

Bioaccessibility of Metals in Soils for Different Liquid to Solid Ratios in Synthetic Gastric Fluid

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The bioaccessible fraction of metals in the stomach has been estimated for two soil materials using laboratory synthesized gastric juice. Heavy metals present in each soil matrix were extracted and measured, as a technique to simulate metal solubilization processes that occur in the human stomach. The results from the synthetic gastric juice extraction were compared to values obtained using a modified EPA concentrated acid extraction procedure, method 3051, on the same soils. The experiments evaluated the effect of varying the liquid to solid ratio on gastric juice extraction efficiency for arsenic (As), chromium (Cr), nickel (Ni), cadmium (Cd), and lead (Pb). Soil samples employed were a National Institute of Standards and Technology (NIST) standard soil (Montana SRM 2710) and a composite hazardous waste contaminated soil from Jersey City, NJ. The current work demonstrates that bioaccessibility is not the same for each metal within a given soil, nor between the soils. Estimates of the bioaccessible fraction of the metals studied ranged from 2 to 61% of the NIST certified values for Montana soil, and from 3% to 58% of EPA method 3051 values in contaminated Jersey City soil. The experiments indicated that the bioaccessibility of metals in the soils extracted by the *in vitro* synthetic gastric juice will only be affected slightly by changes in gastric fluid liquid to solid ratios for the range 100:1 to 5000:1 (mL/g). Further, the masses of each soil (0.05 or 0.5 g) used did not affect the bioaccessibility at the 1000:1 ratio.

Introduction

Contaminated soils pose a particular hazard to children because of activities that involve frequent hand-to-mouth behavior and the subsequent ingestion of soil containing metals and other contaminants (1). Children also have the ability to absorb higher percentages of metals through the digestive system into the blood stream than adults, which may leave them susceptible to adverse health effects (2–6). The amount of metal absorbed is dependent on the individual, the form of the metal within the soil, and soil's physical characteristics (7). Estimating the amount of metal that is

available for absorption after ingestion is therefore a key variable in estimating potential human exposure.

The total extractable metal concentration in the soil fraction is routinely measured by leaching the soil with concentrated nitric acid (HNO₃) in a laboratory microwave extraction procedure (EPA method 3051) or with a mixture of nitric and hydrochloric (HCl) acids on a hot plate (EPA method 3050) (15). A toxicity leaching protocol (TLP) (EPA method 3015) for assessing potential risk to soils at hazardous waste sites employs a concentrated HCl solution and a low liquid to solid ratio (16). Unfortunately, as recently discussed by Ruby (17), these methods do not provide an adequate model for estimating the amount of metal that can be absorbed after the soil is ingested by a child. Although the human digestive system does contain hydrochloric acid, the solution is dilute (0.042–0.067 M) compared to the EPA extraction methods (e.g., 14.3 M for HNO₃, in method 3051). A system, therefore, that provides a better simulation of gastric juice release of metals should reduce the uncertainty in estimates of the bioaccessible fraction. Further, since the amount of soil introduced to the stomach is small with respect to the volume of gastric juice encountered, liquid to solid ratios presented will be significantly different than those used in routine extraction protocols.

Recent leaching studies have shown that the soil/contaminant matrix will affect the availability of a contaminant (8–10). It has also been demonstrated for both dioxin and chromium (2, 11–13) that actual bioavailability of contaminants can vary with soil matrix. Each study has illustrated that major uncertainty exists with most estimates of human exposure and/or dose that is associated with the bioavailability of a contaminant in soil matrix. Current methods for evaluating potential risk use total leachable metals content and extract the soil using rigorous acid digestions or low liquid to solid ratios in an effort to obtain a worst case scenario. However, these are not reasonable approaches for estimating the bioaccessible fraction in the human digestive system because the results, yielding high metal concentrations, were obtained under unlikely physiological conditions (14).

The bioaccessibility of a metal is defined for the present work as the maximal amount of metal that is soluble in a synthetic gastric fluid and therefore potentially available for uptake across the intestinal lumen, while bioavailability is the amount that actually is taken across the cell membranes. Gastric juice was chosen for the current experiments because it is the most acidic of the digestive juices and, to a first approximation, is likely to yield the greatest fractions of soluble metal compounds or complexes. A synthetic model of this component of the digestive system was developed to provide a better estimate of the amount of contaminant available for absorption from the stomach into the body. A more complete sequential extraction system is under development to mimic the entire digestive process.

