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Trends of PM_{2.5} pollution and its mutagenic properties in Turin: a 7 years study

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AIR, WATER AND SOIL POLLUTION SCIENCE AND TECHNOLOGY

AIR POLLUTION
SOURCES, PREVENTION
AND HEALTH EFFECTS

RAJAT SETHI
EDITOR

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Chapter 15

TRENDS OF PM_{2.5} POLLUTION AND ITS MUTAGENIC PROPERTIES IN TURIN: A 7-YEAR STUDY

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ABSTRACT

Fine aero-dispersed particles can be relevant carriers of toxic compounds into the lung alveoli where blood exchange takes place. These compounds include numerous mutagens and carcinogens, especially organic highly and moderately polar chemicals. The fine particles are more numerous, and they have a large available surface for the adhesion of toxic chemicals. Moreover, specific European Community attention has addressed fine particle indicators, such as PM_{2.5}, only since 2008. In this study, the mutagenic properties of urban PM_{2.5} were evaluated during a consecutive seven full years. We collected daily PM_{2.5} samples and then applied a consolidated *in vitro* test. The mutagenic properties were assessed for each month of sampling with the *Salmonella typhimurium* TA98 strain. The annual measured mean levels of PM_{2.5} ranged from 18.1±12.9 to 54.1±29.7 µg/m³. The mutagenicity evaluations ranged from 17±15 to 51±42 net revertants/m³. Seasonal differences are statistically significant both for gravimetric data (p<0.01) and for *Salmonella* assay results (p<0.01). Moreover, the PM_{2.5} organic extract mutagenicity is correlated to the quantity of PM_{2.5} per cubic meter (Spearman's rho=0.637, p<0.01), even if the trend during the years – sorting out the seasonal effect - of the levels and mutagenic properties are quite different. We observed a PM_{2.5} level reduction but an increment for the mutagenicity per cubic metre air, highlighting that there is a wide reduction of the quantity of the PM_{2.5} aero-dispersed over time but the same improvement was not observed for the PM_{2.5} mutagen content.

Keywords: PM_{2.5}, air pollution, mutagenicity, lung cancer

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1. INTRODUCTION

Increased mortality has been associated with ambient air pollution exposures in several population-based cohort studies (WHO, 2006). The majority of these studies have estimated air pollution exposures based on nearby ambient monitors. The associated causes of death have been mainly respiratory disease, cardiovascular disease and lung cancer (Chen et al. 2008; Pelucchi et al. 2009). The effects are significant on susceptible individuals, including individuals with asthma (Ueda et al. 2010), chronic obstructive pulmonary disease patients (Mariani et al. 2010), and the elderly (Faustini et al. 2011). The qualitative review describes possible sources of difference in air pollution responses between women and men of different life stages, life styles, co-exposures and other factors (Clougherty 2010).

Since the major events (such as London 1952) occurred in the 1950s, in which air pollution lead visibly public health, levels of urban air pollution decreased (Kelly et al. 2009; Schwartz 1994). However several studies conducted in cities of the North America and Europe indicate, even now, that particulate air pollution, especially fine particulate, is linked to increased morbidity and mortality (Peacock et al. 2011; Solomon 2010). The observable health effects changed from acute to chronic and chronic degenerative (Ito et al. 2010). The nature and the sources of the air pollutions is widely different today in Europe from 1950s. For example oxygen disulphides are broadly reduced (Gulliver et al. 2011) while nitrogen oxides are not under control in numerous urban environment (Hesterberg et al. 2009; Kelly et al. 2009) and particulate matter becomes even finer, so the scientific focus moves over Total Suspended Particles (TSP) to PM₁₀ (with an aerodynamic diameter below 10 μm), to PM_{2.5} (with an aerodynamic diameter below 2.5 μm) and also to Ultra Fine Particles (UFP, with an aerodynamic diameter below 0.1 μm) (Politis et al. 2008). The PM_{2.5} can be directly emitted from sources such as forest fires, or it can form when gases emitted from power plants, industries and auto mobiles, react in the air. It is able to overtake the alveolar region and from here to diffuse through the blood flow into the whole organism. The PM_{2.5} pollution ranged widely around the world, from 15 $\mu\text{g}/\text{m}^3$ yearly mean level, recorded in place that can be considered background site (Shrestha et al. 2010), to above 90 $\mu\text{g}/\text{m}^3$ recorded in the Asiatic mega-cities (Chang et al. 2009).

