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Gastrointestinal Microbes Increase Arsenic Bioaccessibility of Ingested Mine Tailings Using the Simulator of the Human Intestinal Microbial Ecosystem

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It is widely accepted that the use of total metal concentrations in soil overestimates metal risk from human ingestion of contaminated soils. *In vitro* simulators have been used to estimate the fraction of arsenic present in soil that is bioaccessible in the human digestive track. These approaches assume that the bioaccessible fraction remains constant across soil total metal concentrations and that intestinal microbiota do not contribute to arsenic release. Here, we evaluate both of these assumptions in two size fractions (bulk and <38 μm) of arsenic-rich mine tailings from the Goldenville, Lower Seal Harbour, and Montague Gold Districts, Nova Scotia. These samples were evaluated using an *in vitro* gastrointestinal model, the Simulator of the Human Intestinal Ecosystem (SHIME). Arsenic bioaccessibility, which ranged between 2 and 20% in the small intestine and 4 and 70% in the colon, was inversely related to total arsenic concentration in the mine tailings. Additionally, arsenic bioaccessibility was greater in the bulk fraction than in the <38 μm fraction in the small intestine and colon while colon microbes increased the bioaccessibility of arsenic in mine tailings. These results suggest that the practice of using a constant percent arsenic bioaccessibility across all metal concentrations in risk assessment should be revisited.

Introduction

Three million tons of mine tailings were generated within 64 gold-mining districts in Nova Scotia through mercury-amalgamation and cyanidation techniques between 1861 and the mid-1940s (*1*, *2*). Exposure to arsenic through the ingestion of mine tailings, crushed rock waste produced during the extraction of gold from ore, poses a potential human health risk to communities in Nova Scotia, Canada. Inorganic arsenic consumption is linked to blackfoot disease (*3*), neonatal death, and spontaneous abortions (*4*) as well as skin, bladder, and colon cancer (*5*, *6*). The potential for arsenic exposure is exacerbated by the small particle size of tailings since the probability of ingestion often increases with decreasing particle size (*7*). Current practice is to assume that the bioaccessible fraction, the fraction of an ingested contaminant solubilized in the gastrointestinal tract, is close to 100%, or at least equal to the material used to derive the Toxicological Reference Value (*8*, *9*). Since metal bioaccessibility varies between matrices (i.e., mine tailings, soils, etc.), there is a need to develop bioaccessibility estimates for use in site-specific risk assessments (*8*, *10*).

Arsenic bioaccessibility has been measured using several *in vitro* gastrointestinal models to estimate human health risk from the ingestion of arsenic-contaminated soils and mine tailings (*8*, *9*, *11*−*13*). The bioavailable fraction, that which is absorbed across the intestinal epithelium into systemic circulation, of metal (loid) contaminants may decrease with increasing exposure because of saturation of absorption mechanisms (*8*, *13*). However, the role of arsenic concentration in determining arsenic bioaccessibility using *in vitro* models has been less conclusive. Two studies utilizing different *in vitro* gastrointestinal models demonstrated that arsenic bioaccessibility is independent of arsenic concentration (*8*, *11*). However, when the bioaccessible arsenic concentration was greater than the detection limit, decreasing bioaccessibility was observed for increasing concentrations of arsenate adsorbed to the iron oxide mineral ferrihydrite in the stomach stage of an *in vitro* model (*14*). In contrast, arsenic bioaccessibility in soils increased with increasing concentrations using a different *in vitro* model (*9*). These conflicting results may be due to *in vitro* model differences which may have facilitated or limited arsenic dissolution. For example, the pH, residence times, liquid to solid (LS) ratios, and bioaccessible fraction separation procedures vary with *in vitro* models and may influence the dissolution of contaminants from soils (*15*, *16*). Alternatively, varying soil properties (i.e, pH and iron concentration) and mineralogy influence arsenic release from soil particles (*17*) as several selective sequential extraction studies have demonstrated (*18*, *19*).

