v. 4, n. 74, 2024

International Journal of Health Science

EVALUATION OF CYTOTOXICITY AND GENOTOXICITY OF CHEMICAL MATERIAL IN PM2.5 FILTERS FROM THREE MONITORING STATIONS OF THE AIR QUALITY NETWORK OF THE ABURRÁ VALLEY

Alejandra Betancur Sánchez
National University of Colombia, Faculty of Sciences

Juan Bautista López Ortiz
National University of Colombia, Faculty of Sciences

Carmen Elena Zapata Sánchez
National University of Colombia, Faculty of Mines

Abstract: The increase in air pollution and its adverse effects has raised concerns about regulatory policies and has encouraged the development of new air quality standards. Environmental toxicants can have various effects ranging from irritation to cell and tissue death; however, some genotoxic compounds produce alterations in the genetic material or its associated components, causing mutations or interfering with some repair or polymerization processes involved in chromosome segregation. The objective of this study was to evaluate the effect on the viability and genotoxic potential of $PM_{2.5}$ particulate matter obtained from filters collected from three monitoring stations of the air quality network in the Aburrá Valley, Colombia, on the CHO-K1 and Jurkat cell lines. The tests carried out revealed a reduction in cell viability and punctual damage to DNA. The results suggest that $PM_{2.5}$ material has genotoxic potential and can induce cancer development, as has been suggested in other studies.
Keywords: particulate matter, cell viability, DNA damage, Comet assay.

- Evaluation of particulate matter in cell lines with tumor and non-tumor phenotype
- Biological assessment of air pollution endosomes
- Relationship between cell proliferation and pollution

INTRODUCTION

Economic growth and globalisation have brought benefits to humanity and at the same time have generated new risks. Measuring exposure to many environmental factors is complex because we do not have adequate health information and surveillance systems that allow us to assess the magnitude and severity of the risks, as well as the causal relationship between environment and health (Vargas, 2005). This has increased concern about regulatory policies and has encouraged the development of new standards on air quality (Coronas et al., 2009). High concentrations of fine particle pollution are related to increased mortality from heart disease, respiratory conditions and cancer. The evaluation of particles with a diameter equal to or less than 2.5 μm ($PM_{2.5}$) is the most appropriate indicator to calculate the risks of air pollution (CORDIS, 2014). Particulate matter (PM) has been associated with death and cardiopulmonary disease in elderly patients with pre-existing respiratory conditions (smokers and asthmatics) (Gillespie et al., 2013). Numerous animal studies show that inhalation of PM causes inflammatory airway toxicity (Chen et al., 2010).

Air pollution increases the risk to public health, mainly due to the difficulties in identifying the toxic components of the complex mixture that constitutes PM and the limited knowledge of the genotoxic substances associated with it (Zuluaga et al., 2009). The emission of fine particles from industrial processes into the atmosphere induces damage to cellular DNA, however, evaluations of the chemical composition, size, shape of the particles and the toxicity of the specific sources and study areas are very limited (Jayasekher, 2009).

Particle pollution is a major problem in urban centers due to the high population density, which makes the surface of cities rough, hindering the movement of atmospheric flow and the dispersion of pollutants and their accumulation in certain points of the city. The increase in pollutants in urban areas such as the Aburrá Valley increases public health risks due to the topographic characteristics of being a semi-closed and narrow valley in which thermal inversion phenomena occur, since during the day the atmosphere near the earth's

surface heats up and at night the temperature drops and the cold descends down the slopes, raising the hot air and accumulating pollutants in the Valley.

Thermal inversion, therefore, stops the rise and dispersion of pollutants causing a localized air problem (Enkerlin et al., 1997). Herrera et al (2006 and 2007) established the association between the concentration of various atmospheric pollutants in the city of Medellín and the presence of respiratory pathologies in school children. Pollutants such as $PM_{2.5}$, PM_{10}, soot and lead in the air were found to increase the risk (49.3%) of suffering from respiratory infections or asthma attacks.

PM is a complex and heterogeneous mixture of chemical and biological elements bound to a carbon nucleus that differ in their chemical composition, reaction properties, disintegration time and the ability to diffuse over long or short distances, in addition to the effects they produce on public health (Kampa & Castanas, 2008).

In accordance with the above, the objective of this study is to evaluate the cytotoxic and genotoxic effect of PM in PM2.5 filters from three monitoring stations in the Aburrá Valley in order to correlate this effect with the risk to public health.