In this paper, the heavy metal extraction capabilities of artificial gastric juice at liquid to soils ratios approximating a range of physiological conditions are examined for two soils which have detectable and/or significant concentrations of heavy metals. The estimated bioaccessible fraction is calculated for two different soils at five specific liquid (fluid) to solid ratios and for two different masses of extracted soil. Previous studies have used synthetic digestive juices to estimate the bioavailable fraction of metal contaminants, however, they have not investigated the effects of liquid to soil ratios on the extraction of metals from soils by synthetic gastric juice (11).

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Derivation of the Model. Most previous research estimated a bioavailable fraction using animal experiments (7, 13, 19). Animals were fed known quantities of a contaminated soil and assayed for a marker or sentinel contaminant, as well as the mass of the contaminant passing through the animal. The contaminant was not found to be 100% bioavailable in any study, and the age and physical conditions of the soil had significant impact the bioavailable fraction obtained in each experiment (4–6, 8, 11, 12, 14, 15).

Animal studies are expensive, and animal handling and care are time consuming. Protocols also require digestive characteristics close to humans, and animal models have varied digestive components and retention times. Although the acquired information is valuable, a good synthetic digestive fluid model may better mimic human gastrointestinal function. Work has begun to move from *in vivo* studies methods to *in vitro* methods (11); however, a reliable reproducible extraction method for many soils still needs development. Further, this protocol will be easily used by many laboratories.

Synthetic biofluids have been utilized in various physiological models, for prediction of drug uptake, nutritional values of food and food supplements (2), and metal solubility of orthodontic appliances (20, 21). The present model employs human gastric juice at physiologically based pH and temperature to extract soils at approximately the amount of time soil remains in the human stomach.

Experimental Section

Soil Samples. The National Institute of Standards and Technology (NIST) Montana Soil SRM 2710 has the consistency of powder and is certified for high lead and arsenic concentrations. It was dried and size fractionated by NIST to $<74\ \mu\text{m}$ diameter prior to shipment. The Montana soil was selected because a standardized soil establishes a reference point for intercomparing extraction methods used by other investigators.

The locally collected material, termed Jersey City Composite, is granular and contaminated with chromium (20). The Jersey City soil contains a high concentration of residual metals from chromate ore processing. It is an aged soil/waste matrix typically encountered during cleanup or restoration of hazardous waste sites. The soil/waste material was sieved and dried. The soil was sieved (U.S. Standard Sieve Series, Dual Manufacturing Co. Chicago, IL), and a size fraction of $150 < d < 250\ \mu\text{m}$ in diameter was used in these experiments. It was well characterized by Kitsa et al. (22). Her results demonstrated large differences in chromium extraction among the various vigorous acid extraction techniques. Hydrofluoric acid yielded greater extraction of chromium than did sulfuric acid or nitric acids. These results suggested the need to further examine the bioaccessibility of metals in soils.

For the liquid to solid ratio experiments, 0.05 and 0.5 g of each soil were used to create ratios (mL/g) of 100, 200, 500, 1000, 2000, and 5000:1. The 100, 200, 500, and 1000:1 ratios were prepared using 0.5 g of sample material and between 50 and 500 mL of fluid, with the 1000, 2000, and 5000:1 ratios made using 0.05 g of the soils. In order to evaluate consistency of the extraction method for different sample masses, the 1000:1 ratio overlapped the two soil sample masses. All experiments were performed using class A volumetric glassware.

Extraction. Using U.S. Pharmacopeia methodology, 2.0 g of NaCl [suprapur, EM Science (7647-14-5)] were dissolved in a solution of 7 mL of ultrapure sub-boiling, distilled hydrochloric acid (High Purity Standards, Charleston, SC), and approximately 250 mL of deionized water in a 1 L volumetric flask. A total of 3.2 g of pepsin [from porcine

stomach mucosa, Sigma (9001-75-61)] was added, and the solution was brought to 1000 mL for immediate use.

Soil samples were placed in new Nalgene bottles, and artificial gastric fluid was added to the system. A blank of 100 mL artificial gastric fluid was run, as a control. The bottles were capped and placed in a water bath (Magni Whirl constant temperature bath) at 37 °C and allowed to shake for 2 h. Two hours were chosen as a representation of the average time that HCl comes into contact with solids in the stomach at body temperature (37 °C) following ingestion (3, 23). Ten milliliters of each sample was decanted into 15 mL conical polystyrene centrifuge tubes (Falcon, Becton/Dickinson, Franklin Lakes, NJ) and spun in a Metpath Centrifuge at 3400 rpm (maximum force 1380g) for 10 min. The supernatant was then decanted and analyzed for metals.

