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Genotoxic effects in the buccal cells of students exposed to season-associated increase of air pollution in Prishtina urban area: a preliminary study

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Abstract

The available monitoring data from Kosovo Environmental Protection Agency show concerning levels of the air pollution in Prishtina - the capital city of Kosovo and the most populated city in the country. Due to the air pollutants emitted mostly from the heavy traffic and the coal-fired power plants located in the vicinity, the residents of urban area in Prishtina are exposed to unhealthy air. It is reported that the situation worsens during the autumn and winter months due to more frequent smog episodes. Based on the concerns raised, the aim of the study was to assess the eventual genotoxic effects of air pollution among residents of Prishtina during the autumn and winter months. For this purpose, 29 healthy female non-smoker students (aged 20-26) were involved in this preliminary study. The first sampling of buccal cells took place beginning of November 2019 whereas the second sampling took place by the end of January 2020. Buccal cell samples were analyzed for the frequency of micronuclei and the obtained data demonstrate increased genotoxicity in a sample population as an effect of the exposure to increased air pollution levels in Prishtina urban area during the autumn and winter period. On the other hand, these preliminary data clearly indicate the need for continuing with bio-monitoring studies by extending the timeframe and increasing the number of seasons under investigation.

Keywords

Prishtina, Kosovo, air pollution, buccal cells, micronuclei

Introduction

The actual "*Kosovo Report*" (European Commission, 2023), states that the air quality in Kosovo *"continues to be a major health threat*". While earlier investigations were more focused on industrial sources and areas, more recent reports (World Bank, 2013; World Bank 2019; MESPI, 2021; MESPI, 2022) have emphasized that air pollution is a critical problem also for urban areas in Kosovo. In most of the air monitoring stations in Kosovo, annual average concentrations of particulate matter (PM10 and PM2.5) are exceeded (WB, 2019). The PM10 limit values (for 24 hours) for the protection of human health is 50 μg/m^3 whereas the annual limit values for PM10 and PM2.5 are 40 μg/m^3 and 25 μg/m^3 respectively (MESPI, 2022).

Concerning levels of the air pollution are evidenced in many cities in the Kosovo whereas Prishtina (capital city) is sometimes listed among the most polluted cities in the world (World Bank, 2019). According to the same report, beside the emmision from industrial facilites (Kosovo Electroenergetic Corporation (KEC)) and traffic, the use of solid burning fuels for heating in Prishtina further "contributes" to urban areas to face smog episodes during winter. Geospatial position of Prishtina (in relation to power plants) is reported to have an impact on the pollution levels (Bajcinovci, 2017).

Increased levels of the air pollution (especially PM concentrations) during the winter months (when Prishtina urban area is exposed more often to smog episodes) are reported to be responsible for acute health effects and increased number of the hospital admissions due to cardiovascular and respiratory problems (Ukëhaxhaj et al., 2013; World Bank, 2019; MEI, 2020; Shabani Isenaj et al., 2022).

Recently, there are improvements as regards to the monitoring of several of the pollutants (PM10, PM2.5; CO (Carbon monoxide); O_3 (Ozone); SO_2 (Sulfur Dioxide); NO_2 (Nitrogen dioxide) and the availability of the monitoring data, but the interventions still do not address these issues appropriately. It is known that air pollution is one of the main concerns related to the adverse health effects (Sørensen et al., 2003) therefore, there is a need for monitoring the human exposure in the aim to detect early effects (Anwar, 1994) and assessing the potential human health risks.

Previous researches have investigated the genotoxic effects of poor air quality in industrial zones across Kosovo (Alija et al., 2015; Alija et al., 2016). However, there remains a significant gap in understanding the effects of urban air pollution exposure, a crucial area requiring urgent investigation. Taking into consideration the reported seasonal and annual variation in the emission of the various pollutants in Prishtina, we aimed to perform a preliminary study for assessing the potential genotoxic effects due to the environmental exposure. One of the methods used to assess the genetic damage in many organisms is the Micronucleus assay (Heddle et al., 2011) which, applied to buccal cells as a minimal invasive assessment method is increasingly used to detect the cytogenetic effect due to various exposures, diseases etc., (Holland et al., 2008; Thomas et al., 2009).