In Europe the PM_{2.5} annual mean level is around 25 $\mu\text{g}/\text{m}^3$. In various Nations there were annual values above the 35 $\mu\text{g}/\text{m}^3$ such as Bosnia and Herzegovina, Italy, Czech Republic, Poland (EEA, 2007). These values were generally exceeded in particular urban environment, where the orography and the meteorological characteristics are involved in the determination of an higher pollution (Nemery et al. 2001; Traversi et al. 2008).

The decrease in life expectancy due to PM_{2.5} has been assessed for Europe using accepted relationships between mortality rates and the modelled annual average PM_{2.5} concentrations (WHO, 2006). The loss of life expectancy assessments for the Padana Plain region ranged from 9-12 months in 2000 to the 4-6 months in 2020 (EEA, 2010). A portion of this loss is due to lung cancer (Tainio et al. 2007). The epidemiological relationship between lung cancer and PM pollution is widely debated (Vineis and Husgafvel-Pursiainen 2005), and various studies have discussed the significance of this kind of correlation (Brunekreef et al. 2009; Cohen 2000; Katanoda et al. 2011). Borderline results are justified by the complexity of the factors involved in this environmental problem (Papathomas et al. 2011). On the other hand, a large amount of evidence indicates the genotoxic (Buschini et al. 2001; Kouassi et al.

2010; Topinka et al. 2011) and mutagenic (Claxton et al. 2004; Claxton and Woodall 2007; Gilli et al. 2007 a) properties of the PM_{2.5} mixture. These seem to be mainly associated with the organic compounds contained in the mixture (Claxton et al. 2004; Gutierrez-Castillo et al. 2006). With regard to the mutagenic properties, mainly evaluated with the Ames test, some compounds seem to predominate the toxic burden, including nitro-PAHs (Pereira et al. 2010). Turin is one of the most PM_{2.5} polluted cities in the south of Europe, as other cities placed in the north Italy inside or near the Padana Plain (EEA, 2012). The actual relevant sources of PM_{2.5} are traffic and building-heating (TNO, 2007). It is observable that, during the last 30 years, various historic industrial activities were moved out from the urban territory so the major PM_{2.5} emission sources are not industrial combustion. Additionally a high PM_{2.5}/PM₁₀ ratio was observed during the years, equal to around 79% with a PM_{2.5} mean value from the 2000 to the 2010 of around 65 µg/m³ (Provincia di Torino, 2010). In this polluted contest, after the collaboration within a multi-centres project (Hazenkamp-von Arx et al. 2003), we continued the sampling activity for various years. This work evaluates the environmental PM_{2.5} pollution in Turin during a seven-year period before the publication of the new European Regulation (2008/50/CE) and its Italian introduction (D. Lgs. 155, 30/07/2010). Moreover, the mutagenic properties of PM_{2.5} organic extracts were determined monthly. The air levels and mutagenic properties of the PM_{2.5} are discussed to preview additional human health effects.

2. MATERIALS AND METHODS

2.1. PM_{2.5} Sampling and Gravimetric Analysis

Our equipment included a Basel PM_{2.5} Sampler from BGI (BGI incorporated US) and the sampling procedures were previously detailed (Hazenkamp-von Arx et al. 2003). The sampler and rigid tripod were located in south-east Turin. From 2002 to 2006, a traffic place was chosen, and from 2007 to 2008, an analogue meteorological and chemical station of the Environmental Protection Regional Agency (Piedmont A.R.P.A.) was chosen. The change of sampling station was due to the closure of the first station. The second sampling point is also located south of Turin not far from the first sampling point; the second site, Lingotto, is located outdoors near a small green area (also near the first sampling station there was a green area) within an enclosure zone classified and it is also classified as urban station. The figure 1 showed the first and successive sampler positions on the urban territory. Turin has a population density of 7,000 inhabitants per km², so the impact correlated to human activity on the territory is very high (ISTAT, 2008).

