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In vitro assessments of bioaccessibility and bioavailability of $PM_{2.5}$ trace metals in respiratory and digestive systems and their oxidative potential

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ABSTRACT

Air pollution is a serious environmental issue. As a key aerosol component, $PM_{2.5}$ associated toxic trace metals pose significant health risks by inhalation and ingestion, but the evidences and mechanisms were insufficient and not well understood just by their total environmental concentrations. To accurately assess the potential risks of airborne metals, a series of *in vitro* physiologically based tests with synthetic human lung and gastrointestinal fluids were conducted to assess both the bioaccessibility and bioavailability of various $PM_{2.5}$ bound metals in the respiratory and digestive systems from both urban and industrial areas of Nanjing city. Moreover, the chemical acellular toxicity test [dithiothreitol (DTT) assay] and source analysis were performed. Generally, the bioaccessibility and bioavailability of investigated metals were element and body fluid dependent. Source oriented metals in $PM_{2.5}$ showed diverse bioaccessibility in different human organs. The $PM_{2.5}$ induced oxidative potential was mainly contributed by the bioaccessible/bioavailable transition metals such as Fe, Ni and Co from metallurgic dust and traffic emission. Future researches on the toxicological

mechanisms of airborne metals incorporating the bioaccessibility, bioavailability and toxicity tests are directions.

Key words: Aerosol pollution; Transition metals; Inhalable bioaccessibility; *In vitro* bioavailability; Human health risk assessments

1. Introduction

Owing to the rapid industrialization and urbanization, air pollution has been one of the severe environmental problems in developing countries, including China. Fine particulate matters ($PM_{2.5}$) are main atmospheric pollutant and attracting great concern. As a complex mixture, $PM_{2.5}$ containing a variety of toxic components, such as heavy metals, may directly enter the human bronchi and alveolar areas and threaten human health [1]. Although heavy metals are trace-level component of $PM_{2.5}$, it is the priority environmental pollutant due to both the toxic and carcinogenic characteristics. Airborne trace metals are also reported closely related to the respiratory, cardiovascular and cerebrovascular diseases [2,3].

Airborne trace metals exist in various species with different physical and chemical properties, therefore have diverse impacts on human health, such as inducing ROS (reactive oxygen species) [4]. Most studies generally considered the total $PM_{2.5}$ bound metal concentrations but ignored their actual bioaccessible/bioavailable fractions, which may influence the human health risk assessment of aerosol pollution exposure [5,6]. Thus the bioaccessiblity and bioavailability of $PM_{2.5}$ bound metals become significant issues in air quality evaluation [7]. Ordinarily, after a clearing process by physical mechanism, the substances inhaled or ingested into human body may react with the body via absorption, distribution, metabolism and elimination. However, during the particle exposure, bioaccessible fraction is the key constituent that can be dissolved by human fluids [8]. *In vitro* physiologically based extraction tests (PBET) with synthetic agents comparable to human body fluids were generally used for bioaccessibility of airborne trace metals [9], simulating their solubility in respiratory and digestive systems after the exposure pathways of inhalation and ingestion [10,11]. Among various synthetic agents of respiratory system, the classical Gamble's solution and artificial lung fluid (ALF) or just modifications were usually adopted [12]. For digestive system, the Unified Bioaccessibility Research Group of Europe (BARGE) Method (UBM) validated with *in vivo* model were developed to measure the gastrointestinal bioaccessibility of metals [13]. Although the bioaccessible fraction was often determined by extraction with various artificial/simulated human fluids, the

bioavailable processes were neglected. Bioavailability was defined as the fraction that is absorbed by human, reaches the bloodstream, and finally is transported to an organ with toxicity effects in the body [14]. However, there was not available method for *in vitro* bioavailability test. Because the Diffusive Gradients Thin-films (DGT) technique was widely used to study the *in-situ* bioavailability of trace metals in aquatic system [15], it was assumed as an *in vitro* method to simulate the absorption process (bioavailability) in respiratory and digestive fluid systems following the dissolution process (bioaccessibility).

A number of epidemiologic studies have evidenced the links between exposure of $PM_{2.5}$ pollution and increasing risk of cardiopulmonary diseases and mortality [16,17]. The $PM_{2.5}$ induced inflammation and oxidative stress were proposed to explain this relevance [18,19]. The dithiothreitol (DTT) assay as a chemical acellular test could be used in evaluating $PM_{2.5}$ induced ROS generation, which may be the mechanism of oxidative stress [20,21]. Therefore, the bioaccessible and bioavailable fractions of $PM_{2.5}$ bound metals in the respiratory and digestive systems should be significant in such toxicological effects, but the principle factors and mechanisms need investigation.

In this study, both the *in vitro* bioaccessibility and bioavailability of $PM_{2.5}$ bound metals in human respiratory and digestive systems from two functional areas of a megacity in China were assessed by PBET with imitated pulmonary and digestive fluids, and the oxidative potential of $PM_{2.5}$ was indicated by DTT assay. It aims to estimate the bioaccessibility/bioavailability of $PM_{2.5}$ bound metals in respiratory and digestive systems from various sources and their relations with $PM_{2.5}$ induced ROS generation, thus differentiating the key toxic species of airborne metals.

2. Materials and methods

2.1 Sampling and chemical analyses

The ambient $PM_{2.5}$ samples were collected simultaneously at an urban area (UA) and industrial area (IA) of Nanjing city, eastern China (Fig S1). The urban site (N32°03′, E118°47′) was in the downtown area surrounded by residential and commercial areas with heavy road traffic nearby. The industrial site (N32°12′, E118°43′) was situated on

a campus impacted by large petrochemical and metallurgical industries.

The sampling period was from January to December 2016 for one year with continuous 23h-sample one day every month. $PM_{2.5}$ samples were collected on quartz microfiber filters (QMA, 203mm×254mm, Whatman, UK) by a high-volume sampler (1000 $L \cdot min^{-1}$). Filters were prebaked at 400 °C for 4 h to remove organic substances before sampling. The daily $PM_{2.5}$ mass was obtained by the gravimetric method using a high-precision electronic balance (Sartorius, QUINTIX 124-1 CN) under a constant temperature and humidity condition. Then the filter $PM_{2.5}$ samples were cut into subsamples by ceramic scissors and stored in refrigerator for following tests.

The total metal concentrations in $PM_{2.5}$ samples were determined by inductively coupled plasma-optical emission spectrometer (ICP-OES, Optima 8000, PerkinElmer) and ICP Mass Spectrometer (ICP-MS, NexION300X, PerkinElmer) for low level concentrations after a heating acid digestion procedure. One-eighth filter was digested by being immersed in concentrated HNO_3-$HClO_4$-HF acids with a progressive heating program and finally dissolved in 5% (v/v) high-purity HNO_3. The procedural blanks, sample replicates, and standard reference materials (NIST SRM 1648a, urban PM) were randomly set for quality control. Differences of metal concentration in replicates (n=4) were < 10%. The concentrations of metals in reagent blanks were < 1% of the average analyte concentrations, and their recoveries in the SRM ranged from 90 to 110%.

2.2 In vitro metal bioaccessibility tests

The bioaccessibility of $PM_{2.5}$ trace metals in respiratory and digestive systems were evaluated respectively by the *in vitro* extraction methods based on physiology. To estimate the bioaccessibility of $PM_{2.5}$ trace metals inhaled through respiratory system [22,23], two simulated pulmonary fluids were applied: the artificial lung fluid (ALF, pH = 4.5) representing the intracellular acidic lung fluid after phagocytosis by alveolar macrophages, and Gamble's solution (pH = 7.4) imitating the extracellular healthy fluid. The compositions of these imitated fluids were summarized in Table S1. Briefly, 1/16 sampled filters of known mass were cut into pieces in the plastic bottle, then 30 ml pulmonary fluids were added and shaken for 24 h with 200 rpm in an incubator at a

constant temperature (37 ℃). Solid to liquid (S/L) ratio was about 6 mg/30 ml. Then 5 ml of the extracts were filtrated into centrifuge tubes by a 0.45 μm cellulose microporous membrane, acidized with high-purity concentrated HNO_3 and stored in refrigerator for metal concentration analysis.