Material and methods

Participants

A group of 29 female students from the University of Prishtina (mainly from the Department of Biology) participated in the study. The age was 20 – 26 years. Since most of the students are

originating from different parts of Kosovo, the criteria for inclusion was their plan to stay in urban area of Prishtina (at least 5 days per week) in the forthcoming period (after the first sampling). The stay (at least 5 days/week) was confirmed upon the second sampling. Through this approach, we aimed to assess the genotoxic effect of exposure to the air pollution during the autumn and winter period in Prishtina. Excluding criteria were smoking and any disease known by participants. None of the participants was taking any medication or nutritional supplements up to 40 days before sampling. None of them was having alcohol drinking habits. The investigation was carried out based on the approval issued by the HUCSK (Hospital and University Clinical Service of Kosovo) Ethical Committee. Participation was voluntary and all participants signed a letter of consent.

Buccal cell sampling and slide preparation

The first sampling took place beginning of November 2019 whereas the second sampling took place by the end of January 2020. Since the sampling was not carried out within the same day for all participants, the period between the two samplings was around two and a half months (73 to 83 days).

The buccal cell sampling and lab work was carried out in the Laboratory of Genetics-Department of Biology (University of Prishtina). The sampling was performed according to Faccioni et al. (2003) with some modifications. Buccal cells were sampled using a sterile cytobrush in inner parts of both cheeks (ten times in each side). Buccal cell samples were transferred to phosphate buffered saline (PBS) (in 15mL tubes) and kept in the refrigerator (4°C) until the beginning of the processing (within two hours). For this, the samples were centrifuged at 2500 rpm (for 10 min), the supernatant discarded and the sedimented cells were re-suspended in 100µl PBS. From this cell suspension, 20µl were dropped on a microscope slide. Two slides were prepared for each individual. The slides were air dried and fixed in the ethanol (96%) for 15 minutes.

Staining and microscopical analysis

Slides were stained with Giemsa 10 % (for 25 minutes), rinsed under tap water and air dried. The Giemsa stained slides were analyzed according to Goud et al., (2004) at 400x magnification using the light microscope (Motic). For each individual, 2000 cells were analyzed (1000 from each of two prepared slides) for the frequency of micronucleated cells.

Statistics

The Shapiro-Wilks tests revealed a significant ($p<0.05$) deviation from normality distribution of the micronucleus frequencies. For this reason, significance of the difference between the November and January samples within the cohort was determined by using the Paired-samples Wilcoxon signed ranks tests. Descriptive statistics for age and body mass index (BMI) of the participant cohort was applied.

Results and Discussion

Taking into account the mean age and BMI values of the participant cohort (Table 1) as well as the proper health status (i.e. no disease, no smoking, no regular or excessive alcohol consumption), the observed increase of the micronucleus formation over the winter season (Fig.1) seems not to be attributable to the individual health status. More likely, the effect has to be considered indicative of environmental factors such as the continuous

exposure of the participants to the air pollution in urban area of Prishtina. In general, the observed micronucleus levels (< 3‰; Fig.1A) are locating to the baseline range of micronucleus levels seen in buccal cells in healthy donors (i.e. 0.05 – 11.5‰ as reported by (Holland et al., 2008). Nevertheless, the differences between both sampling times indicate a moderate but significant ($p<0.05$) elevation over the winter season (Fig.1B). In addition, the micronucleus frequencies show a moderate, significant positive correlation (Spearman's rho: +0.584; $p<0.05$) with sampling time. This provides support to the aforementioned causal context of a genotoxic potential exerted by the exposure to the air pollution in urban area of Prishtina. The increased concentrations of the monitored pollutants observed in two monitoring stations in Prishtina (*KHMI* and *Rilindja*) during the period after the first sampling (November 2019 – end of January 2020) shown in Table 2, further support this attribution.

