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Original Research

Evaluation of nutrients effect on the arsenic bioaccessibility of contaminated soil

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Abstract

Soil is considered the primary source of heavy metals. Even at very low concentrations, chronic exposure to harmful heavy metal (arsenic) has a significant negative impact on human health. In this study, we evaluated the effect of nutrients including glucose, proteins, calcium and control (fasted condition) on soil arsenic (As) bioaccessibility by PBET (Physiologically Based Extraction Test) technique with SHIME (simulator of the human intestinal microbial ecosystem). As bioaccessibility of the NJY soil sample was 4.43 to 8.28%, 2.56 to 8.55% and 5.66 to 23.49% in gastric phase, intestine phase and colon phase respectively with different nutrients. CFI soil sample's As bioaccessibility varied depending on the nutrients used, and was 5.78 to 23.86%, 2.32% to 12.54% and 1.06 to 13.85% in gastric phase, intestine phase and colon phase correspondingly. As bioaccessibility of the ZZH soil sample was 2.26 to 25.16%, 24.38% to 57.27% and 9.92 to 23.10% in gastric phase, intestine phase and colon phase with varying nutrients. The outcomes showed that, As bioaccessibility of the soil samples was greatly influenced with plant protein, animal protein, calcium and glucose in the three phases of the digestive system. Therefore, nutrients have a considerable effect on As bioaccessibility and assessment of human health risk.

1. Introduction

Soil is thought as a main source for contaminants originated from diverse origins including trade activities, automobile discharge, waste dumping and construction activities. Maximum ecological pollution control research in the world focus on soil As because it is seriously alarming for human health and ecological security and it is considered as a geogenic and anthropogenic liberated contaminant (Shen *et al.*, 2017; Bundschuh *et al.*, 2021; Song *et al.*, 2022). According to reports, around 20 million people in China reside in regions where there is a high danger of As contamination in the soil as a result of industrial processes like plating and smelting (Fu *et al.*, 2016; Yang *et al.*, 2022; Liu *et al.*, 2022). Inhalation and ingestion of wind-blown soil dust are two ways that people can become exposed to certain heavy metals from the mining area (Loh *et al.*, 2016). A study conducted in the vicinity of an abandoned mining site revealed that over 30% of children (ages 8–13) had levels of arsenic contamination that were higher than average (Kunwittaya *et al.*, 2022). The people have been seriously concerned about arsenic's toxicity, ubiquity and existence. Exposure to low to high quantities of arsenic (10 to 300 μgL^{-1}) from drinking water have negative consequences such as skin diseases, neurological complicacy, circulatory disorders, respiratory complexity, diabetes, malfunction of the liver and kidneys, as well as death from chronic illnesses (Chen *et al.*, 2009). Exposure to drinking water containing As has been related to several issues in adults and children, including neurological effects, cardiovascular effects, pulmonary illnesses and skin diseases (Argos *et al.*, 2010; Chen *et al.*, 2011; Dauphiné *et al.*, 2013; Sohel *et al.*, 2010). It is well known that arsenic causes cancer and has been linked to a number of cancers, including skin, liver, lung, bladder, and prostate cancers (Zhou and Xi, 2018).

Long-term exposure to As can cause a range of malignancies and noncancerous illnesses, including internal and skin cancers, cardiovascular problems, and other ailments (Jomova *et al.*, 2011). The entire As concentration in the surrounding matrix, which includes food, water, soil and can be utilized to calculate possible health hazards (Yusa *et al.*, 2018). In general, the assessment of the risks to human health is based on the overall concentrations of pollutants found in soil. This assumes that, in the absence of site-specific data, all chemicals in soil can be 100% bioavailable and absorbed into the systemic circulation through the gastrointestinal tract. When combined with other soil constituents like organic matter and minerals, metals and metalloids, however, may have a reduced bioavailability (Park *et al.*, 2011). Therefore, only a certain percentage of metals and/or metalloids can solubilize in the human digestive system and be made available for additional absorption; which is known as bioaccessibility (Ng *et al.*, 2015). The percentage of a metal that is soluble and absorbable in the human gastrointestinal system is known as bioaccessibility. In other words, bioaccessibility evaluation tells us how much of a contaminant can enter the bloodstream and be absorbed. A 100% bioavailability assumption could lead to overestimation of dangers and higher cleanup costs at

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contaminated areas. For this reason As bioaccessibility evaluation is necessary.