To assess the bioaccessibility of $PM_{2.5}$ trace metals in digestive system, the modified UBM was conducted [24-26]. The sequential test was performed with three simulated fluids to imitate three processes through mouth, stomach and intestine. Firstly, simulated saliva (pH=6.8): mouth phase, near neutral, enzyme-rich. Secondly, simulated gastric fluid (SGF, pH=2.5): acidic gastric fluids in the stomach. Thirdly, simulated intestinal fluid (SIF, pH=7.0): near neutral, pancreatic and bile juice. The compositions of the simulated digestive fluids were listed in Table S2. The 1/8 filter sample (about 12 mg $PM_{2.5}$) was added to 15 ml simulated saliva, shaken for 5 min, then 15 ml SGF was added and the pH of the solution was adjusted to 2.5, shaken for 2 h, 5 ml of the gastric fluid extract was removed and centrifuged for metal analysis. Finally, 5ml SIF was added and adjusted the pH to 7.0, also shaken for 2 h, then 5 ml gastrointestinal fluid extract was removed and centrifuged for trace metal analysis.

All extraction tests were conducted in dark and maintain at 37 ℃, and the simulated fluids were prepared freshly. The concentrations of bioaccessible and bioavailable trace metals were determined by ICP-OES and ICP-MS. The standard reference material NIST SRM 1648a (urban PM) was extracted by simulated solutions same to sample for quality control.

2.3 In vitro metal bioavailability tests

Bioavailability (effective concentration) is defined as the succedent absorption processes immediately occurring after the dissolution of pollutants in human fluids. In this study, DGT [15] water samplers (LSNM) which consist of 0.78 mm diffusive gel and 0.40 mm Chelex gel, as a model to simulate human alveolar sac wall [46] or intestinal wall were used for bioavailability assay. After the antecedent bioaccessibility tests, the DGT devices were immediately put into those remained mixture of body fluid and $PM_{2.5}$ with the membrane window facing down, shaken for 4h with 150 rpm at a

159 constant temperature (37 °C) in the dark. The Chelex resins with adsorbed metals were
160 then eluted by 1 mol·L^{-1} HNO_3 for at least 24h. The concentrations of bioavailable trace
161 metals were finally determined by ICP-MS.
162
163 *2.4 Oxidative potential of $PM_{2.5}$*
164 The DTT assay is an *in vitro* chemical acellular method for measuring the ROS
165 formation [27-30]. Briefly, 1/32 of filter samples (about 3 mg $PM_{2.5}$) or blank filter were
166 added into 15 ml 100 μM DTT in 0.1 M of potassium phosphate buffer, keeping
167 reactions in a shaker incubator for 30 min at 37 °C in the dark. 500 μl of the reacting
168 mixture was taken every 5 min for monitoring DTT consumption after being filtered
169 through 0.45 μm and quenched by 1 ml TCA (10% v/v). Subsequently, 50 μl of 10 mM
170 5,5'-Dithiobis-2-nitrobenzoic acid (DTNB) in water was added for reacting 5 min, then
171 2 ml of 0.4 M Tris-Base (pH 8.9) with 20 mM of EDTA was added. The resulting 5-
172 mercapto-2-nitrobenzoic acid was determined by a spectrophotometer at 412 nm. All
173 reaction measurements were conducted in a low light exposure environment. The DTT
174 consumption rate was corresponding to the slope of a straight line obtained by several
175 data points (0, 5, 10, 15, 20, 25, 30 min). The average DTT consumption rate of the
176 filter blank and reagent blank was 0.008 and 0.006 μM min^{-1}, respectively. The DTT
177 consumption rates of $PM_{2.5}$ were calculated by the difference between the samples and
178 filter blanks.
179
180 *2.5 Source identification of $PM_{2.5}$ bound bioaccessible metals*
181 According to the characteristics of pollution sources, the Pb isotopes are accurate and
182 intuitive in source apportionments and widely used in qualitative and quantitative
183 analysis of atmospheric Pb sources [31,32]. Lead stable isotope ratios of $^{207}Pb/^{206}Pb$
184 and $^{208}Pb/^{206}Pb$ in simulated pulmonary and gastrointestinal extracts of twelve
185 representative $PM_{2.5}$ samples were determined using ICP-MS. Instrumental parameters
186 were set as: 190 sweeps/reading, 1 reading/replicate, 10 replicates/sample solution,
187 dwell time of ^{204}Pb (40 ms) and ^{206}Pb, ^{207}Pb, ^{208}Pb (25 ms). Filter blank and SRM NIST
188 981were set as quality control. The analytical precision for samples was generally <

189 0.5% for $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$.

190

191 *2.6 Data analysis*

192 SPSS Statistics 21 was used for statistical analysis and Origin 9.1 for plotting.
193 Principal component analysis (PCA) was conducted for source identification.

194

195 **3. Results**

196 *3.1 Trace metals distributed in urban $PM_{2.5}$*

197 Results of the total concentrations of trace metals in $PM_{2.5}$ samples from IA and UA
198 of Nanjing city were showed in Fig S2. The average daily trace metals in air (μg m^{-3})
199 were observed as order of Fe > Cr > Pb > Cu > Mn > As > Ni > Cd > Co in IA, and Fe >
200 Pb > Cr > Mn > Cu > As > Ni > Cd > Co in UA (Table S3). Spatially, the mean
201 concentrations of most airborne trace metals were greater in IA than UA, especially the
202 Mn, Fe, and Cu. However, the contents of metals accumulated in $PM_{2.5}$ (mg kg^{-1})
203 showed inconsistent distribution patterns.

204

205 *3.2 Bioaccesibility and bioavailability of $PM_{2.5}$ bound metals in respiratory system*

206 Two simulated lung fluids were employed to represent different conditions when
207 particles were inhaled into the lung. As shown in the Fig 1, the overall mean
208 bioaccessibility of metals from both IA and UA extracted by ALF was Pb > Cd > Mn >
209 Cu > As > Cr > Fe > Co > Ni, ranging from 14.5-94.2%, although most extracted metals
210 had higher level in UA than in IA except Pb and Cd. Among these investigated metals,
211 Pb and Cd had higher ALF bioaccessibility, but Co and Ni were lower. However, the
212 metal bioaccessibility was totally different when extracted by Gamble's solution, which
213 was in order of As > Cu > Fe > Co > Cr > Cd > Ni > Mn > Pb, ranging from 3.9 - 33.4%
214 (Table S3). The overall mean Gamble's bioaccessibility of Cu, As, Co and Cd were
215 higher in UA, but Fe, Cr, Ni, Mn and Pb were higher in IA.

216 As shown in Fig 2, the acute pulmonary bioavailability of metals determined by DGT
217 in ALF ranged from 2.2 - 49.3 % in IA and 2.2 - 52.9 % in UA, respectively. The Cu
218 (49.3%) in IA and Ni (52.9%) in UA showed highest ALF bioavailability, while the Fe

(2.4% in IA, 5.2% in UA), Cr (2.4% in IA, 2.8% in UA) and As (2.2% in IA, 2.2% in UA) were low. The mean bioavailability of most metals in UA were higher than that in IA, except Cu and Pb. Meanwhile, the Gamble's solution bioavailability of analyzed metals was 1.6 - 12.8 % in IA and 2.2 - 17.0 % in UA, much lower than their ALF bioavailability. In Gamble's solution, Mn (12.8% in UA and 17.0% in IA), Cu (9.2% in UA and 4.7% in IA) and Co (6.2% in UA and 4.7% in IA) showed higher bioavailability than Pb, As and Cr. Except Cd, the mean Gamble's bioavailability of studied metals were higher in UA than in IA, especially Mn and Cu (Table S3).

3.3 Bioaccesibility and bioavailability of $PM_{2.5}$ bound metals in digestive system

The bioaccesibility of metals in gastric phase were Cd > Pb > Cu > Mn > As > Cr > Co > Ni > Fe in both IA and UA, ranged from 11.1 - 86.8 % in IA and 7.8 - 95.9 % in UA, respectively (Fig S3). The mean gastric bioaccessibility of Fe, Ni, Pb was higher in IA, and other metals were higher in UA. In intestinal phase, the metal bioaccessibility were 5.2-73.1 % in IA and 9.6 - 78.6 % in UA, for which Cd was also highest and Fe was the lowest (Fig 3).

