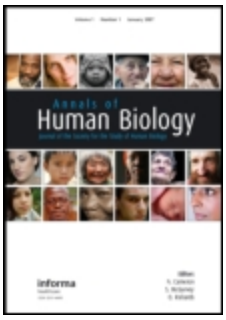
Micronuclei frequency in peripheral blood lymphocytes of healthy subjects living in Turin (North-Italy): contribution of body mass index, age and sex.



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Abstract

Background: Increased micronuclei (MNi) frequencies in human lymphocytes are an indicator of chromosome instability and could be influenced by different exogenous and endogenous factors. The increased exposure to environmental pollutants has led to the awareness of the necessity for constant monitoring of urban human populations.

Aim: We evaluated the MNi frequency in a sample belonging to the non-occupationally exposed population of Turin (North-Western Italy). A possible effect of body mass index, age and sex on the genomic damage levels was also investigated.

Subjects and Methods: The study included 150 subjects. MNi, nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs) were scored in 1,000 lymphocytes per subject.

Results: The MNi, NPBs and NBUDs average frequencies (‰ ± S.D.) were 7.19±2.51, 1.65±1.54 and 2.07±1.76, respectively. Turin shows one of the highest MNi frequencies with respect to other Italian cities and European regions. A significant correlation was found between MNi, NPBs, NBUDs frequencies, age and body mass index.

Conclusion: Baseline MNi frequency was established in a sample of a city, like Turin, exposed to high levels of environmental pollutants. We hope that the results of this study can be used as a stimulus for future biomonitoring programs in other Italian and globally distributed cities.

Key words: environmental pollution; Italian population; genomic damage, genotoxicology

Introduction

Human populations are increasingly exposed to a variety of environmental pollutants that may increase the genomic instability and may affect the baseline values of cytogenetic markers. Populations living in urban areas resulted particularly exposed, principally due to the increase of pollutants generated by industrial activities and motor vehicle exhausts (Liu et al. 2019; Hankey and Marshall 2017; Brugha and Grigg 2014). Moreover, the rise of population and a consumer-oriented economic approach have contributed to increasing atmospheric contamination with harmful substances. The consequence of this negative synergy is that about 90% of European citizens residing in urban areas are exposed to air pollution exceeding EU limit values (EC 2008) and WHO guideline levels (Ceretti et al. 2014; WHO 2006, 2007; EAA 2012).

One of the objectives for public health is to construct systems that allow the biomonitoring of the health status of people living in urban areas and, in general, of the biome. In this sense, biomarkers such as DNA and/or protein adducts, and cytogenetic alterations (e.g., micronuclei, chromosomal aberrations), may help in evaluation of genomic damage and facilitate health risk assessments.

Cytokinesis-block micronucleus (CBMN) cytome is one of the most used assay for the assessment of the level of genomic damage in various human populations (Villarini et al. 2018; Gajski et al. 2018; Nefic and Handzic 2013; Landi et al. 2000). Evaluation of spontaneous MNi frequency can also be useful in order to determine that background level of genomic damage that represents an important comparison value when spontaneous MNi frequency for exposed individuals is not known prior to exposure (Nefic and Handzic 2013).

MNi represent small extranuclear bodies which have not been included in the daughter nuclei at telophase. They may arise from chromosome breakage or if a whole chromosome lags behind at anaphase and fails to be incorporated in the new nuclei (Fenech et al. 2011). Baseline MNi frequencies in cultured human lymphocytes represent accumulated genetic damage appearing during the lifespan of the circulating lymphocytes. However, increased levels of MNi in PBL have been associated prospectively with increased risk of cancer, cardiovascular and neurodegenerative diseases (Bonassi et al. 2011).

MNi do not represent just the products of biological errors, but, recently, other biological effects were associated to their formation. In particular, exposing fragmented DNA to the cytoplasm, MNi triggers the activation of immunity system related genes, suggesting that the presence of MNi can be sensed by the same immunity system (Gekara 2017).
Moreover, MNi also represent a mechanism of elimination of genetic material, such as amplified genes, and contribute to nuclear dynamics and genome chaos (Heng 2019; Ye 2019). This last represents a process of rapid genome re-organization that results in the formation of chaotic genomes, some of which could be selected to establish stable genomes.

Chromosomal instability was also measured by scoring nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs). NPBs represent dicentric chromosomes or the result of a defective separation of sister chromatids at anaphase, whereas NBUDs represent the process of elimination of amplified DNA and/or excess chromosomes from aneuploid cells (Fenech et al. 2011).

