Early biological effects (cytome assay) in children exposed to different levels of PM 0.5 in five Italian cities during winter 2014-2015: MAPEC (Monitoring Air Pollution Effects on Children for supporting public health policy) study

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INTRODUCTION

- Epidemiological studies have shown that air pollution can cause harmful health effects. In particular, it was found a consistent association between exposure to particulate matter (PM) and incidence for several chronic diseases such as lung cancer, cardiovascular diseases and diabetes;
- The World Health Organization's (WHO) International Agency for Research on Cancer (IARC) has classified PM, a major component of outdoor air pollution, as "*Carcinogenic to humans*" (Group 1), and genotoxic damage was indicated as the main mechanism responsible for the toxic effects;
- Children are more vulnerable than adults to the effects of airborne toxic agents for several reasons such as (i) more inhaled air per unit of body weight than adults, (ii) more time spent outside, (iii) more physical activity and (iv) some organs are still in development.

AIM OF STUDY

Evaluation of the associations between urban air pollutants and biomarkers of early biological effect in oral mucosa cells of 1,000 children recruited from first grade schools of 5 Italian towns (Torino, Brescia, Pisa, Perugia and Lecce), which are characterized by different level of particulate matter (PM).

MATERIALS & METHODS

STUDY POPULATION and CITY AIR POLLUTION

- The towns involved in the study are:
  - Brescia and Torino, in Northern Italy;
  - Pisa and Perugia, in Central Italy;
  - Lecce, in Southern Italy.
- The towns in Central and Southern Italy are characterized by lower PM 0.5 values than towns in Northern Italy (Fig.1).
- For each town, 200 children aged 6-8 were recruited from first grade schools.
- Biological samples were collected from the same children at two separate times: winter 2014 and spring 2015.

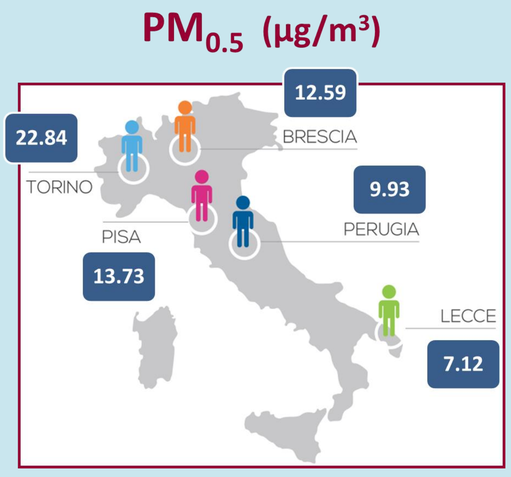


Figure 1. Level of $PM_{0.5}$ in the five Italian cities involved in the study.

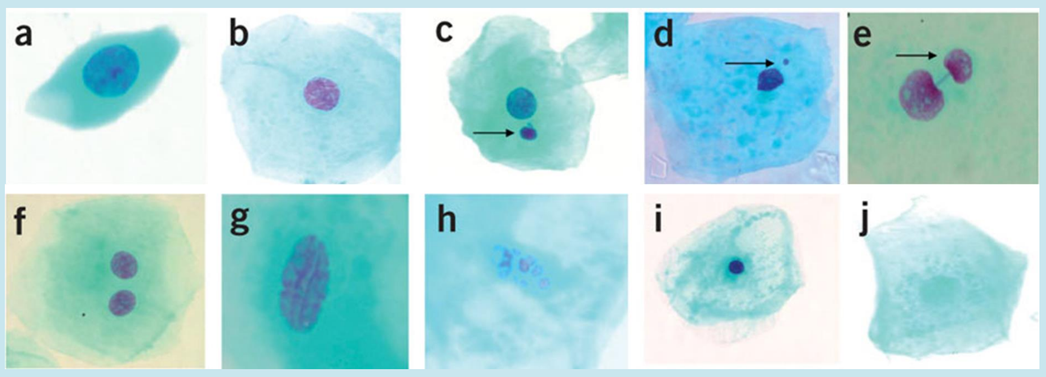
BUCCAL MICRONUCLEUS CYTOME (BMCyt) ASSAY

Sample collection and preparation

- Buccal mucosa cells (BM) were collected by rubbing the inside of the children's cheeks with a toothbrush;
- BM cells were processed, smeared on microscope slide and stained with the Feulgen/Light Green method.

