 doi:10.20944/preprints202111.0022.v1

Article

Genotoxicity of $PM_{2.5}$ and $PM_{1.0}$ Particulates on Human Peripheral Blood Lymphocytes in Manila, Philippines

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Abstract: Urban air quality is increasingly being studied as a fraction of the world's population is now living in megacities. In this study, particulate matter (PM) along Taft Avenue, Manila, Philippines, is investigated in terms of its ability to induce genetic damage on human peripheral blood lymphocytes (PBL). Size-segregated roadside air samples were obtained from 2015-2016 near the university gate and analyzed using *in vitro* micronucleus and cytokinesis-block proliferation tests. While cellular proliferation was unaffected by 0 – 0.1 $kg \cdot m^{-3}$ of $PM_{1.0}$ and $PM_{2.5}$, PBL cells treated with $PM_{2.5}$ displayed significantly higher micronucleus count ($p = 0.03$) compared to the cells treated with $PM_{1.0}$. Atomic Absorption Spectroscopy revealed greater amounts of Cd, Ca, Pb, K, Na, and Zn in $PM_{2.5}$ compared to $PM_{1.0}$. The results indicate the differences in composition of the two size fractions of air particulates are associated with their genotoxicities.

Keywords: Particulate Matter; Genotoxicity; Peripheral Blood Lymphocytes; Atomic Absorption Spectroscopy; *In-vitro* Micronucleus Test

1. Introduction

Particulate matter (PM) are air particles comprising liquid or solid materials that are an *admixture* of organic and inorganic molecules. Common diameter sizes of PM are 1.0 μm, 2.5 μm, and 10 μm. Although the exact mechanisms are still being unravelled, air particulates have been linked to various diseases, from respiratory illnesses, cardiovascular to neurological disorders [1–3]. According to the Environmental Performance Index (EPI), the Philippines is ranked 114th out of 178 countries in poor implementation of environmental policies. In Manila, the average human exposure to $PM_{2.5}$ is 17×10^{-9} $kg \cdot m^{-3}$, exceeding the 10×10^{-9} $kg \cdot m^{-3}$ standard limit for $PM_{2.5}$-exposure by the World Health Organization (WHO) [4]. The Philippines is among the top ten countries globally with the highest death burden due to air pollution which was estimated to account for 64,000 deaths in 2019 [5–6].

Biomonitoring of individuals exposed to high amounts of PM has been a subject of interest in the Philippines due to its heavy air pollution load, especially in its mega-cities [7]. Recolete and Villarino [8] assessed DNA damage to exfoliated buccal cells among

urban street vendors in Iligan City that are considered at health risk due to significant exposures to vehicular exhaust. Vendors who are occupationally exposed to vehicular exhaust showed higher micronucleus frequency compared to a controlled group with 9.40 ± 4.46 and 4.80 ± 3.25, respectively. Similar genotoxicity trends were observed among gasoline station attendants and traffic enforcers in Manila [9]. While there are some studies conducted on DNA damaging effects of air pollution in the Philippines, a more detailed characterization of air particulate components is lacking. Hence, the purpose of this study is to analyze and differentiate the nuclear morphologies of human peripheral blood lymphocytes exposed to $PM_{1.0}$ and $PM_{2.5}$ fractions of air pollutants in Manila.

2. Materials and Methods

2.1. Meteorological and air quality data at the sample site

Average relative humidity (RH) was 63%, and carbon monoxide level was 0.7 ppm throughout July 2015 to May 2016 during air sampling for $PM_{1.0}$. Carbon monoxide and RH levels range from 0.11-0.13 and 69-76%, from December 2016 to March 2017 during sampling for $PM_{2.5}$.

2.2. Collection and Extraction of the PM Samples

The PM samples were collected using a MetOne E-sampler Instrument [10] and a Thermo Scientific Instruments TEOM for $PM_{1.0}$ and $PM_{2.5}$ [11] respectively; both were situated at De La Salle University (DLSU). Filters were readily available in time for the micronucleus assay. The researchers collected the filters of $PM_{2.5}$ on December 1 and 12, 2016; January 23, 2017; February 7 and 28, 2017; and March 21, 2017. For $PM_{1.0}$, the team collected the filters between July 2015 - December 3, 2015, and December 3, 2015 - May 2, 2016. Particulate matter was manually scraped from the air filters and weighed. The recorded weights were 9.5×10^{-6} kg and 6.7×10^{-6} kg for $PM_{1.0}$ and $PM_{2.5}$.