The bioavailability of metals in digestive system evaluated by DGT followed Cd > Mn > Cu > Pb > Co > Ni > Fe > Cr > As in both sampling areas, ranged from 0.78 - 48.6 % in IA and 0.76 - 49.5% in UA, respectively (Fig 4). The mean gastrointestinal bioavailability of As, Ni, Pb were higher in IA, while other metals were more bioavailable in UA.

3.4 Sources of $PM_{2.5}$ bound bioaccessible metals

For tracking the origin of airborne Pb released into the respiratory and digestive systems, Pb isotope ratios in ALF, Gamble's solution, and SIF were compared with the multi natural and anthropogenic sources [33-37] of Pb (Fig 5). Most of the stable Pb isotopes were around the Pb growth curve and in the range of coal combustion, soil, metallurgic dust and unleaded gasoline. $PM_{2.5}$ bound Pb from IA in Gamble's solution mainly overlapped with the range of coal combustion, while Pb of UA was primarily from traffic emission. Most of the bioaccessible Pb in ALF from IA and UA, which

distinguish from that in Gamble's solution, were between metallurgic dust, soil and coal combustion. The Pb isotope ratios in SIF were lower, and the bioaccssible Pb in digestive system was mainly from industrial emission.

To further identify the sources of bioaccessible metals in $PM_{2.5}$, the PCA analysis was conducted and results were shown in Table S5. In the respiratory system, two factors explained 69.8% in variations of ALF bioaccesibility for IA, including Mn, Fe, Ni in PC1 with 39.4% variance attributed to metallurgic dust, and As, Cd, Pb in PC2 with 30.4% variance attributed to coal combustion. Three factors explained 77.2% for UA, including As and Pb in PC1 with 32.4% variance attributed to traffic emission, Cr and Cd in PC2 with 25.4% variance attributed to coal combustion, Ni and Cu in PC3 with 19.4% variance attributed to metallurgic dust. For Gamble's bioaccesibility, three factors explained 80.8% and 79.3% in variance of IA and UA, respectively. Their main sources in IA were metallurgic dust and coal combustion, but in UA were traffic emission, metallurgic dust, and coal combustion. In digestive system, three factors explained that the biaccessible metals in IA were mainly from coal combustion, metallurgic dust and crustal source, but in UA were from traffic emission, natural source, and metallurgic dust.

3.5 Oxidative potential of $PM_{2.5}$ samples and relations with metal bioaccessibility and bioavailability

Fig. 6 showed the results of $PM_{2.5}$-induced ROS activity by DTT assay, the DTT consumption of which ranged from 0.001 - 0.022 $nmol \cdot m^{-3} \cdot min^{-1}$ (average 0.01) in IA and 0.006 - 0.033 $nmol \cdot m^{-3} \cdot min^{-1}$ (average 0.02) in UA, with the average air $PM_{2.5}$ concentrations of 51.9 $\mu g \cdot m^3$ in IA and 62.5 $\mu g \cdot m^3$ in UA, respectively.

Due to the wide scale of the oxidative potential ($nmol \cdot m^{-3} \cdot min^{-1}$) induced by $PM_{2.5}$ [38] and the significance of metal bioaccessibility and bioavailability ($nmol \cdot m^{-3}$) on human exposure, the correlations between DTT consumption and bioaccessible/bioavailable metals of $PM_{2.5}$ via inhalation and digestion were performed (Table 1). Results indicated diverse relations for different metals and various body systems. In ALF of the respiratory system, DTT loss was positively correlated with

bioaccessible Fe ($p<0.01$) and bioavailable Ni ($p<0.05$). In SGF of digestive system, ROS activity was positively correlated with the bioaccessible Co ($p<0.01$).

4. Discussion

4.1 Characteristics of metals in $PM_{2.5}$

Distributions of $PM_{2.5}$ and associated metals at different sites of Nanjing were shown in Fig S2. The average daily $PM_{2.5}$ concentrations were 65.8 and 65.6 $\mu g \cdot m^{-3}$ in IA and UA, respectively, which were close to the China Air Quality Standard (75 $\mu g \cdot m^{-3}$) but exceeded the WHO guideline (25 $\mu g \cdot m^{-3}$). Compared with existing international air quality guidelines, the average daily concentration of As in both IA and UA exceeded the value, and Ni in IA also exceeded guideline for several days (Fig S2 and Table S3), which indicated potential air pollution and health threats. Airborne As in Nanjing was a significant atmospheric pollutant [39]. Concentrations of most airborne metals in IA were greater than in UA, especially Mn, Fe, and Cu. The metals showed different order of concentrations in air ($ng \cdot m^{-3}$) and in $PM_{2.5}$ ($mg \cdot kg^{-1}$), implying the significance of the $PM_{2.5}$ metal accumulation and various sources in aerosol pollution assessments [40].

4.2 Comparisons of bioaccessibility and bioavailability for $PM_{2.5}$ bound metals in respiratory and digestive systems

Increasing evidences reveal that airborne trace metals play a significant role in harming human health [41]. Researches on the health impacts induced by $PM_{2.5}$ have also focused on exposure pathways and effectiveness [42]. On account of the complex situation of human in vivo conditions, there are multiple exposure pathways of airborne $PM_{2.5}$ [43], including inhalation, ingestion and topical absorption. Thus two approaches were considered as the main exposure routes of airborne $PM_{2.5}$ inhalation in current study. The respiratory system resulting pulmonary effects were simulated by the extracellular healthy lung fluid (Gamble's solution) and the intracellular acid lung fluid (ALF). The main three steps of the digestive system were simulated as mouth (saliva phase), stomach (gastric phase, SGF) and intestines (intestinal phase, SIF) [44,45]. Therefore, both the bioaccessiblity and bioavailability of $PM_{2.5}$ metals via respiratory

and digestive systems were comprehensively summarized.

In the respiratory system, pulmonary metal bioaccessibility varied with wide range, and were much higher in ALF than in Gamble's solution, that may be attributed to the ALF acidity and the probable precipitation in the neutral Gamble's solution [46]. Regards to the metal patterns, Pb, Cd, Mn were higher than other metals in ALF, but As, Cu, Fe were higher in Gamble's solution. Spatially, Cu, Co, As showed generally higher SLF bioaccessibility in UA than in IA, but the pulmonary bioaccessibility of Fe, Cr, Ni, Mn, and Cd were determined by fluid type, that the ALF bioaccessibility of Pb and Cd were significantly higher in IA than in UA. The results confirmed that, pulmonary metal bioaccessibility is element and lung fluid dependent [47,48]. For instance, the lower lung bioaccessibility of Pb in Gamble's solution than in ALF indicated that the main speciation of Pb in $PM_{2.5}$ may be PbS, PbO and $PbSO_4$ [49]. The succedent bioavailability tests by DGT showed similar lung fluid pattern of ALF higher than Gamble's solution. Interestingly, some metals, such as Mn, showed inverse order with lower bioaccessibility but higher bioavailability than other metals. Such results implied the necessity to evaluate inhaled metal bioavailability. The bioavailability of $PM_{2.5}$ metals also showed spatial pattern, with higher bioavailability in UA than in IA for most metals.

In digestive system, the metal bioaccessibility in gastric phase increased due to the lower pH comparing with respiratory system [50]. The metal orders of gastric bioaccessibility were the same in IA and UA, but were different from the two SLF bioaccessibility. Most metals had higher SGF bioaccessibility in UA than in IA, except Fe, Ni and Pb. The trend of intestinal metal bioaccessibility was similar to SGF, but the SIF bioaccessibility was lower for all metals owing to its higher pH. The bioavailability of metals in the digestive system showed similar pattern to their bioaccessibility. The differences between respiratory and digestive bioaccessbility and bioavailability are largely ascribed to the fluid composition and pH. Pepsin and NaCl were the main component in the gastric phase and bile salt was added into SIF [51]. The metal cations may react with organics by complexation and change the bioavailability.