Total Extractable Metal Digestion Procedures. The Jersey City composite soil and the Montana NIST soil were digested in nitric acid, and analysis was performed using modified EPA method 3051. Ten milliliters of Ultrex ultrapure concentrated nitric acid was added to 0.1 g of soil, placed into a Teflon digestion vessel, capped, and placed in a laboratory microwave (CEM Corporation, Matthews, NC). The samples were digested for 20 min at 100% power. After the digestion was complete, the samples were allowed to cool, filtered through Whatman 51 filters (hardened and ashless), and placed in 100 mL volumetric flasks. The Teflon liners were rinsed with deionized water, and the rinse was used to wash the filtrate through the filter paper. The volume was then brought to 100 mL with additional water. The samples were analyzed using inductively coupled plasma/mass spectrometry (described below). Twelve samples of each soil were digested, and an average of the concentrations obtained for each metal and used to establish the "total extractable metal concentration".

Inductively Coupled Plasma/Mass Spectrometry (ICPMS). Samples extracted by EPA method 3051 and the synthetic gastric juice were analyzed using a VG Fisons PQ2+ inductively coupled plasma/mass spectrometer (VG Elemental, Danvers, MA) for parts per billion (ppb) sample concentrations of arsenic, lead, chromium, nickel, and cadmium using a modified EPA method 200.8. Standards for instrumental analysis were prepared using solutions from High Purity Standards. Quality assurance checks were run at intervals using National Institutes of Standards and Technology 1643: Trace Elements in Water. For each metal, the detection limit associated with each soil sample solution was 1 ppb.

Results

Bioaccessibility of Each Soil. The bioaccessible fraction was determined by dividing the mass of metal recovered from the gastric juice extraction by the mass of the metal obtained by EPA method 3051 for the Jersey City soil or by the certified value of the metal in the Montana NIST soil.

Accessibility obtained for the Montana soil is reported for all experiments and each metal in Table 1A. The solubility of individual metals in the synthetic gastric juice varied from 2 to 61%. Two of the metals, chromium and nickel, had low bioaccessibility. In contrast, arsenic and cadmium were estimated to be approximately 50% bioaccessible. The results indicate that each metal has different physical and/or chemical binding capacity to the soil.

The synthetic gastric juice extraction of the Jersey City soil results, presented in Table 2, had a range of 3–58% for individual metals. The highest bioaccessible fraction was 58% for Pb, and low bioavailability, 2.8%, was measured for Cr. Cd was not quantifiable in the Jersey City sample.

Liquid (Gastric Fluid) to Solid Ratios. The liquid to solid ratios used in these experiments, 100:1 through 5000:1, were chosen to approximate the mass of soil that might inadvert-

TABLE 1. Montana Soil, Percent Bioaccessibility of Each Metal

	100:1	200:1	(A) Liquid to Solid Ratio			
			500:1	1000:1	2000:1	5000:1
Pb	36 ± 14	46 ± 29	29 ± 5	34 ± 7.5	35 ± 9	42 ± 7
Ni	12 ± 5	13 ± 5	11.5 ± 4	11 ± 3.5	11 ± 8	14 ± 18
As	41 ± 18	56 ± 21	44 ± 6	48 ± 3	46 ± 5	48 ± 4
Cd	51.4 ± 18.5	61 ± 19.6	50.9 ± 14.6	49.5 ± 11	45.7 ± 8	45 ± 13
Cr	2.3 ± 1.8	2.8 ± 2.3	1.4 ± 1.1	1.8 ± 1.2	0.2 ± 0.5	4.2 ± 8.3

(B) NIST Certified Conc of Each Metal	
	certified conc (μg/g)
Pb	5532
Ni	14.3
As	626
Cd	21.8
Cr ^a	39

^a Not certified.

TABLE 2. Jersey City Composite Soil, Percent Bioaccessibility of Each Metal

	100:1	200:1	(A) Liquid to Solid Ratio (mL/g)			
			500:1	1000:1	2000:1	5000:1
Pb	22 ± 5%	30 ± 8%	35 ± 12%	46 ± 16%	58 ± 34%	38 ± 16%
Ni	23 ± 9%	29 ± 11%	31 ± 11%	34 ± 14%	40 ± 15%	34 ± 15%
As	4.5 ± 0.8%	6.0 ± 0.9%	7.7 ± 0.9%	13 ± 4%	18 ± 8%	25 ± 9%
Cr	2.8 ± 1.0%	3.7 ± 1.4%	4.4 ± 1.2%	5.5 ± 2.0%	7.8 ± 2.8%	7.1 ± 4.2%

