ASSESSING THE CYTOTOXICITY AND GENOTOXICITY OF CHEMICAL COMPONENTS IN FINE PARTICULATE MATTERS ($PM_{2.5}$) FROM DIFFERENT AREAS IN TAIWAN

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Introduction

Fine Particulate matter ($PM_{2.5}$) is an important air pollution which can cause adverse health effects through its physicochemical properties such as size, surface area, and chemical composition[1, 2]. $PM_{2.5}$ can pass through the respiratory tract and accumulate in the alveoli. Epidemiological and toxicological researches indicated $PM_{2.5}$ as carcinogen matter and it was categorized as a "Group 1" carcinogen by IARC[1, 3]. Epidemiological studies suggested that long-term exposure to air pollution might increase the risk of cardiovascular diseases and lung cancer mortality. In the air, the compositions of $PM_{2.5}$ are influenced by surrounding environments including natural sources and anthropogenic activities[4, 5]. The contents of pollutants existed in $PM_{2.5}$ is a key to fully understand and assess its impacts on human health. In Taiwan, different anthropogenic activities could lead to different chemical compositions with variety of toxicity. In this study, seven stations affected by specific anthropogenic activities and different air quality area were selected in Taiwan. In vitro experiment was carried out to evaluate the cytotoxic potential of $PM_{2.5}$ composition on human lung carcinoma cell line A549 and genotoxic potential on *Salmonella typhimurium*. Cytotoxic effects of exposure to the $PM_{2.5}$ were measured by MTT assay and reactive oxygen species (ROS) test, when genotoxicity were measured by umu assay. The main goal of this study was to investigate the toxic effects of different chemical compositions in $PM_{2.5}$ and assessing its impacts on cells of respiratory system.

Materials and methods

Polychlorinated dibenzo-p-dioxins and dibenzofuran (PCDD/F), polycyclic aromatic hydrocarbon (PAH) concentration and other chemical compositions in $PM_{2.5}$ were collected in three ambient air sampling sites during 2017 and 2018 including one station (Sites A) in the coastal area which is usually affected by long range transport activities, one traffic sampling site in the center of Taipei basin (Site B), one sampling site in the night market (Site C), two sampling sites in the industial park in northern and central Taiwan (Site D and Site E) and two rural stations in central (Site F) and eastern (Site G) Taiwan. $PM_{2.5}$ compounds were collected using high volume sampling trains (Analitica HVS-$PM_{2.5}$ and Sibata HV-1000R) with the flow rate of 500L/min. In this research, the composition of the ambient fine particles matter in different regions of Taiwan were estimated, and the dose-response relationship of different chemical components in $PM_{2.5}$ was determined by in vitro test and toxicity analysis. Components of the samples including metal elements, organic carbon (OC), elemental carbon (EC), water-soluble cation/anion, PCDD/Fs, and PAHs were extracted and analyzed using the standard techniques, accordingly. The water-soluble ions, metal, and organic components, were exposed to human lung carcinoma cell line A549 for cytotoxicity estimation when genotoxic potential was done with exposure to *Salmonella typhimurium*. In vitro tests including MTT assay, ROS assay, and Umu assay were utilized in this study.