The aim of the present study was to evaluate the MNi frequency, as a biomarker of DNA damage, in peripheral blood lymphocytes (PBL) of non-occupationally exposed subjects

living in Turin (North-West Italy), a city characterized by high levels of air pollution and for which no data are present in literature about background frequency of micronuclei.

Finally, it is known that diet, age and sex may influence genome integrity (Fenech et al. 2003; Nefic and Handzic 2013). Based on these assumptions, we also decided to evaluate the incidence of body mass index, age and sex on the frequency of micronuclei.

Materials and Methods

Population sample

The study population included 150 blood donors randomly sampled in Turin (Italy), during winter 2017. Individuals selected for this study were subjects without any known exposure to specific xenobiotics, except for those of the routine household and traffic. All subjects were living in the city, away from landfills, and their houses were equipped with modern heating systems. However, in the inner suburbs of the city there is an important automotive factory and numerous other smaller industrial installations that significantly contribute to the air pollution of the urban area.

In order to assess the possible influence of the age and body mass index (BMI) on the level of DNA damage, subjects were split into three age groups (21-30, 31-40, 41-55 age groups) and two BMI groups: normal weight, with BMI ranging between 18.5 and 24.99, and overweight with BMI >25.

It is well known that cigarette smoke, alcohol consumption, drugs and X-rays could alter level of genomic damage. For this reason, we excluded from the sample smokers and individuals who reported alcohol consumption, treatment for acute infections and/or chronic non-infectious diseases, history of cancer and exposure to diagnostic X-rays, for at least one year prior the analysis.

Subjects received detailed information about aims and experimental procedures of the study and gave their informed consent. Volunteers, in healthy conditions when sampling was conducted, were selected and anonymously identified by a numeric code.

The study was approved by the University of Turin ethics committee and was performed in agreement with the ethical standards laid down in the 2013 Declaration of Helsinki.

Blood Sample Collection and Cell Cultures

Blood samples were obtained by venipuncture (5-10 mL) and collected in heparinised tubes. All blood samples were coded, stored at 4°C, and processed within 2 hours after collection. Heparinised venous blood (0.3 mL) were cultured in 25 cm^2 flasks containing 6 mL of RPMI-1640 medium, 2 mL of foetal calf serum (FCS), 200 µL of the mitogenic agent Phytohemagglutinin-L (2.3% v/v), and 100 µL of antibiotics solution (100 IU/mL penicillin, and 100 µg/mL streptomycin), for a total of 8.6 mL for each lymphocyte culture. The cultures were successively incubated at 37°C and under 5% of CO_2 in the air in a humidified atmosphere.

After 44 h of incubation, cytochalasin-B was added to the cultures at a concentration of 6 µg/mL to block cytokinesis.

After 72 hrs (for MNi assay) of incubation at 37°C, the cells were collected by centrifugation and treated for 10 min with a pre-warmed mild hypotonic solution (75 mM KCl). After centrifugation and removal of the supernatant, the cells were fixed with a fresh mixture of methanol/acetic acid (3:1 v/v). The treatment with the fixative was repeated three times. Finally, the supernatant was discarded and the pellet, dissolved in a minimal volume of fixative, was seeded on the slides to detect MNi by conventional staining with 5% Giemsa (pH 6.8) prepared in Sörensen buffer.

Microscope analysis was performed at 400x magnification on a light microscope (Dialux 20, Leitz, Germany). MNi, nucleoplasmic bridges (NPB) and nuclear buds (NBUD) were scored in 1,000 binucleated lymphocytes with well-preserved cytoplasm per subject. A total of 1,000 lymphocytes per donor were scored to evaluate the percentage of cells with 1-4 nuclei. The cytokinesis-block proliferation index (CBPI) was calculated, according to the following formula: $[1 \times N1] + [2 \times N2] + [3 \times (N3 + N4)]/N$, where N1-N4 represents the number of cells with 1-4 nuclei, respectively, and N is the total number of cells scored.

Statistical analysis

Statistical analyses were conducted using the SPSS software statistical package programme (version 24.0, Inc., Chicago, Illinois, USA).

Differences between sexes, age and BMI groups were evaluated by non-parametric Wilcoxon Mann-Whitney *U* test and regression analysis. Differences between Turin and other Italian and European populations were evaluated by t-test of the differences between two means.

