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Research Paper

# Hemolysis of $PM_{10}$ on RBCs *in vitro*: An indoor air study in a coal-burning lung cancer epidemic area

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ABSTRACT

Epidemiological studies have suggested that inhalation exposure to indoor ambient air from coal-burning environments is causally associated with respiratory health risks. In order to explore the toxicological mechanisms behind the adverse health effects, the hemolytic activity of $PM_{10}$ (particulate matter with an aerodynamic diameter of 10um or less) samples collected from homes burning coal in the recognized China "cancer village" Xuanwei were evaluated and matched against their trace elemental contents. The results demonstrated that the hemolytic activity of indoor $PM_{10}$ in coal-burning environments ranged from 4.28% to 5.24%, with a clear positive dose-response relationship. Although low dose samples exhibited a reduced hemolytic activity, $PM_{10}$ could have a toxic effect upon people in a coal-burning indoor environment for extended time periods. The concentrations of analyzed trace elements in $PM_{10}$ samples ranged from 6966 to 12,958 ppm. Among the analyzed elements, Zn, Ti, Ni, Cu, Pb, Ba, Mn, Cr and V were found at higher concentrations and accounted for over 95% of the total elements. The concentrations of total analyzed elements in the $PM_{10}$ samples revealed a significant positive correlation with $PM_{10}$ hemolytic activity. Of the analyzed elements, Zn, Pb and Cs positively correlated with hemolysis, while Li, U and V negatively correlated with the hemolysis of human red blood cells (RBCs). Therefore, the heavy metal elements could be one of the main factors responsible for the hemolytic capacity of indoor $PM_{10}$ in coal-burning environments.

## 1. Introduction

In recent years, the impact of airborne particulate matter 10 μm or less ($PM_{10}$) on human health has attracted widespread attention (Li et al., 2020; Silva et al., 2020a; Xing et al., 2020). Epidemiological investigations have indicated a link between increase of the mass levels of airborne particles and adult morbidity and mortality (Kim et al., 2016; Li et al., 2017; Hamanaka and Mutlu, 2018). However, the biological mechanisms underlying the adverse health effects of airborne particles still remains unclear. Many methods have been used to assess the toxicity of $PM_{10}$, including animal tests (Marchini et al., 2016; Jin et al., 2018), single-cell gel electrophoresis (SCGF; Velali et al., 2016; Bahadori et al., 2018), Ames fluctuation test (Klobučar, 2011; Du et al., 2019), plasmid DNA assay (Greenwell et al., 2002; Feng et al., 2020) and the hemolysis assay (Quintana et al., 2011; Faraji et al., 2019).

The hemolysis assay is a practical and rapid method to investigate the cytotoxicity and hemolytic properties (*i.e.* biological membrane damage capacity) of particulate matter (PM) (Hadnagy et al., 2003; Mesdaghinia et al., 2019). Particles dissolved in water exhibit a strong free radical signal of reactive oxygen species (ROS) (Tong et al., 2001), which is known to be cytotoxic (Nash et al., 1966) and interacts with the cell membrane inducing an increase of membrane fluidity and permeability. This interaction may exhaust the protective mechanisms of the cell, and thereby, causes lipid peroxidation of RBCs and their cell components, leading to cell membrane rupture.

Epidemiological investigations have reported that PM pollution induced decreased human red blood cells (RBCs) counts and even anemia (Nikolic et al., 2008; Bahrami et al., 2013; Kargarfard et al., 2015). To further investigate the mechanism, Faraji et al. (2019) analyzed chemical compositions of $PM_{10}$ in two air pollution conditions (*i.e.* dust storms and inversions) and evaluated the toxicological effects of $PM_{10}$ collected in these conditions on the hemolysis of human RBCs *in vitro*. They have identified the significant positive relationship between concentration of PM and hemolytic activity. In addition, Quintana et al. (2011) compared

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the PM collected at an urban-emitting site and a suburban-receptor site and found that the suburban-site induced greater hemolysis. All of these studies indicated that the hemolysis effect is related not only to the concentrations of PM, but also to the composition.

At present, the use of coal for heating and cooking is still the main source of indoor air pollution in rural areas (Zheng and Bu, 2018; Liu and Mauzerall, 2020). Especially in Xuanwei county, Yunnan Province, the mortality and morbidity rate of lung cancer is the highest among the Chinese cities (Mumford et al., 1987). According to the "2012 Chinese Cancer Registry Annual Report", the mean morbidity rate of lung cancer in Xuanwei was 92 per 100,000 which is 4–5 times the national rate (Hao et al., 2012). A number of studies have indicated that lung cancer in Xuanwei was highly associated with the domestic burning of local bituminous coal (Xiao et al., 2012; Seow et al., 2014). The coals for residents in Xuanwei are from the C1 coal seam situated geologically at the end-Permian mass extinction (Large et al., 2009; Shao et al., 2015; Wang et al., 2018) which is characterized by relatively enriched Si together with elevated contents of heavy metals including Zn, Pb, Cd and Cu, in comparison to Chinese coal averages and world averages (Shao et al., 2015). The unusual properties for this coal may result in the special characteristics of compositions and toxicity of the coal-burning particles.