The chemical dissolution of arsenic occurs as a result of desorption of surficially adsorbed arsenic and the solubilization of discrete arsenic-bearing minerals (*12*). In the gastrointestinal tract, chemical dissolution may be limited by the desorption of surficially adsorbed arsenic, rather than solubilization of discrete arsenic minerals (*20*, *21*). However, the low pH observed in the stomach may result in the dissolution of minerals resulting in the release of surficially bound arsenic (*14*). Bioavailable arsenic is likely composed of surficially adsorbed arsenic and a portion of the arsenic incorporated in discrete iron oxide arsenic minerals (*12*) rather than from arsenic incorporated into sulfide-containing minerals (e.g., arsenopyrite) (*20*, *21*). However, not all surficially adsorbed arsenic is bioaccessible since arsenic may be strongly adsorbed to iron oxide minerals (*22*) or iron sulfide minerals (*23*). Microorganisms can directly solubilize arsenic by two different mechanisms involving anaerobic respiration (*24*). First, dissimilatory arsenic-reducing prokaryotes (DARPs) can reduce arsenate, adsorbed to or complexed within soil

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minerals, thereby releasing arsenite into solution (*25*, *26*). Alternatively, dissimilatory iron-reducing prokaryotes (DIRPs) may reduce iron, thus releasing arsenic contained in the iron mineral (*27*). DARPs, such as the *Bacillus sp.* identified in the gastrointestinal tracts of mammals (*28*) may also utilize iron as an electron acceptor (*24*).

The Simulator of the Human Intestinal Microbial Ecosystem (SHIME) is unique from other *in vitro* gastrointestinal models by incorporating the microbial community present in the human colon (*29*). The conditions of the SHIME were selected from the range of physiological pH's and residence times of the human gastrointestinal tract likely to facilitate the dissolution of ingested contaminants. The small intestine stage of the SHIME includes the addition of bile salts, which may impact metal bioaccessibility through alterations of surface tension and the formation of labile bile complexes (*30*). The SHIME is operated under anaerobic conditions with human gastrointestinal microorganisms in the colon stage, thereby facilitating the potential role of DARPs and/or DIRPs in contributing to arsenic release. The assessment of metal bioaccessibility from *in vitro* gastrointestinal fluids often uses centrifugation, microfiltration (0.45 μm), or ultrafiltration for the separation of the bioaccessible fraction (*8*, *14*, *16*, *31*). It is not clear what method best estimates bioaccessibility, but ultrafiltration may be the most appropriate because it represents the defined size fraction known to cross the intestinal lumen (*16*).

For this study, we tested the hypothesis that the percent of arsenic that is bioaccessible to humans from mine tailings depends on the total concentration of arsenic and on the action of gastrointestinal microorganisms. The arsenic bioaccessibility of two size fractions (bulk and <38 μm) of mine tailings from three gold districts in Nova Scotia (Goldenville, Lower Seal Harbour, and Montague) was evaluated in the small intestine and colon stages of the SHIME. Additionally, the analyses were repeated using autoclaved colon SHIME suspension to assess the influence of gastrointestinal microbes on arsenic bioaccessibility.

Materials and Methods

Sample Description. Mine tailings were collected from Goldenville, Lower Seal Harbour, and Montague, all historic gold mine sites near rural or suburban residential communities in Nova Scotia, Canada. This sampling was part of a large multidisciplinary study of Nova Scotia gold districts that has been ongoing since 2003 (*2*, *18*). A bulk sample of near-surface tailings (0−5 cm) was collected from each of the three mine tailings locations and a portion dry-sieved to <38 μm. The <38 μm fraction was selected since small particle sizes are more likely to adhere to hands and be accidentally ingested (*32*) and <38 μm was the finest particle size that could be obtained by dry-sieving. Wet-sieving was avoided since it may dissolve soluble phases and/or cause cementation. In addition to these two size fractions, a cemented material (hardpan) formed by the precipitation of minerals from groundwater was collected from Montague.