All P-values were two tailed and the level of statistical significance was set at $P<0.05$ for all tests.

Results

General characteristics of the studied population

The demographic and genotypic features of the investigated urban population are reported in Table 1. Sixty-six subjects were males, with mean age of 32.71±8.94, Body Height (BH) of 1.72±0.08, Body Mass (BM) of 69.21±10.25 and BMI of 23.41±2.86, whereas 84 were females, with mean age of 29.00±8.72, BH of 1.70±0.09, BM of 66.61±11.28 and BMI of 22.95±2.92). The age of the individuals included in the total sample ranged from 21 to 55

years, with a mean (±S.D.) of 30.63 (±8.979).

The total sample was split into three groups according to the age of the subjects: 21-30 (n = 99, with mean age of 25.12±2.74, BH of 1.69±0.08, BM of 64.90±10.30 and BMI of 22.72±2.90); 31-40 (n = 28, with mean age of 35.79±2.54, BH of 1.73±0.08, BM of 73.04±10.60 and BMI of 24.32±2.93); and 41-55 (n = 23, with mean age of 48.09±3.97, BH of 1.76±0.07, BM of 73.61±9.14 and BMI of 23.61±2.43).

Finally, total mean BH, BM and BMI were 1.71±0.08 m, 67.75±10.88 kg and 23.13±2.91 kg/m^2, respectively. The number of normal weight subjects was 111, with mean age 29.32±8.33, mean BM of 64.34±9.85 kg, mean BH of 1.71±0.08 m and mean BMI 21.83±2.05 kg/m^2. The number of overweight subjects was 39, with a mean age of 34.39±9.79, mean BH of 1.70±0.08 m, mean BM of 77.46±7.28 kg and mean BMI of 26.85±1.36 kg/m^2.

CBMN parameters frequency

Detailed results of the MNi analyses are summarized in Tables 2 and 3. In figure 1 some examples of observed bi-nucleated cells with MNi, NPBs and NBUDs were evident.

A total of 150,000 cells were available for the MNi, NPBs, NBUDs and CBPI analyses, respectively.

The observed MNi, NPBs and NBUDs values (‰) were 7.19±2.51, 1.65±1.54 and 2.07±1.76, respectively, while the mean CBPI value was 1.71±0.08. The regression and non-parametric analyses revealed significant differences ($P<0.001$) in the level of genomic damage between normal weight and overweight subjects, with this last group showing the higher amount of genomic damage. Similarly, significant differences ($P<0.001$) were found between age groups, with the younger group (21-30) showing significantly lower frequencies of all genomic markers with respect to the other two age groups (Tables 2-4). Finally, no

significant differences were found between sexes in the frequency of MNi, NPBs and NBUDs (Tables 2-4).

Comparing our data with those published for other Italian populations (Table 5), we observed for Turin a frequency of micronucleated cells significantly higher ($P<0.001$, t-test) than that reported for Brescia (3.76±2.17, Villarini et al. 2018), Perugia (3.83±1.79, Villarini et al. 2018), Lecce 3.41±1.87, Villarini et al. 2018) and Pisa (3.63±0.08, Landi et al. 2000; 3.83±2.08, Villarini et al. 2018), but significantly lower ($P<0.001$, t-test) with respect to Rome (12.33±5.82, Leopardi et al. 2003).

Similarly, Turin also shows one of the highest MNi frequencies with respect to other European regions, such as Croatia (5.06±3.11, $P<0.001$, t-test, Gajski et al. 2018), Denmark, (2.80±1.90, $P<0.001$, t-test, Look et al. 2014), Crete (2.85±2.15, $P<0.001$, t-test, Look et al. 2014), UK (2.77±2.54, $P<0.001$, t-test, Look et al. 2014), Spain (1.79±1.50, $P<0.001$, t-test, Look et al. 2014) and Norway (1.90±1.46, $P<0.001$, t-test, Look et al. 2014) (Table 5).

Discussion

In their daily activity, people are exposed to a wide variety of environmental pollutants, some of which with known genotoxic properties. In particular, urban air is a complex mixture of many different chemical species (Feretti et al. 2014), most of them with carcinogenic properties (WHO 2013). In this scenario, human biomonitoring programs provide valuable information about environmental exposure and can help to identify potential health risks.

