In vitro ingestion and inhalation bioaccessibility of soilborne lead, cadmium, arsenic and chromium near a chemical industrial park for health risk assessment

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ABSTRACT

Surface soils were collected near a chemical industrial park in the present study and in vitro ingestion and inhalation bioaccessibility of soilborne lead, cadmium, arsenic and chromium was evaluated using various in vitro bioaccessibility procedures including simulated lung fluid (SLF), artificial lysosomal fluid (ALF), modified Gamble solution (MGS), Solubility/Bioaccessibility Research Consortium (SBRC) procedure, and in vitro gastrointestinal (IVG) procedure. The in vitro inhalation and ingestion bioaccessibility of toxic elements showed elemental dependence and differed greatly among SBRC, IVG, SLF, ALF and MGS. The non-carcinogenic and lifetime carcinogenic risks of soilborne toxic elements based on the bioaccessible contents via ingestion and inhalation exposure were within the acceptable level. The present study reveals that the in vitro bioaccessibility of toxic elements was influenced greatly by the current in vitro bioaccessibility procedures, which resulted in great differences on the risk-based assessment via inhalation and oral ingestion exposure.

1. Introduction

Surface soils may contain metal(loid)s from a variety of sources including industrial wastes and emissions, vehicle exhausts, coal-burning emissions, and other activities \[1,2\]. Some metal(loid)s such as cobalt (Co), copper (Cu), manganese (Mn) and zinc (Zn) are essential nutrients with clear physiological functions; however, an excess amount of those metals can result in cellular and tissue damage leading to a variety of adverse effects to humans. Other metal(loid)s such as arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) have no established biological functions and are considered as non-essential metals \[3\]. Those metal(loid)s in surface soils may have toxic effects as a consequence of ingestion or inhalation exposure to humans, particularly children via hand-to-mouth activities \[2\].

In order to predict the potential health risks from toxic pollutants in environmental media, the human health risk assessment receives more and more attention recently \[2\]. The risk-based assessment model advocated by U.S. EPA (Environmental Protection Agency of the United States) has become one of the most widely used screening tools in the human health risk assessment (https://www.epa.gov/risk/human-health-risk-assessment). The previous investigations show that the potential health risks of toxic elements in environmental medium via ingestion exposure may be overestimated based on their total contents, because only a fraction of toxic elements in ingested media can be dissolved after contacting with physiological fluids and then may cause adverse effects to human health \[4,5\]. Therefore, trend in health risk assessment is currently shifting away from total metal(loid) content analyses towards the bioavailable fraction of metal(loid)s that are released from particles after coming into contact with physiological fluids (i.e. bioavailability). As the evaluation of in vivo bioavailability for toxic elements is complex, time-consuming, expensive, and greatly variable on intra- and inter-species of experimental animals, some simple, economic, rapid, and reproducible in vitro procedures are developed and popular as alternative approaches in exposure assessment to measure bioaccessibility (the soluble fraction in the simulated gastrointestinal or lung physiological fluids) \[5\]. Recently a variety of in vitro procedures have been developed to estimate the bioaccessibility of toxic elements by using the simulated human body fluids \[5\]. For example, physiologically based extraction test (PBET), the solubility bioaccessibility research consortium (SBRC), unified bioaccessibility research group of Europe (BARGE) method, in vitro gastrointestinal method (IVG), and the German standard bioaccessibility methodology (Deutsches Institut für Normunge.V., DIN) have been widely used to study the in vitro ingestion bioaccessibility of toxic elements in soils and soil-like materials \[6\]. Initial and modified Gamble’s solution (MGS), simulated lung fluid (SLF) and artificial lysosomal fluid (ALF) are
used to explore the in vitro inhalation bioaccessibility of toxic elements in atmospheric particulate matters and suspended soil particles [7].

