Journal of Hazardous Materials 209–210 (2012) 250–255

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Evaluation of chromium bioaccessibility in chromite ore processing residue using *in vitro* gastrointestinal method

Siwu Yu[a], Jingjing Du[a], Ting Luo[a], Yuying Huang[b], Chuanyong Jing[a,*]

[a] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
[b] Shanghai Synchrotron Radiation Facility, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201214, China

ARTICLE INFO

Article history:
Received 28 September 2011
Received in revised form
29 December 2011
Accepted 4 January 2012
Available online 11 January 2012

Keywords:
Chromite ore processing residue
In vitro gastrointestinal method
Bioaccessibility
Cr speciation
Area under the concentration–time curve

ABSTRACT

Incidental ingestion of Chromite ore processing residue (COPR) particles poses a potential health risk. The purpose of this study was to determine the Cr bioaccessibility from COPR using the *in vitro* gastrointestinal (IVG) procedure. The bioaccessible Cr(VI) was 53.8% and 42.9%, respectively, in the gastric and intestinal phases from a total of 19 490 mg kg^{-1} Cr(VI) in COPR. Food intake including milk, dough, and ascorbic acid resulted in a significant decrease in Cr(VI) bioaccessibility. Some organic acids such as lactic, malic, and citric acid moderately reduced Cr(VI), while acetic acid exhibited no capacity for Cr(VI) reduction. The integrated area under the concentration–time curve (AUC) of the IVG extraction was used to calculate bioaccessibility. Compared with the bioaccessibility conventionally estimated using concentrations at the end of the extraction (CEP), the AUC technique should be implemented to confirm the accuracy of the IVG method when reduction of Cr(VI) occurs during the extraction. The absence of Cr(VI) phases in extracted residues as evidenced by XANES and XRPD analysis confirmed the Cr(VI) release and Cr(VI) reduction by food and ascorbic acid. With readily bioaccessible Cr(VI) and rapid human uptake, reduction of Cr(VI) might not be as effective a detoxification pathway as initially thought.

1. Introduction

Chromite ore processing residue (COPR) is a solid waste generated during the production of hexavalent chromium (Cr(VI)) by alkaline high-temperature roasting and leaching [1]. Millions of tons of COPR have been deposited in urban areas around the world including UK, USA, India, Pakistan and China [2–4]. Cr(VI) continues to leach out from sites abandoned over 40 years ago at concentrations of up to 100 mg L^{-1} [5,6]. Various studies have suggested that consumption of Cr(VI)-contaminated drinking water or inhalation of Cr(VI)-contaminated dust could lead to cancer [7]. Based on the urinary Cr levels following oral administration, the absorption of soluble Cr(VI) (6.9%) in humans is 53 times higher than that of soluble Cr(III) [8]. Besides the lungs and intestinal tract, the liver and kidney are often target organs for Cr(VI) toxicity [7].

In addition to inhalation and drinking, incidental ingestion of Cr-contaminated soils is a potential important exposure route for nearby residents. For example, over 130 COPR contaminated sites existed in Hudson County, New Jersey, the United States in 1991 [3]. Characterization of house dust in the same county in 1992 confirmed that household exposure to Cr occurs near Cr waste sites [9]. Therefore, accurate evaluation of health risks associated with COPR ingestion presents an urgent need [10]. Thus far there have been few studies of Cr speciation and dissolution from COPR in the human gastrointestinal tract.

Recently, chemical *in vitro* extraction methods have been developed as alternatives to the much more expensive *in vivo* approach to determine bioaccessibility [11–13]. Among various *in vitro* techniques, the *in vitro* gastrointestinal (IVG) method proposed by Rodriguez and Basta [14] has been well established to have a close correlation with *in vivo* results [13,15]. Therefore, the *in vitro* gastrointestinal method was expected to be applicable to potentially harmful elements in general. Most IVG studies calculate bioaccessibility using the concentrations at the end of the gastric or intestinal extraction phases [12,15,16]. However, this single time-point IVG method does not explore temporal changes of concentration during the extraction process, suggested as being essential to the accurate determination of bioaccessibility [17].

Chromium speciation presents a unique challenge in precisely deriving the bioaccessible Cr from COPR. Anionic Cr(VI), which readily crosses cellular membranes, could be reduced in the human body to cationic Cr(III), which is incapable of cellular transport and metabolism [18,19]. Simulated synthetic stomach fluids used in bioaccessibility studies could reduce Cr(VI) through

* Corresponding author. Tel.: +86 10 6284 9523; fax: +86 10 6284 9523.
E-mail address: cyjing@rcees.ac.cn (C. Jing).

0304-3894/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2012.01.009

a combination of low pH and soluble organic matter from ingested Cr(VI)-contaminated soil [20]. However, the impact of food ingestion on the rate and extent of Cr(VI) reduction from COPR *per se* is poorly understood.

The objectives of this research were to (1) determine Cr bioaccessibility and speciation in COPR, and (2) study the impact of food and organic acids on the Cr bioaccessibility. To investigate the health risks of ingested COPR, we compared the Cr(VI) bioaccessibility conventionally obtained from the concentration at the end of phase (CEP) and from the integrated area under the concentrations–time curve (AUC). X-ray absorption near edge structure spectroscopy (XANES) and X-ray powder diffraction (XRPD) were used to investigate the impact of different organic acids upon the reduction of Cr(VI) in the simulated gastrointestinal tract.