The frequency of micronucleated cells observed in our sample (7.17‰) falls in the range of 3-12‰ established for non-exposed subjects by the Human Micronucleus (HUMN) international collaborative study, that also established a mean value of 6.5‰ (Nefic and Handzic 2013; Bonassi et al. 2001). However, Turin showed a frequency of micronucleated

cells significantly higher than that reported for other Italian tows and European countries (Table 5). A possible explanation of this relative higher incidence of MNi observed in Turin could be that, because of the heavy industrial activity and the significant increase in the number of cars in the last decades, in Turin a high atmospheric contamination by particulate material and nitrogen oxides has been registered (ARPA 2019; Table 6). Moreover, Turin is a city located in the Po river valley, an area where air exchanges are limited by the surrounding mountains, winds are weak, and air pollutants can accumulate easily. As consequence, Turin is one of the most polluted European cities in terms of particulate matters and ozone (WHO 2013), whose mutagenic potential has been suggested in a number of studies (Alves et al. 2016). Prolonged human exposure to high levels of urban pollution was associated with increased risk of cancer, respiratory and cardiovascular diseases, as well as a relationship was found between high levels of MNi in peripheral blood lymphocytes and increase of cancer risk (Bonassi et al. 2011).

However, the observed differences in the level of cytogenetic damage among different cities could be also related to differences in the local environmental conditions, as well as there are many different unpredictable agents that can cause an increase in the background frequency of genetic end-points (Rossner et al. 2013). Indeed, spontaneous MNi can also be induced by a variety of exogenous and endogenous factors, such as genotoxicant compounds in food (Santovito et al. 2012), age of individuals (Cho et al. 2017), genetic susceptibility and individual damage repair capacity (Santovito et al. 2017). In particular, among these factors, sex, age and BMI seem to play a crucial role (Gajski et al. 2018; Cho et al. 2017; Domnez-Altuntas et al. 2014) and were evaluated in the present study.

As for the role of sex, we observed no significant differences between females and males in the frequencies of MNi, NPBs and NBUDs (Tables 2 and 3). This result contrasts with that recently observed by Gajski et al. (2018) with the same cytogenetic biomarker. A

convincing explanation for a possible gender difference in genomic damage levels is still missing. Different genetic history of populations, as well as polymorphisms in damage repair genes may explain, at least in part, these conflicting data.

It is known that age is an important factor to be taken into consideration when designing human population studies. According to a number of studies on age-related genomic damage incidence in control populations (Cho et al. 2017; Bonassi et al. 2001), the results obtained in this study suggest an influence of the age on MNi rates. Indeed, we observed a significant increase of the frequency of MNi, but not of NPBs and NBUDs, in the older groups compared to the younger ones (Tables 2 and 3). These data could be interpreted as a signal of a potential increase of defects in DNA repair processes with age, resulting in a progressive accumulation of genomic damage and consequent increase risk of cancer (Garcia-Sagredo 2008).

Finally, in our sample, a correlation was found between BMI and genomic damage measured in terms of MNi, NPBs and NBUDs (Tables 2-4), with overweight subjects showing significant higher level of these cytogenetic markers with respect to normal weighted individuals. This result confirms data obtained by Domnez-Altuntas et coll (2014), who observed higher frequencies of MNi, NPBs and NBUDs in obese subjects with respect to normal-weight and over-weight subjects. This increase in the frequencies of MNi, NPBs and NBUDs in over-weight with respect to normal-weight subjects could be related to an increase of oxidative stress levels, and consequently to an increase of genomic damage, that some authors observed in obese and over-weight subjects. For example, Elwakkad et al. (2011), observed higher level of serum 8-hydroxy-2'-deoxyguanosine (8-OHdG), an oxidative stress marker, in severely obese adolescents and, in general, a positive correlation between BMI and serum 8-OHdG concentration. Similarly, Scarpato et al (2011) reported significantly increased MNi frequencies in over-weight and obese children with respect to normal-weight children.

Conclusion

With this paper, we aimed to establish the baseline frequency of MNi in a sample of the urban population of Turin that, like all urban populations, is exposed to high levels of environmental pollutants. This type of data assumes a more important connotation if we consider the fact that, in the present study, as well as in other studied conducted on control populations, we analysed subjects not exposed for professional reasons to xenobiotics, but that are simply living in a city, like Turin, with many problems related to urban pollution (Raaschou-Nielsen et al. 2013; Santovito et al. 2016). In this sense, we hope that the results of this study can be used as a stimulus for future biomonitoring programs in other Italian and globally distributed cities. Finally, it should be emphasize that the results of the present work cannot be generalized for all "Caucasians" because this group is heterogeneous, with differences in the distribution of genetic polymorphisms and in life styles among individuals.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1 - General characteristics of the studied population.