The mineral hosts for arsenic in these tailings samples have been characterized using a variety of techniques including optical mineralogy, electron microprobe, traditional X-ray diffraction (XRD), sequential leach extractions, and synchrotron-based micro-XRD and micro-X-ray absorption near edge structure. Detailed results of these mineralogical investigations are reported elsewhere (*18*). Arsenic occurs naturally in the Nova Scotia gold deposits as arsenopyrite (FeAsS), but extensive near-surface weathering of these mine tailings has oxidized almost all of the sulfide to secondary phases. Minor amounts (<2 vol %) of sulfide minerals were observed in the samples from Goldenville and Lower Seal Harbour, including arsenopyrite, pyrite, pyrrhotite, and chalcopyrite. No sulfides were identified in the Montague sample. The Montague tailings contain abundant hardpan material that has been variably crushed by off-road vehicles. The hardpan cement consists primarily of crystalline scorodite ($FeAsO_4 \cdot 2H_2O$) and amorphous Fe arsenate. Mineralogical analyses of the Goldenville and Lower Seal Harbour tailings samples have identified a more diverse range of secondary arsenic minerals, including Ca−Fe arsenates, and Fe oxyhydroxides containing up to 30 wt.% As_2O_5. These phases occur as discrete grains, reaction rims on oxidized arsenopyrite, and grain coatings on silicate minerals (*18*). In summary, the three samples of historical gold mine tailings evaluated in the present study contain a variety of arsenic-bearing minerals to which individuals may be exposed. The relative abundance of these phases at Goldenville, Lower Seal Harbour, and Montague varies laterally and vertically in the tailings deposits and is not fully represented by the bulk samples collected for this study.

Dynamic SHIME. A three-stage dynamic SHIME model was used to create the colon community used in the batch SHIME colon digests. Stomach nutrition contained per liter: 5 g of $KHCO_3$ (Alfa Aesar), 3 g of NaCl (EM Sci), 0.5 g of arabinogalactan (Sigma), 1 g of pectin (Sigma), 0.5 g of xylan (Sigma), 2 g of potato starch (Nutricia), 0.2 g of glucose (Sigma), 1.5 g of yeast extract (Difco), 0.5 g of pepton (Difco), 2 g of mucin (Sigma), and 0.25 g of cysteine (Sigma). The nutrition solution was acidified to pH 2 by the addition of hydrochloric acid and was then pumped into the stomach compartment and mixed constantly at 37 °C for 2 h. Following the stomach incubation, a pancreatic solution containing 12.5 g L^{-1} NaHCO3, 6 g L^{-1} Oxgall (Difco), and 0.9 g L^{-1} porcine pancreatin powder (Sigma, Belgium) was pumped into the small intestine vessel in a 2:1 (stomach: pancreatic solution) ratio, thereby creating a mixture of pH 6.5. Following a 5 h retention time, the small intestine mixture was pumped into the colon compartment, which was previously inoculated with a fecal culture from a healthy human adult. The fecal culture was generated according to previously published procedures (*33*). In brief, freshly excreted feces from a healthy adult male (10 g) were diluted and homogenized with sterile phosphate buffer (0.1 M, pH 7.0, 100 mL), to which 100 mg of sodium thioglycolate was added. Particulate matter was removed by centrifugation (500*g*, 1 min) (*33*). This fecal culture was stored in 20% glycerol at −80 °C until the time of the experiment. Following the 3 week stabilization period (*33*), the total aerobe and anaerobe concentrations of the colon stage were 9.9 and 10.9 log CFU mL^{-1} and included microbiota such as lactobacilli, bifidobacteria, enterococci, fungi, staphylococci, and clostridia. This colon digest was maintained between pH 5.6 and 5.9, as observed in the human ascending colon, incubated at 37 °C and stirred at 150 rpm for 18 h. Regular flushing of the compartments with nitrogen gas maintained anaerobic conditions. The redox potential of the three SHIME stages were not monitored; however, previous work has demonstrated Eh's (standard redox potential) of −70 mV in the stomach and small intestinal stages and −160 mV in the colon stage. Nutrient and pancreatic solutions were maintained at 4 °C and replaced every 3 days. Aliquots of the suspension from the colon compartment of the dynamic SHIME provided the solutions used in static batch experiments to measure arsenic bioaccessibility.

Static SHIME. Mine tailings sample (150 mg each) was added to two 30-mL glass vessels for the quantification of arsenic bioaccessibility in the small intestine and colon. Fasted stomach solution (6 mL) was added to each vessel. The fasted stomach solution is as described above except that no nutrition was added. The pH was adjusted to 1.5 ± 0.05 with 0.5 M HCl. The anaerobic conditions described for the dynamic SHIME were reproduced in the static SHIME by applying consecutive cycles of overpressure (1 bar N_2-