Results and discussion

The $PM_{2.5}$ concentrations at different regions in Taiwan were estimated to be 39.0 μg/m^3, 43.0 μg/m^3, and 26.3 μg/m^3 from LRT event (Site A), local traffic emission (Site B) and night market (Site C) respectively. SO_4^{2-} was dominant component in all three sources of PM2.5. However, it can be seen the organic/inorganic composition at each sources were not the same (Figure 1). This difference might lead to the difference in the toxic effect toward human health and cell growth. MTT assay for cell viability (Figure 2) found that metal and organic extracts from the cooking smoke in night market caused strongest impact on the viability of A549 cells. Statistical estimation found significant relationship between 18 metal elements and cell viability, three most toxic elements included Vanadium ($r=-0.626$, $p<0.001$), Cadmium ($r=-0.565$, $p=0.009$), and Zinc ($r=-0.606$, $p=0.005$). Comparing to negative control (blank filter), elevated ROS were detected in metal exposure of all sources, in ion exposures in long range transport and traffic sources, and in organic compound exposure in traffic sources and cooking source (night market) (Figure 3). On the other hand, the organic extracts from A, B, and C area, respectively, were applied to *umu* test in *Salmonella typhimurium* (Figure 4). BaP at concentration of 10μM was used as a positive control. The organic extracts obtained from cooking smoke source (C) was found to cause the highest genotoxicity among the three anthropogenic emission. The genotoxicity effect elevated when the exposed dose of PM2.5 increased from 5 μg to 20 μg. It can be explained that the highest Benzo[a]pyrene equivalents (BAPeq) of 3.75 was found in C source. Meanwhile, only the high dose of LRT organic extracts (20 μg of $PM_{2.5}$) caused cytotoxicity on cell viability and genotoxicity when the extracts from traffic emission did not show any significant effect. It can be concluded that the metal and organic extracts of cooking smoke source caused the strongest cytotoxicity on cell viability and ROS determination, and the organic extracts also induced the strongest effect on *umu* genotoxicity test.

In parallel, $PM_{2.5}$ samples collected at two industrial areas (site D and site E) were found to be 27.4±9.90 and 8.92±0.99 μg/m^3, respectively, when that of rural areas (site F and site G) were found to be 16.8±2.39 and 11.0±2.40 μg/m^3, respectively. Cell viabilities of A549 cells were calculated after being exposed to $PM_{2.5}$ at different concentration (Figure 5). There was a clear decline of cell viability in the two rural areas (site F and G). In these two stations, $PM_{2.5}$ collected in winter also showed higher toxicity to cell when $PM_{2.5}$ in winter lead to lower cell viability than that of $PM_{2.5}$ in summer at the same concentration. On the other hand, the different in cell viability between blank sample and $PM_{2.5}$ samples in industrial stations were not notable. This difference might be explained by the content of chemical compounds in $PM_{2.5}$ collected in these two areas. It can seen from the results that even the industrial areas might show higher $PM_{2.5}$ concentration, the impact of cell viability might not be meaningful.

In conclusion, the finding of our research suggested that it is not only PM2.5 concentration but also the chemical composition of those particulate matters influence cell growth.

Acknowledgements

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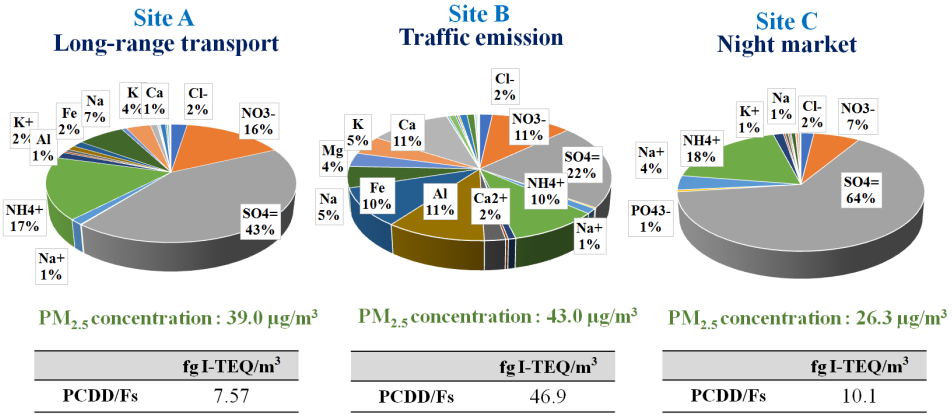

	fg I-TEQ/m^3
PCDD/Fs	7.57

	fg I-TEQ/m^3
PCDD/Fs	46.9

	fg I-TEQ/m^3
PCDD/Fs	10.1

Figure 1. Organic and inorganic component in $PM_{2.5}$ at different area in Taiwan.