In recent years, some investigations have been done on the ingestion bioaccessibility of toxic elements in urban soil, dusts and airborne particulate matters [2,8,9]. For example, the in vitro bioaccessibility of lead in house dust was analyzed by using SBRC, IVG, PBET, and DIN procedures [9]. Some investigations have also been carried out to assess the inhalation and ingestion bioaccessibility of toxic metals in fine size fractions of soil or dusts by using a simulated lung fluid [5,7]. For example, bioaccessible fraction of heavy metals in airborne PM$_{10}$, PM$_{2.5}$, and PM$_{1}$ was evaluated by using artificial lysosomal fluid and Gamble’s solution [10]. Some inconsistent reports were found on the bioaccessibility of toxic metals among different in vitro procedures [11]. Therefore, further investigations should be carried out to compare the ingestion and ingestion bioaccessibility of toxic elements in environmental media based on the risk assessment.

In the present study, surface soils were collected from Pukou district next to Nanjing Chemical Industrial Park in 2014 in Nanjing, a megacity of China, which may be influenced by the local runoff and atmospheric deposition (dry and wet). Toxic elements (As, Cd, Cr, and Pb) in those samples were analyzed. In vitro ingestion and inhalation bioaccessibility of toxic elements were evaluated by using SBRC, IVG, SLF, ALF, and modified MGS, respectively. Environment labile fractions of toxic elements in these soil samples were extracted using a dilute acid solution (0.1 mol L$^{-1}$ HCl) solution. The objective of this study was to evaluate and to compare the potential health risks posed by toxic elements (As, Cd, Cr, and Pb) in the surface samples near a chemical industrial park based on the bioaccessible contents of toxic elements. It will be helpful in understanding the environmental behaviors of toxic elements emitted from a chemical industrial park to its surrounding region.

2. Materials and methods

2.1. Sample collection

Nanjing, the capital city of Jiangsu province, P. R. China, locates in 118°19′ and 119°24′E, 31°13′and 32°36′N and is one of the most important metropolis of the Yangtze River delta with urban permanent population of approximately 6.4 million people in 2014 [12]. Previous investigations have confirmed the contamination of heavy metals in urban surface soils, urban road dusts and atmospheric particles [8,13,14]. Nanjing Chemical Industrial Park established in October 2001 is located in the north of Nanjing. It focuses on petrochemical industries, fine chemicals, polymer materials, new chemical materials, pharmaceutical industries, and basic organic chemical raw materials. Therefore, the emission of contaminants may accumulate in surface soil of surrounding areas, which may result in potential health risks to local residents.

The surface soils were collected from the Pukou district next to Nanjing Chemical Industrial Park in 2014. Each of the soil samples consisted of three sub-samples obtained using a stainless-steel hand auger. All the sampling sites were kept out from special point sources. The soil samples were stored in polyethylene bags and brought back to laboratory. In all, 20 composite samples were collected. The air-dried soil samples were sieved through a 10-mesh polyethylene sieve to remove stones, coarse materials and other debris, and then ground to sieve through a 200-mesh polyethylene sieve for further treatment.

2.2. Extraction of pseudo-total contents of toxic elements using aqua regia procedure (ISO standard 11,466)

The procedure of ISO Standard 11,466 (aqua regia) was used to extract the pseudo-total contents of toxic elements in the soil samples [15]. The aqua regia extractable solutions were diluted with ultrapure water and then stored at 4°C for elemental analyses. An inductively coupled plasma optical emission spectrometry (ICP–OES) (Optima 5300DV, Perkin–Elmer) and an inductively coupled plasma mass spectrometry (ICP–MS) (Elan 9000, Perkin–Elmer) were used for the analyses of elemental concentrations in the resulting solutions. A certified multielement standard solution of 100 mg L$^{-1}$ (SPEx CertiPrep, USA) was diluted to obtain calibration solutions for ICP–OES and ICP–MS analysis.

In order to monitor the drift of the ICP-MS signal due to plasma instability and sample matrix effects during analyses, mixed internal standard (105Rh and 209Bi) was added online through T-junction as our previous report [8].

2.3. Extraction of bioaccessible toxic elements using simulated human body fluids

SLF, ALF, and modified MGS were used to evaluate the inhalation bioaccessibility and SBRC and IVG were used to extract in vitro ingestion bioaccessibility of toxic elements in soil. Components of these in vitro procedures can be found easily in the previous studies [7,16]. A dilute HCl solution (0.1 mol L$^{-1}$) has been used to extract the environmental labile fractions of toxic elements [17] and is also used as a very simplified in vitro procedure to simulate the gastric solution for the bioaccessible toxic elements [18]. The detail operations of these in vitro procedures can be seen in our previous report [8]. Procedural controls for each phase in in vitro procedures were performed in duplicate with
sequential lung and gastrointestinal fluids in the absence of solids. Elemental concentrations in the resulting extraction solutions were analyzed using the ICP–OES and the ICP–MS mentioned above, which can also be seen in our previous report [8].