According to the results of PCA (Table S4) and Pb isotopes, trace metals of $PM_{2.5}$ in

IA were mainly from metallurgic dust and coal combustion, which were also potentially bioaccessible in the respiratory and digestive systems (Table S5). But the metals in UA showed complex sources although mainly from traffic emission and industrial sources (metallurgic dust), which might be influenced by the transport of pollutants from IA through the prevailing east wind in Nanjing. So the bioaccessibility of airborne metals were source oriented. Airborne metals from industrial sources especially the metallurgic dust contributed bioaccessibility significantly in human organs, supplemented with traffic emission to Gamble's bioaccessibility in UA.

In sum, the bioaccessibility and bioavailability in human respiratory and digestive systems were element and body fluid dependent. The different source oriented $PM_{2.5}$ bound metals released various bioaccessibility in different organs.

4.3 Oxidative potential induced by total $PM_{2.5}$

The DTT assay has been proved as an approach to measure the ROS induced by particulates [26,52]. The theory may be electronic transformation between DTT and oxygen because of the intrinsic catalytic capacity of particles [53]. Fig. 6 presented the air concentration and DTT consumption of investigated $PM_{2.5}$ samples, supporting previous researches on oxidative potential of $PM_{2.5}$ [54]. The DTT reactivity showed linear variation with $PM_{2.5}$ concentration, for instance, both were higher in UA than in IA, and both were increased for samples from cold days than from warm days. Further analysis about the influence of $PM_{2.5}$ bound key component on ROS generation appears more significant and meaningful.

4.4 Associations between source-dependent metal bioaccessibility/bioavailability and the $PM_{2.5}$ induced ROS activity

Because $PM_{2.5}$ is a complex mixture with various sources, its components are much complicated. Regarding the toxicological effects [55], airborne trace metals were focused on in current study, but in the aspect of bioaccessibility and bioavailability to human organs. The induced oxidative potential [38] was confirmed closely related to $PM_{2.5}$ and the bioaccessible/bioavailable metals in the respiratory and digestive systems

(Table 1), especially Fe and Ni in the lung and Co in the gastric area, most of which were mainly from industrial source and traffic emission. It has been reported that the exposure to PM, particularly to metals, can induce ROS production [56], which was explained by key metals from varied sources in this study. Some *in vitro* cytotoxicity tests and *in vivo* bioavailability tests have also proved that oxidative stress is one of the important pathways leading to adverse health effects by airborne PM [57]. the oxidative potential of which showed consistent correlations with transition metals [58]. Although these transition metals have been proved to cause ROS, inflammation and then damage DNA and cell function [59-61], their chemical speciation and bioaccessibility/bioavailability in $PM_{2.5}$ and exposure systems should also be key factors, that need be incorporated into both health risk assessments and *in vitro*/*in vivo* toxicology tests.

5. Conclusions

In conclusion, the varied bioaccessibility and bioavailability of airborne metals in the respiratory and digestive systems were element and body fluid dependent. The different source oriented $PM_{2.5}$ bound metals will release diverse bioaccessibility in different human organs. The $PM_{2.5}$ induced oxidative potential showed similar spatial and seasonal distribution patterns with the $PM_{2.5}$ concentrations in air. The bioaccessible/bioavailable transition metals in $PM_{2.5}$ such as Fe, Ni and Co may be the key contributors of ROS generation. Overall, source-oriented metals in $PM_{2.5}$ posed varied bioaccessibility in human body systems and induced diverse oxidative potential due to the different bioaccessibility and bioavailability of transition metals. Future research on the toxicity mechanisms of $PM_{2.5}$ bound metals considering human bioaccessiblity and bioavailability was expected.

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Appendix A

ALF: Artificial Lung Fluid

BARGE: Bioaccessibility Research Group of Europe

DGT: Diffusive Gradients Thin-films

DTT: dithiothreitol

IA: Industrial Area

ICP-OES/MS: inductively coupled plasma-optical emission spectrometer / Mass Spectrometer

PBET: physiologically based extraction tests

PCA: Principal component analysis

ROS: reactive oxygen species

SGF: Simulated Gastric Fluid

SIF: Simulated Intestinal Fluid

UA: Urban Area

UBM: Unified BARGE Method

Appendix B. Supplementary data

The supplementary materials related to this article are found in Figure S1-S3 and Table S1-S5.

Reference

[1] Jin, L., Luo, X.S., Fu, P.Q., Li, X.D., 2017. Airborne particulate matter pollution in urban China: A chemical mixture perspective from sources to impacts. Natl. Sci. Rev. 4, 593–610.

[2] Goix, S., Uzu, G., Oliva, P., Barraza, F., Calas, A., Castet, S., Point, D., Masbou, J., Duprey, J.L., Huayta, C., Chincheros, J., Gardon, J., 2016. Metal concentration and bioaccessibility in different particle sizes of dust and aerosols to refine metal exposure assessment. J. Hazard. Mater. 317, 552–562.

[3] Leclercq, B., Alleman, L.Y., Perdrix, E., Riffault, V., Happillon, M., Strecker, A., Lo-Guidice, J.M., Garçon, G., Coddeville, P., 2017. Particulate metal bioaccessibility in physiological fluids and cell culture media: Toxicological perspectives. Environ. Res. 156, 148–157.

[4] Jin, L., Xie, J.W., Wong, C.K.C., Chan, S.K.Y., Abbaszade, G., Schnelle-Kreis, J., Zimmermann, R., Li, J., Zhang, G., Fu, P.Q., Li, X.D., 2019. Contributions of city-specific fine particulate matter ($PM_{2.5}$) to differential in vitro oxidative stress and toxicity implications between Beijing and Guangzhou of China. Environ. Sci. Technol. 53, 2881–2891.

[5] Talbi, A., Kerchich, Y., Kerbachi, R., Boughedaoui, M., 2018. Assessment of annual air pollution levels with PM_1, $PM_{2.5}$, PM_{10} and associated heavy metals in Algiers, Algeria. Environ. Pollut. 232, 252–263.

[6] Zhou, Q., Wang, L., Cao, Z., Zhou, X., Yang, F., Fu, P., Wang, Z., Hu, J., Ding, L., Jiang, W., 2016. Dispersion of atmospheric fine particulate matters in simulated lung fluid and their effects on model cell membranes. Sci. Total. Environ. 542, 36–43.

[7] Zhong, L., Liu, X., Hu, X., Chen, Y., Wang, H., Lian, H.Z., 2020. In vitro inhalation bioaccessibility procedures for lead in $PM_{2.5}$ size fraction of soil assessed and optimized by in vivo-in vitro correlation. J. Hazard. Mater. 381, 121202.

[8] Guney, M., Chapuis, R.P., Zagury, G.J., 2016. Lung bioaccessibility of contaminants in particulate matter of geological origin. Environ. Sci. Pollut. Res. 23, 24422–24434.

[9] Julien, C., Esperanza, P., Bruno, M., Alleman, L.Y., 2011. Development of an in vitro method to estimate lung bioaccessibility of metals from atmospheric particles. J. Environ. Monit. 13, 621–630.

[10] Plumlee, G.S., Morman, S.A., Ziegler, T.L., 2006. The toxicological geochemistry of earth materials: An overview of processes and the interdisciplinary methods used to understand them. Rev. Mineral. Geochemistry 64, 5–57.

[11] Kastury, F., Smith, E., Juhasz, A.L., 2017. A critical review of approaches and limitations of inhalation bioavailability and bioaccessibility of metal(loid)s from ambient particulate matter or dust. Sci. Total Environ. 574, 1054–1074.

[12] Zereini, F., Wiseman, C.L.S., Püttmann, W., 2012. In vitro investigations of platinum, palladium, and rhodium mobility in urban airborne particulate matter (PM_{10}, $PM_{2.5}$, and PM_1) using simulated lung fluids. Environ. Sci. Technol. 46, 10326–10333.

[13] Juhasz, A.L., Weber, J., Smith, E., 2011. Influence of saliva, gastric and intestinal phases on the prediction of As relative bioavailability using the Unified Bioaccessibility Research Group of Europe Method (UBM). J. Hazard. Mater. 197, 161–168.