Subjects	N (Frequency)	Age (Mean ± S.D.)	BH (m ± S.D)	BM (Kg ± S.D.)	BMI (Kg/m^2± S.D.)
Total	**150**	**30.63±8.98**	**1.71±0.08**	**67.80±10.88**	**23.13±2.91**
Sex					
Males	66 (0.44)	32.71±8.94	1.72±0.08	69.21±10.25	23.41±2.86
Females	84 (0.56)	29.00±8.72	1.70±0.09	66.61±11.28	22.95±2.92
Age groups					
21-30	99 (0.66)	25.12±2.74	1.69±0.08	64.90±10.30	22.72±2.90
31-40	28 (0.19)	35.79±2.54	1.73±0.08	73.04±10.60	24.32±2.93
41-55	23 (0.15)	48.09±3.97	1.76±0.07	73.61±9.14	23.61±2.43
BMI					
Normal weight	111 (0.74)	29.32±8.33	1.71±0.08	64.34±9.85	21.83±2.05
Overweight	39 (0.26)	34.39±9.79	1.70±0.08	77.46±7.28	26.85±1.36

N = number of studied subjects; BH = body height: BM = body mass; BMI = body mass index; S.D. = Standard Deviation

Table 2 – Frequency of MNi in the studied population according to sex, age and BMI.

Groups	N	Cells	MNi (mean± S.D.)	MNC (mean± S.D.)	MNi/BNCs ± S.D. (‰)	MNC/BNCs ± S.D. (‰)	CBPI ± S.D.
Total	**150**	**150,000**	**1,078**	**1,076**	**7.19±2.51**	**7.17±2.47**	**1.71±0.08**
Sex							
Males	66	66,000	479	478	7.26±2.28	7.24±2.25	1.705±0.07
Females	84	84,000	599	598	7.13±2.68	7.12±2.65	1.64±0.15
Age groups							
21-30	99	99,000	614	614	6.20±1.83 *	6.20±1.83 *	1.64±0.14 a,b
31-40	28	28,000	220	220	7.86±1.88 **	7.86±1.88 **	1.70±0.06
41-55	23	23,000	244	242	10.61±2.46	10.52±2.37	1.72±0.05
BMI							
Normal weight	111	111,000	735	734	6.62±2.29 ***	6.61±2.25 ***	1.67±0.13
Overweight	39	38,000	343	342	8.80±2.43	8.77±2.40	1.65±0.12

BMI = Body mass index; BNCs = Binucleated cells; MNi = micronuclei; MNC = cells with 1 or more micronuclei;
CBPI = Cytokinesis-Block Proliferation Index; S.D. = Standard Deviation;

* Significant different with respect to other age groups (P <0.001, Mann-Whitney test)

** Significant different with respect to 41-55 age group (P <0.001, Mann-Whitney test)

*** Significant different with respect to overweight group (P <0.001, Mann-Whitney test)

[a] Significant different with respect to 31-40 age group (P = 0.008, Mann-Whitney test)

[b] Significant different with respect to 41-55 age group (P <0.001, Mann-Whitney test

Table 3 - Frequency of NPBs and NBUDs in the studied population according to sex, age and BMI.

	N	Cells	NPBs	NBUDs	Frequency of BNCs with NPBs ± S.D. (‰)	Frequency of BNCs with NBUDs ± S.D (‰)
Total	**150**	**150,000**	**248**	**310**	**1.65±1.54**	**2.07±1.76**
Sex						
Males	66	66,000	115	154	1.74±1.42	2.33±1.78
Females	84	84,000	133	156	1.58±1.63	1.86±1.72
Age groups						
21-30	99	99,000	111	138	1.12±1.42 *	1.39±1.43 *
31-40	28	28,000	69	87	2.46±1.14	3.11±1.71
41-55	23	23,000	68	85	2.96±1.22	3.70±1.40
BMI						
Normal weight	111	111,000	163	211	1.47±1.57	1.90±1.77
Overweight	39	39,000	85	99	2.18±1.32 **	2.54±1.64 **

BMI = Body Mass Index; NPBs = nucleoplasmic bridges; NBUDs = nuclear buds; S.D. = Standard Deviation;
*P<0.001 (Mann-Whitney test) significantly different with respect to the other two age groups
** P<0.001, (Mann-Whitney test) significantly different with respect to normal-weight group

Table 4. - Multiple regression analysis of confounding factors on MNi, NPBs and NBUDs values in lymphocytes of the study groups.