gas; 2 min) and underpressure (1 bar vacuum; 2 min) for 30 min. Thereby, the vessels were capped with Teflon-coated lids and flushed with nitrogen gas prior to stomach incubation at 37 °C for 2.5 h with rotary-mixing (10 rpm). Following stomach incubation, 3 mL of pancreatic solution was added to each vessel and the pH corrected to 6.2 ± 0.05 with 0.5 M HCl. The vessels were then flushed with nitrogen gas and incubated for an additional 3 h. Following incubation, the small intestine vessels were removed for separation of the bioaccessible arsenic fraction. Colon SHIME suspension (6 mL) from the Dynamic SHIME was added to each active colon vessel while 6 mL of previously autoclaved and cooled colon SHIME suspension was added to each sterile colon vessel. The pH of the colon vessels were adjusted to 5.9 ± 0.05 with 0.5 M HCl. The vessels were then flushed with nitrogen gas for 30 min and incubated at 37 °C for 18 h.

Separation of Bioaccessible Fraction. The contents of each SHIME vessel were centrifuged at 12 000*g* for 20 min, thereby accelerating the subsequent ultrafiltration of the SHIME solution. Less rigorous centrifugation is typically used in the separation of the bioaccessible fraction (*8*, *34*); however, this strong centrifugation was required to pellet a precipitate formed in the colon stage which, if present, prevented ultrafiltration. The supernatant was decanted into a test tube and weighed. Aliquots of the small intestine supernatant (6 mL) and colon supernatant (9 mL) were weighed and transferred into ultrafiltration devices (10K Microsep; Pall) which were subsequently centrifuged (3000 rpm). The mass of the filtrate of each sample were recorded for the arsenic bioaccessibility calculation. The arsenic contained in the filtrate was defined as the bioaccessible arsenic fraction, containing free arsenic oxyanions and small arsenic-containing complexes. The arsenic bioaccessibility (%) was calculated by:

$$\text{bioaccessibility (\%)} = \frac{[\text{filtrate As}] \times \dfrac{\text{filtrate mass} \times \text{total supernatant mass}}{\text{total ultrafiltration mass}}}{\text{total As concentration} \times \text{sample mass}} \times 100\% \quad (1)$$

where [filtrate As] = As concentration (μg mL^{-1}) in SHIME filtrate, filtrate, total supernatant, and total ultrafiltration mass = mass (g) of each solution fraction, total As concentration = As concentration (mg kg^{-1}) in mine tailings sample, and sample mass = mine tailings sample mass (150 mg). Note: Solution densities were measured to be 1.0 g mL^{-1}

Measurement of Arsenic via HG-AFS. Digestion of the bioaccessible fraction was performed using a CEM-2000 microwave according to a procedure modified from Rahman et al. (*35*). In brief, 0.5 mL of SHIME suspension was added to 12 M HCl (5 mL) and the mixture digested at 40% power for 15 min. Subsequently, hydrogen peroxide (1.0 mL, 30%) was added and the mixture digested at 40% power for an additional 15 min. A further 1.0 mL of hydrogen peroxide was added to the mixture, which was digested for another 15 min. The digested SHIME solution was diluted to 30 mL with MQ water and stored at 4 °C until arsenic analysis.

Arsenic was measured using hydride generation atomic fluorescence spectroscopy (Millenium Excalibur, PS Analytical) (*35*). Amounts of 6 mL of pure OmniTrace HCl (12 N, 37%), 4.5 mL of Milli-Q ultrapure water, and 0.4 mL of 3.01 M potassium iodide (KI), and 0.57 M ascorbic acid ($C_6H_8O_6$) solution were added to 9 mL aliquots of each digested SHIME solution. Potassium iodide served to reduce any arsenate in solution to arsenite, which forms the hydride necessary for spectrometric analysis. Fresh $NaBH_4$ (1.4%) and reagent blank solutions were prepared daily. Standard solutions were prepared by dilution of stock 1000 mg L^{-1} arsenic standard (EMD Chemicals Inc., Darmstadt, Germany). All glassware was soaked in 10% Omnitrace nitric acid (EMD Chemicals Inc., Darmstadt, Germany) prior to use and then rinsed five times with distilled water.