[14] Plumlee, G.S., Ziegler, T.L., 2007. The Medical Geochemistry of Dusts, Soils, and Other Earth Materials, Treatise on Geochemistry.

[15] Davison, W., Zhang, H., 1994. In situspeciation measurements of trace components in natural waters using thin-film gels. Nature 367, 546–548.

[16] Saravia, J., Lee, G.I., Lomnicki, S., Dellinger, B., Cormier, S.A., 2013. Particulate matter containing environmentally persistent free radicals and adverse infant respiratory health effects: A review. J. Biochem. Mol. Toxicol. 27, 56–68.

[17] Sayes, C.M., Reed, K.L., Warheit, D.B., 2007. Assessing toxicology of fine and nanoparticles: Comparing in vitro measurements to in vivo pulmonary toxicity profiles. Toxicol. Sci. 97, 163–180.

[18] Suvarapu, L.N., Baek, S.O., 2016. Determination of heavy metals in the ambient atmosphere: A review. Toxicol. Ind. Health 33, 79–96.

[19] Varshney, P., Saini, R., Taneja, A., 2016. Trace element concentration in fine particulate matter ($PM_{2.5}$) and their bioavailability in different microenvironments in Agra, India: A case study. Environ. Geochem. Health 38, 593–605.

[20] Charrier, J.G., Richards-Henderson, N.K., Bein, K.J., McFall, A.S., Wexler, A.S., Anastasio, C., 2015. Oxidant production from source-oriented particulate matter - Part 1: Oxidative potential using the dithiothreitol (DTT) assay. Atmos. Chem. Phys. 15, 2327–2340.

[21] Wang, J., Lin, X., Lu, L., Wu, Y., Zhang, H., Lv, Q., Liu, W., Zhang, Y., Zhuang, S., 2019. Temporal variation of oxidative potential of water soluble components of ambient $PM_{2.5}$ measured by dithiothreitol (DTT) assay. Sci. Total Environ. 649, 969–978.

[22] Wiseman, C.L.S., 2015. Analytical methods for assessing metal bioaccessibility in airborne particulate matter: A scoping review. Anal. Chim. Acta 877, 9–18.

[23] Wiseman, C.L.S., Zereini, F., 2014. Characterizing metal(loid) solubility in airborne PM_{10}, $PM_{2.5}$ and PM_1 in Frankfurt, Germany using simulated lung fluids. Atmos. Environ. 89, 282–289.

[24] Denys, S., Caboche, J., Tack, K., Rychen, G., Wragg, J., Cave, M., Jondreville, C., Feidt, C., 2012. In vivo validation of the unified BARGE method to assess the bioaccessibility of arsenic, antimony, cadmium, and lead in soils. Environ. Sci. Technol. 46, 6252–6260.

[25] Wragg, J., Cave, M., Basta, N., Brandon, E., Casteel, S., Denys, S., Gron, C., Oomen, A., Reimer, K., Tack, K., Van de Wiele, T., 2011. An inter-laboratory trial of the unified BARGE bioaccessibility method for arsenic, cadmium and lead in soil. Sci. Total Environ. 409, 4016–4030.

[26] Liu, W.J., Xu, Y.S., Liu, W.X., Liu, Q.Y., Yu, S.Y., Liu, Y., Wang, X., Tao, S., 2018. Oxidative potential of ambient $PM_{2.5}$ in the coastal cities of the Bohai Sea, northern China: Seasonal variation and source apportionment. Environ. Pollut. 236, 514–528.

[27] Sauvain, J.J., Deslarzes, S., Riediker, M., 2008. Nanoparticle reactivity toward dithiothreitol. Nanotoxicology 2, 121–129.

[28] Fang, T., Verma, V., Guo, H., King, L.E., Edgerton, E.S., Weber, R.J., 2014. A semi-automated system for quantifying the oxidative potential of ambient particles in aqueous extracts using the dithiothreitol (DTT) assay: results from the Southeastern Center for Air Pollution and Epidemiology (SCAPE). Atmos. Meas. Tech. Discuss. 7, 7245–7279.

[29] Liu, Q., Baumgartner, J., Zhang, Y., Liu, Y., Sun, Y., Zhang, M., 2014. Oxidative potential and inflammatory impacts of source apportioned ambient air pollution in Beijing. Environ. Sci. Technol. 48, 12920–12929.

[30] Liu, Q., Zhang, Y., Liu, Y., Zhang, M., 2014. Characterization of springtime airborne particulate matter-bound reactive oxygen species in Beijing. Environ. Sci. Pollut. Res. 21, 9325–9333.

[31] Kelly, F.J., Fussell, J.C., 2012. Size, source and chemical composition as determinants of toxicity attributable to ambient particulate matter. Atmos. Environ. 60, 504–526.

[32] Li, S.W., Li, H.B., Luo, J., Li, H.M., Qian, X., Liu, M.M., Bi, J., Cui, X.Y., Ma, L.Q., 2016. Influence of pollution control on lead inhalation bioaccessibility in $PM_{2.5}$: A case study of 2014 Youth Olympic Games in Nanjing. Environ. Int. 94, 69–75.

[33] Zhang, G.L., Yang, F.G., Zhao, W.J., Zhao, Y.G., Yang, J.L., Gong, Z.T., 2007. Historical change of soil Pb content and Pb isotope signatures of the cultural layers in urban Nanjing. Catena 69, 51–56.

[34] Tan, M.G., Zhang, G.L., Li, X.L., Zhang, Y.X., Yue, W.S., Chen, J.M., Wang, Y.S., Li, A.G., Li, Y., Zhang, Y.M., Shan, Z.C., 2006. Comprehensive study of lead pollution in Shanghai by multiple techniques. Anal. Chem. 78, 8044–8050.

[35] Cumming, G.L., Richards, J.R., 1975. Ore lead isotope ratios in a continuously changing earth. Earth Planet. Sci. Lett. 28, 155–171.

[36] Mukal, H., Furuta, N., Fujii, T., Ambe, Y., Sakamoto, K., Hashimoto, Y., 1993. Characterization of sources of lead in the urban air of Asia using ratios of stable lead isotopes. Environ. Sci. Technol. 27, 1347–1356.

[37] Mukai, H., Tanaka, A., Fujii, T., Zeng, Y., Hong, Y., Tang, J., Guo, S., Xue, H., Sun, Z., Zhou, J., Xue, D.,

Zhao, J., Zhai, G., Gu, J., Zhai, P., 2001. Regional characteristics of sulfur and lead isotope ratios in the atmosphere at several Chinese urban sites. Environ. Sci. Technol. 35, 1064–1071.

[38] Charrier, J.G., Anastasio, C., 2012. On dithiothreitol (DTT) as a measure of oxidative potential for ambient particles: Evidence for the importance of soluble transition metals. Atmos. Chem. Phys. 12, 9321–9333.

[39] Li, H., Wu, H., Wang, Q., Yang, M., Li, F., Sun, Y., Qian, X., Wang, J., Wang, C., 2017. Chemical partitioning of fine particle-bound metals on haze–fog and non-haze–fog days in Nanjing, China and its contribution to human health risks. Atmos. Res. 183, 142–150.

[40] Luo, X.S., Ip, C.C.M., Li, W., Tao, S., Li, X.D., 2014. Spatial temporal variations, sources, and transport of airborne inhalable metals (PM_{10}) in urban and rural areas of northern China. Atmos. Chem. Phys. Discuss. 14, 13133–13165.

[41] Palleschi, S., Rossi, B., Armiento, G., Montereali, M.R., Nardi, E., Mazziotti Tagliani, S., Inglessis, M., Gianfagna, A., Silvestroni, L., 2018. Toxicity of the readily leachable fraction of urban $PM_{2.5}$ to human lung epithelial cells: Role of soluble metals. Chemosphere 196, 35–44.

[42] Mazziotti Tagliani, S., Carnevale, M., Armiento, G., Montereali, M.R., Nardi, E., Inglessis, M., Sacco, F., Palleschi, S., Rossi, B., Silvestroni, L., Gianfagna, A., 2017. Content, mineral allocation and leaching behavior of heavy metals in urban $PM_{2.5}$. Atmos. Environ. 153, 47–60.