Indoor PM emitted from coal burning in Xuanwei includes high concentration of heavy metals (Shao et al., 2013), crystalline silica (Tian et al., 2008; Large et al., 2009), and polycyclic aromatic hydrocarbons (PAHs; Mumford et al., 1995; Lui et al., 2016). These toxic components of PM can enter the respiratory and circulatory systems and be retained in the body (Pinkerton et al., 2000; Chuang et al., 2016), potentially leading to pneumonia, asthma, cardiovascular disease, and even lung cancer (Zhou et al., 2015; Lin et al., 2016). Different methods have been utilized to better understand the toxicity of indoor particulate matter in coal-burning environments. Mumford et al. (1990) compared mouse skin tumorigenicity induced by indoor air particles from coal combustion and wood combustion in Xuanwei and linked this to human lung cancer. Their results showed that, compared to the particles emitted from wood combustion, the particles emitted from coal combustion were more active in tumor initiation; these findings agreed with the epidemiological data, which showed that the residents using 'smoky' bituminous coal as their major fuel in homes had a higher lung cancer mortality rate (Mumford et al., 1990). Li et al. (2012) measured cell viability by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MMT) method and concluded that the particles emitted from the combustion of bituminous coal had great cytotoxicity. Shao et al. (2013) used the plasmid scission assay to investigate the bioreactivity of $PM_{10}$ in coal-burning environments. The results showed that indoor $PM_{10}$ caused higher oxidative damage than corresponding outdoor $PM_{10}$, and heavy metals could be one of the main factors responsible for the high oxidative capacity of indoor $PM_{10}$. Since few studies have been undertaken on the cytotoxicity of coal-burning particles, understanding hemolysis damage induced by indoor coal-burning $PM_{10}$ in lung cancer village has an important role in studying the toxicity of particles.

In this paper, the coal-burning indoor $PM_{10}$ samples collected in the high lung cancer village in Xuanwei, Yunnan Province, and the hemolysis assay is used to assess the toxicity of these indoor $PM_{10}$. Trace element compositions of $PM_{10}$ were examined by inductively coupled plasma mass spectrometry (ICP-MS). The relationship of the heavy metal compositions with the toxicity of these inhalable particles was further discussed.

## 2. Methods

A sampling campaign was carried out in the Xuanwei area during 2013 and 2014. $PM_{10}$ samples were collected from two homes in Hutou Village, which represents the high lung cancer mortality area in China (Mumford et al., 1987). The sampling site in Home#1 was in the kitchen, which had a chimney. Except cooking with coal during daytime, no daily activity took place in this room. The sampling site of Home#2 was in a connected space between the living room and kitchen. The residents of Home#2 used induction cookers for cooking during daytime and portable stoves burning coal for heating at night in the winter. The two houses both used bituminous coal purchased from Yantang coal mine about 1.4 km away from the village. $PM_{10}$ selective inlet heads (C-30, Thermo Fisher Scientific, USA) and polycarbonate filters (47 mm diameter, 0.67 mm pore size, Millipore, UK) were used to collect $PM_{10}$ at a flow rate of 30 L/min. The sampling points were at the average breathing heights (1.5 m above the floor). Detailed sample information is shown in Table 1.

The hemolysis assay was undertaken in the School of Biosciences, Cardiff University and the School of Health Sciences, Cardiff Metropolitan University. The $PM_{10}$ samples were incubated with isolated RBCs and analyzed by the Tecan Infinite® 200 PRO plate reader at λ 540 nm. Initially 10 mL of blood was drawn from a 40 year old female donor. The blood samples were centrifuged at 3750 rotations per minute (rpm) at 4 °C for 10 min; the supernatant and a thin layer of platelets were removed from the blood and discarded. The blood samples were re-suspended with 0.9% saline to make them back up to 10 mL; this was repeated two more times. Four 1 mL aliquots were stored in Eppendorf tubes and centrifuged again at 1700 rpm for 5 min. The final working solution of diluted RBCs was made by adding 200 μL from each of the four Eppendorf tubes to 7.2 mL of 0.9% saline to make a total volume of 8 mL. The $PM_{10}$ was suspended in the phosphate buffered saline (PBS, Sigma, UK) and diluted to 5 doses: 2 mg/mL, 1 mg/mL, 500 μg/mL, 250 μg/mL, 50 μg/mL. The $PM_{10}$ solution (125 μL) was added to the prepared blood (125 μL) in a 96-well (Cole-Palmer, UK) plate which was then sealed with adhesive plastic and placed on a platform shaker (ThermoFisher Scientific, USA) for 10 min. The plate was then centrifuged at 200 rpm for 5 min at 4 °C, 100 μL of supernatant was removed and placed into a corresponding well of a new 96-well plate. The negative control contained 0.9% PBS and the positive control contained 0.1% Triton X-100 mixed with the prepared blood. The plate containing the supernatant had its optical density read using the Tecan Infinite® 200 PRO plate reader at 540 nm. The absorbance readings in optical density from the plate reader was converted into percentage of hemolysis with the following formula: % hemolysis = [(sample OD-control OD)/(standard OD-control OD) × 100] (Dobrovolskaia et al., 2008).

The trace elements in the $PM_{10}$ samples were analyzed by ICP-MS using a Thermo Elemental X Series (X7; ThermoFisher Scientific, USA). The solution of the whole sample was prepared by digesting pooled samples of polycarbonate filters using concentrated nitric acid (Fisherbrand™ Precision Specific Gravity 1.48). Digestions were carried out in a CEMMDS-200 microwave system, using CEM advanced composite vessels with Teflon liners. The digested samples were then concentrated by evaporating the nitric acid and by re-dissolving in 2 mL of 10% nitric acid. Samples were diluted to 20 mL volume using deionized (>18 MU) water. One milliliter of each sample was combined with a 50 ryam ppb thallium standard (1 mL) and this solution was made up to 10 mL with 2% nitric acid to be analyzed in the ICP-MS. The samples were run with an international basalt standard as a positive control. The detection limits of the elements were in the range of 1 ppt

**Table 1**
Sampling information.

| Number | Sampling season | Site | Sample weight (μg) | $PM_{10}$ concentration ($\mu g/m^3$) |
|---|---|---|---|---|
| 1 | Winter | Home#1 | 3445 | 148.5 |
| 2 | | Home#2 | 2311 | 169.6 |
| 3 | Summer | Home#1 | 1008 | 331.4 |
| 4 | | Home#2 | 935 | 261.5 |

(parts per trillion) to 1 ppb (parts per billion). The final results were reported as the ppm (parts per million) or ppb of each element in the analyzed $PM_{10}$.

