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Characterization and cytotoxicity of PAHs in $PM_{2.5}$ emitted from residential solid fuel burning in the Guanzhong Plain, China☆

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The emission factors (EFs) of polycyclic aromatic hydrocarbons (PAHs) in $PM_{2.5}$ were measured from commonly used stoves and fuels in the rural Guanzhong Plain, China. The toxicity of the $PM_{2.5}$ also was measured using *in vitro* cellular tests. EFs of PAHs varied from 0.18 mg kg^{-1} (maize straw charcoal burning in a clean stove) to 83.3 mg kg^{-1} (maize straw burning in Heated Kang). The two largest influencing factors on PAH EFs were air supply and volatile matter proportion in fuel. Improvements in these two factors could decrease not only EFs of PAHs but also the proportion of 3-ring to 5-ring PAHs. Exposure to $PM_{2.5}$ extracts caused a concentration-dependent decline in cell viability but an increase in reactive oxygen species (ROS), tumor necrosis factor *a* (TNF-α) and interleukin 6 (IL-6). $PM_{2.5}$ emitted from maize burning in Heated Kang showed the highest cytotoxicity, and EFs of ROS and inflammatory factors were the highest as well. In comparison, maize straw charcoal burning in a clean stove showed the lowest cytotoxicity, which indicated a clean stove and fuel treatment were both efficient methods for reducing cytotoxicity of primary $PM_{2.5}$. The production of these bioreactive factors were highly correlated with 3-ring and 4-ring PAHs. Specifically, pyrene, anthracene and benzo(a)anthracene had the highest correlations with ROS production (R = 0.85, 0.81 and 0.80, respectively). This study shows that all tested stoves emitted $PM_{2.5}$ that was cytotoxic to human cells; thus, there may be no safe levels of exposure to $PM_{2.5}$ emissions from cooking and heating stoves using solid fuels. The study may also provide a new approach for evaluating the cytotoxicity of primary emitted $PM_{2.5}$ from solid fuel burning as well as other $PM_{2.5}$ sources.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), which are the principal organic pollutants from incomplete combustion of fuel, are of special interest due to their toxicity, carcinogenicity and ubiquitous presence in the environment (Niu et al., 2017; Shen et al., 2011b). Generally, compared to industrial combustion processes equipped with pollution control systems, the domestic cooking/heating process in rural areas without pollution control has much higher emission factors (EFs) of PAHs (Oanh et al., 1999). An estimated 504 Gg PAHs were emitted in 2007 globally and nearly half originated from residential solid fuel burning (RSFB) (Shen et al., 2013b).

High emission of PAHs from combustion sources is related to several factors. The first of these is the type of residential solid fuels (such as branches, crop residues, charcoal and bituminous coal). Approximately 41% of total households globally in 2010 relied on solid fuels for cooking and space heating, and crop residue was the most popular fuel used (Bonjour et al., 2013; Xu et al., 2016b). In developing countries, this proportion reached 80% (Zhang and Tao, 2009). Solid fuels with high volatile matter (VM) contents (i.e., straw, branch and bitumite) lead to a higher possibility of incomplete combustion than those having low VM content (Shen, 2017; Shen et al., 2014a). The second reason for high PAH emission from RSFB is low energy efficiency for the traditional combustion stove. Worldwide, three-stone stoves with an energy efficiency of 8–12% and traditional domestic cookstoves with an energy efficiency of

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10–15% are still widely used in rural areas (MacCarty and Bryden, 2015). Moreover, a wide range of traditional space-heating cookstoves with energy efficiencies in the range of 25–30% are used as main heating measures in the rural areas where individual residential heating is popular (Zeng et al., 2007). The extremely low combustion efficiencies lead to high energy wastage and high emissions of contaminants such as particulate matter (PM), carbon monoxide and PAHs (Shen et al., 2011a; Shen et al., 2010; Sun et al., 2017).

After two "energy conservation and emission reduction" campaigns implemented by the Chinese government (National Improved Stove Program, 1982–1992; 11th to 12th Five-year Plan, 2011–2015) (Chowdhury et al., 2013; Mehetre et al., 2017), the residential stove market in China is currently (2017) selling an unprecedented number of units. Consequently, various types of stoves coexist in rural areas of China. This situation suggests the effectiveness of the emission reduction policy, but causes difficulties for documenting environmental impacts because the EFs statistics for different stoves vary enormously (Shen et al., 2014a; Zhi et al., 2008). For instance, EF of PM and PAHs from traditional stoves could be 1–2 orders of magnitudes higher than those of modern stoves that have a secondary air supply (Shen et al., 2010; Sun et al., 2017). Accurate measurements of EFs from different solid fuels and stoves could reduce the high uncertainty in emission inventories (Gullett et al., 2004; Johnson et al., 2010; Lu et al., 2009).

The majority of previous studies on emissions from cookstoves and heating stoves focused on the exposure assessment of PM or equivalent PAHs; however, these metrics are only indicative of carcinogenic and noncarcinogenic risks (Bostrom et al., 2002; Dorne et al., 2011; Zhang et al., 2009). Organic matter in PM is capable of causing cellular oxidative stress (Li et al., 2010) and some PAHs can cause pro-inflammatory effects (Lin et al., 2013). Furthermore, production of reactive oxygen species (ROS), which can induce inflammatory reactions in human cells, is closely related with some PAHs (Benbrahim-Tallaa et al., 2012). The research on estimating ROS production triggered by primary PM from RSFB is rare, but it could be useful for visualizing and evaluating the cytotoxicity of PM from RSFB.