Moreover, the topographical characteristics of the area contribute to the critical air pollution (Hazenkamp-Von Arx et al. 2004). Turin is surrounded by the Alps to the West and North, and to the East, there are hills. Only a part of the Southern territory connects without a significant headland to the Padana Plain. The pump was programmed and calibrated to 16.67 L/min at 20 °C and 1013 mbar. We used Teflon filters (47 mm, 2 mm pore size, by Pall).

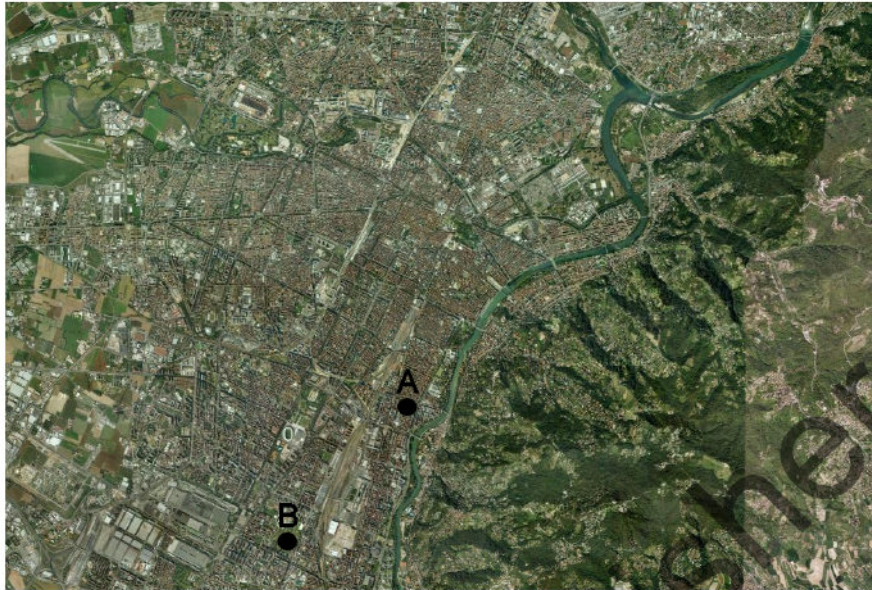


Figure 1. Map of the Turin's urban territory on which the sampler A and B positions are indicated.

The sample period started in January 2002 and lasted 84 months; seven full years were analysed. PM_{2.5} sampling was conducted over 24- and 48-hours periods on weekdays and weekends, respectively. The time always started at midnight on a Monday, and measurements followed on Wednesday, Friday, and in the successive week on Tuesday, Thursday, the weekend and, during the following week.

This procedure resulted in 6 samples per month, representing 8 days (48 hours) of measurement distributed over a two-week period. From these filters, a monthly mean concentration was calculated by accounting for the effective sampling time of each pump. The filters were conditioned for 48 hours and were weighted with an analytical balance ($\pm 10 \mu\text{g}$) before and after sampling to calculate the PM mass concentration.

The procedures were conducted according to the European Committee for Standardization (CEN, 1998), as previously described (Gilli et al. 2007 b).

2.2. Extractions and Biological Assays

Daily filters were pooled to obtain one monthly sample. Extractions of each pooled sample were performed with a Soxhlet apparatus for at least 80 cycles with acetone. Subsequent evaporation was induced by a Rotavapor instrument, and the re-suspension of the sample was performed with dimethyl-3-sulfoxide (DMSO) to obtain an equivalent concentration of $0.1 \text{ m}^3/\mu\text{l}$. The mutagenicity assay was executed according to Maron and Ames (1983). The slope of the dose-response curve (revertants/ m^3) was calculated by the least squares linear regression from the first linear portion of the dose-response curve (Gilli et al. 2007 b). All experiments were done in triplicate with at least three doses. The results were expressed as total revertants minus spontaneous revertants to obtain net revertants per cubic meter (rev/m^3), and they were calculated by the dose-response curve (Buschini et al. 2001;