Table 1. Cohort descriptive statistics for age and body mass index (BMI)

	mean ± 2SE	minimum – maximum	median	N
Age [years]	22.38 ± 0.58	20.0 – 26.0	22.0	29
BMI	22.06 ± 1.06	18.4 – 29.7	21.5	29

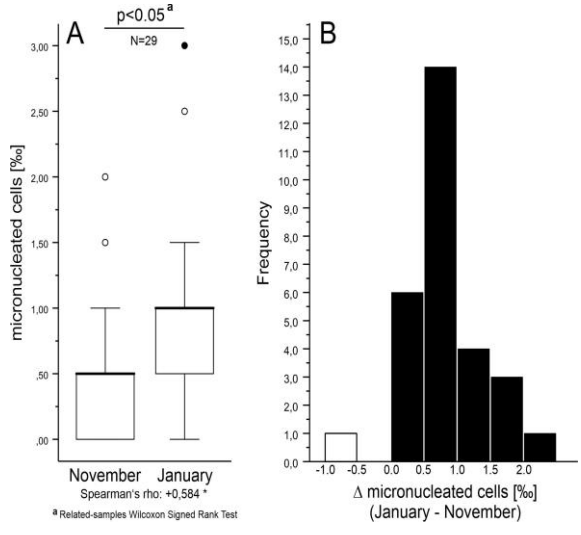


Fig. 1. Assessment of micronucleus frequencies in buccal cells collected from the donors in two consecutive seasons (November 2019 and January 2020). **(A)** Results shown as Box-Whisker plots. Circles represent mild (open) and strong (closed) outliers. **(B)** Histogram (0.5% intervals) for the differences of the micronucleus frequencies observed between the sampling seasons. Note, that the majority (N=14) of donors show an increase of the micronucleus frequencies within the + 0.5 – 1‰ interval.

The data from these two monitoring stations *(KHMI* and *Rilindja*) listed as "*urban background*" (MESPI, 2022) are presented and discussed. Here, it is important to underline that emissions from the industrial facilities in the vicinity also have an impact on the pollution in Prishtina (Bajcinovci, 2017). Thus, it is expected that industry-derived pollution also has an impact on the exposure of the participants in the study (whose inclusion criteria was the stay in urban area of Prishtina for at least 5 days per week) in the forthcoming period (after the first sampling). As regard to the suitability of the micronucleus assay, it is important to underline the strong linkage between exposure to a given genotoxic compound and increased MN frequencies which can be seen already at short exposure intervals that are markedly shorter than the interval monitored in our study (Moore et al., 1996; Holland et al., 2008). The data presented in Table 2 (extracted from KEPA reports published in their web site) are important in relation to the observed genotoxic potential in this preliminary investigation. The data covering the six-month period (August 2019 – January 2020) of the monitoring (SO_2, CO**,** NO_2, O_3, PM10 and PM 2.5) at two stations in Prishtina (*KHMI* and *Rilindja*) were extracted and taken into consideration. The mean values of SO_2 in August, September and October 2019 showed no big difference compared to following three months (November and December 2019 and January 2020) in monitoring stations (*KHMI* and *Rilindja*). Similar situation is observed for NO_2 in *KHMI* station. For *Rilindja* station the data (for NO_2) for the August and September 2019 were not available in the KEPA reports. Ozone (O_3) mean values were higher in the first three months. Higher ozone mean values

Table 2. Average and maximum monthly values for selected pollutants measured at *KHMI* and *Rilindja* monitoring stations in Prishtina during the period September 2019- January 2020[1]