2.3. In-Vitro Micronucleus Assay

Each PM sample was combined with PB Max Karyotyping Medium (Gibco) to produce a stock solution of the PM. The stock solution was diluted to different PM treatment doses, namely 0.05 $kg \cdot m^{-3}$, 0.1 $kg \cdot m^{-3}$, and 0.15 $kg \cdot m^{-3}$. A volume of 0.5×10^{-6} m^3 of blood was added to 4.5×10^{-6} m^3 of PB-Max Karyotyping Medium with the PM sample. Peripheral blood lymphocytes were cultured in a 5% CO_2 environment at 37°C. After 24 hours, 21×10^{-9} m^3 of Cytochalasin B was introduced to the sample, and after 68-72 hours, the culture was harvested. Samples were then placed in a centrifuge at 1200 RPM for 10 minutes. The supernatant was removed from the culture medium and was treated by 7×10^{-6} m^3 of cold (277.15 K) 0.075 M KCl to lyse red blood cells. Again, it was placed in a centrifuge with the same settings and instead treated with 5×10^{-6} m^3 fixative composed of methanol and acetic acid (10:1 ratio) and combined with Ringer's solution one is to one ratio. Steps were repeated about two to three times until the cell suspension is clear. After removing the supernatant to 1 cm or less above the cell pellet, the suspension was dropped onto a clean glass slide and was stained with 2 - 6% Giemsa after drying. Staining was done for about 10 - 20 minutes. Slides were then rinsed with distilled water.

MN analysis was done through light microscopy using an Olympus BX15 Microscope and Nikon DS-Fi3 with 20x magnification for efficient counting. Criteria given by the TG-487 were followed in this study. As stated in the TG-487 [12], at least 1000 binucleated cells were counted for the MN frequency per treatment, along with the negative control. One thousand cells were scored per replicate per treatment. The equation

$$MN\ Frequency = \frac{Number\ of\ BN\ cells\ with\ MN}{Total\ number\ of\ cells} x100 \quad (1)$$

was used to obtain the MN frequency of each treatment per PM size fraction. Two replicates were done for each dose and each PM size. A review panel at the Center for Natural Science and Environment Research (CENSER), DLSU approved the research protocol following the guidelines of the University Research Ethics Committee. The research was conducted under the supervision of a licensed biomedical practitioner of the Philippine Nuclear Research Institute.

2.4. Statistical Analysis of Micronucleus Assay

One-way ANOVA with Tukey-Kramer HSD test was used to evaluate the results for each dose and PM size from the micronucleus assay. The MN frequencies obtained from the two replicates of each dose of each PM size were used to evaluate the genotoxicity of each concentration of PM. The MN frequencies of each dose were then compared to the negative control with a confidence level of 99% ($p \leq 0.01$). Tukey-Kramer was then used to identify which treatment pairs were significantly different. A confidence level of 99% ($p \leq 0.01$) was also used for the post hoc test.

To compare MN frequencies of all the concentrations of $PM_{1.0}$ and $PM_{2.5}$, two-way ANOVA was used with a statistical significance of less than 0.05 ($p \leq 0.05$). Note that the 0.15 $kg \cdot m^{-3}$ of $PM_{1.0}$ was not included in the two-way ANOVA because no binucleate cells were found. Binucleate cells with micronucleus were found in the 0.15 $kg \cdot m^{-3}$ concentration of $PM_{2.5}$. STATISTICA was used for all the statistical analyses done for this study.

3. Results

The size and chemical composition of PM play differentiating roles in the adverse health effects of air pollution. Since the penetration depth and deposition of PM in the pulmonary and circulatory system is particle-size dependent, we evaluated the DNA damaging effects between the two respirable size fractions: $PM_{2.5}$ (fine particulates) and $PM_{1.0}$ (ultrafine particulates). In a review by *Xing et al.* [13], several studies point out that $PM_{2.5}$ is harmful to the respiratory system. A meta-analysis conducted by *Huang et al.* [14] studying the relationship between $PM_{2.5}$ exposure and lung cancer incidence revealed that Asia has the highest lung cancer incidence correlated with $PM_{2.5}$.

On the other hand, $PM_{1.0}$ is deposited in the alveolar, tracheobronchial compartments and can even enter the bloodstream. With such a biological fate, $PM_{1.0}$ is likely to present a more complex set of health risks. For example, *Sanchez-Guerra et al.* [15] showed that exposure of human blood to $PM_{1.0}$ sampled from air pollutants in Beijing induced DNA methylation that has the potential to trigger carcinogenesis. DNA methylation events can inhibit the expression of tumor-suppressing genes that eventually initiate cancer development [16].

