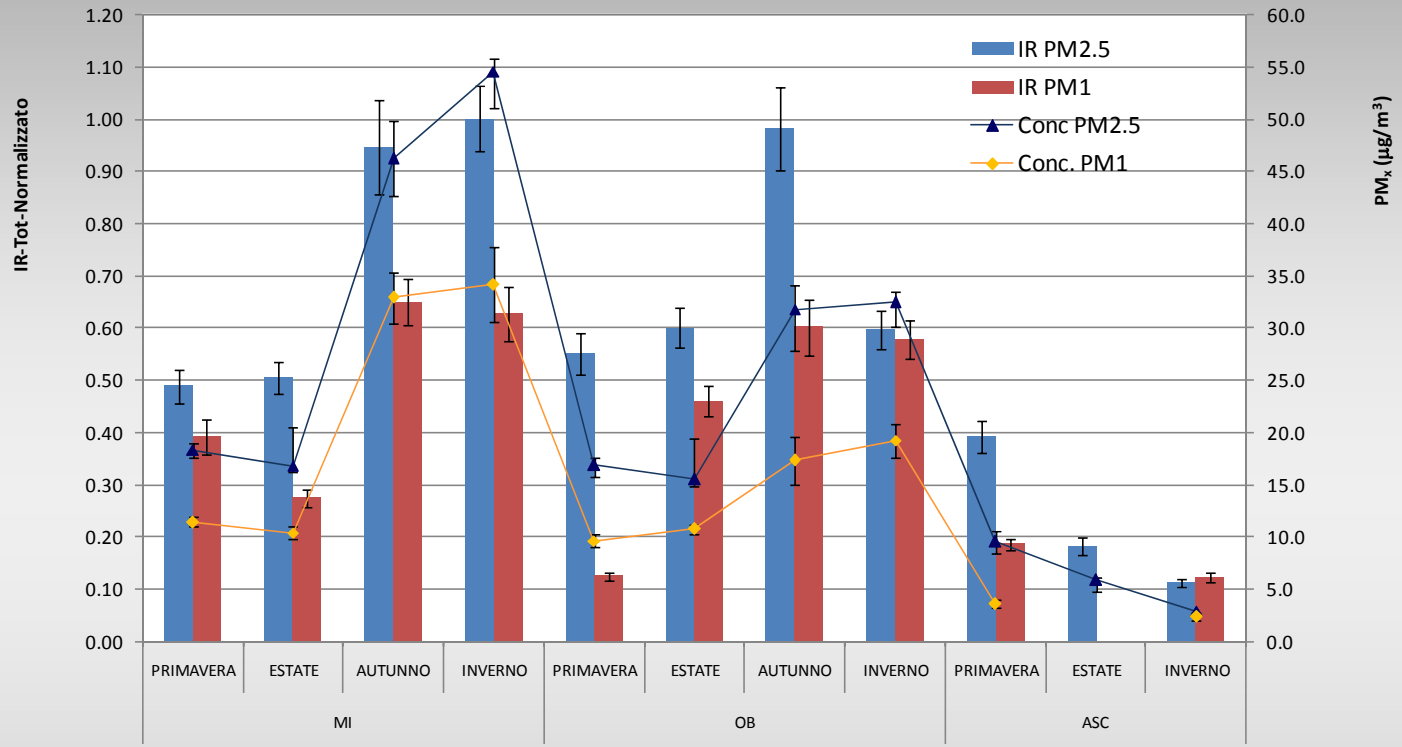
citotoxicity Index Risk

genotoxicity Index Risk

IRtot = INDEX RISK (0-1) calculated for the j-th PM sample (PM1 or PM2.5) for each site of the Lombardy Region (MI, OB, ASC) and season.

PM health risk

IR and PM1 , PM2.5 concentrations, for MI, OB and ASC, in the 4 seasons :average values and standard deviation



IR-tot PM2.5>PM1: PM1 concentrations are 60-70% of PM2.5

IR-tot max: PM2.5 MI and OB in autumn-winter

IR-tot autumn/winter > spring/summer: sites of MI e OB in spring/summer have a IR which is 40-50% of the winter one.

In spring/summer PM concentrations are lower than in winter but at these concentration the biological effects are higher.

How to conclude

- Fine PM chemical composition has a biological impact, which is seasonal dependent. The sites sampled and the PM dimensions are less relevant.

- The most significant effects are on cell viability and DNA damage in spring and summer for MI and OB and in winter for ASC

- The risk index is higher for MI and OB during autumn/winter

How to continue …….

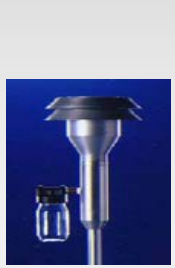
- ….by the chemical characterization of different emission sources
- …by the definition of the toxicity threshold value of the PM components
- …by the reception of the results from the control air quality Institutional Organizations

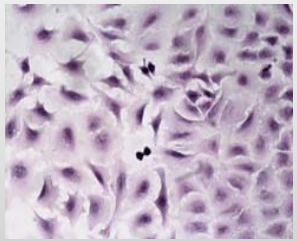
Research Center
POLARIS
Particulate Matter and Health Risk

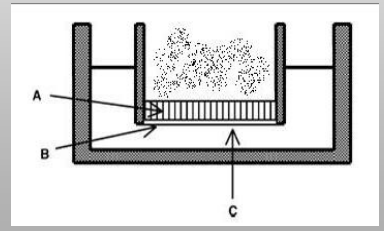
VESPA project received a financial support by Lombardy Region, Milan Municipality and Province of Milan. ARPA Lombardy and Lombardy Foundation were in the Scientific Commettee

The PM samples, the characterization, and the IR were performed by

Ezio Bolzacchini
Grazia Perrone
Luca Ferrero
Claudia LoPorto
(Lab of Atmospheric Chemistry, University Milano-Bicocca)







The cell experiments were performed by:

Marina Camatini
Viviana Corvaja
Maurizio Gualtieri
Paride Mantecca
Eleonora Longhin
(Lab. Cell Biology, Universisy Milano-Bicocca)