MATERIALS AND METHODS

STUDY SITES

For the collection of samples, BGI PQ200 semi-automatic air sampling equipment and Partisol Plus 2025 were used, which capture PM with a PM2.5 separator with 47 mm diameter Teflon filters and 0.2 µm pore size exposed for 24 hours. The three sampling points of the Aburrá Valley Air Quality Monitoring Network evaluated were:

Station 1: MED-UNFM (Faculty of Mines, National University of Colombia, Medellin Campus). Strategic location due to its proximity to Carrera 80, one of the city's main roads with the largest traffic flow and exit to the municipalities of Western Antioquia and the Atlantic Coast, a site of great influx of people to academic work. In addition, it is a residential area and in general, multiple activities take place in this sector. From Station 1, 3 filters were analyzed from May to October 2015.

Station 2: MED-PJIC (Jaime Isaza Cadavid Polytechnic). Site whose purpose is to monitor areas of direct influence by vehicular traffic. From Station 2, 5 filters were analyzed from May to October 2015.

Station 3: BAR-PDLA (Parque de las Aguas, municipality of Barbosa (Antioquia)) evaluated as a reference control, is a background station whose objective is to determine the levels of pollutants entering the Aburrá Valley. From Station 3, 3 filters were analyzed from May to October 2015.

REMOVING PM 2.5 FROM FILTERS

A Soxhlet extraction procedure was performed, the evaluated protocol was the one reported by Meléndez et al. (2012). For the Soxhlet extraction, the solvents used were acetone and dichloromethane (160 to 170 mL (1:1)) for 4 hours in cellulose thimbles. Then, the extract was rotary evaporated to approximately 2 mL, at 30°C and 150 rpm; it was diluted in pure Dimethyl Sulfoxide (DMSO) and kept refrigerated at 4°C in the dark until further analysis. Some filters were subjected to an ultrasound extraction process; the methodology was that reported by Sato et al. (1995) with modifications.

PM TREATMENTS

From the extracts obtained dissolved in pure DMSO, working solutions were obtained by dilution in PBS. The concentrations evaluated in the MTT reduction and neutral red uptake tests were: 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/mL and in the Trypan Blue and genotoxic tests the concentrations of 10, 30 and 50 µg/mL were studied.

CELL CULTURES

In the cytotoxicity and genotoxicity tests (Comet Assay), the CHO–K1 cell lines (ATCC® CCL-61™) derived from Chinese hamster ovary, which grows attached to the substrate (Ahn & Antoniewicz, 2013) and the Jurkat cell line (ATCC® TIB152™) of leukemoid origin (Cai et al., 2010) were used.

The cell lines were maintained in RPMI-1640 medium (Sigma) supplemented with 5% fetal bovine serum (FBS, Gibco), in a humidified atmosphere with 5% CO2 and incubated at 37°C. In the experiments, exponential phase cultures were used, with confluence greater than 80%.

The CHO-K1 cell line was selected because it is accepted as a biological model to study the behavior of certain cellular variables on non-tumorous tissue of metabolites of industrial, biotechnological, environmental, among others, interest (Kang et al., 2007); the CHO-K1 cell line represents the healthy population. Likewise, Jurkat was used as a tumor biological model to represent the population with comorbidities or susceptible to developing diseases due to poor air quality.

CELL VIABILITY TESTS

The experiments to evaluate viability were performed in triplicate and each treatment was subjected to three replicates. The controls used were: negative control (untreated cells), solvent control (DMSO 10%) and positive control (pure DMSO).

MTT reduction test: This test allows to evaluate the effect on mitochondrial metabolism.

This assay was performed using the protocol of Ulukaya et al. (2008) with some modifications. The MTT assay was evaluated in 96-well plates, in each well $6x10_3$ Jurkat cells or $7x10_3$ CHO-K1 cells were added in RPMI-1640 medium supplemented with 5% FBS at 37°C and 5% CO2. After 20 hours, the cells were treated and incubated under the same conditions described for 20 hours. Then, 10 µl of the MTT solution (5 mg/ml) was added to each well, they were incubated for 3.5 hours in the dark and 100 µl of isopropanol-acid was added to each well to dilute the formazan crystals formed, shaken and finally read in an ELISA reader at 570 nm. The viability percentage was determined from the relationship between the absorbance of each treatment and the absorbance of the control.