Cassoni et al. 2004; Claxton et al. 2004). The ratio between net revertants and spontaneous revertants was calculated for each test (Mutagenicity Ratio, MR) (Gilli et al. 2007 a). The MR indicates weak mutagenicity with a value of 1.5-3, sufficient mutagenicity for values in the range of 3-5 and high mutagenicity for values above 5 (Horn et al. 1983; Roller and Aufderheide 2008).

The mutagenic activity of airborne particulate extracts was studied using the TA98 *Salmonella typhimurium* strain. The spontaneous revertants obtained during 42 bioassay sessions ranged from 13 to 20 (18±2) for TA98. The genotype of each tester strain was routinely confirmed, and in each assay session, positive and negative controls were included. In each assay, 2-nitrofluorene (1 µg/plate) was tested as a known mutagen positive control.

Generally more than one strain is necessary for mutagenicity evaluation and moreover also metabolic activation is employed, but we hadn't the possibility to apply always during the time these requirements. We included only the TA98 *Salmonella typhimurium* results because these is the unique homogeneous and continuous data over the 7 years period.

Moreover this strain showed a good sensibility to the mutagens collected in this kind of urban environment (Gilli et al. 2007 a) even if its response is not exhaustive of the whole mutagenicity.

2.3. Statistics

Statistical analyses were performed using the SPSS Package, version 17.0, and R version 2.13. In particular, the Spearman rank order correlation coefficient was used to assess relationships between variables. A Mann-Whitney's U test was used to compare means, and a test of Kruskal-Wallis was used for multivariate analysis. Both PM_{2.5} gravimetric and TA98 mutagenicity time series were decomposed into trend, seasonal and irregular components using a local polynomial regression model (LOESS). The mean differences and correlations were considered significant at $p < 0.05$ and highly significant at $p < 0.01$. Various cited data are downloaded from Ariaweb data-base, a Regional on-line system to share the monitoring data produced by the Environmental Protection Agency.

3. RESULTS AND DISCUSSION

Table 1 displays a descriptive analysis of the total collected data. The PM_{2.5} pollution level in the outdoor air is very variable, and the mean of all the recorded values is nearly 40 µg/m³, far above the WHO annual air quality value. Approximately 6 months during the monitoring period presented mean values over 60 µg/m³, almost double the WHO suggested maximum daily mean level of 35 µg/m³ (Krzyzanowski 2008). These data were comparable to those obtained in other north Italian cities, such as Milan, where the PM_{2.5} ranged between 7.8 and 133.4 µg/m³ (Lonati et al. 2008); other studies showed a comparable PM_{2.5} pollution situations to the Padana Plain (Traversi et al. 2008). In the largest central Italian cities, the levels decreased; for example, Rome presented PM_{2.5} levels ranged between 10 and 65 µg/m³ (Cattani et al. 2010). The Spanish cities, such as Barcelona, presented minor levels of contamination analogous to the cities of southern Italy (Pey et al. 2008).

Table 1. Descriptive analysis of the collected data at the two sampling stations

	Number of samples	Min	Max	Mean	Standard Deviation
TA98 net revertants/m ³	84	0	128	32	32
PM2.5 µg/m ³	84	3.1	139.1	38.3	23.0

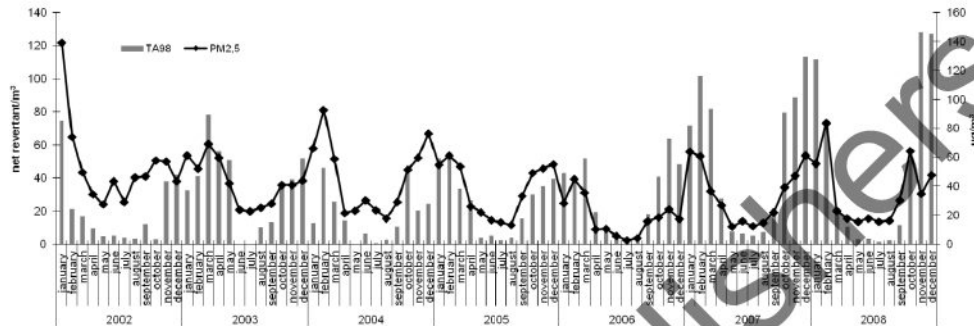


Figure 2. Mutagenicity evaluations and airborne levels of PM2.5 measured during the study period.