Statistical evaluation was performed by SPSS Statistics 17.0. Pearson correlation tests was used to assess the relationship between the concentrations of trace elements and the hemolysis under the dose of 500 μg/mL. $P < 0.05$ and $P < 0.1$ was regarded as significant for analysis.

## 3. Results

The values of hemolysis at the five doses; 50, 250, 500, 1000, 2000 μg/mL are shown in Table 2. The highest doses were 2000 μg/mL in winter and 1000 μg/mL in summer. Hemolysis ranged from 4.28% to 5.24%, with Home#1 having the lowest value in winter, with 4.28% at 250 μg/mL, and Home#2 having the highest value in summer, with 5.24% at 1000 μg/mL. Hemolysis in Home#2 [$(4.66 \pm 0.34)\%$] was higher than Home#1 [$(4.5 \pm 0.20)\%$], but this difference was not significant ($p = 0.45$).

The concentrations of 42 trace elements in the $PM_{10}$ were analyzed by ICP-MS (Table 3). Fig. 1 shows the mean value of trace element concentrations (over $10 \times 10^{-6}$) under the different sampling conditions (Ramirez et al., 2020). Total trace elements concentrations of indoor $PM_{10}$ in Xuanwei ranged from 6966 to 12,958 ppm, averaged 9462 ppm. Zn, Ti, Ni, Cu, Pb, Ba, Mn, Cr, V had the highest concentrations, and accounted for over 95% of the total trace elements. Zn had the highest concentrations ranging from 1926 to 7720 ppm, with the mean value 3494 ppm. The trace element concentrations of PM in Xuanwei showed a significant higher value than other cities (Silva et al., 2020b; Zhang et al., 2020; Lima et al., 2021). Compared with airborne particles in Beijing (Shao et al., 2019), the trace element concentrations of indoor $PM_{10}$ in Xuanwei showed 3–5 times higher. It should be noted that Sc and W were not found in Beijing. The heavy metals, such as Ti, V, Co, Ni, Eu, Cu, Cd, Pr, Nd, and In, had noticeably higher concentrations, being 5–10 times higher in Xuanwei than in Beijing.

## 4. Discussion

The results of linear fitting (Fig. 2) showed that the hemolysis was positively correlated with the doses of $PM_{10}$ ($R^2 = 0.77–0.90$), except the sample collected during winter in Home #2. A similar result in Iran has been reported that hemolysis raised with increased PM concentration (Faraji et al., 2019). The positive dose-response relationships between hemolysis and the sample concentrations indicated that the indoor airborne particles in Xuanwei could induce toxic effects if the particulate matter reached a high enough mass concentration. Previous studies have shown that particle matter, once inhaled, cannot be completely removed from the body in a short term, and some may be retained in the lungs (Oberdörster, 1988). Although low dose samples showed a low hemolytic activity, it can be inferred that if people are exposed to a higher concentration of coal-burning emissions for a longer time, the toxic effects of particles to human health would become significance.

A number of studies have shown that heavy metal elements in particulate matter have strong oxidative capacity (Huang et al., 2015; Song et al., 2015; Gallego-Cartagena et al., 2020; Oliveira et al., 2020). Shao et al. (2013) demonstrated that the concentration of soluble trace elements in the coal-burning indoor $PM_{10}$ samples in Xuanwei

**Table 2**
Quantification of hemolysis induced by indoor $PM_{10}$ in the Xuanwei coal-burning environments.

| Dosage(μg/mL$^{-1}$) hemolysis(%) | | 50 | 250 | 500 | 1000 | 2000 |
|---|---|---|---|---|---|---|
| Winter | Home#1 | 4.31 | 4.28 | 4.36 | 4.31 | 4.51 |
| | Home#2 | 4.37 | 4.4 | 4.39 | 4.35 | 4.38 |
| Summer | Home#1 | 4.53 | 4.69 | 4.68 | 4.87 | |
| | Home#2 | 4.71 | 5.02 | 5.07 | 5.24 | |