Exclusion of trypan blue dye: With this test it is possible to evaluate the effect of the contaminant on the integrity of the membrane and cell morphology.

In this assay, 80,000 cells/well were seeded in 24-well dishes (CHO-K1 and Jurkat) and incubated for 24 hours, then the cells were treated for 24 hours. The cell suspension was incubated with trypan blue (0.04%) and counted in a Neubauer chamber. Cell viability was determined as the number of live cells over the number of dead cells, expressed as a percentage.

Neutral red uptake: This test is used to determine the effect of particulate matter on the integrity of the membrane of lysosomes and endosomes. This assay is carried out in a similar way to the MTT, except that after 20 hours of treatment the culture medium was removed, each well was washed with PBS and 50 µl of neutral red (0.1 mg/mL) was added, and then incubated for 1.5 hours. Then, the neutral red was removed, washed with medium and 100 µl of ethanol (50%)-acetic

acid (0.1%) was added for 10 minutes. Finally, it was read in an Elisa reader at 540 nm. Viability was determined as in the MTT test.

GENOTOXICITY TESTS

Alkaline single cell gel electrophoresis (Comet assay): This test evaluates the effect on the genome at the level of breaks or punctual ruptures.

The protocol used was the one reported by Singh, et al (1988). In this assay, $1x10^5$ CHO-K1 cells were taken in 2250 µl of medium and treated for 24 hours with the chemical component of the PM2.5 filters. Then, the percentage of DNA damage was analyzed and compared with the untreated control. In addition, DMSO controls (0.03%) and a positive control with hydrogen peroxide (25 µM) were made.).

After treatment, cell suspensions were centrifuged at 2400 rpm for 5 min, the supernatant was removed, and cells were mixed with 90 µl of low-melting-point agarose (LMA 0.05%, SIGMA) and plated on slides pretreated with normal-melting-point agarose (NMA 0.5%, SIGMA). They were allowed to solidify at 4°C for 8 min. Subsequently, plates were immersed in lysis buffer (1% sodium sarcosinate, 2.5 M NaCl; 100 mM EDTA disodium salt solution, 10 mM Tris; pH 10; and Triton X-100, 1%).

Plates were washed with PBS and incubated for 20 minutes in an electrophoresis chamber with running buffer (300 mM NaOH, 1 mM EDTA) and then the samples were run at 25 V and 300 mA for 30 minutes at pH 13. After electrophoresis, plates were washed with neutralizing buffer (0.4 M Tris pH 7.5) for 15 minutes. Subsequently, the slides were dehydrated with methanol and stored for one day to be stained with Ethidium Bromide (2 µg/mL). Finally, they were observed under a fluorescence microscope (ZEISS) with a 20X objective. 50 nucleoids per plate were randomly analyzed and with the help of the CASP program, the percentage of damage in the tail of the comets formed in the cells with the treatments was measured. All steps were performed in the dark after exposure to lysis buffer, to prevent additional DNA damage.

STATISTIC ANALYSIS

Data were expressed as mean ± standard error. Statistical significance analysis was performed using ANOVA; standard error is represented by $p<0.001$ for multiple comparison analysis using Tukey's test, results were compared with untreated control in all tests.

RESULTS AND DISCUSSION

MTT REDUCTION

The MED-UNFM station showed the greatest decrease (50%) in cell viability at the highest concentration (100 µg/mL). The data that do not follow the straight line trend could be explained by considering that the samples analyzed are complex mixtures, for this reason, some compounds may present synergism or antagonism with other molecules. In addition, the points of the MED-UNFM and MED-PJIC traffic stations appear further away from the trend line, possibly reflecting a greater heterogeneity in the components of the mixture (Fig. 1).

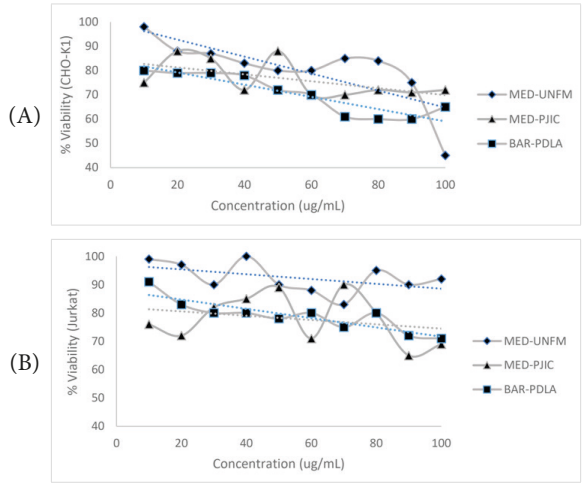


Figure 1: Percentage of viability by MTT in (A) CHO-K1 cells (B) Jurkat cells treated with different concentrations of PM2.5 filter extracts obtained from three monitoring stations in the Aburrá Valley.