2. Materials and methods

2.1. Reagents

Pepsin (cat. no. P7000), bile salt (cat. no. B8631) and pancreatin (cat. no. P1500) from Sigma, Chemical Co., St. Louis, MO were used to simulate the human gastrointestinal fluids. Two kinds of food, powdered milk (Inner Mongolia Yili Industrial Group Co., Ltd., China) and dough (Beijing Guchuan Food Co., Ltd., China) were purchased from a local supermarket. Five organic acids were used in the study, where ascorbic, lactic and malic acids were purchased from Sinopharm Chemical Reagent Co., Ltd. (China), and glacial acetic and citric acids were from Beijing Chemicals Corporation (China). The chemical formulas of these organic acids are shown in Supplementary Data (SD) Fig. S1. All reagents were of analytical reagent grade and were used without further purification. The standard reference material (SRM) of COPR (GSB07-1019-1999) was obtained from the Institute for Environmental Reference Materials of Ministry of Environmental Protection (Beijing, China). Milli-Q water was used in all experiments.

2.2. COPR sample

COPR samples were collected from the upper-most layer at an open-air disposal site in Jinan, China. Before its closure in June 2006, the chromate production company had been in business for about 38 years and produced 40 000 metric tons per year of chromate using the high-lime procedure, and 60 000 metric tons per year of COPR as waste. The samples were thoroughly mixed on a rotator at 40 rpm for 2 h, and then passed through an 80 mesh sieve to obtain small particles (<200 μm) which may adhere to the hands for incidental ingestion [14]. The sieved samples were stored in capped containers before use.

2.3. Characterization

Total metal contents in COPR were determined according to USEPA method 3051A [21]. The accuracy and precision of this microwave digestion method were validated with the COPR SRM. The soluble metal concentrations were determined using graphite furnace atomic absorption spectrometry (AAS800, Perkin Elmer Co., USA) and inductively coupled plasma optical emission spectrometry (ICP-OES Optima 2000 DV, Perkin Elmer Co., USA). An alkaline digestion test was conducted to determine Cr(VI) content in solid samples following USEPA method 3060A [22]. Soluble Cr(VI) concentrations were determined using a Hach DR 2800 spectrophotometer based on USEPA method 7196A [23]. X-ray absorption near edge structure (XANES) spectroscopy was employed to study the Cr speciation in COPR and in the post-extraction residues. X-ray powder diffraction (XRPD) was applied to examine the mineralogical compositions in COPR and in the extracted residues. The details of XRPD and XANES are summarized in the SD.

2.4. In vitro gastrointestinal extraction procedure

Chromium bioacccessibility was evaluated using the IVG method proposed by Rodriguez and Basta [14]. Briefly, 1.2 g COPR was mixed with 180 mL synthetic gastric fluids in a 250 mL flask in a water bath at 37 °C. The synthetic gastric juice was composed of 0.15 M NaCl and 1% (w/v) pepsin. Then, the gastric solution pH was adjusted to and controlled at 1.8 with concentrated HCl during the entire gastric extraction (1 h). The solution was purged with nitrogen gas and 1 mL of antifoam A (Sigma, US) was added to remove the excessive foam. Samples (1-mL for each time) were collected at 0, 3, 5, 7, 10, 15, 20, 30, 45, and 60 min in the gastric phase. The samples were centrifuged at 10 000 rpm for 5 min, and the supernatant was used to determine soluble Cr, Cr(VI), and Fe concentrations. After a 1-h gastric extraction, the solution was adjusted to simulate intestinal fluid by increasing the pH to 5.5 with saturated $NaHCO_3$ solution and adding 0.35% porcine bile extract and 0.035% porcine pancreatin. This intestinal extraction was sustained for another hour. Samples (1-mL for each time) were collected at 3, 5, 7, 10, 15, 20, 30, 45, and 60 min in the intestinal phase, and processed as described above.

Triplicate COPR samples were extracted in parallel experimental settings for quality control purposes. The relative standard deviation (RSD) was calculated as an indication of method precision; generally RSDs of less than 15% are deemed acceptable. The Cr bioaccessibility was calculated using the following Eq. (1) [14]:

$$\textit{In vitro}\ \text{bioaccessible Cr}\,(\%) = \left[\frac{\textit{in vitro}\ \text{extracted Cr}}{\text{total Cr}}\right] \times 100 \qquad (1)$$

where *in vitro* extracted Cr is the Cr concentration at the end of the gastric or intestinal phase.

2.5. Cr(VI) reduction in IVG solution

To investigate the possible reduction of Cr(VI) by the IVG fluid itself, 1 and 30 mg L^{-1} Cr(VI) as K_2CrO_4 was added into the IVG fluid. Samples were collected at the designated intervals as described above. The samples were centrifuged and the supernatant was used for Cr(VI) measurements.

2.6. Impact of food and organic acids

The effects of dough, milk, and various organic acids on the Cr(VI) bioaccessibility were examined by adding 60 g dough [12], 9 g powdered milk [13], 0.5 g ascorbic acid, 0.5 g citric acid, 0.42 mL lactic acid, 0.5 g malic acid, and 0.5 mL acetic acid [24,25], respectively, to separate samples at the beginning of the IVG process.

3. Results and discussion

3.1. COPR characterization

The metal contents in COPR are shown in Table 1 with the mean value and the standard deviation of triplicate samples. Total Cr concentrations were $57\,570 \pm 660$ mg kg^{-1} determined using microwave-assisted acid digestion. The acid digestion method was validated as evidenced by the good agreement between detected and certified values for SRM (Table 1). The Cr(VI) content of 9400 ± 200 mg kg^{-1} was obtained using the alkaline digestion. On the other hand, XANES analysis in Fig. 1 shows that 19 490 mg kg^{-1}, *i.e.* approximately 34% of total Cr, was present as Cr(VI) in COPR. Consistent with our results, Geelhoed et al. [1] have reported that approximately 30% of the Cr in COPR is

Table 1
Content of major elements in COPR and SRM (mg kg^{-1}).