Many scientists have evaluated the risk of ingesting polluted soil that contains heavy metals by using animal models, such as mice and young pigs (Denys *et al*., 2012; Juhasz *et al*., 2010). However, these models are costly, time-consuming and ethically problematic (Ting *et al*., 2015) that's why we use PBET (Physiologically Based Extraction Test) in vitro method. The colon is the terminal segment of the gastrointestinal system, which have a wide variety of microbiological species. It is claimed that the types and amounts of components that are dissolved may be significantly affected by gut bacteria entering the colon (Sun *et al*., 2012). The amount of dissolved and types of components that are present may be significantly affected by gut microbiota entering the colon.

Previous research verified that soil pollutants bioaccessibility depended on a number of factors, including pH, in vitro tests, and soil physicochemical parameters (Smith *et al*., 2014). However, under real-world circumstances, diet had an impact on the bioaccessibility of pollutants. In light of this, diet context plays a crucial role in determining bioaccessibility and the potential health risks associated with exposure to oral contaminants (Alava *et al*., 2013). Human diets contain diverse nutrients. The main fuel for the human body is glucose. Glucose provides the human brain with energy (Mergenthaler *et al*., 2012). For humans, protein is an essential nutrition. Lack of protein can result in anemia, heart failure, and hypertension (Wu, 2016). The integrity of the skeleton would be compromised by a calcium deficit (Nordberg *et al*., 2009). Getting enough calcium during infancy is vital since puberty is a critical period for preventing osteoporosis (Moitra *et al*., 2013). The aim of this experiment was to evaluate the effect of nutrients such as glucose, plant protein, animal protein and calcium on soil arsenic's bioaccessibility the Gastric, Intestine and Colon phases by the use of PBET technique with SHIME model.

2. Materials and Methods

2.1 Ethical approval

No ethical approval is required for this study.

2.2 As-contaminated soils and nutrients

The experiment was carried out in the College of Resources and Environment laboratory at the University of Chinese Academy of Sciences in Beijing, China. Three samples of surface (0–20 cm) soil were taken from three different province of China (Table 1). Each sample of soil was crushed, air-dried as well as sieved using a 2 mm net size to evaluate the physicochemical characteristics of the soil. Every soil sample was sieved to less than 0.25 mm before the in vitro test since human hands are more prone absorbing particles this size. All the chemical compounds used in this investigation, unless otherwise noted, were bought from the chemical company Sigma-Aldrich. The entire investigation was conducted using MilliQ water. Prior to use, all the glassware was immersed for at least 24 hours into 10% HNO3 acid (v/v) and then thoroughly cleaned three times using MilliQ water. Treatments included four nutrients, animal protein (casein), plant protein (soybean), calcium (as calcium carbonate), and glucose. Beijing, China-based online retailer was the source of both plant and animal protein. Calcium had a 99.99% purity and glucose had a purity of ≥99.5%. Protein makes up 99.0% purity of plant protein powder and 96.6% of animal protein powder.

Table 1. Soil sampling locations.

Soil sampling locations	**Type / nature of collected soil**
Nujiang, Yunnan province, China (NJY)	Mining soil
Chifeng, Inner Monglia province, China (CFI)	Mining soil
Zhuzhou, Hunan province, China (ZZH)	Farming soil

2.3 Characteristics of soil

The physicochemical parameters of the soil were determined in three separate samples. After 0.5 hours of equilibration in water extract, the soil pH was estimated with a pH meter. The amount of soil organic matter (OM) was determined using acid dichromate oxidation process (Bao, 2000). A laser diffractometer was used to measure soil particles size (Blott and Pye, 2006). Ammonium oxalate (0.2 mol L^{-1}) was used to extract the oxalate-extractable aluminum, manganese oxides and iron (Yin *et al*., 2014). Total As concentrations in soils were measured following Wang *et al*. (2018).