The PM2.5 levels observed in northern Italy were comparable to East European cities (Pastuszka et al. 2010), and in northern Europe, the PM2.5 pollution is less relevant. In the atmosphere of the UK, the annual mean levels are nearly 11 µg/m³ (Harrison and Yin 2010).

The measured mutagenicity is comparable to other European evaluations and less than the mutagenicity recorded in South America and Asia (Claxton et al. 2004; Claxton and Woodall 2007). On the other hand, the PM2.5 mutagenicity is higher than the PM10 mutagenicity recorded in the same city (Gilli et al. 2007 c), as also confirmed in the literature (Claxton et al. 2004). Figure 2 shows the collected PM2.5 pollution data and its evaluated mutagenicity. A marked seasonal trend both for gravimetry and mutagenicity is distinguishable. Multivariate non parametric model of gravimetric data by month is significant ($p < 0.01$). Moreover, also the multivariate model of mutagenic data by month is significant ($p < 0.01$). These seasonal variations are widely confirmed in the literature and are probably due to the greater numbers of emission sources in winter, such as heating combustion, and to the meteorological conditions favourable to thermal inversion phenomena (Cattani et al. 2010). The PM2.5 level and its mutagenic properties are directly and significantly correlated (Spearman's $\rho = 0.637$; $p < 0.01$), as clearly showed in Figure 3. This correlation is due mainly to the seasonal variations both of the PM2.5 levels and its mutagenic properties. Moreover the analysis of the temporal variation living off the seasonal effect, during the years, showed divergent trends. Figure 4 illustrates the measured annual means with a trend summarizing line for gravimetric data (A) and for mutagenicity data (B). Different variations are clearly distinguishable over time. The data analysis considering a potential site-effect was conducted but no influence was recognized. The levels of the aero-dispersed PM2.5 in the Turin environment decreased almost constantly (with the exception of 2004 and the years after 2006). Some innovations realised in Turin produced a positive effect on air quality, and particulate pollution decrement.

