Mineralogy affects geoavailability, bioaccessibility and bioavailability of zinc

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ABSTRACT

We correlated mineralogical and particle characteristics of Zn-containing particles with Zn geo-availability, bioaccessibility, and bioavailability following gavage and intranasal (IN) administration in rats. We compared samples of Zn/Pb mine waste and five pulverized pure-phase Zn minerals (<38 μm). Particles were neutron-activated to produce radioactive ^{65}Zn. We assessed geoavailability using sequential extractions and bioaccessibility using in vitro extraction tests simulating various pH and biological conditions. Zn in vivo bioavailability and in vitro bioaccessibility decreased as follows: mine waste > hydrozincite > hemimorphite > zincite ≈ smithsonite >> sphalerite. We found significant correlations among geoavailability, bioaccessibility and bioavailability. In particular, Zn bioavailability post-gavage and post-IN was significantly correlated with bioaccessibility in simulated phagolysosomal fluid and gastric fluid. These data indicate that solid phase speciation influences biological uptake of Zn and that in vitro tests can be used to predict Zn bioavailability in exposure assessment and effective remediation design.

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1. Introduction

Remediation of contaminated sites requires accurate assessments of risk, yet total contaminant concentrations alone are often poor predictors of uptake and toxicity. Human health risks from contaminants are determined by inherent chemical toxicity, extent of exposure and contaminant bioavailability (fraction of a contaminant in exposure media that can be absorbed by organisms). Thus, evaluating bioavailability can improve dose estimation from exposure assessment (Kobayashi and Okamura, 2005; Milton and Johnson, 1999; Ruby et al., 1996). Metals are common contaminants at mining and other contaminated locations. Children are especially vulnerable to metal-contaminated soils and indoor dust. Hand-to-mouth and inhalation exposures in playgrounds and home environments are common.

In addition to bioavailability, metal mobility in the environment and biological uptake are influenced by two other related parameters: geoavailability and bioaccessibility. Geoavailability describes the release of metals from solid phases under environmentally relevant conditions. It is the fraction of total metals that can be released to the biosphere through mechanical, chemical and biological processes (Smith, 2007). Bioaccessibility describes the ability of a metal to be solubilized in a biological fluid after ingestion, such as gastric fluid (following oral ingestion) or phagolysosomal fluid (following macrophage uptake after inhalation).

Multiple mechanisms control absorption of particle-bound metals. Oral bioavailability is influenced by physiological factors such as fasted/fed state, associated motility, gastric emptying, intestinal transit time, variability in gastrointestinal (GI) contents and nutritional status (Amidon et al., 1995). For instance, iron (Fe) status regulates expression of divalent metal transporter 1 (DMT1), which mediates absorption of Fe and many other divalent metals such as manganese (Mn), zinc (Zn), cadmium (Cd), and lead (Pb) (Au et al., 2008). In the respiratory tract, inhaled airborne particles may be deposited depending on their aerodynamic size. The majority (92%) of larger particles (>8 μm) deposit in nasal passages and upper airways, while inhaled smaller particles (≤2 μm) deposit in the deep lung (93–97%) where they are phagocytized by macrophages (Brain and Valberg, 1979; Heyder and Svarten, 2002).

Bioavailability also varies widely depending on properties of the metal, metal speciation (distribution among various chemical forms), particle size, morphology, and solubility. Metal sulfides and some other primary metal ores tend to have low bioavailability (Ruby, 2004). However, metal ores undergo temporal shifts in speciation that can affect bioavailability. For example, weathering of sulfide minerals in the presence of oxygen can result in speciation changes and the formation of secondary mineral phases that often have greater bioavailability than the parent ore. Metals can also decrease in bioavailability as they become more strongly incorporated into the soil matrix. Temporal variability in metal bioavailability highlights the value of repeated sampling over time.

In vivo determination of metal bioavailability in animal models is time-consuming and expensive. Moreover, the results must be extrapolated to humans. Thus, simpler in vitro bioaccessibility tests to estimate bioavailability would be valuable. Several studies have estimated oral bioavailability of metals in contaminated soils and mine wastes using in vitro tests with a single extraction or series of extractions designed to mimic the GI system (Bradham et al., 2011; Casteel et al., 2006; Juhasz et al., 2009a, 2009b; Navarro et al., 2006; Ruby et al., 1996; Schroder et al., 2004). These studies concluded that the characteristics of each metal (Navarro et al., 2006), pH of the extraction solution (Juhasz et al., 2009a, 2009b; Ruby et al., 1996), mineralogical composition of samples (Bradham et al., 2011; Navarro et al., 2006), matrix properties and presence of other organic or inorganic components (Casteel et al., 2006) significantly influenced bioaccessibility.

Validation of in vitro tests as predictors of bioavailability requires evaluating both bioavailability and bioaccessibility on the same materials. Correlations between bioaccessibility and bioavailability have been established for Cd (Juhasz et al., 2010; Schilderman et al., 1997), Pb (Casteel et al., 2006; Kelley et al., 2002; OSWER, 2004; Ruby et al., 1996) and As (Bradham et al., 2011; Juhasz et al., 2009b). Studies of Pb- and As-contaminated soils showed that in vitro extraction results successfully predicted Pb bioavailability in rats, but over-predicted As bioavailability in rabbits and primates (Ruby et al., 1996). Bioaccessibility of Cd in contaminated soils extracted in simulated gastric or intestinal fluids was significantly correlated with relative bioavailable Cd in swine (Schroder et al., 2004).