**Table 3**
The contents of trace elements in the indoor $PM_{10}$ in the Xuanwei coal-burning environments (in ppm).

| Elements | Summer | | Winter | | Mean | SD[*] |
|---|---|---|---|---|---|---|
| | Home#1 | Home#2 | Home#1 | Home#2 | | |
| Li | 8.51 | 9.16 | 12.92 | 13.70 | 11.07 | 2.27 |
| Be | 1.47 | 1.75 | 2.36 | 3.02 | 2.15 | 0.60 |
| Sc | 3.76 | 2.23 | 1.77 | 5.15 | 3.23 | 1.33 |
| Ti | 1620.00 | 1237.00 | 1581.00 | 3071.00 | 1877.25 | 705.14 |
| V | 82.30 | 26.40 | 134.77 | 172.00 | 103.87 | 54.92 |
| Mn | 333.00 | 663.00 | 230.67 | 561.00 | 446.92 | 172.81 |
| Cr | 128.00 | 277.00 | 250.00 | 536.00 | 297.75 | 148.57 |
| Co | 51.40 | 18.90 | 13.47 | 30.80 | 28.64 | 14.56 |
| Ni | 3169.00 | 1058.00 | 672.33 | 835.00 | 1433.58 | 1011.25 |
| Cu | 757.00 | 876.00 | 977.00 | 226.00 | 709.00 | 289.53 |
| Zn | 2013.00 | 7720.00 | 1926.00 | 2319.00 | 3494.50 | 2443.96 |
| Ga | 5.14 | 9.82 | 5.62 | 10.00 | 7.65 | 2.27 |
| Rb | 53.10 | 39.30 | 36.70 | 27.40 | 39.13 | 9.20 |
| Y | 5.99 | 4.24 | 3.09 | 12.10 | 6.35 | 3.47 |
| Nb | 3.83 | 2.64 | 2.56 | 7.16 | 4.05 | 1.87 |
| Mo | 3.97 | 6.97 | 5.38 | 5.20 | 5.38 | 1.07 |
| Cd | 66.00 | 132.00 | 73.97 | 120.00 | 97.99 | 28.47 |
| Sb | 9.92 | 34.00 | 18.27 | 18.80 | 20.25 | 8.69 |
| Cs | 2.75 | 4.34 | 2.08 | 2.64 | 2.95 | 0.84 |
| Ba | 298.00 | 205.00 | 517.67 | 172.00 | 298.17 | 134.89 |
| La | 8.74 | 6.15 | 4.39 | 17.80 | 9.27 | 5.16 |
| Ce | 16.60 | 12.50 | 9.19 | 35.80 | 18.52 | 10.31 |
| Pr | 1.95 | 1.50 | 1.06 | 4.35 | 2.21 | 1.27 |
| Nd | 8.17 | 5.07 | 3.47 | 14.90 | 7.90 | 4.38 |
| Sm | 1.30 | 1.51 | 0.62 | 3.38 | 1.70 | 1.02 |
| Eu | 7.12 | 0.24 | 0.18 | 0.64 | 2.04 | 2.94 |
| Gd | 1.30 | 0.91 | 0.67 | 2.60 | 1.37 | 0.74 |
| Tb | 0.18 | 0.14 | 0.08 | 0.43 | 0.21 | 0.13 |
| Dy | 1.49 | 0.86 | 0.69 | 2.80 | 1.46 | 0.83 |
| Ho | 0.24 | 0.18 | 0.10 | 0.44 | 0.24 | 0.12 |
| Er | 0.78 | 0.34 | 0.36 | 1.69 | 0.79 | 0.55 |
| Tm | 0.12 | 0.05 | 0.05 | 0.13 | 0.08 | 0.04 |
| Yb | 0.68 | 0.32 | 0.29 | 1.38 | 0.66 | 0.44 |
| Lu | 0.14 | 0.08 | 0.07 | 0.25 | 0.13 | 0.07 |
| W | 1.67 | 1.93 | 1.22 | 3.16 | 1.99 | 0.72 |
| Tl | 2.00 | 2.74 | 4.58 | 4.73 | 3.51 | 1.17 |
| Pb | 390.00 | 524.00 | 398.00 | 343.00 | 413.75 | 67.03 |
| Bi | 6.76 | 6.42 | 5.77 | 6.32 | 6.32 | 0.35 |
| Th | 1.91 | 1.17 | 1.18 | 2.94 | 1.80 | 0.72 |
| U | 1.66 | 1.24 | 2.90 | 2.15 | 1.99 | 0.62 |
| Sr | 157.00 | 60.50 | 62.13 | 100.00 | 94.91 | 39.18 |
| In | 0.81 | 2.54 | 1.47 | 3.26 | 2.02 | 0.95 |
| Total | 9226.74 | 12,958.14 | 6966.09 | 8700.11 | 9462.77 | 2184.52 |

[*] Standard deviation.

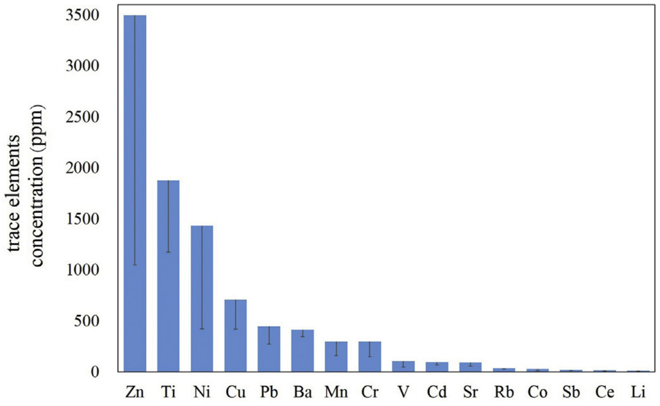

**Fig. 1.** Histogram showing the concentrations of trace elements in the indoor $PM_{10}$ in the Xuanwei coal-burning environments.

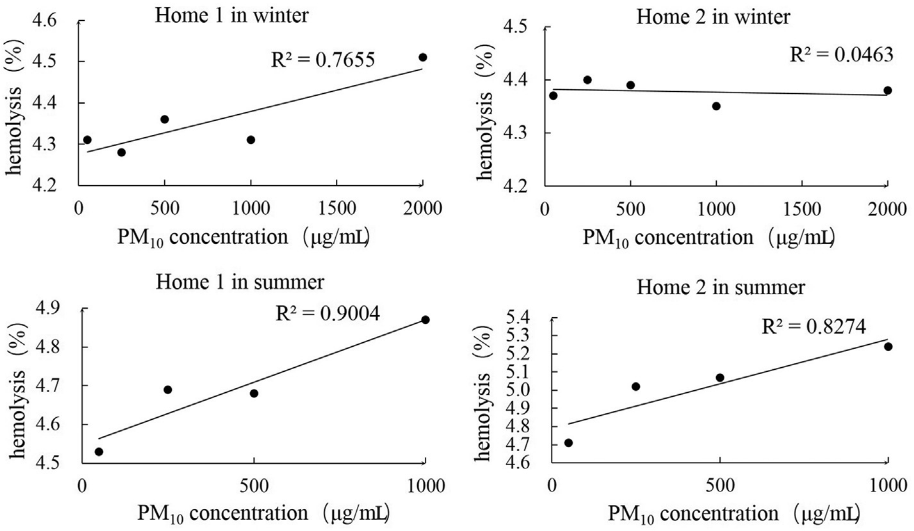

**Fig. 2.** Correlation analysis between the $PM_{10}$ concentrations and the hemolysis of $PM_{10}$ samples from two homes in the Xuanwei coal-burning environments.