Element	COPR	SRM	
		Detected	Certified[b]
Total Cr	57 570 ± 660	34 240 ± 1 220	32 100 ± 1 300
Fe	55 360 ± 2 500	69 920 ± 980	71 600 ± 2 600
Ca	168 700 ± 7 400	194 500 ± 1 600	197 000 ± 7 000
Mg	69 210 ± 2 750	141 700 ± 800	145 000 ± 7 000
Na	30 930 ± 550	10 720 ± 130	11 400 ± 700
Al	28 630 ± 1 450	31 490 ± 530	32 400 ± 1 600
Si	29 110 ± 1 520	28 600 ± 390	28 100 ± 1700
Mn	550 ± 40	1 070 ± 10	1 500 ± 130
Cr(VI)[a]	19 490[a]		n/a[c]

[a] Determined with XANES analysis.
[b] Obtained from SRM.
[c] Non-available.

in the Cr(VI) form. The difference in Cr(VI) percentage obtained with alkaline digestion (16%) and XANES (34%) suggests that the alkaline digestion may underestimate the Cr(VI) content. The observation was in agreement with recent reports comparing the alkaline digestion and XANES analysis [26,27]. Therefore, the Cr(VI) content determined with XANES (19 490 mg kg^{-1}) was used in the following bioaccessibility calculations. The mineral assemblage of COPR in this study (Fig. 2) was consistent with COPR mineralogy reported previously for COPR materials from Glasgow and New Jersey [1,27]. Cr(VI)-bearing minerals identified in COPR include stichtite ($Mg_6Cr_2CO_3(OH)_{16}{\cdot}4H_2O$), hydrotalcite ($Mg_6Al_2(CO_3)(OH)_{16}{\cdot}4H_2O$), katoite ($Ca_3Al_2(OH)_{12}$), and calcium aluminum chromium oxide hydrate (CAC) ($3CaOAl_2O_3CaCrO_4{\cdot}14H_2O$), which are demonstrated hosts for Cr(VI) in COPR samples through anionic substitution [28–30].

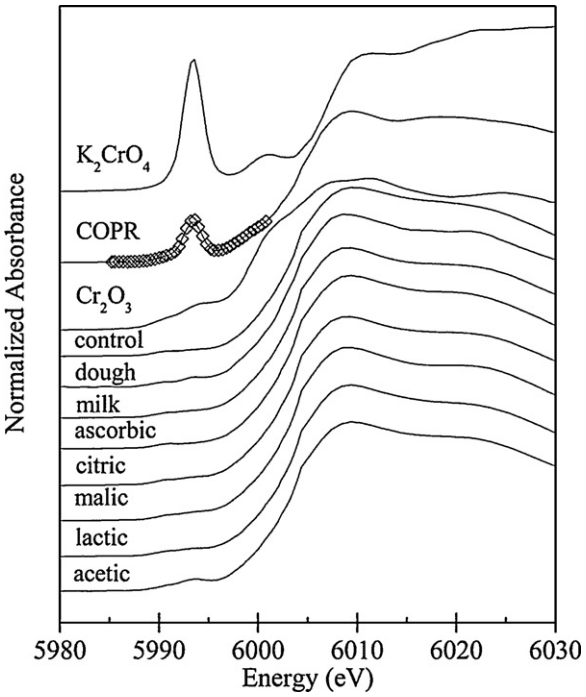

Fig. 1. Chromium K-edge XANES spectra for K_2CrO_4, COPR, Cr_2O_3, IVG extracted residues (control) and various food addition samples including dough, powdered milk, and organic acids such as ascorbic, citric, malic, lactic, and acetic acids. LCF fitting result (◊) for COPR: 33% Cr(VI), 67% Cr (III).

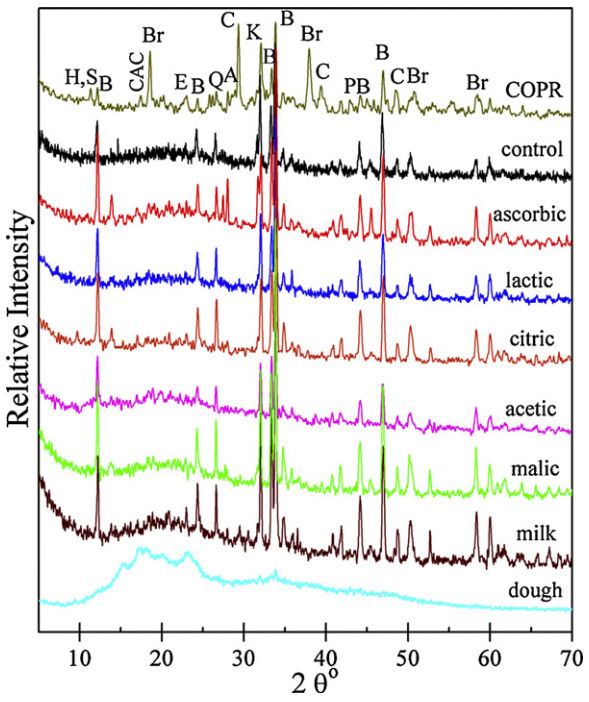

Fig. 2. The XRPD patterns for COPR and IVG-extracted residues with the addition of milk, dough, and ascorbic, citric, malic, lactic, and acetic acids. B: brownmillerite; Br: brucite; Q: quartz; S: stichtite; H: hydrotalcite; CAC: calcium aluminum oxide chromium hydrate; E: ettringite; K: katoite; C: calcite; A: albite; P: periclase.

