MUTAGENIC/GENOTOXIC EFFECT OF $PM_{0.5}$ COLLECTED IN FIVE ITALIAN TOWNS IN TWO SEASONS: RESULTS OF THE MAPEC STUDY

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INTRODUCTION

PM can be considered as the atmospheric pollutant that mostly affects human health. The International Agency for Research on Cancer (IARC) has recently classified air pollution and fine PM as carcinogenic to human (1 Group). Different studies showed that PM induces several kinds of adverse cellular effects as cytotoxicity, mutagenicity, DNA damage and stimulation of cytokine production.

MAPEC STUDY: AIM

- Evaluate the associations between air pollution (in particular PM) and biomarkers of early biological effects in oral mucosa cells of 6-8 year old children recruited from first grade schools
- Propose a model for estimating the global risk of early biological effects due to air pollutants and other factors in school children

STUDY PURPOSE

- Evaluate children exposure to urban air pollution ($PM_{0.5}$) in 5 Italian towns characterized by different levels of airborne PM
- Investigate the mutagenic and genotoxic effects of $PM_{0.5}$ samples

MATERIALS AND METHODS

- PM_{10} with a HiVol multistage cascade impactor (72 h) in the school area, during biological sampling.

Different fractions: 10.0-7.2, 7.2-3.0, 3.0-1.5, 1.5-0.95, 0.95-0.49, and <0.5 µm

- 2 season (winter 2014 and spring-summer 2015)
- 5 Italian towns (2-4 schools for each town)

TORINO (3 schools: TO1-TO2-TO3)
BRESCIA (4 schools: BS1-BS2-BS3-BS4)
PISA (4 schools: PI1-PI2-PI3-PI4)
PERUGIA (4 schools: PG1-PG2-PG3-PG4)
LECCE (3 schools: LE1-LE2-LE3)

✓ Gravimetric analysis
✓ Sample extraction ($PM_{0.5}$) (Soxlhet)

→ CHEMICAL ANALYSES (PAHs, Nitro-PAHs…)

→ BIOLOGICAL ANALYSES

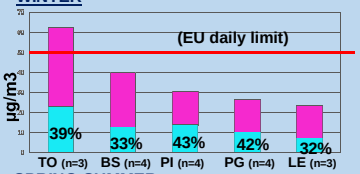
COMET ASSAY (+/-Fpg)
MICRONUCLEUS TEST
CITOTOXICITY TEST
Human cells
- A549

AMES TEST
S.typhimurium
-Strain TA 98
-Strain TA 100 (+/- S9)
-Strain TA 98NR
-Strain YG1021

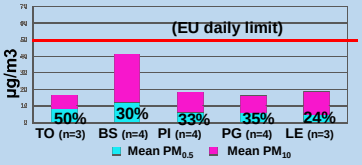
RESULTS AND DISCUSSION

GRAVIMETRIC ANALYSIS

WINTER



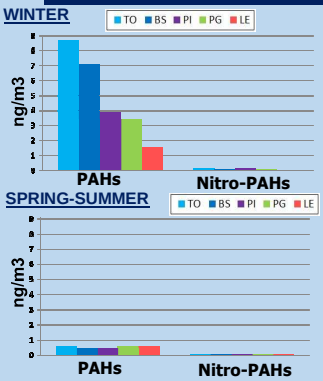
SPRING-SUMMER



- $PM_{0.5}$ represents a very variable percentage of PM_{10}:
  Winter 19,6-63%
  Spring-Summer 9,9-55,9%
- PM_{10} concentration → generally lower than 50 µg/m³
- The highest values of PM_{10} were observed in the towns of the Padania Plain (Torino and Brescia) in winter
- Lower values of PM_{10} and $PM_{0.5}$ in spring-summer

CHEMICAL ANALYSIS

WINTER



SPRING-SUMMER

- The highest values of PAHs were found in $PM_{0.5}$ sampled in Torino, Brescia and Pisa (in particular BS2, TO1 and PI3)
- The highest values of Nitro-PAHs were found in $PM_{0.5}$ sampled in Pisa and Torino (in particular PI3 and TO2)
- PAHs and Nitro-PAHs were lower in spring-summer with values comparable among the towns