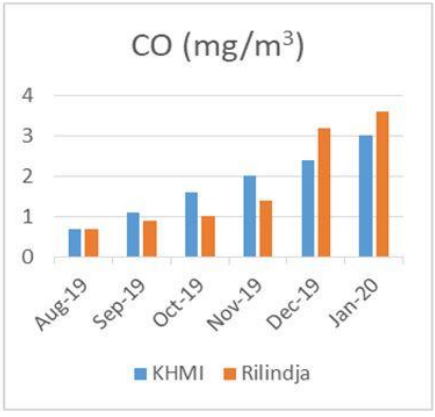
Stations	Monitored pollutants	August 2019	September 2019	October 2019	November 2019	December 2019	January 2020
KHMI	SO_2 (µg/m^3)	7.4 (60.1)	8.1 (99.6)	11.2 (83.8)	6.8 (21.6)	11.1 (81)	17.3 (78.4)
	CO (mg/m^3)	0.7 (1)	1.1 (1.8)	1.6 (2.6)	2 (2.3)	2.4 (6.4)	3 (5.7)
	NO_2 (µg/m^3)	31 (102.5)	26.3 (105.5)	36.7 (150.9)	18.6 (58.1)	23.7 (74.6)	32.6 (110.8)
	O_3 (µg/m^3)	59.9 (149)	50.8 (129.6)	29.9 (119)	18 (68.4)	16.5 (49.9)	15.4 (54.4)
	PM 10 (µg/m^3)	19.4 (25.9)	18.5 (31.1)	38 (82.7)	23.3 (65.1)	33.6 (94.4)	58.4 (139.7)
	PM 2.5 (µg/m^3)	10.2 (35.4)	11 (45.2)	22.6 (86.2)	16.9 (89.2)	27 (158)	47.5 (179.1)
Rilindja	SO_2 (µg/m^3)	10.7 (31.8)	9.5 (74)	11 (86)	10.4 (21)	12.4 (95.5)	15.7 (76.6)
	CO (mg/m^3)	0.7 (1.4)	0.9 (1.5)	1 (2.1)	1.4 (3.2)	3.2 (6.6)	3.6 (6.3)
	NO_2 (µg/m^3)	n.a.	n.a	56.7 (194.5)	40.4 (95.8)	39.9 (99.7)	47.5 (206.5)
	O_3 (µg/m^3)	55.5 (114.5)	44 (98.9)	29.2 (87.8)	21.4 (71)	18.3 (57.8)	19.1 (52.2)
	PM 10 (µg/m^3)	14.7 (17.2)	14.5 (24.3)	25.7 (59.4)	22 (45.5)	35.8 (70.6)	60.8 (148.4)
	PM 2.5 (µg/m^3)	7.9 (19.3)	8.9 (22.4)	15.6 (71)	14.3 (84.3)	27.5(135.3)	48 (234.5)

[1]*KEPA, monthly reports https://www.ammk-rks.net/al/mjedisi/20/raportet/20mujore (last visited April 20, 2024). Given are the average values; monthly maximum values are given in brackets. PM refers particulate matter; CO (Carbon monoxide); O_3 (Ozone); SO_2 (Sulfur Dioxide); NO_2 (Nitrogen dioxide). KHMI (Kosovo Hidro-meteorological Institute).*

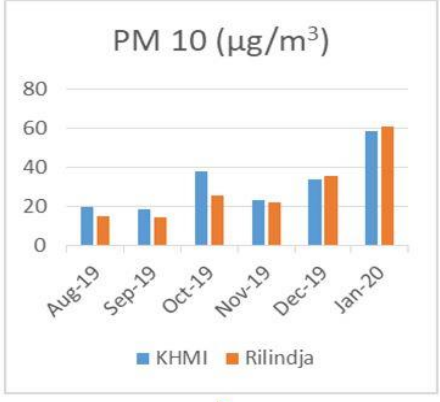
are reported to be characteristic for summer days (longer and warmer days) whereas the opposite situation is observed for SO_2, CO**,** NO_2, and PM (Cichowicz et al., 2017). On the other hand, an obvious increase in the concentration of the pollutants such as CO**,** PM10 and PM 2.5 during