3.2. Reduction of Cr(VI) in IVG fluids

No Cr(VI) reduction occurred when the prepared 1 and 30 mg L^{-1} Cr(VI) solutions underwent the IVG extraction (Fig. 3). The observation demonstrates that pepsin, bile, and pancreatin could not reduce Cr(VI). Our results were also in contrast to a previous hypothesis that ingested Cr(VI) might be reduced to Cr(III) because the extreme low pH condition of the stomach favours the Cr(VI) reduction [31,32]. The Eh-pH diagram (SD Fig. S2) shows that the Cr(VI) reduction under experimental conditions is thermodynamically feasible. However, the system was not at equilibrium status because of the slow rate of the redox reactions in the absence of foodstuff. Our different findings indicate that any reduction of Cr(VI) in our bioaccessibility studies on COPR will not have resulted from the IVG fluids themselves. The results highlight the need for further investigation into Cr speciation and bioaccessibility.

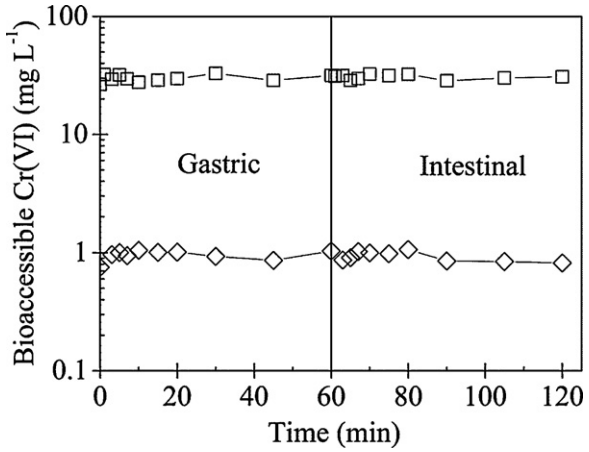

Fig. 3. Change of Cr(VI) concentrations during the *in vitro* gastrointestinal extraction with (□) 1 and (◊) 30 mg L^{-1} Cr (VI).

3.3. Chromium bioaccessibility

The bioaccessible Cr(VI) and Cr(III) was 53.8% and 22.6%, respectively, in the gastric phase from the original COPR sample used as a control (Table 2). Nevertheless, the bioaccessibility in the intestinal phase was reduced to 42.9% for Cr(VI) and 3.0% for Cr(III). The decrease of the Cr bioaccessibility from the gastric to intestinal phase was coupled with the marked diminution of bioaccessible Fe (Table 2). This observation could be the result of three concurrent reactions as pH increased from 1.8 in the gastric phase to 5.5 in the intestinal phase: Cr adsorption on *in situ* formed iron oxides, Cr(III) precipitation, and coprecipitation of Cr and Fe. Meanwhile, our bioaccessibility results were higher than reported values for some Cr(VI)-contaminated soils where reduction by soil organic matter may have played a part [20], suggesting that COPR *per se* poses a greater health risk.

In the presence of powdered milk and dough, the bioaccessible Cr(III) was increased in the gastric and intestinal phases (Table 2). On the other hand, the Cr(VI) bioaccessibility was dramatically reduced by an order of magnitude with the addition of dough and powdered milk. The decrease of bioaccessible Cr(VI) might be attributed to the reduction of Cr(VI) by organic components in milk and dough as shown in SD Tables S1 and S2.

The addition of organic acids such as acetic, ascorbic, citric, lactic, and malic acid increased the bioaccessibility of Cr(III) as compared with the control sample (Table 2). No significant difference was observed for bioaccessible Cr(VI) between control and acetic acid samples ($n = 3$, $p > 0.05$). With the exception of acetic acid, however, all tested organic acids reduced the Cr(VI) bioaccessibility. The distinct effects of organic acids could be attributed to their molecular structures (SD Fig. S1). Ascorbic acid resulted in the lowest bioaccessible Cr(VI) most probably because of its reduction capacity *via* its hydroxyl groups [33]. Cr(VI) may be catalytically reduced in the acidic gastric solution by α-OH carboxylic acids including lactic, citric, and malic acids [34–36]. No Cr(VI) reduction was observed in the presence of acetic acid because acetic acid contains no α-OH group [35].

3.4. Digestion kinetics

The changes of Cr(VI), Cr(III), and Fe concentrations as a function of digestion time are shown in Fig. 4 for control and food addition samples. A modified calculation method using the integrated area under the concentration–time curve (AUC) was used to determine the bioaccessibility, and the results are listed in Table 2 for comparison with the conventional method using the concentration at the end of phase (CEP). The impact of food ingestion on Cr(VI) bioaccessibility obtained using AUC was similar to that of CEP (Table 2). Foods could be categorized into three groups based on cluster analysis (SD Fig. S3): foods inert to Cr(VI) bioaccessibility such as acetic acid; moderate Cr(VI) reducers such as lactic, citric, and malic acids; and Cr(VI) scavengers including milk, dough, and ascorbic acid.