Quality Assurance/Quality Control. Blank SHIME suspension (without mine tailings) was microwave digested and measured for arsenic according to the procedures described above and returned an average concentration of 0.27 μg L^{-1} (SE = 0.008). Duplicate SHIME subsamples were digested and measured for arsenic for every ten samples processed. Additionally, a calibration blank, standard, and an analytical duplicate were measured for every ten samples analyzed to ensure instrumental accuracy and precision. The USEPA defines the methodological detection limit as the minimum concentration that can be determined with 99% confidence that the true concentration is greater than zero (*36*). The methodological detection limit for the measurement of arsenic in microwave-digested SHIME solution is calculated as follows:

$$\text{MDL} = (t_{n-1,0.01}) \times \text{sd} \quad (2)$$

where the Student's *t*-value at the 0.01 level of probability for seven samples is 3.143, and 'sd' is the standard deviation of seven microwave-digested blank SHIME solutions. The standard deviation was 21.5 ng L^{-1} for a methodological detection limit of 68 ng As L^{-1}.

Statistical Analysis. Nested factorial Analysis of Variance (ANOVA) was used to compare mean arsenic bioaccessibility from the bulk and <38 μm mine tailings fractions from Goldenville, Lower Seal Harbour, and Montague in the small intestine and microbially active and sterile colon. ANOVA assumptions of normality and homogeneous variance were verified using a Type I error rate of 0.05. Fisher's protected Least-Significant Difference (LSD) (GLM, $\alpha = 0.05$) was used to test for significant differences.

Results

Percent arsenic bioaccessibility was inversely related to sample arsenic mass despite increasing arsenic release (Figure 1); however, this trend is not observed in the <38 μm fraction if the size fractions are considered separate distributions. Bioaccessibility ranged between 2.5 and 20% in the small intestine compared to 4 and 70% in the colon. Arsenic bioaccessibility was log-normally distributed (*W*-Test, $P > 0.05$) with homogeneous variance (Levene's Test, $P > 0.05$). In the small intestine, there was little difference between the mass of bioaccessible arsenic in the 5000 and 17000 μg arsenic samples, suggesting that arsenic release may have peaked by 5000 μg. In contrast, the mass of bioaccessible arsenic appeared to continue increasing with increasing total arsenic in the colon.

Arsenic bioaccessibility in bulk tailings was greater (Fisher protected LSD, $P = 0.001$) than in the <38 μm fraction in both the small intestine and colon (Figure 2). No significant interaction in arsenic bioaccessibility existed between mine tailings location and size fraction (GLM, $P = 0.814$). Thus, the arsenic bioaccessibility of the mine tailings locations were pooled for both the small intestine ($n = 9$) and colon ($n = 9$) stages of the SHIME. Total arsenic mass was consistently less in the bulk than in the <38 μm fraction of all mine tailings (Goldenville: bulk = 62 μg As, < 38 μm = 455 μg As; Montague: bulk = 4608 μg As, < 38 μm = 16764 μg As; Lower Seal Harbour: bulk = 97 μg As, < 38 μm = 791 μg As). The bioaccessible arsenic concentration in the SHIME fluid ranged between 1.3 and 16.2 mg L^{-1} in the small intestine and 2.9 and 27.3 mg L^{-1} in the colon.

Small intestine arsenic bioaccessibility of the bulk and <38 μm mine tailings fractions was greater from Goldenville than Lower Seal Harbour which was greater than Montague (Fisher protected LSD, $P < 0.001$) (Figure 3). The same order