The decrease in the percentage of cell viability was observed in both the CHO-K1 and Jurkat cell lines, however, the effect was more variable in the Jurkat cell line. In both cell lines, the effect of the mixture obtained from the BAR-PDLA station was the most homogeneous. The statistical analysis showed a highly significant difference ($p<0.001$) with the treatments at 60 µg/mL. These results agree with the studies carried out by Orona et al., (2014) who reported changes in cell viability in the lung cell line at concentrations greater than 50 µg/mL.

EXCLUSION OF TRYPAN BLUE DYE

Based on the results obtained in the MTT test, three intermediate concentrations were selected to be evaluated with Trypan Blue. The results observed in the CHO-K1 and Jurkat cell lines treated with the extract from the filters of the MED-UNFM station showed a lower cell viability at the concentration of 30 µg/mL, however, the effect is not significant compared to the untreated control (96.9% CHO-K1 line and 94.89%, Jurkat cells).

The results of Figure 2 (B) from the MED-PJIC station show that the extract from the filters does not affect viability by rupture of the cell membrane in CHO-K1 cells while the Jurkat cells show a decrease in the percentage of cell viability by 55% with the concentration 50 µg/mL. The extract from the filters collected at this station exhibited greater damage to the leukemoid cell line, which could be related to the greater susceptibility of the tumor line and in turn to individuals with cancer exposed to MP, as stated by de Kok et al., (2006) and Pope et al., (2002).

The results of the particulate matter taken from the BAR-PDLA station (Figure 2 (C)) showed lower viabilities compared to the other two stations. Again, a lower effect on viability was observed with the 50 µg/mL concentration in the Jurkat cell line and a greater effect in the CHO-K1 cell line compared to the untreated controls (94% and 98% viability for CHO-K1 and Jurkat, respectively).

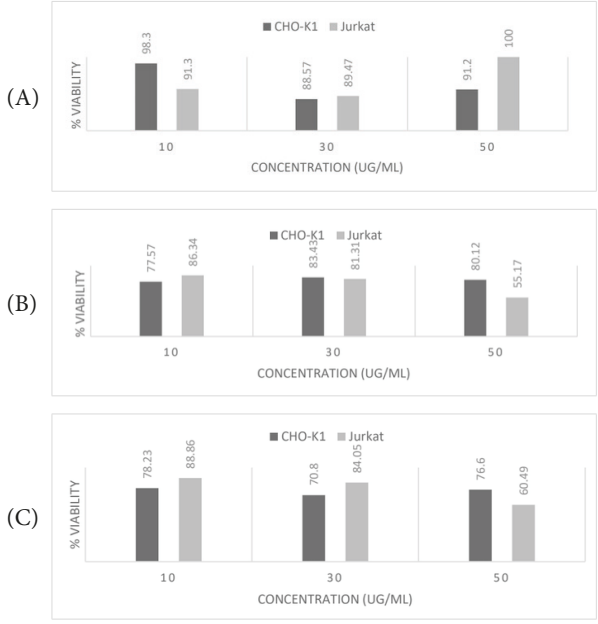


Figure 2: Viability by Trypan Blue of CHO-K1 and Jurkat cells treated with different concentrations of PM 2.5 filter extracts obtained from (A) MEDUNFM Station (B) MED-PJIC Station (C) BAR-PDLA Station

NEUTRAL RED UPTAKE

Figure 3 shows the results of neutral red uptake in the CHO-K1 cell line treated with extracts from the MED-UNFM and MED-PJIC stations, similar to what was observed in the MTT test. The peaks that deviate from the trend could be explained by the heterogeneity of the complex mixture for the traffic stations. At the BAR-PDLA station, the effect was different from that reported in the other cell viability tests, since an inverse effect is observed: as the concentration of particulate matter increases, cell viability increases. It is likely that the composition of the mixture for this season generates a loss of cell viability at low concentrations and that this damage decreases as the concentration increases. The statistical analysis showed high significance with concentrations of 80, 90, and 100 µg/mL.