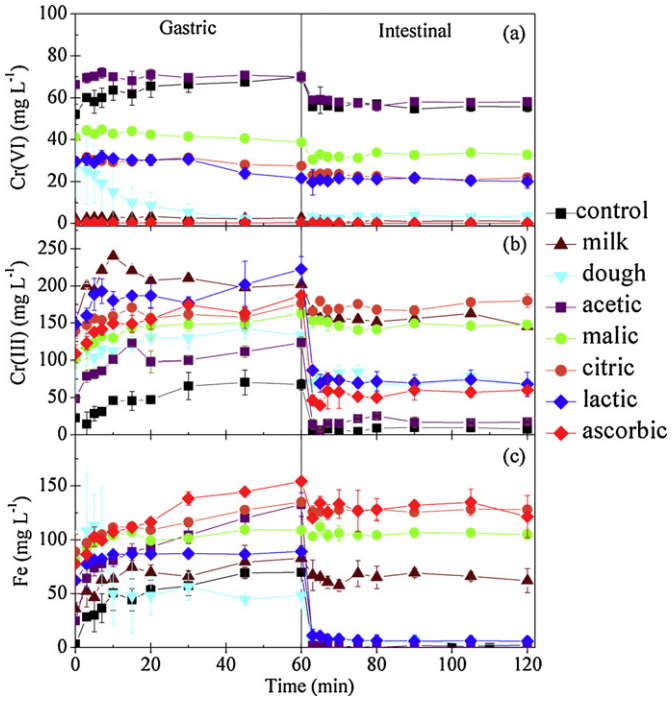

Fig. 4. Kinetics of Cr(VI), Cr(III) and Fe with COPR during the *in vitro* gastrointestinal extraction. Gastric phase was during 0–60 min; intestinal phase was during 60–120 min. Error bars represent the standard deviation ($n = 3$).

A side-by-side comparison between AUC and CEP results demonstrates that the CEP method may inaccurately estimate the Cr bioaccessibility in COPR (Table 2). For example, the CEP method underestimated the bioaccessible Cr(VI) by an order of magnitude for the dough sample. A close examination of the kinetic data in Fig. 4 suggests that the Cr(VI) concentration decreased from 27.7 mg L^{-1} at the beginning of the gastric phase to 0.5 mg L^{-1} at the end of the intestinal phase. The CEP method assumes that any Cr(VI) reduction is instantaneous; however, this assumption resulted in an underestimation when redox transformation occurred in Cr(VI) samples. On the other hand, the CEP method may overestimate the Cr(III) bioaccessibility when concentrations of Cr(III) and Fe increased with time (Fig. 4). In conclusion, the AUC method, which is universally accepted as characteristic of the extent of drug absorption [37,38], should be implemented to confirm the accuracy of the CEP method.

The rate and extent of Cr release are of equal importance. Cr(VI) was readily bioaccessible as evidenced by its instantaneous occurrence and unchanged concentrations when COPR was exposed to the simulated gastric juice except for the dough sample (Fig. 4). A

Table 2
Bioaccessibility in gastric and intestinal phases evaluated using CEP and AUC methods.

Samples	Gastric bioaccessibility (%)						Intestinal bioaccessibility (%)					
	Cr(VI)		Cr(III)		Fe		Cr(VI)		Cr(III)		Fe	
	CEP	AUC	CEP	AUC	CEP	AUC	CEP	AUC	CEP	AUC	CEP	AUC
Control	53.8 ± 2.0	50.3 ± 1.4	22.6 ± 2.7	22.1 ± 3.5	18.5 ± 0.2	17.0 ± 1.4	42.9 ± 1.8	46.0 ± 0.2	3.0 ± 2.9	13.0 ± 1.6	0.3 ± 0.1	0.2 ± 0.1
Milk	2.1 ± 1.0	2.1 ± 0.4	79.7 ± 3.5	81.4 ± 3.2	22.5 ± 3.2	56.7 ± 2.3	1.0 ± 0.1	1.5 ± 0.4	57.4 ± 14.3	71.7 ± 1.6	16.8 ± 3.0	49.8 ± 2.8
Dough	0.4 ± 0.3	6.2 ± 4.1	52.4 ± 1.1	50.7 ± 4.1	13.1 ± 3.4	35.6 ± 2.1	2.8 ± 2.3	4.4 ± 2.5	26.3 ± 3.8	40.1 ± 0.9	0.8 ± 0.6	28.0 ± 0.7
Acetic	54.0 ± 1.0	54.0 ± 0.9	48.7 ± 3.1	41.1 ± 1.5	36.0 ± 3.0	47.2 ± 1.2	44.8 ± 1.6	49.4 ± 0.5	6.8 ± 1.3	24.6 ± 1.2	0.6 ± 0.4	34.2 ± 1.0
Malic	29.8 ± 1.5	32.1 ± 0.4	64.3 ± 2.7	57.0 ± 2.0	29.5 ± 0.4	50.5 ± 1.5	25.3 ± 0.5	28.7 ± 0.5	58.2 ± 0.6	57.5 ± 1.1	28.4 ± 1.3	49.6 ± 0.8
Citric	21.2 ± 0.1	22.8 ± 0.6	69.5 ± 3.2	63.3 ± 1.2	35.6 ± 1.1	51.6 ± 1.0	16.9 ± 1.0	19.9 ± 0.4	70.9 ± 3.5	65.7 ± 06	34.7 ± 0.9	52.2 ± 0.6
Lactic	16.7 ± 1.6	20.2 ± 1.4	87.7 ± 6.7	75.2 ± 3.7	24.1 ± 0.1	59.2 ± 2.6	15.5 ± 2.4	18.8 ± 1.3	26.8 ± 6.5	52.6 ± 3.0	1.7 ± 1.1	42.8 ± 1.9
Ascorbic	0.3 ± 0.1	0.3 ± 0.1	73.7 ± 4.8	63.3 ± 1.7	41.8 ± 0.2	43.6 ± 1.2	0.1 ± 0.0	0.2 ± 0.0	23.8 ± 0.8	43.3 ± 1.0	32.9 ± 5.3	29.9 ± 0.7

Table 3
Carcinogenic risk (CR) and hazard quotient (HQ) of chromium for children.