the period between two samplings was observed (Table 1). In Fig. 2 (data extracted from Table 1) it can be seen that there was a continuous increase of the monthly average values for CO in both monitoring stations. Similar situation was observed also for PM10 and PM2.5 (except for October which average values were higher compare to November). This increase of the monthly average values for CO, and PM puts emphasis on these compounds in context with the presumptive pollution – genotoxicity relationship. This strengthens the concept that seasonal shifts of urban air – pollutants correspond with an increase of micronucleus formation, albeit the responsible compounds as well as underlying genotoxic mechanism needs to be investigated in future research.

It is worthy to emphasize that the increased levels of the pollutants and exceedances of limit values are recorded years earlier in two stations in Prishtina (*KHMI* and *Rilindja*) World Bank, 2013) and the situation is showed to be similar also recently. According to the Annual Report on the State of the air 2019 (MEI, 2020), *KHMI* monitoring station registered an increase in the number of days exceedances for PM10 (exceedances of the allowed norm) during the winter months showing that of the total of 51 days (of exceedances in 2019), 39 days were in the months January, February, March and December. During the same period (according to the same report) the number of exceedances in *Rilindja* station was 29. Also, in 2020 (according to Annual Report on the State of the Environment for 2020), there was a high number (57) of exceedances for *KHMI* whereas in *Rilindja*, the number was 78 (MESPI, 2021). Annual Report on the State of the Air 2022 (MESPI 2023) shows 59 days of exceedances in Rilindja station and 25 exceedances in *KHMI* (mostly during the winter months except for *Rilindje* station which showed high number of days (10) of exceedances also in November.



a



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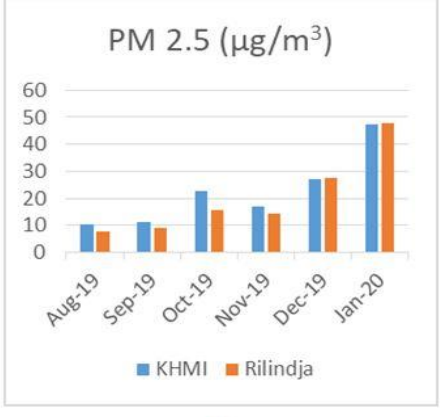

c

Figure 2. Monthly average values of three pollutants as observed in two monitoring stations (*KHMI* and *Rilindja*): **a)** CO; **b)** PM10; **c)** PM2.5. Data extracted from the Table 2.

Related to the effect of air pollution in urban areas, De Donno et al., (2016) reported an association between the frequencies of the micronuclei in buccal mucosa cells of the children exposed to pollution from the heavy traffic. Higher frequency of the micronuclei was observed in buccal cells of pre-school children exposed to the heavy air pollution in urban area compared to the micronucleus frequencies found in children living in less polluted areas. On the other hand, seasonal differences (higher effect in winter compare to summer season) in buccal micronucleus-cytome assay (BMCyt) biomarkers (micronuclei, binuclear cells, karyolysis, condensed chromatin and pyknosis) are reported by Cetkovic et al. (2023) as an effect of air pollution in Sarajevo (urban area). In this context, it is important to mention that autumn and winter urban PM samples showed mutagenicity in *Salmonella typhimurium* strains (Bocchi et al., 2016). Higher genotoxic effect of the winter samples (compare to those collected in summer) was reported also by other authors (Abou Chakra et al., 2007; Çakmak et al., 2019).

Beside the limited timeframe and limited number of seasons under investigation, the preliminary findings from this study, combined with existing pollution monitoring data in Prishtina, underscore the necessity for further investigation into urban pollution exposure, particularly concerning particulate matter (PM). In this context, it is important to underline that Particulate Matter (PM) are known as human carcinogen and for the association to mortality and morbidity (Lippmann, 2012).