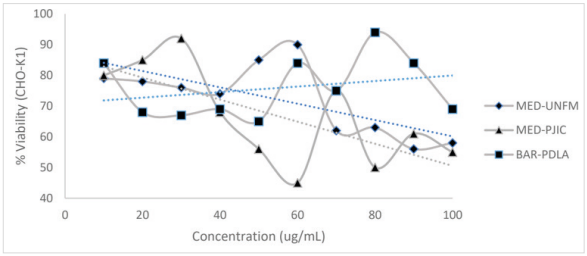


Figure 3: Viability by neutral red uptake in CHO-K1 treated with PM2.5 filter extracts sampled at the MED-UNFM, MED-PIJC and BAR-PDLA stations in the Aburrá Valley.

GENOTOXIC EVALUATION OF PARTICULATE MATTER

Figure 4 shows the percentage of DNA damage in CHO-K1 cells treated with PM from filters evaluated at the three monitoring stations. The data from the traffic stations are similar to each other, while the results from the background station (BAR-PDLA) are contrary to the other two stations. At the traffic stations, greater DNA damage is observed for the concentration of 30 µg/mL, while for the BAR-PDLA station a dose response effect is shown, i.e., as the concentration increases, DNA damage increases, however, this value does not approach the critical points reported at the traffic stations. The comet assay data obtained on the CHO-K1 cell line treated with PM2.5 are similar to those reported by Vargas et al., (2014) in peripheral blood lymphocytes. All data showed a significant difference compared to the untreated control (% DNA damage <10%). From the above, it can be suggested that particulate matter from the MED-UNFM and MED-PJIC monitoring stations have an acute genotoxic effect, which could accumulate over time, generating potential damage at the whole genome level.

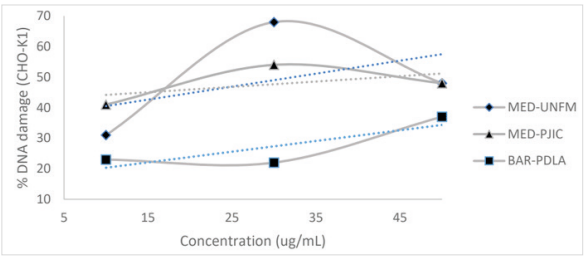
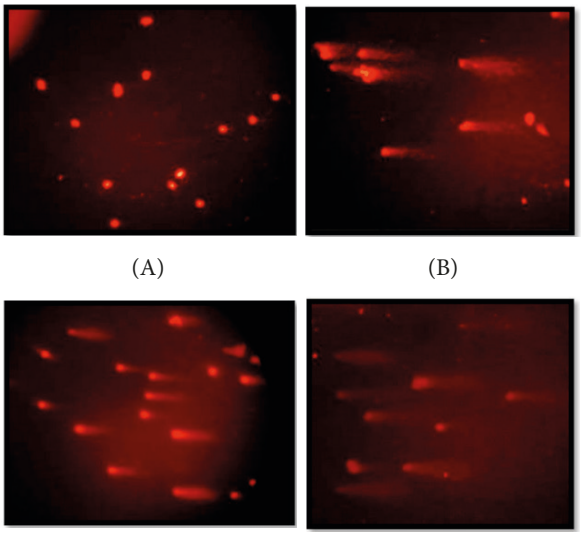


Figure 4: Percentage of DNA damage obtained by comet assay in CHO-K1 cells treated with PM2.5 from the filters of three stations in the Aburrá Valley.



(A) (B)

(C) (D)

Figure 5: Effect of particulate matter on DNA (A) Negative control (B) MED-PJIC (30µg/mL) (C) MED-UNFM (30µg/mL) (D) BAR-PDLA (70µg/mL)

In Figure 5 it is possible to observe the results of the comet assay for the negative control and for each of the stations at the concentration where the greatest damage due to DNA breakage occurred.

CONCLUSION

Using the MTT and neutral red assays, a dose-response cytotoxic effect was observed for both the CHO-K1 and Jurkat lines, indicating that particulate matter has a negative effect on mitochondrial metabolism and on lysosomes and endosomes, both for non-tumor cells and tumor cells, which may result in an increased risk for people suffering from cancer and exposed to this type of pollutant.