Samples	Gastric		Intestinal	
	Cr(VI) CR ($\times 10^{-4}$)	Cr(III) HQ ($\times 10^{-3}$)	Cr(VI) CR ($\times 10^{-4}$)	Cr(III) HQ ($\times 10^{-3}$)
Control	18.6 ± 0.5	37.3 ± 5.8	17.3 ± 0.3	21.9 ± 2.8
Milk	0.8 ± 0.2	137.4 ± 5.4	0.6 ± 0.1	121.0 ± 7.1
Dough	2.3 ± 1.5	85.6 ± 6.9	1.6 ± 0.9	67.7 ± 1.6
Acetic	20.0 ± 0.5	69.4 ± 2.6	18.3 ± 0.2	41.4 ± 2.0
Malic	11.9 ± 0.2	96.1 ± 3.4	10.6 ± 0.2	97.0 ± 1.8
Citric	8.4 ± 0.3	106.9 ± 2.0	7.4 ± 0.1	110.9 ± 1.1
Lactic	7.9 ± 0.8	126.9 ± 6.2	7.0 ± 0.5	88.7 ± 5.0
Ascorbic	0.1 ± 0.0	106.9 ± 2.9	0.1 ± 0.0	73.1 ± 1.7

previous study demonstrated a greater absorption of Cr(VI) than Cr(III) in the human gastrointestinal tract [8]. With a spontaneous Cr(VI) release from COPR and the following rapid human uptake, reduction of Cr(VI) might not be as effective a detoxification pathway as initially thought.

3.5. Risk assessment

The chemical daily intake (CDI) was calculated to evaluate the worst case scenario for potential COPR risks to children. The CDI values in the unit of mg kg^{-1} d^{-1} were determined using the following Eq. (2) [39]:

$$\mathrm{CDI} = \mathrm{C} \times \frac{\mathrm{IR} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{BW} \times \mathrm{AT}} \quad (2)$$

where C is the Cr concentration in COPR (mg kg^{-1}); IR is the ingestion rate (200 mg d^{-1}) [40]; EF is the exposure frequency (182 d y^{-1}) [41]; ED is the exposure duration (4 years for preschool children) [42]; BW is the average body weight (15 kg) [40]; and AT is the averaging time (for non-carcinogens, AT = ED × 365 d; for carcinogens, AT = 70 y × 365 d y^{-1} = 25 550 d) [39].

The CDI was 7.4×10^{-3} mg kg^{-1} d^{-1} for Cr(VI), and 0.25 mg kg^{-1} d^{-1} for Cr(III). The results suggest that the Cr(VI) intake from COPR was over two times higher than the USEPA oral reference dose (R_fD_o) of 3×10^{-3} mg kg^{-1} d^{-1} for Cr(VI) [43], while the Cr(III) intake was about an order of magnitude lower than the Cr(III) R_fD_o of 1.5 mg kg^{-1} d^{-1} [43]. However, the above CDI calculation did not consider the impact of food and organic acids on the Cr bioaccessibility. To include bioaccessibility in the risk assessment, the nondimensional carcinogenic risk (CR) for Cr(VI) and non-carcinogenic hazard quotient (HQ) for Cr(III) were calculated using Eqs. (3) and (4), respectively [39,44]:

$$\mathrm{CR} = \mathrm{CDI} \times \mathrm{B} \times \mathrm{SF_o} \quad (3)$$

$$\mathrm{HQ} = \frac{\mathrm{CDI} \times \mathrm{B}}{R_fD_o} \quad (4)$$

where B is the bioaccessibility, and oral cancer slope factor (SF_o) is 0.5 (mg kg^{-1} d^{-1})$^{-1}$ [43]. The results listed in Table 3 indicate that the Cr(VI) CR values from COPR were 18.6×10^{-4} in the gastric phase and 17.3×10^{-4} in the intestinal phase, more than 15 times higher than the safe value (1×10^{-4}) determined by the USEPA [44]. Among the foodstuffs and organic acids, milk and ascorbic acid could significantly reduce the CR of Cr(VI) to a safe value (Table 3). Meanwhile, the Cr(III) HQ values from COPR were 37.3×10^{-3} in the gastric phase and 21.9×10^{-3} in the intestinal phase (Table 3). Although food and organic acids increased the HQ for Cr(III), their values were still much lower than the safe level of 1 established by the USEPA [44].

3.6. Cr speciation

The XANES analyses shown in Fig. 1 indicate that no Cr(VI) was detected in the residues of the IVG extraction. The absence of Cr(VI) may be due to two reactions. First, Cr(VI) was released from the solid phase to the artificial gastrointestinal juice. Second, Cr(VI) was reduced to Cr(III) with the addition of food and organic acids.

The XRPD patterns for COPR and the IVG-extracted residues are presented in Fig. 2. During extraction, Cr(VI)-bearing minerals, as well as brucite ($Mg(OH)_2$), ettringite ($Ca_6Al_2(SO_4)_3(OH)_{12}\cdot 26H_2O$), periclase (MgO), and calcite ($CaCO_3$) were dissolved in the human gastrointestinal tract. Therefore, their characteristic peaks cannot be observed in the XRPD spectra.

Brownmillerite ($Ca_2(Al,Fe^{3+})_2O_5$), a Cr(III)-bearing mineral, was persistently detected in COPR and extraction residue samples (Fig. 4). Furthermore, no new Cr(III) phases were observed in the residues, which suggests the formation of amorphous rather than crystalline Cr(III) minerals where Cr(VI) was reduced.