[43] Hernández-Pellón, A., Nischkauer, W., Limbeck, A., Fernández-Olmo, I., 2018. Metal(loid) bioaccessibility and inhalation risk assessment: A comparison between an urban and an industrial area. Environ. Res. 165, 140–149.

[44] Pelfrêne, A., Douay, F., 2018. Assessment of oral and lung bioaccessibility of Cd and Pb from smelter-impacted dust. Environ. Sci. Pollut. Res. 25, 3718–3730.

[45] Perrone, M.G., Gualtieri, M., Ferrero, L., Porto, C. Lo, Udisti, R., Bolzacchini, E., Camatini, M., 2010. Seasonal variations in chemical composition and in vitro biological effects of fine PM from Milan. Chemosphere 78, 1368–1377.

[46] Luo, X.S., Zhao, Z., Xie, J.W., Luo, J., Chen, Y., Li, H., Jin, L., 2019. Pulmonary bioaccessibility of trace metals in $PM_{2.5}$ from different megacities simulated by lung fluid extraction and DGT method. Chemosphere 218, 915–921.

[47] Tang, Z.J., Hu, X., Chen, Y.J., Qiao, J.Q., Lian, H.Z., 2019. Assessment of in vitro inhalation bioaccessibility of airborne particle–bound potentially toxic elements collected using quartz and PTFE filter. Atmos. Environ. 196, 118–124.

[48] Turner, A., Radford, A., 2010. Bioaccessibility of trace metals in boat paint particles. Ecotoxicol. Environ. Saf. 73, 817–824.

[49] Huang, H., Jiang, Y., Xu, X., Cao, X., 2018. In vitro bioaccessibility and health risk assessment of heavy metals in atmospheric particulate matters from three different functional areas of Shanghai, China. Sci. Total Environ. 610–611, 546–554.

[50] Denys, S., Tack, K., Caboche, J., Delalain, P., 2009. Bioaccessibility, solid phase distribution, and speciation of Sb in soils and in digestive fluids. Chemosphere 74, 711–716.

[51] Hu, X., Xu, X., Ding, Z., Chen, Y., Lian, H.Z., 2018. In vitro inhalation/ingestion bioaccessibility, health risks, and source appointment of airborne particle-bound elements trapped in room air conditioner filters. Environ. Sci. Pollut. Res. 25, 26059–26068.

[52] Lu, Y., Su, S., Jin, W., Wang, B., Li, N., Shen, H., Li, W., Huang, Y., Chen, H., Zhang, Y., Chen, Y., Lin, N., Wang, X., Tao, S., 2014. Characteristics and cellular effects of ambient particulate matter from Beijing. Environ. Pollut. 191, 63–69.

[53] Patel, A., Rastogi, N., 2018. Oxidative potential of ambient fine aerosol over a semi-urban site in the Indo-Gangetic Plain. Atmos. Environ. 175, 127–134.

[54] Cho, A.K., Sioutas, C., Miguel, A.H., Kumagai, Y., Schmitz, D.A., Singh, M., Eiguren-Fernandez, A., Froines, J.R., 2005. Redox activity of airborne particulate matter at different sites in the Los Angeles Basin. Environ. Res. 99, 40–47.

[55] Xie, J.W., Jin, L., Cui, J.L., Luo, X.S., Li, J., Zhang, G., Li, X.D., 2020. Health risk-oriented source apportionment of $PM_{2.5}$ associated trace metals. Environ. Pollut. 262, 114655.

[56] Shuster Meiseles, T., Shafer, M.M., Heo, J., Pardo, M., Antkiewicz, D.S., Schauer, J.J., Rudich, A., Rudich, Y., 2016. ROS-generating/ARE-activating capacity of metals in roadway particulate matter deposited in urban environment. Environ. Res. 146, 252–262.

[57] Saffari, A., Daher, N., Shafer, M.M., Schauer, J.J., Sioutas, C., 2014. Global perspective on the oxidative potential of airborne particulate matter: A synthesis of research findings. Environ. Sci. Technol. 48, 7576–7583.

[58] Yuan, Y., Wu, Y., Ge, X., Nie, D., Wang, M., Zhou, H., Chen, M., 2019. In vitro toxicity evaluation of heavy metals in urban air particulate matter on human lung epithelial cells. Sci. Total Environ. 678, 301–308.

[59] MØller, P., Loft, S., 2010. Oxidative damage to DNA and lipids as biomarkers of exposure to air pollution. Environ. Health. Perspect. 118, 1126–1136.

[60] Chen, Y., Luo, X.S., Zhao, Z., Chen, Q., Wu, D., Sun, X., Wu, L.C., Jin, L., 2018. Summer winter differences of $PM_{2.5}$ toxicity to human alveolar epithelial cells (A549) and the roles of transition metals. Ecotoxicol. Environ. Saf. 165, 505–509.

[61] Uzu, G., Sauvain, J.J., Baeza-Squiban, A., Riediker, M., Sánchez Sandoval Hohl, M., Val, S., Tack, K., Denys, S., Pradère, P., Dumat, C., 2011. In vitro assessment of the pulmonary toxicity and gastric availability of lead-rich particles from a lead recycling plant. Environ. Sci. Technol. 45, 7888–7895.

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Table 1

Pearson correlation coefficients (R) for DTT loss with bioaccessible and bioavailable $PM_{2.5}$ metals in respiratory and digestive systems.

	ALF (pH=4.5)		Gamble's solution (pH=7.4)		SGF	SIF	
	R (bioaccessibility)	R (bioavailability)	R (bioaccessibility)	R (bioavailability)	R (bioaccessibility)	R (bioaccessibility)	R (bioavailability)
Cr	-0.086	-0.180	-0.289	-0.277	-0.160	-0.315	-0.203
Mn	-0.026	-0.319	-0.194	0.072	-0.598*	-0.690*	-0.619*
Fe	**0.796****	-0.280	0.101	-0.105	-0.348	-0.382	-0.313
Co	0.340	0.570	-0.043	-0.293	**0.765****	0.156	0.034
Ni	0.358	**0.586***	-0.157	0.205	0.049	-0.064	-0.072
Cu	-0.241	-0.102	-0.151	0.049	-0.424	-0.469	-0.613*
As	-0.615*	-0.557	-0.577*	-0.609*	-0.623*	-0.621*	-0.657*
Cd	-0.316	-0.373	-0.083	-0.306	-0.310	-0.325	-0.361
Pb	-0.515	-0.595*	0.422	0.026	-0.595*	-0.439	-0.685*

Notes: *p<0.05, **p<0.01. The significant correlation coefficients were in bold.