2.4 Production of colon microbiota for SHIME

The five chambers that comprise the SHIME (simulator of the human intestinal microbial ecosystem) are the small intestine, ascending colon, transverse colon, descending colon, and stomach. Typically, one volunteer's fresh fecal microorganisms were used to insert into the colon compartment (Laird *et al*., 2007; Sun *et al*., 2012). A 28-year-old Chinese male volunteer who was in good health and had not taken antibiotics during the six months before the study was conducted provided fresh fecal microorganisms to be injected into each of the three colon compartments. Colon microbial solution in the SHIME was prepared following Sultana *et al*. (2020).

2.5 Determination of bioaccessible As

In this research, we have determined soil As bioaccessibility in the gastric phase, small intestinal phase and colon phase using the PBET method with SHIME model due to use of various nutrients. The synthetic gastric solution was prepared following Sultana *et al*. (2020). In each 50 mL polypropylene tube, a 0.3 g soil sample and 30 ml gastric solution were added, following a 1:100 soil/solution ratio (Yin *et al*., 2015). Each vessel had only gastric solution for the control treatment. Gastric fluid was combined with 1.0 g of glucose, powdered plant and animal protein for the glucose, plant and animal protein treatment. For calcium treatment, 1.55 g of powdered calcium carbonate was added to the gastric solution. After that, the tubes holding the nutrients and gastric fluid were placed in a shaker setting the temperature at 370°C and 150 rpm for one hour to finish the gastric phase. After completing the gastric phase, intestinal phase and colon phase were maintained following the procedure of Yin *et al*. (2016 and 2015). Using a syringe, 10 mL of sample were taken after completion of each phase, and the sample was centrifuged for 20 minutes at 4000 rpm. After passing through a 0.45 µm filter, each sample was kept at a temperature of -20°C. ICP OES/ICP MS was used to determine the As concentration (diluted with 3% HNO_3) three times for each treatment. Arsenic bioaccessibility was evaluated following the formula (Cui *et al*., 2011),

$$\text{Bioaccessibility } (BA) = \frac{Cb}{Ct} * 100$$

Where, C_b indicate bioaccessible fraction of As in the gastric or intestine or colon phase, C_t indicate the amount of total As in soil.

2.6 Statistical analysis

The mean bioaccessibility of As for different nutrients were compared with control condition using one-way analysis of variance (ANOVA). Other statistical analyses were carried out in Microsoft Excel 2010, and DMRT (Duncan multiple range tests) were done with SPSS software (Version 23.0).

3. Results and Discussion

3.1 Soil characteristics

Soil properties including total As content of 3 soils are shown in Table 2. The pH of soil samples varied from 6.70 to 7.85 which indicate that one neutral soil (pH 6.70), two slightly alkaline soil (pH 7.53, 7.85) (Cui and Chen, 2010; Yin *et al*., 2015). The amount of soil organic matter content ranged from 1.72 to 4.32% (*w*/*w*). The amount of clay varied from 0.77 to 14.50% (*w*/*w*). The concentration of oxalate-extractable Fe, Mn, and Al ranged from 22.30 to 29.05 gkg^{-1}, 1.35 to 2.25 gkg^{-1}, 9.99 to 46.30 gkg^{-1} respectively. The total concentrations of As in soils varied from 33.90 to 110.90 $mgkg^{-1}$ respectively.

Table 2. Physicochemical characteristics of the soil samples.

Soil properties	NJY	CFI	ZZH
pH	6.70	7.53	7.85
OM %	3.04	1.72	4.32
Clay Content %	14.50	0.77	6.10
Fe g/kg	29.05	25.79	22.30
Mn g/kg	1.35	2.25	1.46
Al g/kg	9.99	18.40	46.30
As mg/kg	71.24	110.90	33.90