AMES TEST

Net revertants/m³

SITE	-S9 TA100 W	S	TA98 W	S	TA98NR W	S	YG1021 W	S	+S9 TA100 W	S	TA98 W	S	TA98NR W	S	YG1021 W	S
BRESCIA																
BS 1	-	-	0.5	-	-	-	7.7	0.8	-	-	-	-	-	-	12.9	0.7
BS 2	-	-	0.4	-	-	-	10.7	1.8	-	-	0.9	-	-	-	16.8	2.5
BS 3	-	-	-	-	-	-	9.7	0.9	-	-	0.6	-	-	-	14.6	1.1
BS 4	-	-	0.6	-	-	-	7.6	0.8	-	-	1.0	-	-	-	20.0	1.0
TORINO																
TO 1	4.8	-	1.9	-	1.0	-	90.8	1.7	-	-	1.5	-	0.9	-	94.9	1.6
TO 2	3.0	-	1.5	-	1.2	-	16.5	2.3	-	-	1.9	-	0.9	-	35.8	1.5
TO 3	-	-	0.9	-	0.5	-	17.7	0.7	-	-	1.0	-	0.7	-	36.6	0.8
PISA																
PI 1	-	-	-	-	-	-	1.9	0.9	-	-	-	-	-	-	3.0	1.0
PI 2	-	-	-	-	-	-	2.9	0.4	-	-	0.7	-	-	-	7.0	0.6
PI 3	-	-	-	-	-	-	7.4	2.3	-	-	0.9	-	-	-	14.3	3.5
PI 4	-	-	0.8	-	-	-	5.8	1.0	-	-	0.8	-	-	-	19.8	0.9
PERUGIA																
PG 1	-	-	0.5	-	-	-	7.2	7.1	-	-	0.9	-	-	-	15.4	1.5
PG 2	-	-	0.3	-	-	-	7.1	0.6	-	-	0.6	-	-	-	17.8	17.8
PG 3	-	-	-	-	-	-	3.0	0.8	-	-	-	-	-	-	7.2	7.2
PG 4	-	-	0.4	-	-	-	3.4	0.4	-	-	-	-	-	-	10.1	0.1
LECCE																
LE 1	-	-	0.4	-	-	-	1.7	1.7	-	-	-	-	-	-	4.8	4.7
LE 2	-	-	0.5	-	0.4	-	4.5	4.5	-	-	0.6	-	-	-	8.2	8.2
LE 3	-	-	-	-	-	-	1.4	1.4	-	-	-	-	-	-	2.5	2.5

WINTER

TA100
- Mutagenic effect in TO1,TO2 samples

TA98
- All samples with at least one mutagenic dose
- Low mutagenic effects
- The highest effect in TO1 and TO2

Slight increase +S9 → presence of indirect mutagens (PAHs)

TA98NR
- Slight decrease of the effect in BS1, BS2, BS4, TO1, TO2, TO3, PI4, PG1, PG2, PG4, LE1, LE2 samples
→ presence of nitroaromatic compounds (Nitro-PAHs)

YG1021
- All sample showed mutagenic effect
- The highest effect in TO1, TO2 and TO3
→ presence of nitroaromatic compounds (Nitro-PAHs)

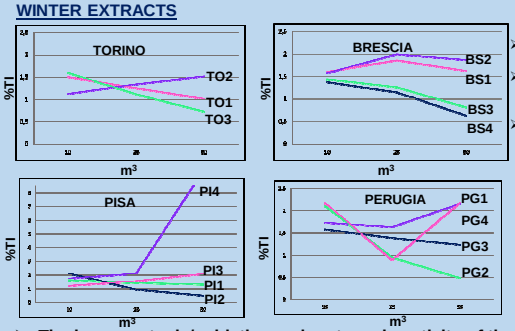
SPRING-SUMMER

Low mutagenic effect (no response with TA100,TA98 and TA98NR)

YG1021
- All sample showed mutagenic effect
- Values lower than winter samples

COMET ASSAY

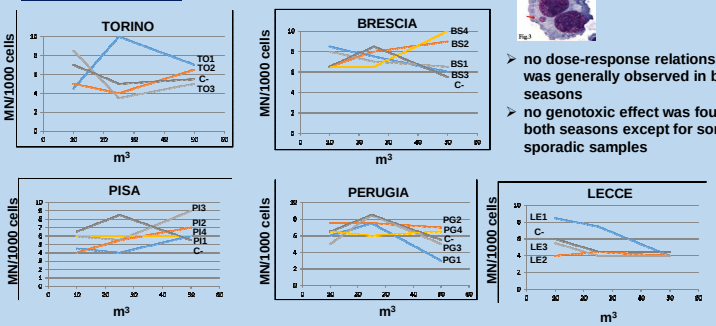
WINTER EXTRACTS



- no dose-response relationship was generally observed in both seasons
- no genotoxic effect was found in both seasons except for some sporadic samples
- the Fpg treatment did not increase the genotoxic effect → no oxidative activity of the samples analysed in both seasons

MICRONUCLEUS TEST

WINTER EXTRACTS



- no dose-response relationship was generally observed in both seasons
- no genotoxic effect was found in both seasons except for some sporadic samples

- The low genotoxic/oxidative and mutagenic activity of the $PM_{0.5}$ winter extracts could be related to the low level of air pollution observed in this winter sampling associated to a high atmospheric instability.
- The low level of mutagenic effect of the $PM_{0.5}$ extracts in summer-spring could be related to the lower chemical contamination observed in the samples

CONCLUSIONS

- The high variability of $PM_{0.5}$ observed in this study should be more investigated.
- For a greater understanding of the relationship between PM size, composition and biological effects, the results obtained in this study suggest to investigate also the biological effect of the other PM fractions and in particular of the $PM_{0.5-1}$ fraction.