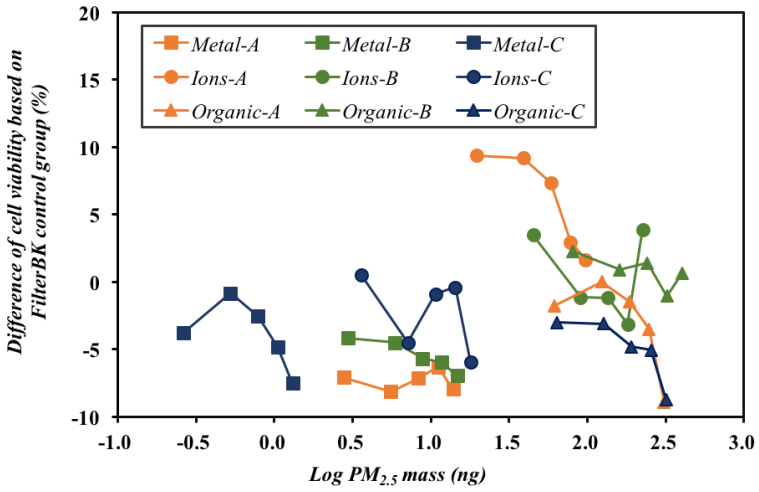

Figure 2 MTT assay for cell viability via $PM_{2.5}$ exposure at different area in Taiwan.

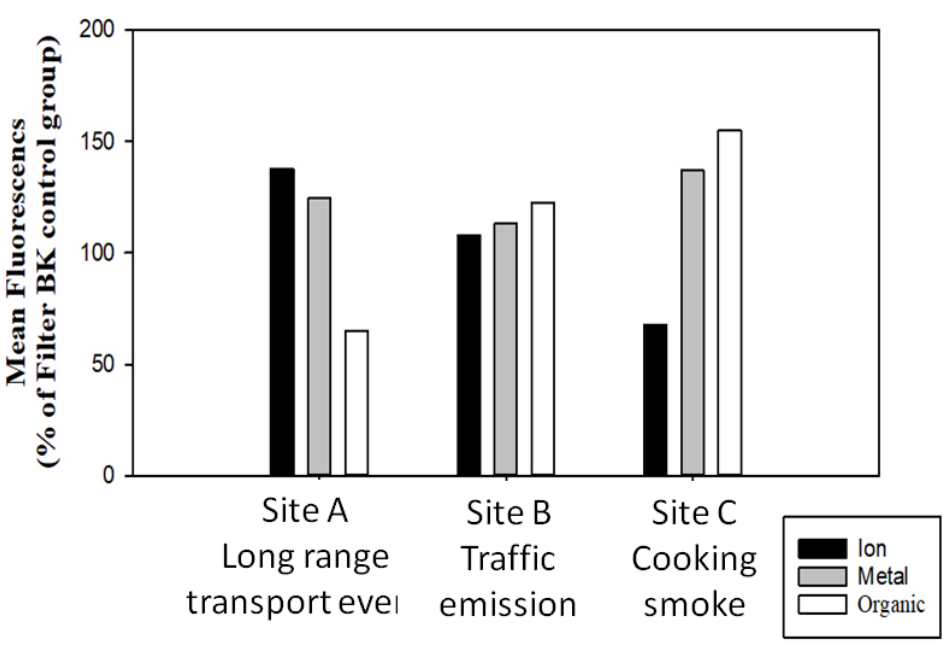

Figure 3 Reactive oxygen species (ROS) detection via $PM_{2.5}$ exposure at different area in Taiwan.