3.1. Differential Genotoxicity of $PM_{2.5}$ and $PM_{1.0}$

Figure 1 shows the MN frequencies of human peripheral blood lymphocytes that were exposed to different concentrations of $PM_{1.0}$ and $PM_{2.5}$. Based on the p-values obtained, there was a significant difference in the induction of micronuclei between $PM_{1.0}$ and $PM_{2.5}$. Two-way ANOVA showed that $PM_{2.5}$ was more genotoxic compared to $PM_{1.0}$ at $p = 0.03$. No result was obtained at 0.15 $kg \cdot m^{-3}$ concentration for $PM_{2.5}$ because the level resulted in cytotoxicity.

Figure 2, on the other hand, shows the results of the CBPI (cytokinesis-block proliferation index) count at each concentration of the PM size fractions. There was no significant difference between the CBPI at each treatment concentration, regardless of the size-fraction, indicating that the range of concentrations used for $PM_{1.0}$ did not affect cell proliferation. $PM_{2.5}$, on the other hand, was also non-toxic to the cells from 0.05-0.01 kg m^{-3}.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 November 2021 doi:10.20944/preprints202111.0022.v1

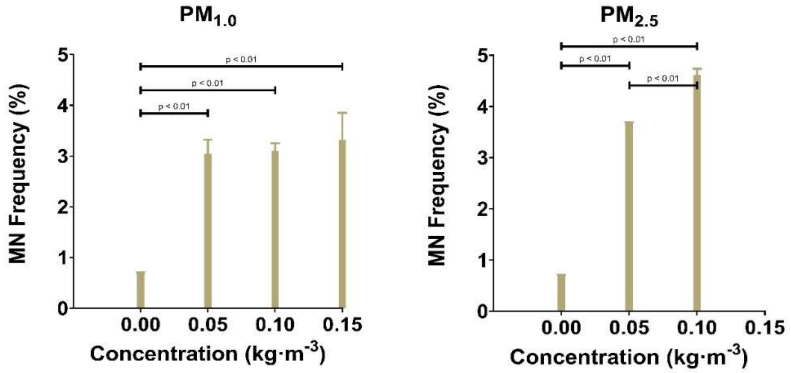

Figure 1. MN frequency of the two PM size fractions. Only the p-values which yielded a significant difference are shown.

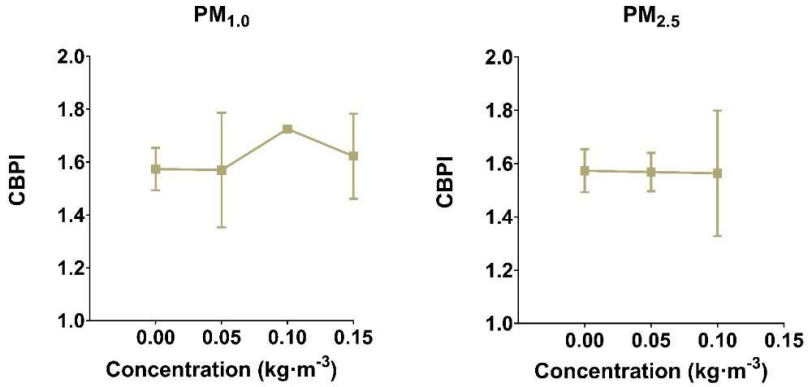

Figure 2. CBPI counts obtained from both PM size fractions.

To further confirm cell proliferation was not affected throughout the concentration range, nuclear morphologies of the cells were assessed. Cell counts of mono, bi-, and poly-nucleated lymphocytes did not significantly differ across the different concentrations for both $PM_{1.0}$ and $PM_{2.5}$ (Figure 3). Thus, except for the highest concentration used for $PM_{2.5}$, cell viability was not affected by the level of analyte used in the experiment. The observations were attributed to the differential genotoxic effects of the two classes of PMs.