**Table 4**
Correlation coefficients between the concentration of trace elements and the hemolysis of the indoor $PM_{10}$ in the Xuanwei coal-burning environments.

| Li | Be | Sc | Ti | V | Mn | |
|---|---|---|---|---|---|---|
| −0.81* | −0.698 | −0.316 | −0.622 | −0.954** | 0.61 | |
| Cr | Co | Ni | Cu | Zn | Ga | |
| −0.365 | 0.026 | 0.234 | 0.361 | 0.892* | 0.313 | |
| Rb | Y | Nb | Mo | Cd | Sb | |
| 0.404 | −0.364 | −0.457 | 0.552 | 0.448 | 0.646 | |
| Cs | Ba | La | Ce | Pr | Nd | |
| 0.948** | −0.454 | −0.361 | −0.371 | −0.363 | −0.343 | |
| Sm | Eu | Gd | Tb | Dy | Ho | |
| −0.185 | 0.09 | −0.368 | −0.334 | −0.397 | −0.253 | |
| Er | Tm | Yb | Lu | W | Tl | |
| −0.475 | −0.347 | −0.441 | −0.421 | −0.139 | −0.743 | |
| Pb | Bi | Th | U | Sr | In | Total |
| 0.895* | 0.527 | −0.459 | −0.894* | −0.098 | 0.018 | 0.955** |

* $p < 0.1$.
** $p < 0.05$.

had a significant positive correlation with DNA damage in the plasmid scission assay, and the heavy metal elements, especially Cd, Pb, Sb, Tl and Zn, were suggested to be the main cause of high oxidative capacity. The plasmid scission assay with the individual soluble metals demonstrated the direct bioreactivity of soluble Cu, Zn, Pb, and Fe (Merolla and Richards, 2005).

In this study, the relationship between the concentrations of trace elements and the hemolysis under the dose of 500 μg/mL were analyzed by SPSS Statistics. The correlation coefficients are given in Table 4. The results showed that the correlation coefficient between hemolysis and concentration of total trace elements of indoor $PM_{10}$ in Xuanwei was 0.955, higher than the critical value 0.878 under the 0.05 significance level. This positive correlation suggested that the trace elements of $PM_{10}$ in Xuanwei were involved with hemolytic activity. This result implied that the trace elements in particulate matter could not only cause DNA damage (Shao et al., 2013), but also possess a hemolytic effect. A significant positive correlation also existed between the individual elements Zn, Pb, and Cs and the hemolysis, indicating these specific elements could be causing the hemolytic effects of the $PM_{10}$ samples. A significant negative correlation was observed between the elements Li, U, and V, and the hemolysis, implying that these elements may be able to play an inhibitory role.

Several previous studies investigated the relationships between the heavy metal compositions and the oxidative capacities of the coal-burning particles, including the particles emitted in the coal-burning indoor environment in Xuanwei lung cancer village, in the laboratory coal-combustion system, and also in a coal-mining area. The metal elements which showed positive correlations with the oxidative capacity of the PM were summarized in Table 5. Of the analyzed elements, Zn, Pb, Cd, Cu, As, Tl, Mn, Sb, and Bi showed a significant positive correlation with the oxidative capacity of PM in coal-burning environments. Among them, Zn, Pb, Cd, As and Tl were most frequently identified to be significantly correlated with PM oxidative capacity. Therefore, Zn, Pb, Cd, As

**Table 5**
Heavy metals showing a most significant positive correlation with the oxidative capacity of airborne particles in different coal-burning environments.

| Site | Type | Smapling Year | Zn | Pb | Cd | As | Tl | Cu | Mn | Sb | Bi | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Xuanwei | Indoor $PM_{10}$ | 2007 | + | + | + | | | | | | | Shao et al., 2008 |
| | | 2008 | + | + | + | + | | + | + | | | Zhou et al., 2009 |
| | | 2011 | + | + | | + | + | | | + | + | Wang, 2012 |
| | | 2013,2014 | + | + | | | | | | | | This study |
| | Indoor and outdoor $PM_{10}$ | 2007 | + | + | + | + | + | | | + | | Shao et al., 2013 |
| | Indoor $PM_{2.5}$ | 2016 | + | + | | | | + | + | | + | Xi, 2018 |
| | Indoor PM | 2016,2019 | + | | + | | + | + | | | | Feng et al., 2020 |
| Laboratory of coal-combustion system | $PM_{10}$ | 2016 | + | + | + | + | + | | + | + | | Shao et al., 2016 |
| Pingding-shan coal-mining area | Outdoor $PM_{10}$ | 2014 | + | + | + | + | + | + | | | | Song et al., 2015 |

and Tl could be regarded as the main toxic elements in PM emitted from coal combustion.

## 5. Conclusions

(1) Hemolysis of RBCs by indoor $PM_{10}$ over a dose range from 50 to 2000 μg/mL ranged from 4.28% to 5.24%. The hemolytic activities of $PM_{10}$ showed a rising trend with increasing doses. Although low dose samples exhibited a low hemolytic activity, $PM_{10}$ could have a toxic effect upon people in a closed indoor environment for extended time periods.

(2) The total trace elements in $PM_{10}$ had a positive correlation with the hemolytic properties, in which the Zn, Pb and Cs could contribute more obvious hemolysis. In contrast, Li, U and V demonstrated negative correlations with the hemolysis, suggesting these elements may have inhibited the hemolysis of human RBCs. In combination with the results from previous studies, Zn, Pb, Cd, As and Tl could be regarded as the main toxic elements in PM emitted from coal-combustion.

(3) The hemolysis assay as a new, simple method in assessing the particle toxicity could be used in the toxicological study of a variety of environmental pollutants.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## References

Klobučar, R.S., 2011. Editor results of an international round robin study with the Ames fluctuation test. International Conference on Data Storage & Data Engineering, Bangalore, India, pp. 169–172.