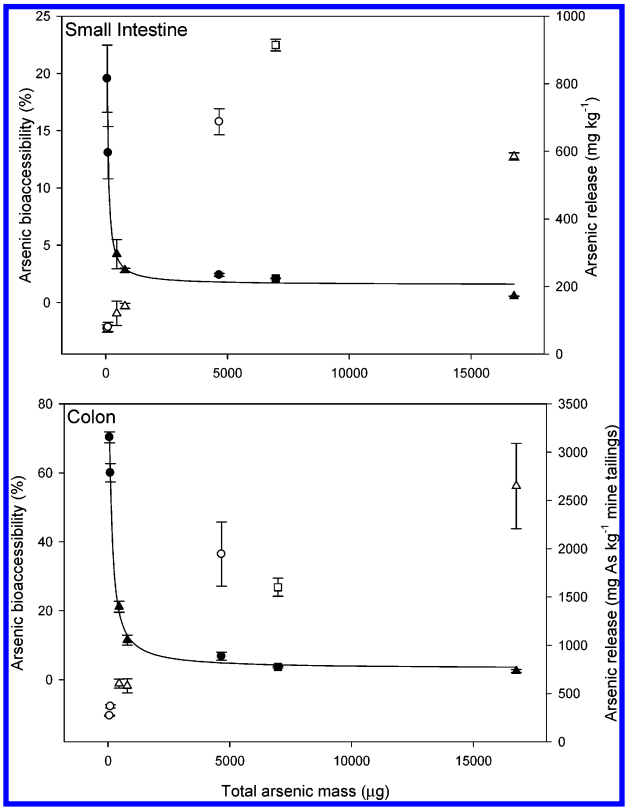


FIGURE 1. Relationships between arsenic bioaccessibility (%) (left *Y*-axis, closed symbols) and arsenic release (mg kg^{-1}) (right *Y*-axis, open symbols) and total arsenic mass (μg) in the small intestine and colon stages of the SHIME following exposure to the bulk (circles), <38 μm (triangles), and hardpan (squares) fractions of mine tailings from Nova Scotia, Canada. Notice the difference in both *Y*-axis scales between the small intestine and colon panels. The bioaccessible fractions of the SHIME suspension are operationally defined as the <10 kDa filtrate of the supernatant of a 12 000*g* (20 min) centrifugation. Each data point is the mean of three mine tailing replicates. Error bars denote standard error of the estimate.

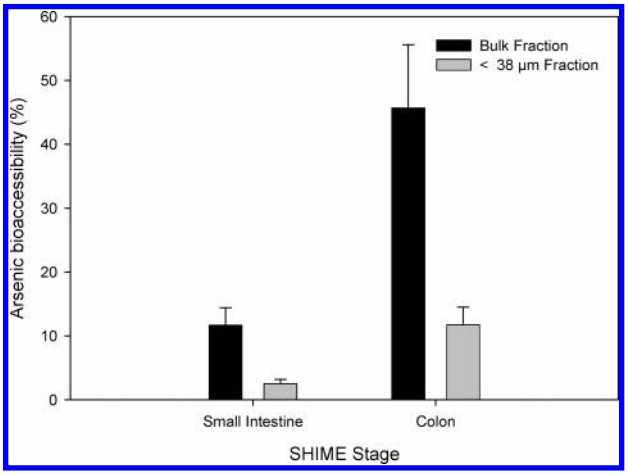


FIGURE 2. Arsenic bioaccessibility (%) of two size fractions (bulk and <38 μm) of mine tailings from Nova Scotia, Canada in the small intestine and colon stages of the SHIME. Treatments from each location, Goldenville (n = 3), Montague (n = 3), and Lower Seal Harbour (n = 3), were pooled to generate mean arsenic bioaccessibility estimates of the bulk and <38 μm size fractions in the small intestine and colon. The bioaccessible fractions of the SHIME suspension are operationally defined as the <10 kDa ultrafiltrate of the supernatant of a 12 000*g* (20 min) centrifugation. Error bars denote standard error of the estimate.

of arsenic bioaccessibility was observed in the colon stage; however, the colon arsenic bioaccessibility of the bulk fraction of tailings from Goldenville and Lower Seal Harbour were both greater (Fisher protected LSD, $P < 0.001$) than that of tailings from Montague. No difference (Fisher protected LSD, $P > 0.05$) was observed between the colon arsenic bioaccessibilities of the bulk fraction of tailings from Goldenville and Lower Seal Harbour. In contrast, all pairwise comparisons of colon arsenic bioaccessibility from the <38 μm from the three locations were significant.

The arsenic bioaccessibility of the active colon stage of the SHIME was greater than the small intestine (Figure 4) for all three locations (Fisher-Protected LSD, $P < 0.001$). Sterilization of the colon SHIME suspension prior to incubation decreased arsenic bioaccessibility to levels similar to the small intestine. A black precipitate was formed following the active colon incubation of mine tailings but not in the absence of mine tailings. In contrast, no precipitate was formed when the colon suspension was autoclaved prior to colon incubation with mine tailings.