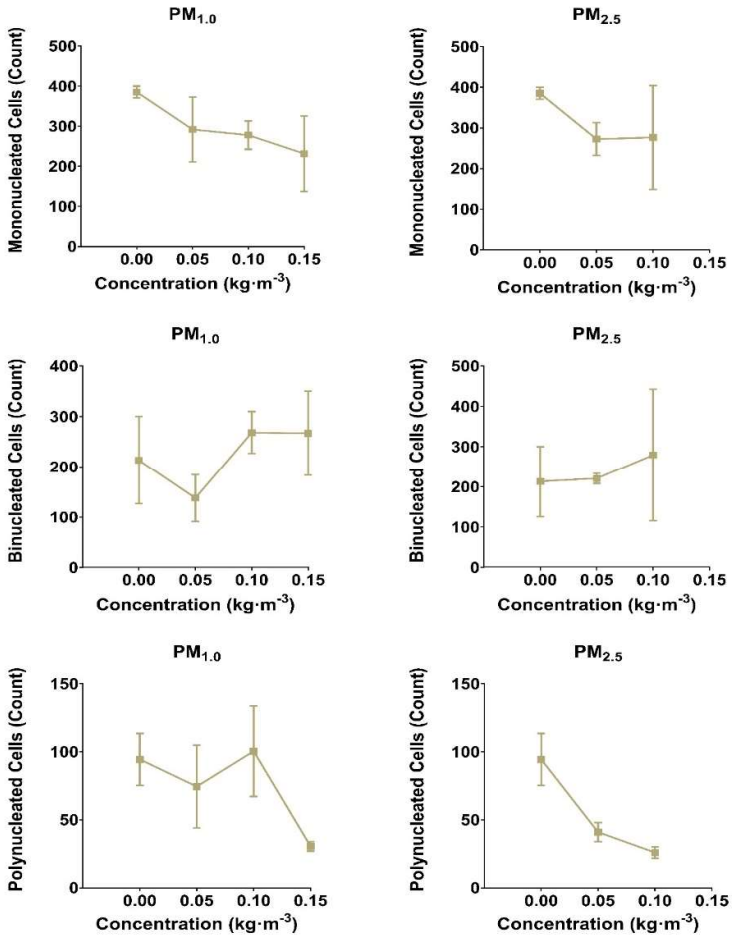

Figure 3. Cell count profile for both $PM_{1.0}$ and $PM_{2.5}$.

3.2. Differences in the heavy metal compositions

The exact chemical nature of the PM fractions that showed different genotoxic activities is still unclear. Since trace metal species from vehicular exhaust emitted into the atmosphere can cause various health-related problems, the presence of heavy metals in the samples was studied. Cadmium (Cd), Lead (Pb), and Zinc (Zn) were found in both PM size fractions. Calcium (Ca), Potassium (K), and Sodium (Na) were also found in the samples used for the AAS analysis. Consistent with the pattern of genotoxicity, the concentrations of the heavy metals, particularly of Pb, are higher in $PM_{2.5}$ compared to $PM_{1.0}$ (Table 1). Since most vehicle exhaust is already lead-free in the Philippines, it is suspected that the trace amounts of lead may originate from second-and third-hand smoke, resuspended soil, and lead-acid car batteries [17].

Table 1. Comparison of the elemental composition. Estimated Method Detection Limit (EMDL) indicates the lowest possible concentration that can be detected. Estimated Limits of Quantitation (ELOQ) is an indication of a high probability of the element in the sample.

Elements	$PM_{1.0}$	$PM_{2.5}$
Cd ($mg \cdot L^{-1}$)	< EMDL (EMDL = 0.5)	< EMDL (EMDL = 0.5)
Ca ($mg \cdot L^{-1}$)	50 (ELOQ = 50)	40 (ELOQ = 50)
Pb ($mg \cdot L^{-1}$)	< EMDL (EMDL = 2)	5 (ELOQ = 7)
K ($mg \cdot L^{-1}$)	1440	2100
Na ($mg \cdot L^{-1}$)	2800	2770

Zn (mg·L^{-1})	3 (ELOQ = 4)	3 (ELOQ = 4)

4. Conclusions

Based on the dose-dependent increase in micronuclei frequency of peripheral blood lymphocytes exposed to $PM_{1.0}$ and $PM_{2.5}$, air pollutants in Manila may have the potential to lead to long-term DNA damage. $PM_{2.5}$ appears to show higher levels of cell toxicity relative to $PM_{1.0}$. This finding is consistent with the elemental signature of the samples where heavy metal concentrations, particularly of lead, are higher in $PM_{2.5}$ compared to $PM_{1.0}$.

Author Contributions: Conceptualization, M.E., J.C., C.V., E.V. and C.D.; methodology, G.C., G.R., C.A.; formal analysis, M.E., J.C., C.V., E.V., M.G. and C.D.; investigation, M.E., J.C., C.V., E.V. and C.D.; resources, M.E., J.C., C.V., and E.V.; writing—original draft preparation, M.E., J.C., C.V.; writing—review and editing, E.V., M.G. and C.D.; supervision, E.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Ethical review and approval were waived for this study.

Informed Consent Statement: Informed consent was obtained from the subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

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