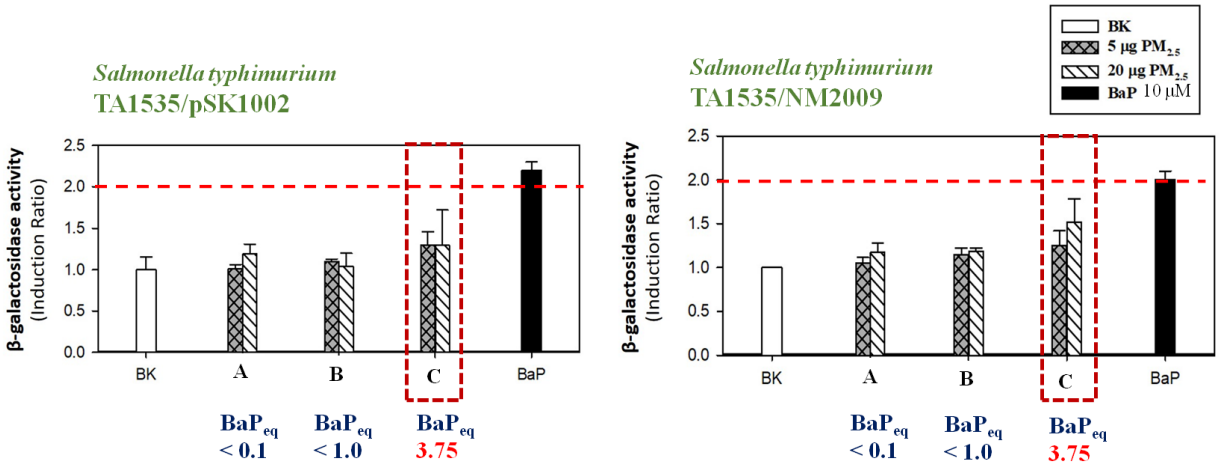

Figure 4 Genotoxicity result via $PM_{2.5}$ exposure at different area in Taiwan

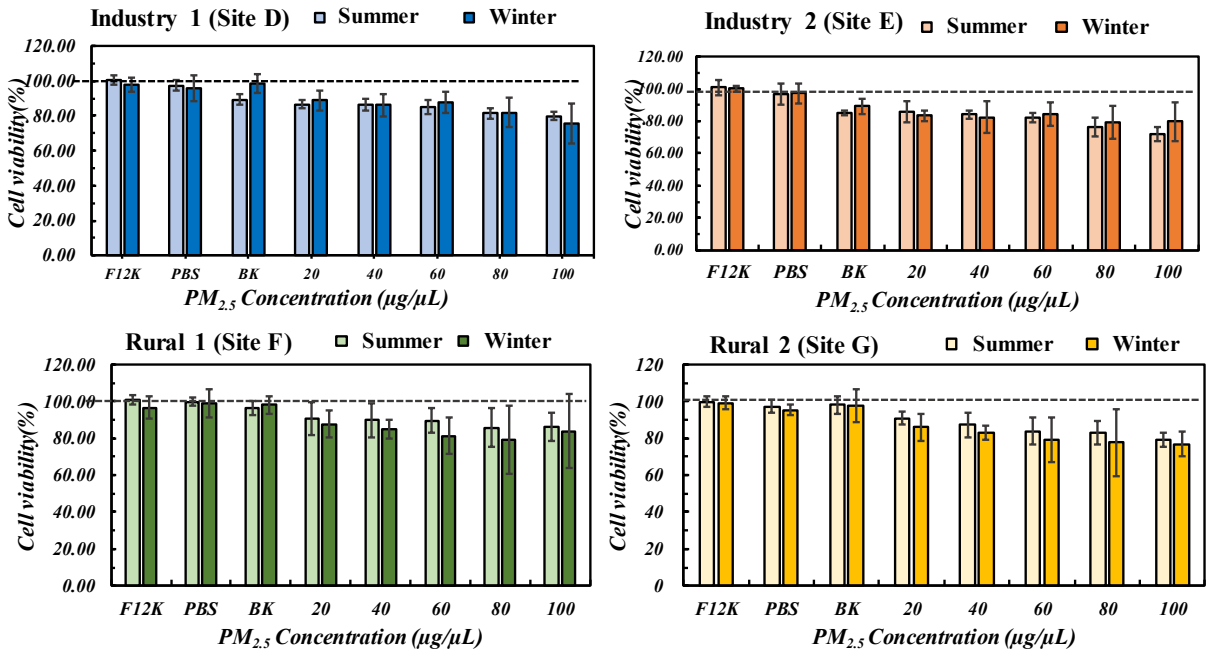

Figure 5: Cell viability of A549 cells exposed to $PM_{2.5}$ collected from industrial and rural areas