2.4. Data analysis

SPSS 13.0 for Windows was used for the descriptive statistics and mean comparisons of bioaccessible contents among the in vitro procedures. Differences of the bioaccessibility among in vitro procedures were carried out by using one-way analysis of variance (ANOVA) and post hoc multiple comparisons of means were done by using the least significance difference (LSD) test. Statistical significance was set as p < 0.01.

3. Results and discussion

3.1. Elemental contents and enrichment of toxic elements

The descriptive statistics of element contents (Cr, Pb, As, and Cd) in the surface soils are summarized in Table 1. Element contents varied greatly and their average contents were in the order of Cr > Pb > As > Cd (Table 1). Coefficients of variation (CV) show the differences of a certain element among sites (meaning the spatial variation). Cd showed the highest CV value (50.0%) while CV values of other elements were less than 30%, meaning the spatial homogeneity of elemental distribution (Table 1).

The anthropogenic inputs of Cr, Pb, As, and Cd were distinguished using enrichment factors (EFs), ratios of a certain elemental contents (Ci) to a reference element (Cfe) in the studied soil sample and the reference material (EFs = (Ci/Cfe)sample/(Ci/Cfe)reference) [19,20]. Element background values of soil in Nanjing were selected as the reference material [21]. EF values were from 0.58 to 0.82 (average value of 0.65) for Cr, from 0.77 to 2.23 (1.18) for Pb, from 0.82 to 1.55 (1.01) for As, and from 0.69 to 4.45 (1.66) for Cd. EF values for the studied elements in soil samples were all below 2 except them for Cd in some sampling sites, which were below 5. According to the categories defined by Sutherland et al. [19], EF < 2 indicates deficiency to minimal enrichment and EF = 2–5 is moderate enrichment. Blaser et al. [20] reported that EF > 1 meant element enrichment in soils. Therefore, the anthropogenic input of As, Cd, and Pb was confirmed in surface soil in most of the sampling sites, which may result from the emission of the Chemical Industrial Park.

3.2. Ingestion bioaccessibility of toxic elements

The ingestion bioaccessibility is the ratio of the soluble fraction of a certain element in the in vitro simulated gastrointestinal physiological solution to its total content in the environmental medium [6]. The ingestion bioaccessibility of Cr, Pb, As, and Cd was shown in Figure 1. Figure 1(a) shows that the gastric bioaccessibility of Cr, Pb, and Cd between SRBC procedure and IVG procedure had no significant differences except for the higher bioaccessibility of As in IVG procedure. Figure 1(b) shows that there were no significant differences on intestinal bioaccessibility of Cr, Pb, and Cd between SRBC procedure and IVG procedure except for higher bioaccessibility of As in IVG procedure. So As showed higher bioaccessibility extracted by using IVG procedure than that of SRBC procedure. The average values for diluted HCl extraction were 8.45% (range: 4.89 ~ 12.5%) for Cr, 63.9% (42.5 ~ 86.8%) for Pb, 23.8% (16.7 ~ 34.8%) for As, and 59.2% (34.5 ~ 79.9%) for Cd. They were higher than them extracted by using SRBC and IVG procedure. The bioaccessibility of Pb in the stomach phase solution for SRBC procedure was significantly higher than that in the intestinal phase solution; while Cr, As, and Cd in the intestinal phase solution were higher than them in the stomach phase solution (Figure 1). To the IVG procedure, As, Cd, Cr, and Pb in intestinal phase and stomach phase solution were no significant differences (Figure 1). These suggested that the ingestion bioaccessibility varied greatly among toxic elements and the in vitro procedures also influenced elemental bioaccessibility.