(B) Mean Concentration (n = 12) of Each Element Released by EPA Method 3051 (μg/g)	
Pb	972
Ni	496
As	1120
Cr	7020

ently enter the mouth during normal outdoor activities (4) and the resulting mixture that would be presented to the stomach of a child. Roughly 2.5 L of gastric juice is released to the stomach (23), which can be divided into 24 h to approximately yield of 105 mL/h. Since the amount of fluid in the stomach will vary depending on the time of day, level of activity, and amount of food ingested, the range was chosen to encompass a variety of conditions that could be plausible in the human stomach (e.g., 100 mL/0.1 g = 1000:1 mL/g).

The variability in metal extractability of the Montana NIST soil across the range of liquid to solid ratios is shown in Table 1A. Most of the metals in the Montana NIST soil had a consistent percentage of each metal extracted across all the liquid to solid ratios used in the experiments, with the 1000:1 ratio providing representative extractability for most metals, though Cr and Pb had some differences in highly dilute solutions. The solubility of each metal for the liquid to solid ratios employed was independent of masses used, 0.5 and 0.05 g, suggesting that the synthetic gastric juice is releasing the metal uniformly from the soil over this range.

The extractability of the Jersey City soil for the five liquid to solid ratios was different than observed for the Montana NIST soil. As seen in Table 2, the range in percent extractable metal changed with the different ratios. For example, lead values ranged from 22 to 58% and chromium ranged from 2.8 to 7.8% with the higher extractions appearing with the more dilute solution. There was an increase in the bioaccessible arsenic concentration as the liquid to solid ratio increased. The results suggest that complete extraction of the metals had not been achieved at the lower ratios for the Jersey City composite; if solution saturation has occurred, a decrease of bioaccessible arsenic would be expected as higher liquid to solid ratios are employed in extraction of the soil

sample. The values are still within +12% or -9% of the value recorded at the 1000:1 ratio.

Discussion

Statistical analyses were performed on the mean values of three replicate experiments on the Jersey City and Montana NIST SRM 2710 soils using lead, arsenic, and chromium values obtained at the different liquid to solid ratios. There was a statistically significant difference in the bioaccessible fraction of Pb, As, and Cr measured for each ratio within a soil, as well as for the individual metals between soils, suggesting that soil matrix, element, and/or compound play a role in bioaccessibility. These results are consistent with previous observations made at single liquid to solid ratios (12, 17, 24, 25). The current work, however, demonstrates that bioaccessibility is not the same for each metal within a given soil, nor between the soils.

A fasting ratio of 1000:1 (mL/g) was used as a reference value for the ratios examined, as future experiments are a developed using this ratio. Compared to the values obtained at the 1000:1 ratio, the solubility of the metals in the synthetic gastric juice was not altered substantially within the range 100:1 to 5000:1 mL/g for either soil (significance level 0.05). The trend, however, of increased solubility of arsenic for higher liquid to soil ratios in the Jersey City composite and the higher variability in extractability of specific metals across the range of liquid to solid ratios suggests that investigators may need to know information about the composition of the metallic compounds in a soil to establish the chemical mechanisms of release. A linear regression, Figure 1, of the arsenic versus liquid to soil data yielded an r^2 of 0.58, supporting the hypothesis that extractability increases with dilution for arsenic.