A high number of inhabitants in Prishtina is exposed to the pollution, therefore authorities should pay more attention to the air pollution in urban areas in Prishtina and other cities in Kosovo where exceeding values of the pollutants are also evidenced (MESP, 2023; MESP, 2021; MEI 2020). When planning the future research work, it is important to underline that employing more biomarkers and monitoring of the individual exposure are considered of an outmost importance for better understanding of the underlying mechanisms of the effect of the particulate air pollution on human health (Sørensen et al., 2003).

Conclusion

A season-associated increase of the pollution in Prishtina urban area is associated to an increase of the genotoxic effects as shown by the elevated frequencies of the micronucleated buccal cells of the individuals from the exposed population.

Based on the genotoxicity data obtained in this study and the available monitoring data, there is a need for more monitoring and bio-monitoring studies in the Kosovo and especially in the areas with the high concentration of the population which is exposed to various sources of the pollution. The investigations on the environmental pollution monitoring and bio-monitoring (with the focus on human bio-monitoring) should be prioritized and based on a well-designed program and well established network of researchers and institutions. Another important issue is that (besides the monitored pollutants) there are also pollutants of concern (such as polycyclic aromatic hydrocarbons) which are not monitored. Complete information on the pollutants is important for bio-monitoring studies to reveal the causal basis for the evidenced health problems.

Authors' contributions

Djellza Panxhaj:

Conceptualization, Formal Analysis, Investigation, Writing - Original Draft.

Fisnik Asllani:

Conceptualization, Methodology, Formal Analysis.

Avdulla Alija:
Conceptualization, Methodology, Supervision, Writing – Review and Editing.

Shemsedin Dreshaj:
Conceptualization, Methodology.

Nikolaus Bresgen:
Conceptualization, Formal Analysis, Visualisation, Writing – Review and Editing.

Conflict of interest

Authors declare no conflict of interest.

References

Abou Chakra OR, Joyeux M, Nerrière E, Strub MP, Zmirou-Navier D (2007) Genotoxicity of organic extracts of urban airborne particulate matter: an assessment within a personal exposure study. Chemosphere 66(7):1375–1381.

Alija AJ, Asllani F, Bajraktari ID, Collins AR, Dreshaj S, Bresgen N, Eckl PM (2015) Atmospheric Pollution in Kosovo Is Associated with Increased DNA Damage in the Human Population. Biomonitoring 2(1):35–41.

Alija AJ, Collins AR, Dreshaj S, Asllani F, Bajraktari ID, Bresgen N, Eckl PM (2016) Differences in basal DNA damage in blood cells from men and women. Biomonitoring 3(1):1–4.

Anwar WA (1944) Monitoring of Human Populations at Risk by Different Cytogenetic End Points. Environ Health Perspect 102(4):131–134.

Bajcinovci B (2017) Environment Quality: Impact From Traffic, Power Plant and Land Morphology, a Case Study of Prishtina. Environmental and Climate Technologies 19:65-74.

Bocchi C, Bazzini C, Fontana F, Pinto G, Martino A, Cassoni F (2016) Characterization of urban aerosol: seasonal variation of mutagenicity and genotoxicity of $PM_{2.5}$, PM_1 and semi-volatile organic compounds. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 809:16-23.

Çakmak G, Arı PE, Emerce E, Arı A, Odabaşı M, Schins R, Burgaz S, Gaga EO (2019) Investigation of spatial and temporal variation of particulate matter in vitro genotoxicity and cytotoxicity in relation to the elemental composition. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 842:22-34.

Cetkovic T, Haveric A, Behmen S, Omanovic MH, Klacar LC, Dzaferspahic A, Durmisevic I, Mehanovic M, Haveric S (2023) A pilot biomonitoring study of air pollution in the urban area of Sarajevo, Bosnia and Herzegovina: genotoxicity assessment in buccal cells. Mutagenesis 38(1):33-42.