PM2.5-induced DNA breaks were found at the three monitoring stations evaluated. The concentration of 30 μg/mL showed greater genotoxic damage (65%) in the comet assay for particulate matter captured at high traffic stations. In each of the samples analyzed by the comet assay, DNA damage was observed, which could have a negative impact on health through the manifestation of cancer when exposure is prolonged.

According to the results of this study, there is greater heterogeneity of the complex mixture at the traffic stations than at the background station.

It is necessary to identify the compounds present in $PM_{2.5}$, analyze the effect on cell viability and the genotoxic potential of each compound and compare them with the results obtained with the complex mixture from which they were isolated.

It is recommended to evaluate the effect on the cell cycle of tumor and non-tumor cells treated with PM2.5 and other particles such as PM10 by means of cloning efficiency, in addition to complementing with cytotoxic tests such as the glutathione assay to estimate the oxidative damage that PM can generate.

REFERENCES

Abbas, I., Saint-Georges, F., Billet, S., Verdin, A., Mulliez, P., Shirali, P., & Garçon, G. (2009). Air pollution particulate matter (PM2.5)-induced gene expression of volatile organic compound and/or polycyclic aromatic hydrocarbon-metabolizing enzymes in an in vitro coculture lung model. Toxicology in Vitro, 23(1), 37–46.

Azqueta, A., Gutzkow, K. B., Brunborg, G., & Collins, A. R. (2011). Towards a more reliable comet assay: Optimising agarose concentration, unwinding time and electrophoresis conditions. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 724(1-2), 41–45.

Baldacci, S., Maio, S., Cerrai, S., Sarno, G., Baïz, N., Simoni, M., ... & Study, H. E. A. L. S. (2015). Allergy and asthma: Effects of the exposure to particulate matter and biological allergens. Respiratory medicine, 109(9), 1089-1104.

CORDIS. (2014). La contaminación atmosférica, uno de los mayores riesgos para la salud mundial. Retrieved from http://cordis.europa.eu/news/rcn/36549_es.htmll

de Kok, T. M., Driece, H. A., Hogervorst, J. G., Briedé, J. J. (2006). Toxicological assessment of ambient and traffic-related particulate matter: a review of recent studies. Mutation Research/Reviews in Mutation Research, 613(2), 103-122.

Ebada S., Edrada R., Lin W., Proksch P. 2008. Methods for isolation, purification and structural elucidation of bioactive secondary metabolites from marine invertebrates. Nature Protocols 3:1820 -1831.

Enkerlin E. C., Cano, G., Garza Cuevas, R. A., Vogel Martínez, E. (1997). Ciencia ambiental y desarrollo sostenible. In Ciencia ambiental y desarrollo sostenible. International Thomson.

Gillespie, P., Tajuba, J., Lippmann, M., Chen, L. C., Veronesi, B. (2013). Particulate matter neurotoxicity in culture is size-dependent. Neurotoxicology, 36, 112-117.

Golub, E. (2014). Environmental health costs in Colombia: The changes from 2002 to 2010.

Herrera A., Echeverri C., Maya G., Ordóñez J.2011. Patologías respiratorias en niños preescolares y su relación con la concentración de contaminantes en el aire en la ciudad de Medellín (Colombia). Revista Ingenierías Universidad de Medellín 10 (19): 21-32.

IDEAM, Estado de la Calidad del Aire en Colombia 2007-2010 Bogotá, D.C., 2012.

Kang, S. G., Lee, D. Y., Kang, M. L., & Yoo, H. S. (2007). Biological characteristics of Chinese hamster ovary cells transfected with bovine Prnp. Journal of Veterinary Science, 8(2), 131.

Kato, F., Tanaka, M., & Nakamura, K. (1999). Rapid fluorometric assay for cell viability and cell growth using nucleic acid staining and cell lysis agents. Toxicology in vitro, 13(6), 923-929.

Kampa, M., & Castanas, E. (2008). Human health effects of air pollution. Environmental pollution, 151(2), 362-367

Laumbach, R. J. (2010). Outdoor air pollutants and patient health. American family physician, 81(2), 175

Li, N., Sioutas, C., Cho, A., Schmitz, D., Misra, C., Sempf, J., Nel, A. (2003). Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environmental health perspectives, 111(4), 455.

MAVDT. 2010. Política de Prevención y Control de la Contaminación del Aire. Viceministerio de Ambiente.