Bahadori, F., Kocyigit, A., Onyuksel, H., Dag, A., Topcu, G., 2018. Cytotoxic, apoptotic and genotoxic effects of lipid-based and polymeric nano micelles, an in vitro evaluation. Toxics 6 (1), e0007. https://doi.org/10.3390/toxics6010007.

Bahrami, F., Esfarjani, F., Marandi, S.M., 2013. Effects of intermittent exercise in polluted and clean air on hemolysis of red blood cells in Endurance Runners. J. Isfahan Med. School 30 (212), 1845–1855.

Chuang, H.C., Hsiao, T.C., Wang, S.H., Tsay, S.C., Lin, N.H., 2016. Characterization of particulate matter profiling and alveolar deposition from biomass burning in northern Thailand: the 7-SEAS study. Aerosol Air Qual. Res. 16 (11), 2897–2906.

Dobrovolskaia, M.A., Clogston, J.D., Neun, B.W., Hall, J.B., Patri, A.K., Mcneil, S.E., 2008. Method for analysis of nanoparticle hemolytic properties in vitro. Nano Lett. 8 (8), 2180–2187.

Du, X.M., Gao, S.X., Hong, L.L., Zheng, X., Zhou, Q.Y., Wu, J.H., 2019. Genotoxicity evaluation of titanium dioxide nanoparticles using the mouse lymphoma assay and the Ames test. Mutat Res-Gen. Tox. En. 838, 22–27.

Faraji, M., Pourpak, Z., Naddafi, K., Nodehi, R.N., Nicknam, M.H., Shamsipour, M., Osornio-Vargas, A.R., Hassanvand, M.S., Alizadeh, Z., Rezaei, S., Mazinani, M., Soleimanifar, N., Mesdaghinia, A., 2019. Chemical composition of PM10 and its effect on in vitro hemolysis of human red blood cells (RBCs): a comparison study during dust storm and inversion. J. Environ. Health Sci. 17, 493–502.

Feng, X.L., Shao, L.Y., Xi, C.X., Jones, T., Zhang, D.Z., BeruBe, K., 2020. Particle-induced oxidative damage by indoor size-segregated particulate matter from coal-burning homes in the Xuanwei lung cancer epidemic area, Yunnan Province, China. Chemosphere 256, e127058. https://doi.org/10.1016/j.chemosphere.2020.127058.

Gallego-Cartagena, E., Morillas, H., Maguregui, M., Patino-Camelo, K., Marcaida, I., Morgado-Gamero, W., Silva, L.F.O., Madariaga, J.M., 2020. A comprehensive study of biofilms growing on the built heritage of a Caribbean industrial city in correlation with construction materials. International Biodeterioration & Biodegradation 147, e104874. https://doi.org/10.1016/j.ibiod.2019.104874.

Greenwell, L.L., Moreno, T., Jones, T.P., Richards, R.J., 2002. Particle-induced oxidative damage is ameliorated by pulmonary antioxidants. Free Radical Bio. Med. 32 (9), 898–905.

Hadnagy, W., Marsetz, B., Idel, H., 2003. Hemolytic activity of crystalline silica – separated erythrocytes versus whole blood. Int. J. Hyg. Envir. Heal. 206 (2), 103–107.

Hamanaka, R., Mutlu, G., 2018. Particulate matter air pollution: effects on the cardiovascular system. Front. Endocrinol. 16 (9), e00680. https://doi.org/10.3389/fendo.2018.00680.

Hao, J., Zhao, P., Chen, W.Q., 2012. Chinese Cancer Registry Annual Report. Mil. Med. Sci. Press, Beijing, pp. 100–101 (in Chinese).

Huang, J., Lim, M.Y., Hwang, C.X., Zhao, B.X., Shao, L.Y., 2015. Contrasts in spatial and temporal variability of oxidative capacity and elemental composition in moxibustion, indoor and outdoor environments in Beijing. Environ. Pollut. 202, 78–84.

Jin, S.P., Li, Z., Choi, E.K., Lee, S., Kim, Y.K., Seo, E.Y., Chung, J.H., Cho, S., 2018. Urban particulate matter in air pollution penetrates into the barrier-disrupted skin and produces ROS-dependent cutaneous inflammatory response in vivo. J. Dermatol. Sci. 91 (2), 175–183.

Kargarfard, M., Shariat, A., Shaw, B.S., Shaw, I., Lam, E.T.C., Kheiri, A., Eatemadyboroujeni, A., Tamrin, S.B.M., 2015. Effects of polluted air on cardiovascular and hematological parameters after progressive maximal aerobic exercise. Lung 193 (2), 275–281.

Kim, K.E., Cho, D., Park, H.J., 2016. Air pollution and skin diseases: adverse effects of airborne particulate matter on various skin diseases. Life Sci. 152, 126–134.

Large, D.J., Kelly, S., Spiro, B., Tian, L.W., Shao, L.Y., Finkelman, R., Zhang, M., Somerfield, C., Plint, S., Ali, Y., Zhou, Y.P., 2009. Silica-volatile interaction and the geological cause of the Xuan Wei lung cancer epidemic. Environ. Sci. Technol. 43 (23), 9016–9021.

Li, G., Huang, Y., Liu, Y., Guo, L., Zhou, Y., Yang, K., Lei, Y., 2012. In vitro toxicity of naturally occurring silica nanoparticles in C1 coal in bronchial epithelial cells. Chinese J. Lung Cancer 15 (10), 561–568 (in Chinese with English abstract).

Li, Z.S., Wen, Q.M., Zhang, R.L., 2017. Sources, health effects and control strategies of indoor fine particulate matter ($PM_{2.5}$): a review. Sci. Total Environ. 586, 610–622.