3.3. Inhalational bioaccessibility of toxic elements

Inhalation exposure to suspended soil particles may result in the accumulation of soil particles in the bronchial and alveolus region. Toxic elements in those particles may be dissolved in the lung physiological solution and enter the blood system. The bioaccessibility of soilborne As, Cd, Cr, and Pb was investigated by using three in vitro procedures (SLF, MGS, and ALF) in the present study. Inhalational bioaccessibility of toxic elements is listed in Table 2. Table 2 shows that the average value of elemental bioaccessibility was in the order of As > Cd > Pb = Cr (SLF), Cd = As > Pb = Cr.

**Table 1.** Elemental contents in surface soil (mg kg⁻¹).

|    | Pb    | Cr    | As    | Cd    |
|----|-------|-------|-------|-------|
| Min| 11.6  | 28.5  | 7.45  | 0.0595|
| Max| 27.4  | 36.6  | 13.4  | 0.333 |
| Ave| 16.1  | 32.5  | 9.03  | 0.136 |
| SD | 4.4   | 2.4   | 1.61  | 0.069 |
| CV | 27.3  | 7.38  | 17.8  | 50.0  |

Min: minimum; Max: maximum; Ave: average value; SD: standard deviation; CV: coefficient of variation.
(MGS), and Cd> As> Pb> Cr (ALF). The inhalational bioaccessibility of toxic elements using ALF was generally higher than them using SLF and MGS (Table 2). Therefore, the bioaccessibility of As, Cd, Cr, and Pb was influenced by the in vitro procedures of ALF, SLF, and MGS (Table 2).

3.4. Comparisons of the in vitro inhalation and ingestion bioaccessibility procedures

Figure 1 and Table 2 show that the bioaccessible concentrations or bioaccessibility of toxic elements varied greatly among different in vitro inhalation and ingestion bioaccessibility procedures such as ALF, SLF, MGS,
Table 2. In vitro inhalation bioaccessibility of toxic elements in the soils (%).

| Simulated lung fluid | Modified Gamble solution | Artificial lysosomal fluid |
|----------------------|---------------------------|---------------------------|
| Range                | Range                     | Range                     |
| Pb                   | 0.173 ± 0.062 a            | 1.19 ± 2.87 a             | 0.405 ± 1.12 c            |
|                      | 2.04 ± 0.484 a             | 5.04 ± 3.07 a             | 1.07 ± 0.57 b             |
| Cr                   | 0.412 ± 0.558 b            | 2.17 ± 2.85 b             | 0.420 ± 0.726 c           |
|                      | 0.768 ± 3.056 b            | 3.55 ± 0.32 a             | 2.62 ± 0.474 b            |
| As                   | 15.2 ± 3.95 b              | 19.4 ± 32.2 ± 8.63 ± 13.8 ± 2.9 |
|                      | 29.9 ± 41.5 a              | 6.0 ± 17.7 a              | 5.24 b 55.0 ± 11.9 a 49.0 a |
| Cd                   | NA ~ 23.9 ± 20.2 ± 14.0 ± 33.3 ± 17.8 ± 32.6 ± 9.5 |

Average values with different letters in same row differ significantly by the Duncan’s test (p < 0.05); NA: not available.

SBRC and IVG procedure. The differences may be due to the components and the pH value of these in vitro procedures. For example, values of total dissolved solid (TDS) of ALF, SLF, and MGS are 30, 10, and 20 mg L⁻¹, respectively, differing greatly among the in vitro procedures. NaCl (6.4 g L⁻¹) and NaHCO₃ (2.7 g L⁻¹) are the main components of SLF while NaCl (6.4 g L⁻¹), NH₄Cl (5.3 g L⁻¹), NaHCO₃ (2.3 g L⁻¹) and NaH₂PO₄ (1.7 g L⁻¹) are for MGS. However, citric acid (22.75 g L⁻¹, about two thirds of the TDS) and an organic phase reagent, is the dominant component for ALF and then NaOH (6.0 g L⁻¹) and NaCl (3.21 g L⁻¹) in IVG procedure; the main components for the stomach phase solution are glycine in SBRC procedure and pepsin and NaCl in IVG procedure; the intestinal phase solution in SBRC and IVG procedure has the same components (bile and pancreatin) but different concentrations. Moreover, ALF (pH4.5), MGS (pH7.4), and SLF (pH7.6) have different pH values. The pH values for the stomach/intestinal phase solution are 1.5/7.0 in SBRC procedure and 1.8/5.5 in IVG procedure. The influence of pH and extractant compositions on As bioaccessibility in contaminated soils has been confirmed [22]. Therefore, differences on the electrolytes, chelate ability of ligands and solution pH may be the key parameters for the variation of bioaccessibility of toxic elements in our study.