Less is known about relationships between bioaccessibility and bioavailability for micronutrient metals such as Zn, Cu and Mn. While the consequences of Zn deficiency are well known (Otten et al., 2006), Zn is also among the top 100 hazardous substances on the U.S. Agency for Toxic Substances and Disease Registry's Substance Priority List (ATSDR, 2012). Toxicity from excessive intake of Zn is increasingly recognized, including inhalation of zinc oxide fumes in workplaces or polluted environments (El Safty et al.,

2008). Consequences include Cu deficiency, immune dysfunction, anemia and other hematological abnormalities (Fosmire, 1990). To our knowledge, there have been no systematic comparisons of Zn bioaccessibility and bioavailability across a range of chemical forms and routes of exposure.

The goals of this study were to determine whether Zn bioavailability and bioaccessibility are influenced by mineralogy and particle characteristics and the extent to which these in vitro bioaccessibility tests and geochemically-based sequential extractions may predict in vivo bioavailability. We determined Zn geo-availability, in vitro bioaccessibility, and in vivo bioavailability following gavage and intranasal administration of five Zn minerals and a Zn-rich mine waste sample. These exposure pathways were selected since larger airborne particles from piles of mine wastes are more likely deposited in the nose than in the lungs and are likely to be ingested via hand-to-mouth transfer, especially by children playing in contaminated sites. We compared common pure-phase Zn minerals with Zn-rich mine waste containing a complex mixtures of different phases. Studying pure phase Zn minerals allows us to directly evaluate the effect of mineralogy on bioavailability and bioaccessibility, and other factors that may affect metal mobility such as organic matter, iron oxides, and cation exchange capacity.

2. Materials and methods

2.1. Particle characterization

We obtained samples of smithsonite [$ZnCO_3$], hydrozincite [$Zn_5(CO_3)_2(OH)_6$], hemimorphite [$Zn_4Si_2O_7(OH)_2 \cdot H_2O$], sphalerite [$(Zn,Fe)S$] and zincite [$(Zn,Mn)O$] (Table 1) from the Harvard University Mineralogical Museum (Cambridge, MA). Samples were ground with a mortar and pestle and then sieved through a 38 μm pore-sized brass sieve with stainless steel wire. In addition, a sample of weathered mine waste collected from a pile at the Tar Creek Superfund Site (Oklahoma, U.S.A.) was air-dried at room temperature and sieved to <38 μm without grinding. This site was a former Pb and Zn mine where the major ores produced were sphalerite and galena (PbS) (McKnight and Fischer, 1970). The high Zn concentrations (up to 22% Zn by mass in <1 μm particles) in mine waste (Schaider et al., 2007) raise concerns about elevated Zn exposures for residents. Previous analyses of the <38 μm mine waste samples using X-ray diffraction showed that 20—35% of Zn was present as hemimorphite and sphalerite and the majority in poorly crystalline secondary mineral forms (Schaider et al., 2007). These particles are small enough to be transported by wind (Duggan et al., 1985) and deposited in the nose and oral pharynx (Brain and Valberg, 1979).

All samples were divided into three aliquots. One was analyzed for specific surface area (SSA) by the Brunauer—Emmett—Teller (BET) method of N_2 adsorption at the National Institute for Occupational Safety and Health (Morgantown, WV) (Brunauer et al., 1938). The second set of aliquots was analyzed for size, morphology and elemental composition. Aqueous suspensions of particles were analyzed for size distribution in a Beckman—Coulter LS 13 320 Laser Diffraction Particle Size Analyzer (LDPSA) (Beckman Coulter, Inc., Brea, CA). Similar suspensions of particles were spread onto carbon adhesive tabs on aluminum scanning electron microscopy (SEM) specimen mount stubs (Electron Microscopy Sciences, PA). After drying under

Table 1
Characterization of pure-phase Zn minerals and Zn-rich mine waste samples.

Particle	Source	Zn (%)[a]	Specific activity ^{65}Zn μCi/mg[b]	BET SSA (m²/g)[c]	LDPSA analyses				
					Mean Dia. (μm)	Mode Dia. (μm)	90th % Dia. (μm)	<2.5 μm (%)	<10 μm (%)
Mine waste	Picher Mining Field, Oklahoma, U.S.A.	10.4	3.58	7.1 ± 0.2	9.91	13.6	24.2	25	63
Hydrozincite	Numa Mine, Cantabria, Spain	59.5	1.58	93 ± 0.3	18	28.7	34.3	13	33
Hemimorphite	Unknown source, 1916	54.3	1.24	3.9 ± 0.2	19.8	26.1	48.6	20	44
Zincite	Franklin Mine, New Jersey, U.S.A	73.2	2.89	0.8 ± 0.1	18.7	28.7	37.6	15	34
Smithsonite	Carroll County, Arkansas, U.S.A.	52.1	1.34	2.7 ± 0.2	13.9	19.8	33.2	24	55
Sphalerite	Las Manforas Mine, Cantabria, Spain	64.1	1.48	1.5 ± 0.0	12.5	21.7	29.4	27	51

[a] Zn concentrations in Zn mineral particles were calculated based on chemical formula.
[b] Specific activity of ^{65}Zn varied depending on length of irradiation and Zn concentration. Neutron activation analysis of mine waste particles (data not shown) revealed the presence of multiple metals including Zn (10.36%), Fe (1.93%), Mg (1.39%), Cd (0.027%) and Mn (0.024%). Despite lower concentration (10.4%) of Zn in mine waste particles, specific activity was higher due to longer (20-fold) irradiation than Zn mineral particles.
[c] BET SSA — Specific surface area from BET analysis.