Li, Y.W., Shao, L.Y., Wang, W.H., Zhang, M.Y., Feng, X.L., Li, W.J., Zhang, D.Z., 2020. Airborne fiber particles: Types, size and concentration observed in Beijing. Sci. Total Environ. 705, e135967. https://doi.org/10.1016/j.scitotenv.2019.135967.

Lima, B.D., Teixeira, E.C., Hower, J.C., Civeira, M.S., Ramírez, O., Yang, C.X., Oliveira, M.L.S., Silva, L.F.O., 2021. Metal-enriched nanoparticles and black carbon: a perspective from the Brazil railway system air pollution. Geosci. Front. 12 (3), e101129. https://doi.org/10.1016/j.gsf.2020.12.010.

Lin, H.L., Tao, J., Du, Y.D., Liu, T., Qian, Z.M., Tian, L.W., Di, Q., Rutherford, S., Guo, L.C., Zeng, W.L., Xiao, J.P., Li, X., He, Z.H., Xu, Y.J., Ma, W.J., 2016. Particle size and chemical constituents of ambient particulate pollution associated with cardiovascular mortality in Guangzhou, China. Environ. Pollut. 208, 758–766.

Liu, H., Mauzerall, D.L., 2020. Costs of clean heating in China: evidence from rural households in the Beijing-Tianjin-Hebei region. Energ. Econ. 90, e104844. https://doi.org/10.1016/j.eneco.2020.104844.

Lui, K.H., Bandowe, B.A.M., Tian, L.W., Chan, C.S., Cao, J.J., Ning, Z., Lee, S.C., Ho, K.F., 2016. Cancer risk from polycyclic aromatic compounds in fine particulate matters generated from household coal combustion in Xuanwei, China. Chemosphere 169, 660–668.

Marchini, T., Wolf, D., Michel, N.A., Mauler, M., Dufner, B., Hoppe, N., Beckert, J., Jackel, M., Magnani, N., Duerschmied, D., Tasat, D., Alvarez, S., Reinohl, J., von Zur Muhlen, C., Idzko, M., Bode, C., Hilgendorf, I., Evelson, P., Zirlik, A., 2016. Acute exposure to air pollution particulate matter aggravates experimental myocardial infarction in mice by potentiating cytokine secretion from lung macrophages. Basic Res. Cardiol. 111 (4), e44. https://doi.org/10.1007/s00395-016-0562-5.

Merolla, L., Richards, R.J., 2005. In vitro effects of water-soluble metals present in UK particulate matter. Exp. Lung Res. 31 (7), 671–683.

Mesdaghinia, A., Pourpak, Z., Naddafi, K., Nodehi, R.N., Alizadeh, Z., Rezaei, S., Faraji, M., 2019. An in vitro method to evaluate hemolysis of human red blood cells (RBCs) treated by airborne particulate matters ($PM_{10}$). MethodsX 6, 156–161.

Mumford, J.L., He, X.Z., Chapman, R.S., Cao, S.R., Harris, D.B., Li, X.M., Xian, Y.L., Jiang, W.Z., Xu, C.W., Chuang, J.C., 1987. Lung cancer and indoor air pollution in Xuan Wei, China. Science 235 (4785), 217–220.

Mumford, J.L., Helmes, C.T., Lee, X., Seidenberg, J., Nesnow, S., 1990. Mouse skin tumorigenicity studies of indoor coal and wood combustion emissions from homes of residents in Xuan Wei, China with high lung cancer mortality. Carcinogenesis 11 (3), 397–403.

Mumford, J.L., Li, X., Hu, F., Xu, B., Chuang, J., 1995. Human exposure and dosimetry of polycyclic aromatic hydrocarbons in urine from Xuan Wei, China with high lung cancer mortality associated with exposure to unvented coal smoke. Carcinogenesis 16 (12), 3031–3036.

Nash, T., Allison, A.C., Harington, J.S., 1966. Physico-chemical properties of silica in relation to its toxicity. Nature 210 (210), 259–261.

Nikolic, M., Nikic, D., Stankovic, A., 2008. Effect of air pollution on red blood cells in children. Pol. J. Environ. Stud. 17 (2), 267–271.

Oberdörster, G., 1988. Lung clearance of inhaled insoluble and soluble particles. J. Aerosol Med. Pulm. D. 1 (4), 289–330.

Oliveira, M.L.S., Flores, E.M.M., Dotto, G.L., Neckel, A., Silva, L.F.O., 2020. Nanomineralogy of mortars and ceramics from the Forum of Caesar and Nerva (Rome, Italy): the protagonist of black crusts produced on historic buildings. Journal of Cleaner Production 278, e123982. https://doi.org/10.1016/j.jclepro.2020.123982.

Pinkerton, K.E., Green, F.H., Saiki, C., Vallyathan, V., Plopper, C.G., Gopal, V., Ménache, M.G., 2000. Distribution of particulate matters and tissue remodeling in the human lung. Environ. Health Persp. 108 (11), 1063–1069.

Quintana, R., Serrano, J., Gómez, V., de Foy, B., Miranda, J., GarciaCuellar, C., 2011. The oxidative potential and biological effects induced by $PM_{10}$ obtained in Mexico City and at a receptor site during the MILAGRO campaign. Environ. Pollut. 159 (12), 3446–3454.

Ramirez, O., da Boit, K., Blanco, E., Silva, L.F.O., 2020. Hazardous thoracic and ultrafine particles from road dust in a Caribbean industrial city. Urban Clim. 33, 1–11.

Seow, W.J., Hu, W., Vermeulen, R., Hosgood, H.D., Downward, G.S., Chapman, R.S., He, X.Z., Bassig, B.A., Kim, C., Wen, C.J., Rothman, N., Lan, Q., 2014. Household air pollution and lung cancer in China: a review of studies in Xuanwei. China J. Cancer 33 (10), 471–475.