Cichowicz R, Wielgosiński G, Fetter W (2017) Dispersion of atmospheric air pollution in summer and winter season. Environ Monit Assess 189: 1-10.

De Donno A, Grassi T, Ceretti E, Viola GCV, Levorato S, Vannini S, Salvatori T, Carducci A, Verani M, Bonetta SA, Carraro E, Bonizzoni S, Bonetti A, Bagordo F, Serio F, Idolo A, Gelatti U, Mapec Life Study Group (2016) Air Pollution Biological Effects In Children Living In Lecce (Italy) By Buccal Micronucleus Cytome Assay (The Mapec_Life Study). Int J Sus Dev Plann 11(4):500–510.

EC (2023) Progress report for Kosovo. European Commission.

Faccioni F, Franceschetti P, Cerpelloni M, Fracasso ME (2003). In vivo study on metal release from fixed orthodontic appliances and

DNA damage in oral mucosa cells. Am J Orthod Dentofacial Orthop 124(6):687–693.

Goud KI, Hasan Q, Balakrishna N, Rao KP, Ahuja YR (2004) Genotoxicity evaluation of individuals working with photocopying machines. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 563(2):151-158.

Heddle JA, Fenech M, Hayashi M, MacGregor JT (2011) Reflections on the development of micronucleus assays. Mutagenesis 26(1):3–10.

Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S, French M (2008) The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: the HUMN project perspective on current status and knowledge gaps. Mutat Res. 659(1-2):93–108.

Lippmann M (2012) Particulate matter (PM) air pollution and health: regulatory and policy implications. Air Quality, Atmosphere & Health 5:237-241.

MEI (2020) Annual Report on the State of the Air in Kosovo, 2019. KEPA (Kosovo Environmental Protection Agency), MEI (Ministry of Environment and Infrastructure), Kosovo.

MESPI (2023) Annual Report on the State of the Air 2022. KEPA (Kosovo Environmental Protection Agency. Ministry of Environment Spatial Planning and Infrastructure.

MESPI (2021) Annual Report on the State of the Environment for 2020. KEPA (Kosovo Environmental Protection Agency. Ministry of Environment Spatial Planning and Infrastructure.

MESPI (2022). State of the Air Report 2021. Ministry of Environment Spatial Planning and Infrastructure.

Moore LE, Warner ML, Smith AH, Kalman D, Smith MT (1996) Use of the fluorescent micronucleus assay to detect the genotoxic effects of radiation and arsenic exposure in exfoliated human epithelial cells. Environmental and molecular mutagenesis 27(3):176-184.

Shabani Isenaj Z, Berisha M, Gjorgjev D, Dimovska M, Moshammer H, Ukëhaxhaj A (2022) Air Pollution in Kosovo: Short Term Effects on Hospital Visits of Children Due to Respiratory Health Diagnoses. Int J Environ Res Public Health 19(16):10141.

Sørensen M, Autrup H, Møller P, Hertel O, Jonsen SS, Vinzents P, Knudsen LE, Loft S (2003) Linking exposure to environmental pollutants with biological effects. Mutat Res 544:255–271.

Thomas P, Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S, French M (2009) Buccal micronucleus cytome assay. Nat Protoc 4(6):825–837.

Ukëhaxhaj A, Gjorgjev D, Ramadani M, Krasniqi S, Gjergji T, Zogaj D (2013) Air Pollution in Prishtina (2013) Air Pollution in Pristina, Influence on Cardiovascular Hospital Morbidity. Med Arch 67(6):438–441.

World Bank (2019) Western Balkans Regional AQM- Western Balkans: Report –AQM in Kosovo. World Bank.

World Bank (2013) Kosovo Country Environmental Analysis. Cost Assessment of Environmental Degradation, Institutional Review, and Public Environmental Expenditure Review. Washington, DC: World Bank.