	MNi frequency			**NPBs frequency**			**NBUDs frequency**		
CF	**β-co**	***P*-value**	**95% CI** (Lower) - (Upper)	**β-co**	***P*-value**	**95% CI** (Lower) - (Upper)	**β-co**	***P*-value**	**95% CI** (Lower) - (Upper)
Sex	-0.14	0.08	(-3.14) - (0.17)	0.08	0.27	(-0.18) - (0.65)	-0.01	0.91	(-0.49) - (0.43)
Age	0.70	<0.001	(0.16) – (0.23)	0.60	<0.001	(0.08) – (0.13)	0.64	<0.001	(0.10) - (0.15)
BMI	0.38	<0.001	(1.32)-(3.03)	0.20	0.01	(0.16)-(1.27)	0.16	0.05	(0.00)-(1.28)

BMI = Body Mass Index; β-co = β-coefficient; CF = Confounding Factor; MNi = micronuclei; NBUBs = nuclear buds; NPBs = Nucleoplasmic bridges

Table 5 – Frequency of MNi observed in some European populations. All *P*-values represent the statistical result of the comparison with Turin.

Country	N	Cells with MNi Frequency±SD	*P*-value (t-test)	References
Southern Europe				
Bosnia-Herzegovina, Sarajevo	100	7.94±8.44	0.293	Nefic & Handzic 2013
Bosnia-Herzegovina, Sarajevo	30	10.97±6.59	<0.001	Ibrulj et al. 2006
Bosnia-Herzegovina, Sarajevo	44	8.25±3.58	0.023	Haveric et al. 2010
Croatia	200	5.06±3.11	<0.001	Gajski et al. 2018
Croatia	200	6.90±3.32	0.403	Kopjar et al. 2010
Greece, Crete	215	2.85±2.15	<0.001	Look et al. 2014
Italy				
Turin	150	7.17±2.47	==	Present Study
Bologna	31	8.00±1.77	0.078	Maffei et al. 2014
Brescia	237	3.76±2.17	<0.001	Villarini et al. 2018
Lecce	213	3.41±1.87	<0.001	Villarini et al. 2018
Perugia	223	3.83±1.79	<0.001	Villarini et al. 2018
Pisa	159	3.83±2.08	<0.001	Villarini et al. 2018
Pisa	1,429	3.63±3.02	<0.001	Landi et al. 2000
Rome	58	12.33±5.82	<0.001	Leopardi et al. 2003
Spain	123	1.79±1.50	<0.001	Look et al. 2014
Yougoslavia	164	8.03±5.38	0.074	Di Giorgio et al. 1994
Northern Europe				
Denmark	117	2.80±1.90	<0.001	Look et al. 2014
Norway	59	1.90±1.46	<0.001	Look et al. 2014
UK	111	2.77±2.54	<0.001	Look et al. 2014
Western Europe				
France, Marseille	200	9.87 ±3.1	<0.001	Milosevic-Djordjevic et al. 2002
Eastern Europe				
Hungary	188	16.00±7.30	<0.001	Köteles et al. 1993
Czech Republic	49	11.29±3.02	<0.001	Rossnerova et al., 2016

Table 6 - List of the mains pollutants, with their concentrations, measured in the year 2017 in the City of Turin. All values represent mean annual values. In bold are reported the values exceeding the limits set by the Italian law.
Data available on: http://www.cittametropolitana.torino.it/cms/risorse/ambiente/dwd/qualita-aria/relazioni-annuali/relazione2017.pdf
Accessed 02-09-2019.

Pollutant	Observed Concentration ($\mu g/m^3$)*	Limits set by the Italian law ($\mu g/m^{3)}$
SO_2	7.50	125
C_6H_6	1.13	5
CO	1.60	10
NO_2	**54**	40
O_3	68	120
PM10	**42**	40
PM2.5	**30**	25

* Average values from different detection stations.

Figure 1 – Examples of bi-nucleated cells with micronuclei (A-B), tri-nucleated cells with micronucleus (C); tetra-nucleated cell with micronuclei (D); bi-nucleated cells with Nuclear Buds (E) and bi-nucleated cells with Nucleoplasmic Bridges (F).