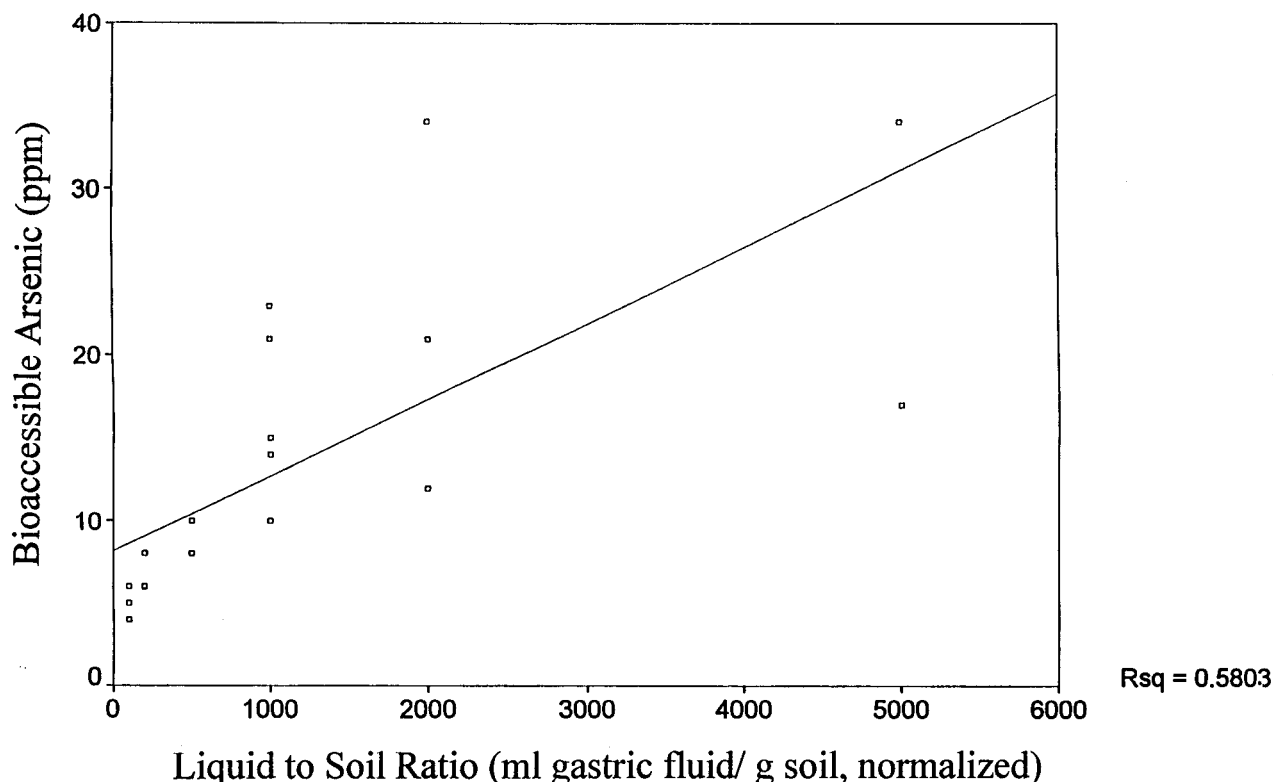


FIGURE 1.

TABLE 3. Comparison of the Mean Bioaccessible Fraction of Metals Extracted Using Synthetic Gastric Juice for both Montana and Jersey City Soils at a 1000:1 Liquid to Soil Ratio for a 0.05 and 0.5 g Sample

	Pb	Cr	Cd	As	Ni
Montana Soil: SRM-2710					
0.5 g mass	0.31 ± (na) ^a	0.01 ± 0.014	0.51 ± 0.11	0.46 ± 0.03	0.10 ± 0.03
0.05 g mass	0.35 ± 0.06	0.018 ± 0.012	0.50 ± 0.18	0.48 ± 0.03	0.11 ± 0.02
Jersey City Composite Soil					
0.5 g mass	0.39 ± 0.23	0.041 ± 0.017	0.29 ± 0.16	0.098 ± 0.024	0.29 ± 0.16
0.05 g mass	0.51 ± 0.10	0.066 ± 0.016	0.37 ± 0.13	0.164 ± 0.034	0.37 ± 0.13

^a Only two values.

The experiments also tested the scale-up capability of the technique for different quantities of extracted soil. Metal solubility can peak from saturation of the solution because of limited fluid volume, or conversely, the larger mass could provide greater soil surface area for release of a metal. Two sample masses, 0.5 and 0.05 g, for each type of soil were extracted for As, Cd, Cr, and Pb at the testing ratio of 1000:1. At this ratio, the bioaccessibility of As, Cd, Cr, or Pb for each mass of soil and each soil sample, Table 3, did not change significantly using the synthetic extraction solution. For metals shown, the results were not statistically significantly different using the *t*-test. The use of a soil mass, therefore, in the range 0.05–0.5 g can be incorporated into the sequential protocol at 1000:1 ratio. For at least the 1000:1 ratio, the amount of mass that can be used in the synthetic gastric juice system could vary by at least a factor of 10 without affecting the overall accessibility.

These experiments provide insight on the influence that one major ingestion/digestive system variable, liquid to solid ratio in the stomach, has on the bioaccessibility of a metal in a synthetic gastric juice system. At ratios from 100:1 to 5000:1, (normalized mL/g), for the standard Montana NIST soil and the Jersey City soil, there were differences in the percent bioaccessibility of each metal across the range of ratios selected. However, the percent bioaccessibilities,

across the range, were still much smaller than the proportion of metals that would be assumed to be bioavailable, based upon the EPA methods, or other aggressive techniques employed by NIST.

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