Discussion

To the knowledge of the authors, this is the first study to demonstrate that intestinal microorganisms increase arsenic bioaccessibility in the human gastrointestinal tract. Yet, increased dissolution of arsenic by anaerobic microorganisms in water and soil is well documented (*37*). The dissolution of arsenic in sediments may be facilitated by the microbial reduction of arsenate to arsenite, which possesses a lower affinity for sorption to iron oxide minerals, or the microbial reduction of iron oxide minerals bearing arsenic (*37*). Both DIRPs and DARPs are capable of liberating arsenic adsorbed to or complexed within iron minerals, thereby increasing dissolved arsenic concentrations (*25*−*27*). Since these bacteria have been identified in the gastrointestinal tracts of mammals (*28*), it is not surprising that the colon microbiota are capable of increasing arsenic bioaccessibility. Increased arsenic mobilization in anaerobic sediments may be coupled to the formation of sulfide precipitates (*27*). Thus, the formation of the black precipitate in the microbially active colon stage of the SHIME adds a further line of evidence that DIRPs and/or DARPs are responsible for the increased arsenic bioaccessibility observed in the colon stage of the SHIME. The common presence of sulfate-reducing bacteria in the colon stage of the SHIME further corroborates this observation.

As Beak et al. (*14*) observed in the gastric phase of an *in vitro* gastrointestinal model, increasing arsenic concentration decreased the percent arsenic bioaccessibility in the small intestine of the SHIME. This same relationship was also observed in the colon stage of the SHIME despite increased solubilization of arsenic due to the activity of colon microbiota. An investigation of the mechanism of the relationship between arsenic bioaccessibility and concentration will be necessary before conclusions may be made regarding why this inverse relationship was observed with the mine tailings in the SHIME but not in studies of other arsenic-contaminated soils (*8*, *9*, *11*). The inverse relationship between arsenic concentration and bioaccessibility in the SHIME may have arisen due to the saturation of the GI fluid or kinetic limitations of arsenic release. Our study used mine tailings from different sites of varying particle sizes and mineralogy (*18*), and thus we are unable to construct an isotherm to assess the K_d of arsenic in GI fluid.

The bioaccessibility of ingested contaminants is typically greater from small particle sizes of soils (*38*). However, the arsenic bioaccessibility from mine tailings in the SHIME was greater in the bulk than in the <38 μm fraction. This discrepancy may be due to differences in the mineralogy of

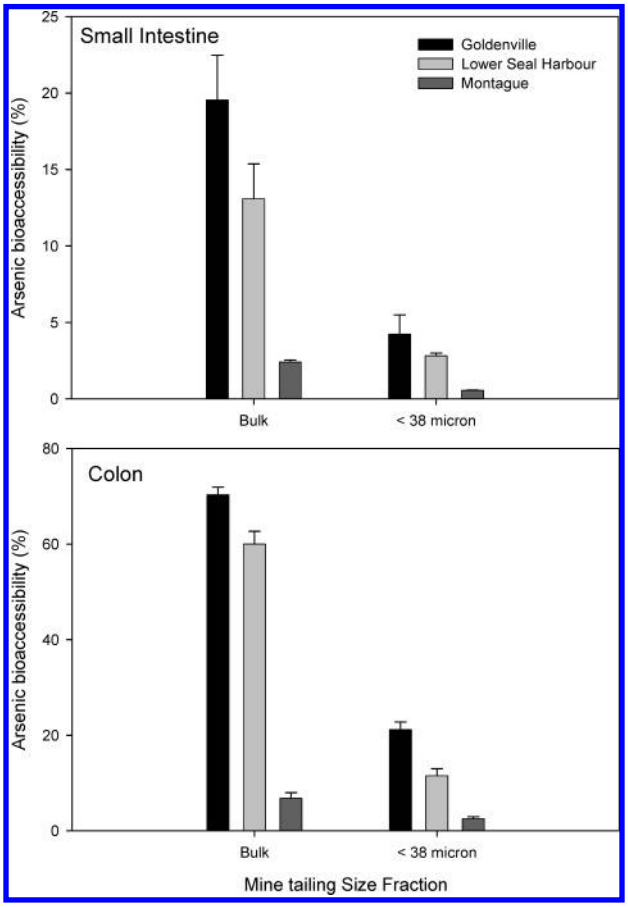


FIGURE 3. Mean arsenic bioaccessibility (%) in the small intestine and colon stages of two size fractions (bulk, < 38 μm) of mine tailings from three locations (Goldenville, Montague, and Lower Seal Harbour) in Nova Scotia, Canada. Arsenic bioaccessibility of three replicate small intestine and colon SHIME treatments were measured for both size fractions from each location. Bioaccessible fractions were separated using ultrafiltration (<10 kDa). Error bars denote standard error of the estimate.