The Guanzhong Plain area in China covers approximately 36,000 km^2 and has a population of almost 24 million. The traditional type of wintertime residential heating in rural areas is burning maize straw in a "Heated Kang", which causes serious rural and urban air pollution problems (Shen et al., 2009, 2014a; 2014b; Sun et al., 2017). The dependency on solid fuels exceeds 80% in the rural Guanzhong Plain (Hou et al., 2017). Sun et al. (2017) described the field and laboratory measurements of $PM_{2.5}$ EFs from RSFB in detail.

The objectives of this study were 1) to measure and characterize the EFs of PAHs from RSFB in different conditions in the Guanzhong Plain, China, and 2) to investigate the cytotoxicity of the $PM_{2.5}$ emitted from RSFB and the correlation between cytokines and particle-bound PAHs.

2. Methodology

2.1. Stoves and fuels

Three types of stoves were evaluated: a Heated Kang (HK), an "old-fashioned" stove (OS) and a clean stove (CS). A brief description and nomenclature for each experiment are given in Table 1. Sampling was conducted both in a laboratory and on site in a typical village in the Guanzhong Plain, China. $PM_{2.5}$ samples were collected on site both HK (Fig. S1a) and OS (Fig. S1b). Maize straw was the typical fuel used in HK. Two scenarios were examined according to usual domestic practices: long smoldering for almost the whole night (MS-HK1) and short smoldering before bedtime (MS-HK2). The smoldering time control was realized by regulating the air supply rate. For OS, bitumite (BI-OS) and anthracite (AN-OS) were the main fuels used for heating, and maize straw (MS-OS) was usually used for ignition.

For CS (Fig. S1c), the experiments were conducted in a laboratory combustion chamber (described by Sun et al., 2017). The CS tested in this study was fitted with a secondary air supply, and was a type that was widely used for cooking and heating in the selected village. The stove could use several types of fuels, including straw, briquettes and charcoal. Three of the main fuels used in the area (maize straw, rice straw and wood branches) were selected for the laboratory tests to simulate the real scenarios. The fuels collected were air-dried under storage, which means they were stored in ambient temperature (~20 °C) and controlled relative humidity (~35–40%) before analyzed and used. Proximate analysis was conducted for the air-dry fuels and the results are shown in Table S1. On an air-dry fuel basis, the sum of moisture, ash, volatile matter and fixed carbon (all in %) were 100%.

A specially fabricated dilution system having a dilution rate from 5- to 50-fold was used to collect the smoke emitted from solid fuel burning stoves in the on-site experiments. A certain number of parallel diluted smoke channels were fitted for online and offline samplers. The air-stream of diluted smoke was drawn into three channels fitted with mini-volume samplers (Airmetrics, Springfield, OR, USA). Each sampler operated at a flow rate of 5.0 L min^{-1} and collected the $PM_{2.5}$ on Quartz-fiber (QM/A, Whatman, Maidstone, UK) and Teflon® filters, which were both pre-treated before being used. For each stove -fuel experiment, replication was conducted at least 3 times to get the average and standard deviation data. The number of replicate experiments is shown in Table 1. The field dilution sampling equipment and procedures are described in detail in Sun et al. (2017).

2.2. PAHs measurements

Collection methods of $PM_{2.5}$ samples, both in laboratory and on site, were the same as described in Sun et al. (2017). One-half of each quartz-fiber filter was extracted with high-purity dichloromethane and methanol (2:1, v/v) under ultrasonication for 15 min. The extraction procedure was repeated three times to ensure the completeness of extraction. Water and debris in the combined extracts were then removed by passing the liquid through Pasteur pipettes filled with sodium sulfate (Na_2SO_4) and glass wool. The extracts were finally concentrated to 1 mL by a rotary evaporator under vacuum. Then the samples were analyzed using a gas chromatography/mass spectrometer (GC/MS) (Model 7890A/5975C, Agilent Technologies, Santa Clara, CA, USA). The settings of GC/MS programs are shown in Niu et al. (2017). In all, 16 preferential controlled PAH species were measured: naphthalene (NAP), acenaphthene (ACE), acenaphthyleme (ACY), fluorene (FLO), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), benzo(a)anthracene (BaA), chrysene (CHR), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo- (a)pyrene (BaP), dibenz(a,h)anthracene (DahA), indeno(l,2,3- cd)pyrene (IcdP), and benzo(g,h,i)perylene (BghiP).

2.3. Filter extraction for cell toxicity studies

One-half of each particle-laden Teflon® filter was immersed in 2 mL of high-purity methanol, and then subjected to ultrasonication in an ice-cooled water bath for 30 min. The extraction procedure was repeated twice, and the combined extracts of each filter were purged under a gentle stream of nitrogen ($N_2 > 99.995\%$) for 2 h to completely remove the solvent. The extracts after