4. Conclusions

Accurate determination of the Cr bioaccessibility and speciation is of great importance in evaluation of health risks associated with ingested COPR. Our results indicated that Cr(VI) would not be reduced by the simulated gastrointestinal juice itself. Ingestion of dough, milk, and organic acids including ascorbic, lactic, malic, and citric acid can facilitate the Cr(VI) reduction in the IVG extraction. Furthermore, XANES and XRPD analysis confirmed the Cr(VI) reduction with food ingestion. In addition, the conventional method to calculate bioaccessibility using the concentrations at the end of extraction phase could be improved by integrating areas under the concentration–time curve of the IVG extraction.

Acknowledgement

The research is supported by the National Natural Science Foundation of China (20921063, 20890112, 41023005).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2012.01.009.

References

[1] J.S. Geelhoed, J.C.L. Meeussen, S. Hillier, D.G. Lumsdon, R.P. Thomas, J.G. Farmer, E. Paterson, Identification and geochemical modeling of processes controlling leaching of Cr(VI) and other major elements from chromite ore processing residue, Geochim. Cosmochim. Acta 66 (2002) 3927–3942.
[2] D. Zhang, S. He, L. Dai, Y. Xie, D. Wu, G. Bu, K. Peng, H. Kong, Impact of pyrolysis process on the chromium behavior of COPR, J. Hazard. Mater. 172 (2009) 1597–1601.
[3] T. Burke, J. Fagliano, M. Goldoft, R.E. Hazen, R. Iglewicz, T. McKee, Chromite ore processing residue in Hudson County, New Jersey, Environ. Health Perspect. 92 (1991) 131–137.
[4] T. Wang, M. He, Q. Pan, A new method for the treatment of chromite ore processing residues, J. Hazard. Mater. 149 (2007) 440–444.
[5] J.G. Farmer, R.P. Thomas, M.C. Graham, J.S. Geelhoed, D.G. Lumsdon, E. Paterson, Chromium speciation and fractionation in ground and surface waters in the