NJY= Nujiang, Yunnan; CFI=Chifeng, Inner Monglia; ZZH=Zhuzhou, Hunan

3.2 Arsenic bioaccessibility of NJY soil in the gastrointestinal phases

The bioaccessibility data of As from the NJY soil samples showed the significant variation due to various nutrients (Table 3). Arsenic bioaccessibility of the NJY soil sample were 4.43–8.28% in the gastric phase with different nutrients. The highest 8.28 % As bioaccessibility were observed for plant protein and the lowest 4.34 % As bioaccessibility were found under fasted condition in the gastric phase. Arsenic bioaccessibility of the NJY soil sample were 2.56 - 8.55% in the in small intestinal phase for nutrients. The highest 8.55% As bioaccessibility were observed for plant protein and the lowest 2.56 % As bioaccessibility were found for calcium in the small intestinal phase. Arsenic bioaccessibility of the NJY soil sample were 5.66 - 23.49% in the colon phase due to different nutrients. The highest 23.49 % As bioaccessibility were estimated for animal protein and the lowest 5.66% As bioaccessibility were found under fasted condition in the colon phase. Short-term changes to protein consumption can affect the intestinal microbiome (David *et al*., 2014), which could accelerate As's breakdown in soil. Few researchers have been noticed higher soil As bioaccessibility in colon phase which is similar with our results (Laird *et al*., 2007; Wang *et al*., 2018).

Table 3. Arsenic bioaccessibility of NJY soil in the gastrointestinal phases.

Treatment	Gastric phase	Intestine phase	Colon phase
Control	4.34±0.51^{b}	8.41±0.47^{a}	5.66±0.74^{d}
Glucose	4.41±0.10^{b}	4.11±0.33^{b}	16.49±0.76^{b}
Plant protein	8.28±0.85^{a}	8.55±0.86^{a}	7.06±1.38^{d}
Animal protein	5.73±1.07^{b}	8.29±1.32^{a}	23.49±1.20^{a}
Calcium	5.00±0.67^{b}	2.56±0.44^{b}	9.89±0.54^{c}

Values= Mean±SD; SD stands for standard deviation; statistically significant differences with varying treatments are shown by various letters (*P*<0.05).

3.3 Arsenic bioaccessibility of CFI soil in the three phases

As bioaccessibility data from the CFI soil samples demonstrated the significant variation for various nutrients (Table 4). In the gastric phase, the CFI soil sample's arsenic bioaccessibility ranged from 5.78 to 23.86% depending on the nutrients. Fasted condition (control) had the highest As bioaccessibility 23.86% and the lowest 5.78 % As bioaccessibility were estimated for calcium in the gastric phase. The CFI soil sample's arsenic bioaccessibility ranged from 2.32% to 12.54% in the small intestine phase for nutrients. The maximum 12.54% As bioaccessibility were observed for plant protein and the minimum 2.32 % As bioaccessibility were found for calcium in the intestine phase. The arsenic bioaccessibility of CFI soil sample ranged from 1.06 - 13.85% in colon phase due to different nutrients. The highest 13.85 % As bioaccessibility were estimated for glucose and the lowest 1.06% As bioaccessibility were found in the colon phase due to use of plant protein. When glucose was dissolved in organic carbon (DOC), it enhanced the amount of As released from the soil (Mohapatra *et al*., 2007). Arsenic is not confined to the hydrophobic micelles of carbohydrates, as demonstrated by Moreda *et al.* (2012). As a result, in the liquid phase, As is more bioavailable than free As. Similar results have been reported for soil Cd bioaccessibility the three phases with nutrients (Sultana *et al*., 2020).