Meléndez Gélvez, I., Martínez Montañez, M. L., Quijano Parra, A. (2012). Actividad mutagénica y genotóxica en el material particulado fracción respirable MP2, 5 en Pamplona, Norte de Santander, Colombia. Iatreia, 25(4), 347-356.

Miller, K. A., Siscovick, D. S., Sheppard, L., Shepherd, K., Sullivan, J. H., Anderson, G. L., & Kaufman, J. D. (2007). Long-term exposure to air pollution and incidence of cardiovascular events in women. New England Journal of Medicine, 356(5), 447-458.

Orona, N. S., Astort, F., Maglione, G. A., Saldiva, P. H. N., Yakisich, J. S., Tasat, D. R. (2014). Direct and indirect air particle cytotoxicity in human alveolar epithelial cells. Toxicology in Vitro, 28(5), 796-802.

Pereira, T. S., Gotor, G. N., Beltrami, L. S., Nolla, C. G., Rocha, J. A., Broto, F. P., Vargas, V. M. (2010). Salmonella mutagenicity assessment of airborne particulate matter collected from urban areas of Rio Grande do Sul State, Brazil, differing in anthropogenic influences and polycyclic aromatic hydrocarbon levels. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 702(1), 78-85.

Piraino, F., Aina, R., Palin, L., Prato, N., Sgorbati, S., Santagostino, A., & Citterio, S. (2006). Air quality biomonitoring: assessment of air pollution genotoxicity in the Province of Novara (North Italy) by using Trifolium repens L. and molecular markers. The Science of the Total Environment, 372(1), 350–359.

Pope, C. A. (2004). Air pollution and health - good news and bad. The New England Journal of Medicine, 351(11), 1132–1134.

Pope III, C. A., Burnett, R. T., Thun, M. J., Calle, E. E., Krewski, D., Ito, K., & Thurston, G. D. (2002). Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. Jama, 287(9), 1132-1141.

Quintero, M. Z., Ruiz, A. M. V., Trujillo, I. C. O. (2009). Efecto genotóxico y mutagénico de contaminantes atmosféricos. Medicina UPB, 28(1), 33-41.

Reinecke, S. A., Helling, B., & Reinecke, A. J. (2002). Lysosomal response of earthworm (Eisenia fetida) coelomocytes to the fungicide copper oxychloride and relation to life-cycle parameters. Environmental toxicology and chemistry, 21(5), 1026-1031.

Rodrigo, G., Almanza, G. R., Cheng, Y., Peng, J., Hamann, M., Duan, R. D., & Åkesson, B. (2010). Antiproliferative effects of curcuphenol, a sesquiterpene phenol. Fitoterapia, 81(7), 762-766.

Silva, M. J., Carothers, A., Castelo, B. N., Dias, A., & Boavida, M. G. (1999). Sister chromatid exchange analysis in workers exposed to noise and vibration. Aviation, space, and environmental medicine, 70(3 Pt 2), A40-5.

Torres, A. M. H., Londoño, C. A. E., Vasco, G. J. M., & Molina, J. E. O. (2011). Patologías respiratorias en niños preescolares y su relación con la concentración de contaminantes en el aire en la ciudad de Medellín (Colombia). Revista Ingenierías Universidad de Medellín, 10(19), 21-31.

Ulukaya, E., Ozdikicioglu, F., Oral, A. Y., & Demirci, M. (2008). The MTT assay yields a relatively lower result of growth inhibition than the ATP assay depending on the chemotherapeutic drugs tested. Toxicology in Vitro: An International Journal Published in Association with BIBRA, 22(1), 232–239.

Vineis, P. (2005). Air pollution and cancer: biomarker studies in human populations. Carcinogenesis, 26(11), 1846–1855.

Vargas, M., Parra, A., Gelvez, I. (2014). Genotoxicidad en el aire de Cúcuta-Colombia en muestras del PM 2.5. Revista Ambiental Agua, Aire Y Suelo, 5(1).

WHO. (2014). Calidad del aire (exterior) y salud. Nota descriptiva N°313. Retrieved from http://www.who.int/mediacentre/factsheets/fs313/es/

Zhang, R., Wang, G., Guo, S., Zamora, M. L., Ying, Q., Lin, Y., Wang, Y. (2015). Formation of urban fine particulate matter. Chemical Reviews, 115(10), 3803-3855.

Zuluaga, M., Valencia, A. M., & Ortiz, I. C. (2009). Efecto genotóxico y mutagénico de contaminantes atmosféricos, 28, 33–41.