vicinity of chromite ore processing residue disposal sites, J. Environ. Monit. 4 (2002) 235–243.
[6] J.S. Geelhoed, J.C.L. Meeussen, M.J. Roe, S. Hillier, R.P. Thomas, J.G. Farmer, E. Paterson, Chromium remediation or release? Effect of iron(II) sulfate addition on chromium(VI) leaching from columns of chromite ore processing residue, Environ. Sci. Technol. 37 (2003) 3206–3213.
[7] National Institute of Health, NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate, Washington, DC, 2007.
[8] B.D. Kerger, D.J. Paustenbach, G.E. Corbett, B.L. Finley, Absorption and elimination of trivalent and hexavalent chromium in humans following ingestion of a bolus dose in drinking water, Toxicol. Appl. Pharmacol. 141 (1996) 145–158.
[9] P.J. Lioy, N.C.G. Freeman, T. Wainman, A.H. Stern, R. Boesch, T. Howell, S.I. Shupack, Microenvironmental analysis of residential exposure to chromium-laden wastes in and around New Jersey homes, Risk Anal. 12 (1992) 287–299.
[10] J.G. Farmer, R. Jarvis, Strategies for improving human health in contaminated situations: a review of past, present and possible future approaches, Environ. Geochem. Health 31 (2009) 227–238.
[11] M.V. Ruby, R. Schoof, W. Brattin, M. Goldade, G. Post, M. Harnois, D.E. Mosby, S.W. Casteel, W. Berti, M. Carpenter, D. Edwards, D. Cragin, W. Chappell, Advances in evaluating the oral bioavailability of inorganics in soil for use in human health risk assessment, Environ. Sci. Technol. 33 (1999) 3697–3705.
[12] J.L. Schroder, N.T. Basta, J.T. Si, S.W. Casteel, T. Evans, M. Payton, In vitro gastrointestinal method to estimate relative bioavailable cadmium in contaminated soil, Environ. Sci. Technol. 37 (2003) 1365–1370.
[13] B. Marschner, P. Welge, A. Hack, J. Wittsiepe, M. Wilhelm, Comparison of soil Pb in vitro bioaccessibility and in vivo bioavailability with Pb pools from a sequential soil extraction, Environ. Sci. Technol. 40 (2006) 2812–2818.
[14] R.R. Rodriguez, N.T. Basta, An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and solid media, Environ. Sci. Technol. 33 (1999) 642–649.
[15] J.L. Schroder, N.T. Basta, S.W. Casteel, T.J. Evans, M.E. Payton, J. Si, Validation of the in vitro gastrointestinal (IVG) method to estimate relative bioavailable lead in contaminated soils, J. Environ. Qual. 33 (2004) 513–521.
[16] P. Pouschat, G.J. Zagury, In vitro gastrointestinal bioavailability of arsenic in soils collected near CCA-treated utility poles, Environ. Sci. Technol. 40 (2006) 4317–4323.
[17] D.G. Beak, N.T. Basta, K.G. Scheckel, S.J. Traina, Bioaccessibility of arsenic(V) bound to ferrihydrite using a simulated gastrointestinal system, Environ. Sci. Technol. 40 (2006) 1364–1370.
[18] S. DeFlora, A. Camoirano, M. Bagnasco, C. Bennicelli, G.E. Corbett, B.D. Kerger, Estimates of the chromium(VI) reducing capacity in human body compartments as a mechanism for attenuating its potential toxicity and carcinogenicity, Carcinogenesis 18 (1997) 531–537.
[19] C. Pellerin, S.M. Booker, Reflections on hexavalent chromium – health hazards of an industrial heavyweight, Environ. Health Perspect. 108 (2000) A402–A407.
[20] A. Broadway, M.R. Cave, J. Wragg, F.M. Fordyce, R.J.F. Bewley, M.C. Graham, B.T. Ngwenya, J.G. Farmer, Determination of the bioaccessibility of chromium in Glasgow soil and the implications for human health risk assessment, Sci. Total Environ. 409 (2010) 267–277.
[21] US EPA, Method 3051A, Microwave Assisted Acid Digestion of Sludges, Sediments, Soils, and Oils, Washington, DC, 2007.
[22] US EPA, Method 3060A, Alkaline Digestion for Hexavalent Chromium, Washington, DC, 1996.
[23] US EPA, Method 7196A, Chromium, Hexavalent (Colorimetric), Washington, DC, 1992.
[24] M.V. Ruby, A. Davis, T.E. Link, R. Schoof, R.L. Chaney, G.B. Freeman, P. Bergstrom, Development of an in vitro screening-test to evaluate the *in vivo* bioaccessibility of ingested mine-waste lead, Environ. Sci. Technol. 27 (1993) 2870–2877.
[25] M.V. Ruby, A. Davis, R. Schoof, S. Eberle, C.M. Sellstone, Estimation of lead and arsenic bioavailability using a physiologically based extraction test, Environ. Sci. Technol. 30 (1996) 422–430.
[26] M. Wazne, A.C. Jappilla, D.H. Moon, S.C. Jagupilla, C. Christodoulatos, M.G. Kim, Assessment of calcium polysulfide for the remediation of hexavalent chromium in chromite ore processing residue (COPR), J. Hazard. Mater. 143 (2007) 620–628.
[27] D. Dermatas, M. Chrysochoou, D.H. Moon, D.G. Grubb, M. Wazne, C. Christodoulatos, Ettringite-induced heave in chromite ore processing residue (COPR) upon ferrous sulfate treatment, Environ. Sci. Technol. 40 (2006) 5786–5792.
[28] S. Hillier, M.J. Roe, J.S. Geelhoed, A.R. Fraser, J.G. Farmer, E. Paterson, Role of quantitative mineralogical analysis in the investigation of sites contaminated by chromite ore processing residue, Sci. Total Environ. 308 (2003) 195–210.
[29] S. Hillier, D.G. Lumsdon, R. Brydson, E. Paterson, Hydrogarnet A host phase for Cr(VI) in chromite ore processing residue (COPR) and other high pH wastes, Environ. Sci. Technol. 41 (2007) 1921–1927.
[30] M. Chrysochoou, D. Dermatas, Application of the Rietveld method to assess chromium(VI) speciation in chromite ore processing residue, J. Hazard. Mater. 141 (2007) 370–377.
[31] D.J. Paustenbach, B.L. Finley, F.S. Mowat, B.D. Kerger, Human health risk and exposure assessment of chromium (VI) in tap water, J. Toxicol. Environ. Health – Part A 66 (2003) 1295–1339.
[32] R.E. Wolf, J.M. Morrison, M.B. Goldhaber, Simultaneous determination of Cr(III) and Cr(VI) using reversed-phased ion-pairing liquid chromatography with dynamic reaction cell inductively coupled plasma mass spectrometry, J. Anal. Atom. Spectrom. 22 (2007) 1051–1060.
[33] S. Salovaara, A.S. Sandberg, T. Andlid, Organic acids influence iron uptake in the human epithelial cell line Caco-2, J. Agric. Food Chem. 50 (2002) 6233–6238.
[34] B. Deng, A.T. Stone, Surface-catalyzed chromium(VI) reduction: reactivity comparisons of different organic reductants and different oxide surfaces, Environ. Sci. Technol. 30 (1996) 2484–2494.
[35] J. Sun, J.D. Mao, H. Gong, Y. Lan, Fe(III) photocatalytic reduction of Cr(VI) by low-molecular-weight organic acids with α-OH, J. Hazard. Mater. 168 (2009) 1569–1574.
[36] C. Li, Y.-Q. Lan, B.-L. Deng, Catalysis of manganese(II) on chromium(VI) reduction by citrate, Pedosphere 17 (2007) 318–323.
[37] K. Yamaoka, T. Nakagawa, T. Uno, Statistical moments in pharmacokinetics, J. Pharmacokinet. Pharmacodyn. 6 (1978) 547–558.
[38] J.D. Ma, E.T. Nguyen, S.M. Tsunoda, H.E. Greenberg, J.C. Gorski, S.R. Penzak, L.S. Lee, Assessment of oral midazolam limited sampling strategies to predict area under the concentration time curve (AUC) during cytochrome P450 (CYP) 3A baseline, inhibition and induction or activation, Int. J. Clin. Pharmacol. Ther. 48 (2010) 847–853.
[39] X. Hu, Y. Zhang, J. Luo, T. Wang, H. Lian, Z. Ding, Bioaccessibility and health risk of arsenic, mercury and other metals in urban street dusts from a mega-city, Nanjing, China, Environ. Pollut. 159 (2011) 1215–1221.
[40] US EPA, Supplemental Guidance for Developing Soil Screening Levels for Superfund Sites, OSWER 9355, Washington, DC, 2002, pp. 4–24.
[41] US EPA, Exposure Factors Handbook, Volume I: General Factors, EPA 600-P-95-002Fa, Washington, DC, 1997.
[42] US EPA, Cancer Risk Calculations, Handbook for Implementing the Supplemental Cancer Guidance at Waste and Cleanup Sites. Available at: http://www.epa.gov/oswer/riskassessment/sghandbook/riskcalcs.htm.
[43] US EPA, Region 9, Regional Screening Levels 2011 Table (Industrial Soil Supporting), Available at: http://www.epa.gov/region9/superfund/prg/index.html.
[44] US EPA, Guidance for Evaluating the Oral Bioavailability of Metals in Soils for use in Human Health Risk Assessment, OSWER 9285, Washington, DC, 2007, pp. 7–80.