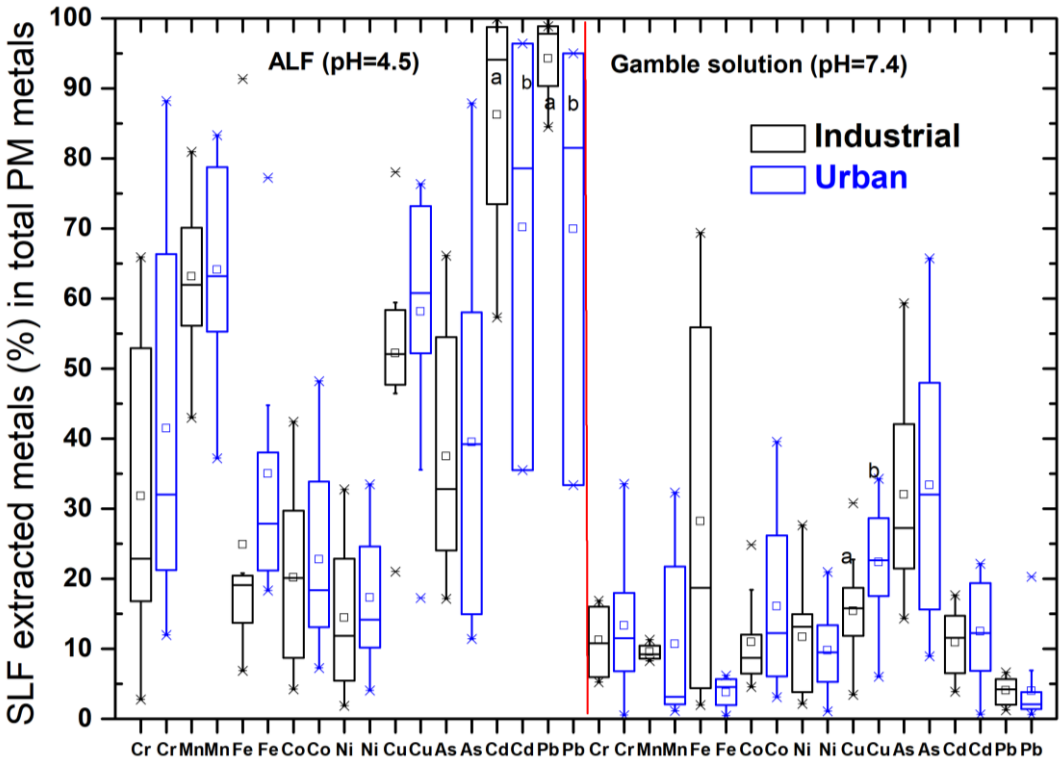


Fig. 1. Bioaccessibility (%) of metals extracted by SLF (ALF and Gamble's solution) in $PM_{2.5}$ from industrial and urban areas of Nanjing city. The significant difference was found at $p<0.05$. The central mark indicates the median, and the bottom and top edges of the box indicate the 25^{th} and 75^{th} percentiles, respectively.

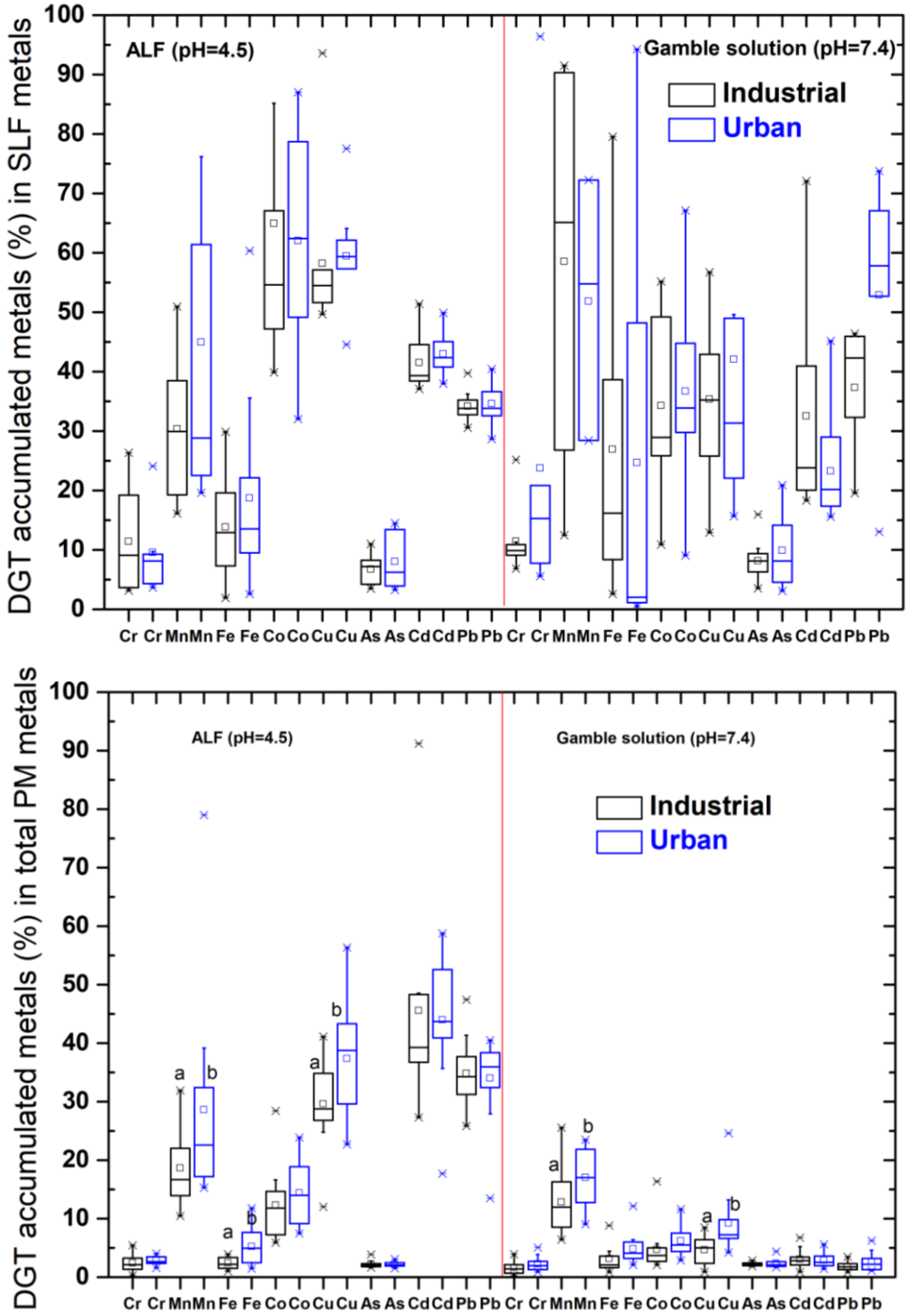


Fig. 2. Bioavailability (%) of metals accumulated by DGT in SLF extracts (ALF and Gamble's solution) and in total PM metals from industrial and urban areas of Nanjing city. The significant difference was found at $p<0.05$. The central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively.

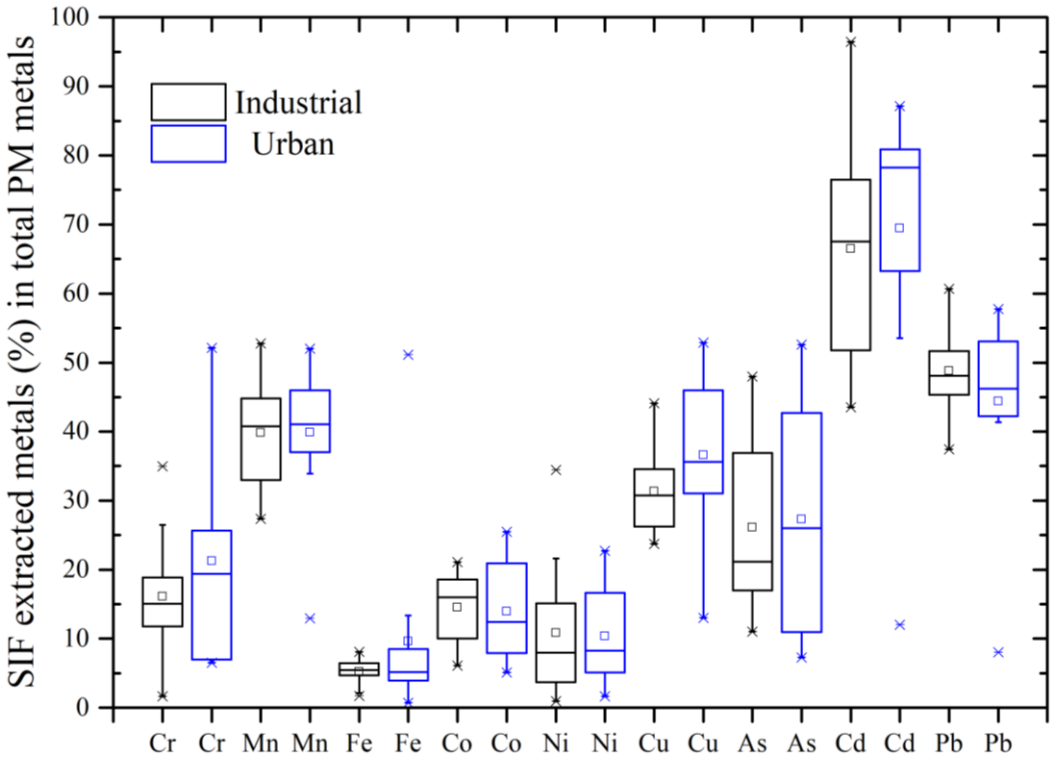


Fig. 3. Biaccessibility (%) of metals extracted by simulated intestinal fluid (SIF) in $PM_{2.5}$ from industrial and urban areas of Nanjing city. The central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively.