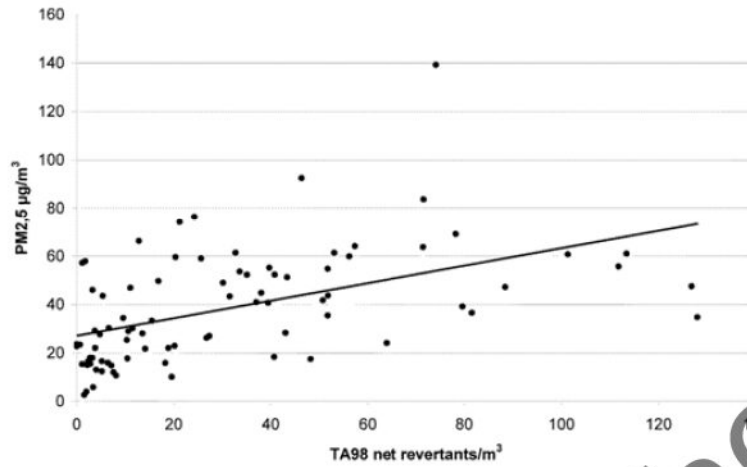
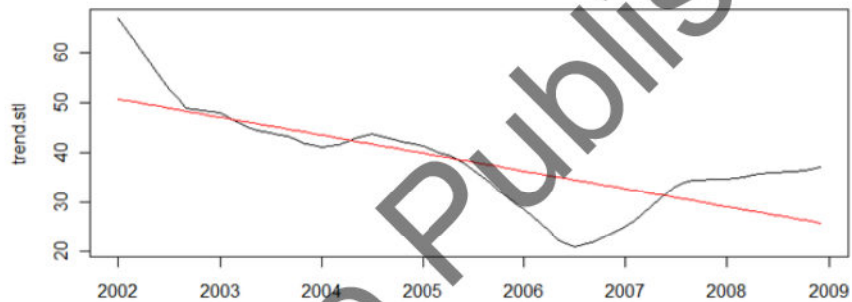
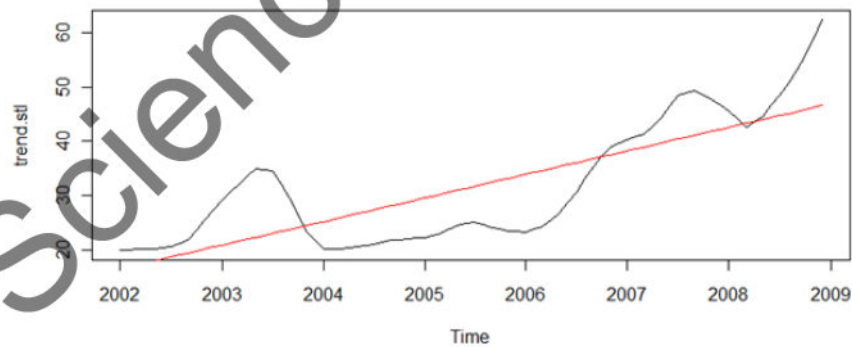


Figure 3. Correlation between mutagenicity and levels of PM_{2.5}. Spearman's $\rho = 0.637$ ($p < 0.01$).



a)



b)

Figure 4. Secular trends calculated using a Loess smoother for PM_{2.5} gravimetry (A) and TA98 mutagenicity (B). The decomposed linear trend for each time series was over-imposed (red lines).

For example, a centralised tele-heating system operated and cover the majority of the urban growth, and an underground transport system is significantly reducing the traffic burden (PUMS, 2008).

Table 2. Number of monthly sample classified in base of the mutagenicity evaluation by year

Mutagenic evaluation	2002	2003	2004	2005	2006	2007	2008
No effect (ratio <1.5)	9	3	9	5	6	5	7
Weak (1.5 <ratio < 3)	2	7	3	7	5	1	0
Significant (3 <ratio < 5)	1	2	0	0	1	4	2
High (5 <ratio)	0	0	0	0	0	2	3

A same decline is confirmed from other series of published data (EEA, 2010), and it is higher than 40%. The PM_{2.5} air quality 2000-2009 national trend, published in the US and based on 724 cities, also showed a 27% PM_{2.5} decrease (EPA, 2011).

For the mutagenicity, we can observe a quite different variation, even an increase. The table 2 showed the samples classification according to the Mutagenicity Ratio criteria. During the time a shift toward appreciable mutagenicity was observed. Turin had a weak mutagenic burden during 2002, and then mutagens present in the PM_{2.5} mixture increased and worsened the mutagenic air quality. Very few data were collected in Turin's environment to describe the PM_{2.5} composition. Today after the introduction of the 2008 European Directive, various contaminants are determining on the PM_{2.5}. Meanly during the year benzo(a)anthracene represented the $1.56 \cdot 10^{-5}$ part in mass of the total PM_{2.5}, the indeno(1,2,3-cd)pyrene the $1.43 \cdot 10^{-5}$, benzo(a)pyrene the $1.26 \cdot 10^{-5}$, the benzo(b)fluoranthene, benzo(j)fluoranthene and benzo(k)fluoranthene the $3.13 \cdot 10^{-5}$, while among the metal contributions we observed the lead ($2.2 \cdot 10^{-4}$ part in mass of the measured PM_{2.5}), nickel ($9.08 \cdot 10^{-5}$), arsenic ($2.09 \cdot 10^{-5}$) and cadmium ($5.52 \cdot 10^{-6}$) (ariaweb).