Shao, L.Y., Yang, Y.Y., Wu, M.Y., Xiao, Z.H., 2008. The oxidative damage capacity of indoor PM 10 and its relationship with trace elements in lung cancer epidemic area in Xuanwei. J. Environ. Health 25 (12), 1094–1096 (in Chinese).

Shao, L.Y., Hu, Y., Wang, J., Hou, C., Yang, Y.Y., Wu, M.Y., 2013. Particle-induced oxidative damage of indoor $PM_{10}$ from coal burning homes in the lung cancer area of Xuan Wei, China. Atmos. Environ. 77, 959–967.

Shao, L.Y., Wang, J., Hou, H.H., Zhang, M.Q., Wang, H., Spiro, B., Large, D., Zhou, Y.P., 2015. Geochemistry of the C1 coal of the latest Permian during mass extinction in Xuanwei, Yunnan. Acta Geol. Sinica 89, 163–179 (in Chinese with English abstract).

Shao, L.Y., Hou, C., Geng, C.M., Liu, J.X., Hu, Y., Wang, J., Jones, T., Zhao, C.M., BeruBe, K., 2016. The oxidative potential of $PM_{10}$ from coal, briquettes and wood charcoal burnt in an experimental domestic stove. Atmos. Environ. 127, 372–381.

Shao, L.Y., Chang, L.L., Zhang, M.Y., 2019. Trace element compositions in $PM_{2.5}$ after the action for comprehensive control of air pollution in Beijing. Earth Sci. Front. 26 (6), 298–308 (in Chinese with English abstract).

Silva, L.F.O., Pinto, D., Neckel, A., Oliveira, M.L.S., 2020a. An analysis of vehicular exhaust derived nanoparticles and historical Belgium fortress building interfaces. Geoscience Frontiers 11 (6), 2053–2060.

Silva, L.F.O., Schneider, I.L., Artaxo, P., Núñez-Blanco, Y., Pinto, D., Flores, É.M.M., Gómez-Plata, L., Ramírez, O., Dotto, G.L., 2020b. Particulate matter geochemistry of a highly industrialized region in the Caribbean: basis for future toxicological studies. Geoscience Frontiers 17, e101115. https://doi.org/10.1016/j.gsf.2020.11.012.

Song, X.Y., Shao, L.Y., Yang, S.S., Song, R.R., Sun, L., Cen, S., 2015. Trace elements pollution and toxicity of airborne $PM_{10}$ in a coal industrial city. Atmos. Pollut. Res. 6 (3), 469–475.

Tian, L.W., Lucas, D., Fischer, S.L., Lee, S.C., Hammond, S.K., Koshland, C.P., 2008. Particle and gas emissions from a simulated coal-burning household fire pit. Environ. Sci. Technol. 42 (7), 2503–2508.

Tong, Y., Xinbo, N.I., Zhang, Y., Cheng, F., Qiu, Z., Tiecheng, T.U., Shunhua, Y.E., 2001. The study on free radical toxicological mechanism of aerosols. Acta Sci. Cir. 21 (06), 654–659 (in Chinese with English abstract).

Velali, E., Papachristou, E., Pantazaki, A., Choli-Papadopoulou, T., Planou, S., 2016. Redox activity and in vitro bioactivity of the water-soluble fraction of urban particulate matter in relation to particle size and chemical composition. Environ. Pollut. 208, 774–786.

Wang, J., 2012. A Study of Toxicology of PM10 Generated by Indoor Coal Burning in Xuanwei City of Yunnan Province and in the Control Point of Guizhou Province. M.S. thesis. China University of Mining &Technology, Beijing 64 pp. (in Chinese with English abstract).

Wang, J., Shao, L.Y., Wang, H., Spiro, B., Large, D.J., 2018. SHRIMP zircon U-Pb ages from coal beds across the PermianeTriassic boundary, eastern Yunnan, southwestern China. J. Palaeogeog-English 7 (2), 117–129.

Xi, C.X., 2018. Characteristics of the Composition of Heavy Metal Elements and Toxicity of Indoor $PM_{2.5}$ From Coal Combustion in Xuanwei Area. M.S. thesis. China University of Mining &Technology, Beijing 105 pp (in Chinese with English abstract).

Xiao, Y.Z., Shao, Y., Yu, X.J., Zhou, G.B., 2012. The epidemic status and risk factors of lung cancer in Xuanwei city, Yunnan Province, China. Front. Med. 6 (4), 388–394.

Xing, J.P., Shao, L.Y., Zhang, W.B., Peng, J.F., Wang, W.H., Shuai, S.J., Hu, M., Zhang, D.Z., 2020. Morphology and size of the particles emitted from a gasoline-direct-injection-engine vehicle and their ageing in an environmental chamber. Atmos. Chem. Phy. 20, 2781–2794.

Zhang, Y.X., Shi, Z.B., Wang, Y.Y., Liu, L., Zhang, J., Li, J.F., Xia, Y., Ding, X.K., Liu, D.T., Kong, S.F., Niu, H.Y., Fu, P.Q., Zhang, X.Y., Li, W.J., 2020. Fine particles from village air in northern China in winter: large contribution of primary organic aerosols from residential solid fuel burning. Environ. Pollut. 272, e116420. https://doi.org/10.1016/j.envpol.2020.116420.heng.

Zheng, G.Z., Bu, W.T., 2018. Review of heating methods for rural houses in China. Energies 11 (12), 3402–3420.

Zhou, M.G., He, G.J., Liu, Y.N., Yin, P., Li, Y.C., Kan, H.D., Fan, M.R., Xue, A., Fan, M.Y., 2015. The associations between ambient air pollution and adult respiratory mortality in 32 major Chinese cities 2006–2010. Environ. Res.-New York 137, 278–286.

Zhou, L., Shao, L.Y., Song, X.Y., Liu, Y.X., 2009. Investigation of the bioreactivity of indoor PM. J. Toxicol. 23 (6), 439–441 (in Chinese with English abstract).