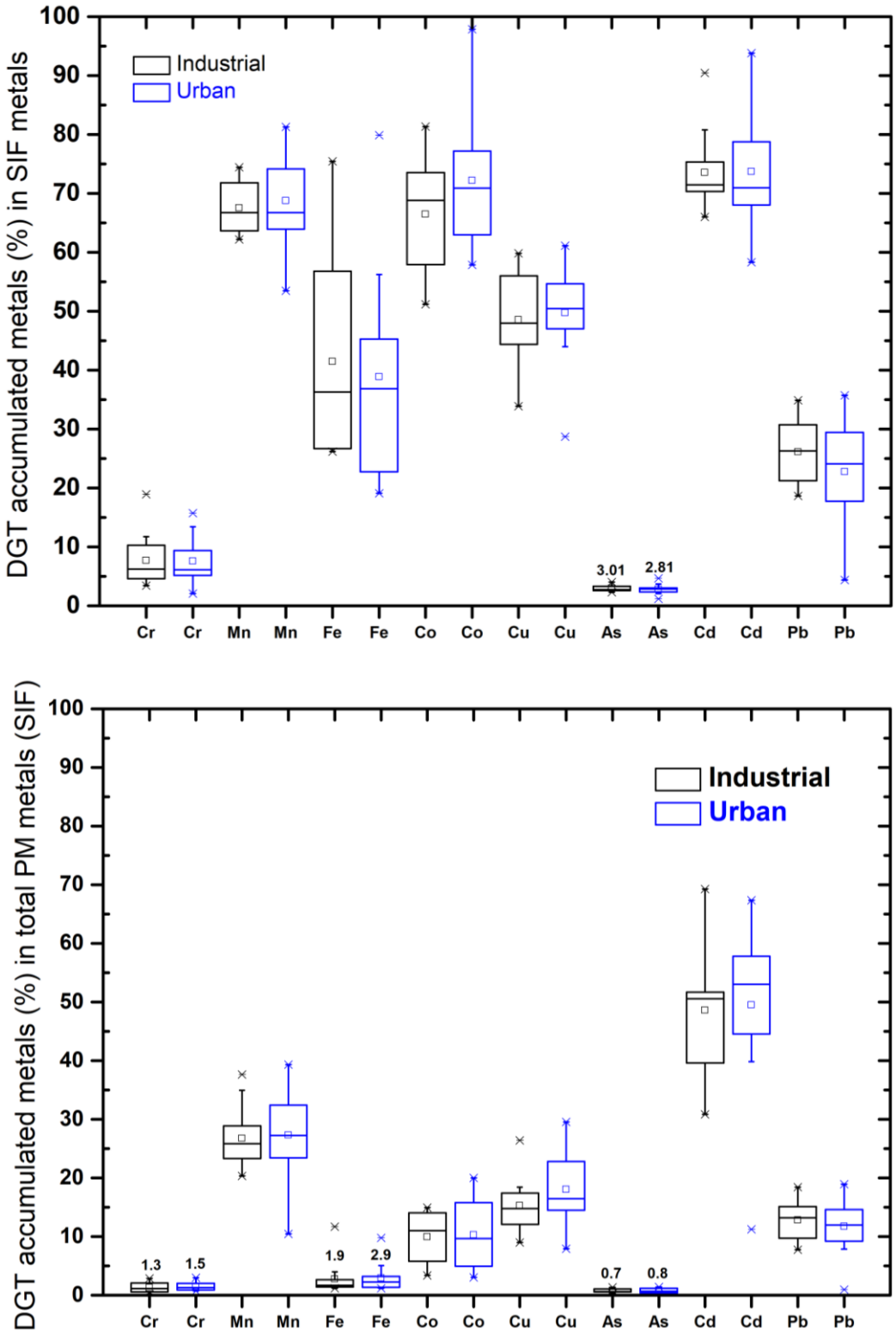
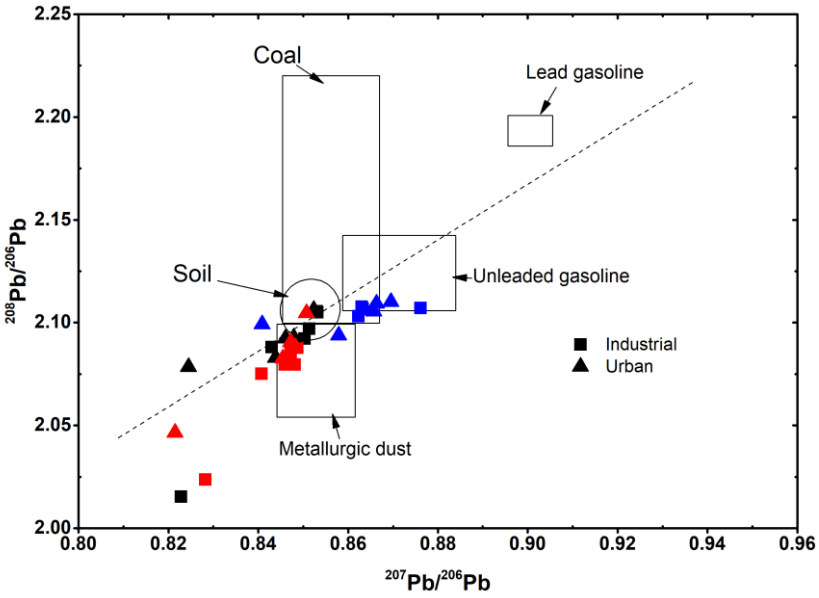


Fig. 4. Bioavailability (%) of metals accumulated by DGT in simulated gastrointestinal fluid extraction and in total PM metals from industrial and urban areas of Nanjing city. The central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively.



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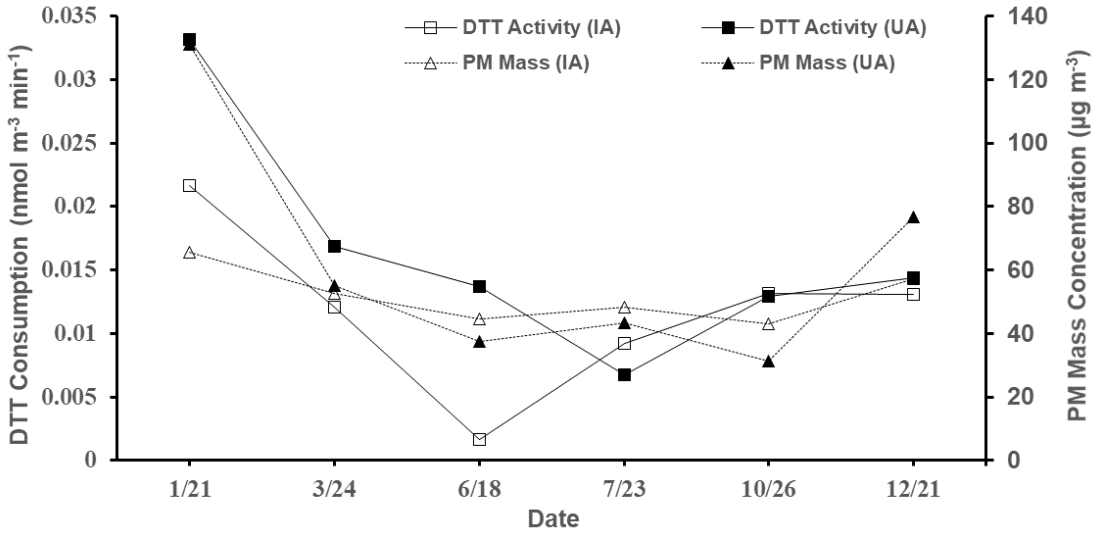

Fig. 6. DTT consumption and ambient air concentrations of $PM_{2.5}$ samples from industrial and urban areas in Nanjing city.