Consequently the 99.97% of the PM_{2.5} mass has not a specific chemical identity and not even mutagenicity characterization.

On the other hands this is generally true also around the world (Claxton et al. 2004).

Other air pollution indicator such as particularly the nitrogen dioxides and the benzo(a)pyrene didn't show in Turin a decrement but a substantial constancy during the time. This could indirectly shows a relevant presence of nitro-coumpounds and aromatic compounds in general. Other studies showed a dominant and marked role of the nitro-compounds action, as direct mutagens, on this experimental system (Traversi et al. 2011). The level of the mutagenic activities recorded during this study is comparable to data obtained in other European countries and published in the literature (Claxton and Woodall 2007; Du Four et al. 2005). These kinds of biological effect determinations are rare for the PM_{2.5} mixture; at the moment, no published time series data exists that is long enough or suitable for an exhaustive comparison with the showed results.

CONCLUSION

During the last few years, after the publication of the EU Regulation (2008/50/CE), the member States were required to align their own regulations, and Italy pursued this action by publishing the D. Lgs. 155 on July 30, 2010. These documents contained a specific regulation for PM_{2.5} and PM₁₀. Starting in 2015, the PM_{2.5} annual mean must equal $25 \mu\text{g}/\text{m}^3$. This

level was not attained in any monitoring year during the study in Turin, but a constant decrease of the annual level was observed. If the decrease calculated by applying a linear regression model to the data in this study will continue, a 7% decline in urban PM_{2.5} concentration every year will occur, and the limit will definitely be reached by 2015.

We observed a different trend for the mutagenic properties of the PM_{2.5} organic extract; the linear regression of all obtained data shows an increment of nearly 20% each year. Less particulate occurs, but more mutagens appear in the air.

In general the Italian emission inventory showed a general decline for various pollution indicators from 2000 to 2007, including SO_x (-55%), NO_x (-20%), CO (-31%), PM₁₀ (-16%) and PM_{2.5} (-20%). But contemporaneously wide variation in the emission sources exists; for example, the decline is more significant for combustion in energy and transformation industries and for road transport, whereas there is an increase in the non-industrial combustion plant emissions and waste treatment and disposal sources (ISPRA, 2009). It was demonstrated that the variation in the emission sources determines the different toxic properties of a mixture such as PM_{2.5} (Cavanagh et al. 2009). Then - looking at the same emission inventory - other pollutants such as polycyclic aromatic hydrocarbons increased during the same time (+21%), and still other emergent chemicals such as dioxins, hexachlorobenzene and polychlorinated biphenyl remained quite constant without a clear time dependency (ISPRA, 2009). Many of these compounds are known mutagens and carcinogens (IARC, 1998), and their nitro derivatives stand out as the most frequent and most relevant aero-dispersed mutagens and carcinogens in the urban environment (Pereira et al. 2010; Traversi et al. 2009). Today the incidence rate of the lung cancer in males equals 75.3 per 100,000 in the Europe, 84.7 in Italy and 90 in Turin (Ferlay et al. 2007; AIRTUM 2008). Evaluating what will happen in the next long-term period remains impossible because the future lung cancer incidence in Turin will be influenced by numerous previous exposure factors. Tobacco smoking remains the first known risk factor for the lung cancer, with a latency time estimated at thirty years (Weiss 1997) on the other hands we have, in the next years, to control the lung cancer incidence also with other - today minor - risk factors such as PM_{2.5} pollution.

According to this point of view an approach on a biological model, such as that applied in this study may assess the mutagenic and genotoxic risk today. The properties of this biological kind of determinations to evaluate mutagenicity as relevant aspect of the toxicity is widely discussed and proved (Kirkland et al. 2011) moreover the collection of multi test evaluation during the time on the PM_{2.5} organic extract is auspicious.

On the other hands the arguments of this study assert that very little current and suitable information exists concerning the mutagens and carcinogens present in the air. An improvement in this direction isn't granted by PM_{2.5} mixture reduction, rather it could be the opposite, so specific research on this field is crucial.

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