The previous studies show the differences on the bioaccessibility extracted using different in vitro procedures [10,23,24]. For example, metal(loid) mobility for airborne PM (PM₁₀, PM₂.₅ and PM₁) extracted with Gamble’s solution was notably lower compared to samples extracted with ALF [10]. Significant differences were found on Pb bioaccessibility in house dust samples among SBRC, IVG, PBET, and DIN procedures [9]. Although these simulated gastrointestinal and lung physiological fluids have been widely used, the comparisons of the bioaccessible concentrations or bioaccessibility in the present study highlighted again the influence of the extraction procedures utilized on metals’ bioaccessibility in the surface soils, which may cause more uncertainties in risk-based assessment.

3.5. Potential health risk from toxic elements

Soilborne toxic elements may pose potential threat to human health via inhalation and ingestion exposure. Therefore, the potential non-carcinogenic and carcinogenic risks to local residents were evaluated based on the risk assessment models advocated and validated by U.S. EPA ([https://www.epa.gov/risk/human-health-risk-assessment](https://www.epa.gov/risk/human-health-risk-assessment)). The default values of the parameters of the models were set as our previous report [8], the non-carcinogenic risks and the lifetime carcinogenic risks were calculated and their values based on elemental total contents were listed in Table 3. The risks based on elemental bioaccessible contents were seen in the supporting materials (Table S1 & S2). Table 3 shows that values of carcinogenic risks form As, Cr, and Pb via ingestion and inhalation exposure were within the acceptable level (1 × 10⁻⁶) advocated by U.S. EPA. For inhalation exposure, the values of hazard quotient (HQ) and hazard index (HI, sum of HQ) for the toxic elements were all within the safe level (= 1) advocated by U.S. EPA, so there were no non-carcinogenic risks posed by a single element and multi-elements (Table 3). Table 2 shows that values of in vitro inhalation bioaccessibility of toxic elements were below 10% except for about 10-30% for As and Cd. Figure 1 shows that values of in vitro ingestion bioaccessibility of toxic elements were below 10% expect for about 10-40% for Pb and Cd. The comparisons of Table 3 with Table S1 & S2 show that all of the potential non-carcinogenic and carcinogenic risks based on elemental bioaccessible contents were within the acceptable level. So risk-based assessment based on total contents of toxic elements overestimated the potential health risks, which may result in the unnecessary control and management. However, Table 2 and Figure 1 show the differences of in vitro bioaccessibility of toxic elements among different in vitro procedures. Values
of risks based on elemental bioaccessible contents differed greatly (Table S1 & S2), suggesting the differences among these in vitro procedures. Therefore, further development of better in vitro procedures for predicting toxic elements’ bioaccessibility in environmental media such as soil, dust, and atmospheric particulate matters are needed for risk-based assessment.

4. Conclusions

In the present study, the inhalation and ingestion bioaccessibility of As, Cr, Cd, and Pb showed obvious elemental dependence and also varied greatly among SBRC, IVG, SLF, ALF, and MGS procedures. Bioaccessible concentrations of As, Cr, Cd, and Pb were lower than their environmental labile concentrations extracted by using a dilute acid solution (0.1 mol L\(^{-1}\) HCl), suggesting the loose binding to soil particles of the bioaccessible fractions. The comparisons of the in vitro procedures showed that the significant differences on the inhalation and ingestion bioaccessibility of As, Cr, Cd, and Pb might be resulted from pH and organic/inorganic components among the in vitro procedures. Moreover, compared to elemental in vitro inhalation/ingestion bioaccessibility, risk-based assessment based element total contents would be overestimated the potential risks posed by toxic elements, while risk-based assessment based element bioaccessible contents were influenced by the in vitro procedures. Therefore, in order to obtain more scientific prediction of the health risks posed by toxic elements, further comparisons and optimization on the in vitro bioaccessibility procedures should be conducted.

Disclosure statement

No potential conflict of interest was reported by the authors.

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