Microscope analysis

- Microscope analysis was performed using both bright field and fluorescence microscope at 1,000x magnification. All slides were analyzed blindly by experienced scorers.
- For each subject, 1,000 BM cells were gathered in the following categories: **basal cells** [a], **normal differentiated cells** [b], **binucleated cells** [f], **apoptotic/necrotic cells** (i.e. condensed chromatin [g], karyorrhectic [h], pyknotic [i], and karyolitic [j]).
- DNA-damaged cells, *i.e.* cells with **micronuclei (MN)**, which are indicative of chromosome loss or fragmentation [c/d] and/or **nuclear buds (NB)**, which may be related to the elimination of amplified DNA or DNA repair [e], were scored in 2,000 normal differentiated cells.



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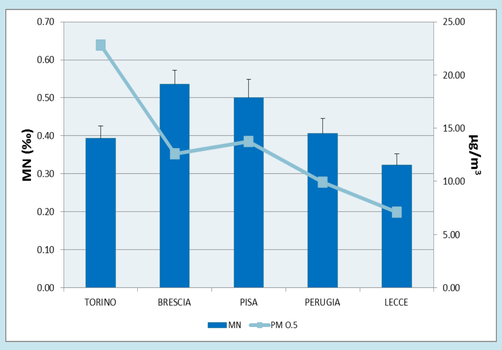
RESULTS

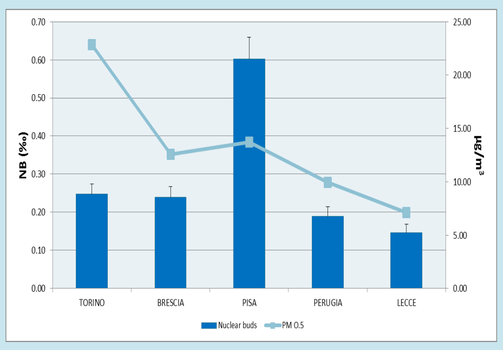
Results relative to samples collected in the winter season are discussed.

Table 1. Frequency (‰) of MB cell type in children recruited in different Italian cities

	Basal cells (B)		Binucleated Cells (BN)		Condensed chromatin (CC)		Karyorrhectic (KHC)		Pyknotic (PYK)		Karyolytic (KYL)	
	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
TORINO	0.36	0.04	3.95	0.14	28.55	1.19	10.38	0.57	0.15	0.03	24.87	1.12
BRESCIA	0.70	0.08	3.78	0.14	34.92	1.34	22.49	1.17	0.38	0.04	33.21	1.07
PISA	0.65	0.09	3.85	0.16	25.56	1.15	12.83	0.77	0.27	0.04	40.07	1.95
PERUGIA	0.34	0.04	3.87	0.12	27.49	1.39	10.76	0.78	0.27	0.05	22.84	0.94
LECCE	0.27	0.04	3.40	0.13	26.80	1.31	11.49	0.78	0.07	0.02	26.82	1.11

Figure 2. Micronuclei and nuclear buds frequencies (‰) in BM of children recruited in different Italian cities and air PM 0.5 level





- The frequencies of the different **BM cell type** where found to be **significantly different** among the five towns ($p<0.05$, Kruskal-Wallis test), with the exception of BN cells (Tab.1).
- Spearman's correlation analysis revealed a **significant positive correlation** between the **geographical distribution (South to North) of town** and:
  - the proportion of **basal cells**, which reflects the proliferative potential, as well as the frequency of cells in different death stages (i.e. **CC**, **KHC** and **PYK**) (rho=0.065-0.148, $p<0.05$);
  - the frequency of DNA damage biomarkers, *i.e.* **MN** (rho=0.79, p< 0.01) and **Nbud** (rho=0.089, $p<0.01$) (Fig.2).
- Unexpectedly, PISA showed the highest frequency of NB and TORINO the lowest frequency of MN (Fig.2)

DISCUSSION

- ✓ Our data showed an increase of the frequency of **DNA damage biomarkers** (MN and NB) and **apoptotic/necrotic cells** with increasing level of **urban air pollution**.
- ✓ The reason why the frequency of NB is greater than expected in PISA and lower than expected in TORINO is unclear, but could potentially be related to population dependent **environmental exposure** or **dietary factors** which **could affect genotoxic effects**.
- ✓ These results will be integrated and correlated to those of the spring season.
- ✓ The BMCyt assay may become a relevant biomonitoring tool in the future for **early detection of genotoxic risk associated with air pollution**.

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