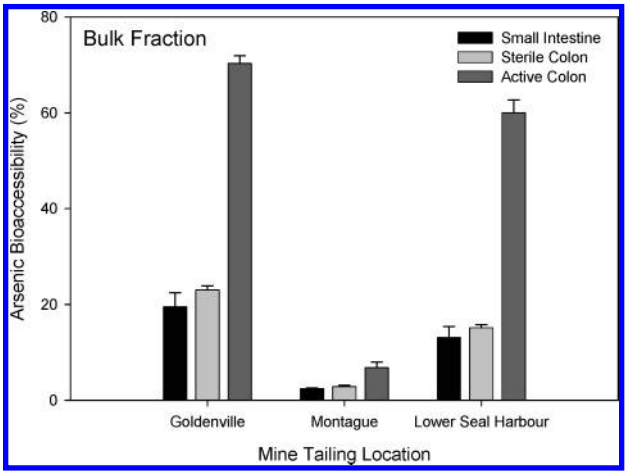


FIGURE 4. Mean arsenic bioaccessibility (%) in three SHIME stages (small intestine, sterile colon, and microbially active colon) treated with the bulk fraction of mine tailings from three locations (Goldenville, Montague, and Lower Seal Harbour). Arsenic bioaccessibility was measured in three replicates of each SHIME stage treated with mine tailings from each location. Sterile colon SHIME suspension was prepared by autoclaving aliquots of active colon suspension prior to colon incubation. The bioaccessible fractions of the SHIME suspension are operationally defined as the <10 kDa ultrafiltrate of the supernatant of a 12 000*g* (20 min) centrifugation. Error bars denote standard error of the estimate.

the particle sizes; however, it may also be due to the high arsenic concentrations present in the <38 μm fraction. Thus, the lower percent bioaccessibility in small size fractions of the mine tailings may arise from the greater arsenic concentrations, thereby confounding the size/ bioaccessibility relationship often observed. Since contaminant distribution among size fractions varies between soils and size fraction affects probability of exposure, this result emphasizes the need to assess both the concentration and bioaccessible fraction distribution among various size fractions for use in human health risk assessment (*7*, *32*).

Inclusion of the colon stage for *in vitro* arsenic bioaccessibility analyses significantly increases the mobilization of arsenic from mine tailings. However, the toxicological importance of this result remains to be demonstrated since the principal site of arsenic absorption is likely in the small intestine (*39*). Although the rate of uptake of arsenic in the colon will be markedly reduced relative to the small intestine, the longer residence time in conjunction with increased arsenic bioaccessibility in the colon may result in appreciable arsenic absorption. Additionally, genotoxic arsenic metabolites have been identified in the colon of mammals (*40*, *41*), and higher mortality rates due to colon cancer have been observed in populations that ingest high concentrations of arsenic (*6*). Previous studies have attempted to validate arsenic bioaccessibility estimates generated with *in vitro* gastrointestinal models with the oral bioavailability of ingested arsenic from animal-dosing experiments (*8*, *13*, *31*). The implicit assumption of this comparison for use in human health risk assessment is that the gastrointestinal microbiota of the animal model is functionally similar to that of humans. Studies investigating the role of DIRPs and DARPs in the toxicokinetics of arsenic ingestion will be necessary before conclusions regarding the validity of bioaccessibility estimates generated with or without the colon stage will be possible.

The use of total arsenic concentration from ingested soils and mine tailings results in the overestimation of human health risk due to the limited dissolution of contaminants from ingested soil particles relative to that of the soluble salts administered in toxicity studies (*10*, *21*). *In vitro* gastrointestinal models such as the SHIME have gained acceptance as a valuable tool for estimating the relative bioavailability of arsenic-contaminated soils (*10*, *15*, *21*). The experiments detailed in this paper demonstrate that assumptions of constant bioaccessibility from varying arsenic concentrations and size fractions could lead to inaccurate exposure estimates for use in risk assessments, thereby agreeing with recent recommendations for the incorporation of bioaccessibility estimates into human health risk assessment (*32*). Furthermore, neglecting to include the colon in previous assessments of arsenic bioaccessibility may have resulted in an underestimation of the risk due to ingestion of arsenic-contaminated soils.

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