3.4. Arsenic bioaccessibility of ZZH soil in the three phases

The As bioaccessibility results from the ZZH soil samples showed a notable difference for different nutrients (Table 5). In the gastric phase, the ZZH soil sample's arsenic bioaccessibility ranged from 2.26 to 25.16% depending on the nutrients. Glucose showed the highest levels of As bioaccessibility, 25.16% and the lowest 2.26 % As bioaccessibility in the gastric phase was discovered while fasted condition. In the small intestine phase for nutrients, the ZZH soil sample's arsenic bioaccessibility ranged from 24.38% to 57.27%. The highest 57.27% As bioaccessibility were estimated for animal protein and the lowest 24.38 % As bioaccessibility were found for calcium in the small intestinal phase. Arsenic bioaccessibility of the ZZH soil sample were 9.92 - 23.10% in the colon phase due to different nutrients. The highest 23.10% As bioaccessibility were found for calcium and the lowest 9.92% As bioaccessibility were estimated under fasted condition in the colon phase. Protein thiol groups have the ability to bind arsenic, which could impact the metal's bioaccessibility (Narukawa and Chiba, 2010). According to Laird *et al.* (2009), in the simulated gastric and intestine fluid, the As bioaccessibility increased by carbohydrate combinations increased and in the small intestine digest, a combinati`on of carbohydrates facilitated the adsorption of phosphate. Similar outcomes have been noticed for the bioaccessibility of As, Cu and Cd in the three phases with nutrients (Sultana *et al*., 2020; Wang *et al*., 2018).

Table 4. Arsenic bioaccessibility of CFI soil in the gastrointestinal phases.

Treatment	Gastric phase	Intestine phase	Colon phase
Control	23.86±0.08^{a}	5.26 ±0.05^{c}	9.70±1.43^{b}
Glucose	8.46±0.21^{c}	8.41±1.05^{b}	13.85± 0.67^{a}
Plant protein	7.21±0.57^{c}	12.54±0.54^{a}	1.06±0.11^{d}
Animal protein	17.81±0.80^{b}	5.45±0.28^{c}	4.11±0.76^{c}
Calcium	5.78±0.87^{d}	2.32±0.04^{d}	14.48±0.68^{a}

Values= Mean±SD; SD stands for standard deviation; statistically significant differences with varying treatments are shown by various letters (*P*<0.05).

Table 5. Arsenic bioaccessibility of ZZH soil in the gastrointestinal phases.

Treatment	Gastric phase	Intestine phase	Colon phase
Control	2.26±0.32^{d}	25.14±2.14^{c}	9.92±0.72^{c}
Glucose	25.16±0.51^{a}	53.51±3.30^{ab}	15.30±0.94^{bc}
Plant protein	4.32±0.11^{d}	49.00±1.04^{b}	14.58±2.51^{bc}
Animal protein	10.87±1.94^{c}	57.27±3.16^{a}	15.36±2.08^{b}
Calcium	14.28±1.98^{b}	24.38±2.23^{c}	23.10±4.16^{a}

Values= Mean±SD; SD stands for standard deviation; statistically significant differences with varying treatments are shown by various letters (*P*<0.05).

4. Conclusions

Arsenic bioaccessibility of Arsenic-contaminated soils affected by different nutrients such as plant protein, animal protein, glucose and calcium during gastrointestinal assimilation of arsenic. Plant protein increased As bioaccessibility in gastric and intestinal phase of NJY soil while animal protein enhanced As bioaccessibility in colon phase. As bioaccessibility also significantly enhanced by plant protein in intestine phase and calcium increased the bioaccessibility of As in colon phase of CFI soil. As bioaccessibility of HZZ soil significantly enhanced in gastric phase by glucose, increased in the intestine phase by animal protein and significantly enhanced in the colon phase by calcium. According to the present study, consideration should be given to the impact of nutrients on As's bioaccessibility when evaluating the health risks associated with soil As.

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Data availability

The data generated from this study might be available on the valid request from the corresponding author.

Informed consent statement

No informed consent was required to conduct the study.

Conflict of interest

The authors declare no conflict of interest.

Authors' contribution

Conceptualization: Md Hafizur Rahman, Mst Sharmin Sultana and Yanshan Cui; **Data collection and methodology preparation:** Md Hafizur Rahman and Mst Sharmin Sultana; **Data analysis:** Md Hafizur Rahman and Mst Sharmin Sultana; **Conducted data analysis and interpretation of results:** Md Hafizur Rahman and Mst Sharmin Sultana; **Reviewed it and revised the final version:** Md Hafizur Rahman, Mst Sharmin Sultana and Yanshan Cui. Md Hafizur Rahman and Mst Sharmin have worked together and contributed equally. So we decided to make both of them as first author. All of the enlisted authors have read and approved the final version of the published article.

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