Methodologies for Estimating Bioaccessibility of Six Metals in Household Dust: Zn, Pb, Cd, Cu, Ni, and Cr

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Abstract

The purpose of this study is to evaluate the relative advantages and disadvantages of two approaches for estimating oral bioaccessibility using a physiologically-based extraction technique (PBET): a simple gastric phase simulation and a two-phase gastrointestinal simulation. Bioaccessibility estimates of six metals prevalent in Canadian contaminated sites (zinc, lead, cadmium, copper, nickel, and chromium) were compared using the gastric phase simulation alone and the complete gastrointestinal simulation. Samples included vacuum dust samples from 33 homes, certified dust and soil reference materials, and a house dust control sample. Bioaccessibility measurements using the gastric phase simulation were greater than or equal to measurements obtained using the gastrointestinal simulation for the six studied metals. This research found that for the six studied metals, a simple simulation of the gastric phase provides the most conservative and cost-effective approach for estimating oral bioaccessibility of ingested metals.

Résumé

Le but de cette étude est d'évaluer les avantages et les désavantages de deux approches expérimentales crées pour estimer la bioaccessibilité en utilisant une extraction physiologique: soit une simulation simple de l'étape gastrique et une simulation gastro-intestinale en deux étapes. Les estimés de bioaccessibilité de six métaux communément retrouvés dans les sites contaminés du Canada (zinc, plomb, cadmium, cuivre, nickel et chrome) ont été comparés en utilisant la simulation de l'étape gastrique et la simulation gastro-intestinale complète. Les échantillons utilisés provenaient de poussières de 33 résidences, de poussière certifiée, de matériaux références de sol et d'un échantillon contrôle de poussière de maison. Les résultats indiquent que les mesures de bioaccessibilité de l'étape gastrique étaient toujours supérieures ou égales à celles obtenues pour l'étape gastro-intestinale, et ce pour les 6 métaux étudiés. Cette étude démontre donc que pour les 6 métaux étudiés, une simulation simple de l'étape gastrique fournit un estimé conservateur et peu couteux pour évaluer la bioaccessibilité de métaux ingérés.

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1. Introduction

The indoor environment is a growing concern in terms of the potential for exposure to harmful contaminants, particularly in Canada, where Canadians spend an average of 90% of their time inside (Leech *et al.,* 2002). With industrial activities such as mining and pollution automobiles and transportation in rural areas as well as construction activities from high populations in urban settings, soil and household dust have become an important source for trace metal exposure (Turner, 2011). For non-volatile contaminants such as trace metals, the driving exposure parameter for most health risk assessments at contaminated sites is the assumed rate of ingestion (Wilson *et al.,* 2013). Bioavailability, in the context of oral ingestion, has been defined as the capability for absorption of a chemical by an organism and uptake into the systemic circulation (Kelley *et al.,* 2002; Broadway *et al.,* 2012). Testing for bioavailability of trace metals in soil is *in vivo* and requires the use of animals such as juvenile swine or rats; human tests have been scarce. These *in vivo* experiments are generally costly and involve a time-consuming testing period as well as important ethical implications. *In vitro* (chemical) approaches, which were developed later, can be used as a tool to estimate exposure of trace metals for risk assessments. This is referred to as the bioaccessibility of given metals, and offer a faster, more ethical and reproducible approach to evaluating the potential of trace metal absorption in a simulated human gastrointestinal tract after oral ingestion. To date, a variety of bioaccessibility extractions have been validated against *in vivo* data as accurate predictors of a metal's bioavailability.

Currently, the EN ISO 17402 (2008) suggests that in routine soil quality assessments, chemical measurements may be substituted for biological tests if it has been shown that there is a correlation between the two and that the precision and the sensitivity are matched: the duration of the tests must be compared and if the correlation is statistically strong, then bioaccessibility measures may be used in lieu of bioavailability testing. Hamel *et al.* (1998) define bioaccessibility as "the maximal amount of metal that is soluble in a synthetic gastric fluid and therefore potentially available for transport across the intestinal lumen" (p. 53). Multiple *in vitro* extractions have been developed to mimic the gastrointestinal tract, with a focus on the physical, chemical, and microbiological processes in the human body. Bioaccessibility provides an upper boundary value—that is, the most conservative value for human protection—as to what could potentially become available to the human systemic circulation and thus available for uptake. The key compartments used in oral bioaccessibility models for trace metals are the mouth, the stomach, the intestine and the colon:

with pH considered one of the main the driving factor controlling bioaccessibility with the mouth pH at a neutral and short residence time it is often omitted from the *in vitro* digestions. Every method is unique and incorporates its own chemical composition in order to mimic the physiological conditions of each digestive compartment, liquid-to-solid ratios, mixing and separation methods, and residence time. When properly determined, bioaccessibility has the potential to make a significant impact on the current practices for risk assessment (Bradham *et al.,* 2014).

1.2 Background

Most risk assessment practices for contaminated soil and dust assume that 100% of the ingested portion is considered to reach target organs and to have a toxic effect (Koch *et al*., 2013). Over the years, through in vivo and in vitro testing, studies have shown that only a portion of the total metal concentration will reach or have the potential to reach the blood stream (Ruby *et al.*, 1996, Oomen *et al*., 2006, Wragg *et al*., 2011). To date, simple gastric simulations omit the mouth compartment and the passage through the intestine and have shown to yield a more conservative estimate of bioaccessible lead but have not been validated with in vivo data for the majority of other metals; there is also a general lack of bioaccessibility data (Turner, 2011). Although Pb and Cd bioaccessibility in soil are known to be elevated in the gastric phase as compared to the intestinal phase (Ruby *et al.,* 1996, Juhasz *et al.,* 2010, Oomen *et al.,* 2002), some authors argue that bioaccessibility for certain other elements (Cr and Ni) may be greater in the intestinal phase than in the gastric phase (Karadas *and Kara* 2011, Poggio *et al.,* 2009, Sialelli *et al.,* 2011). If so, then an intestinal phase assay would be a more conservative (and therefore more appropriate) choice of in vitro assay for these elements.

1.3 Statement of Hypothesis

It is hypothesized that a physiologically based extraction technique that simulates the gastric environment will yield higher bioaccessibility estimates than a simulation of the intestinal environment for six study metals (Zn,Pb, Cd, Cu, Ni, and Cr) in house dust; such results would lead to the conclusion that a gastric extraction yields the more conservative estimate, thus eliminating the need for an intestinal phase.

1.4 Purpose

The purpose of this study is to critically evaluate the oral bioaccessibility of trace metals from household dust in a human gastrointestinal system using a physiologically based extraction technique (PBET) that will mimic both the gastric alone and the gastrointestinal tract. The differences in bioaccessibility from each phase for metals zinc (Zn), lead (Pb), cadmium (Cd), copper (Cu), nickel (Ni), and chromium (Cr) will be analyzed and compared to one another for significant differences. The goal is to find the most conservative value in terms of human protection, bearing in mind that the higher the bioaccessibility, the greater the potential for absorption into the systemic system. Analytical variability (RSD) will be assessed using the NIST certified reference materials (CRMs), and causes of variability therein will be discussed. A separate analysis will be performed with the data from the 33 selected dust samples collected in Montreal homes from a previous study evaluating blood lead levels (Levallois *et al.,* 2014) which will address the spatial variability. The relative standard deviation (RSD) from the home samples reflects the true variability caused by differences in proximity to outside metal sources, building materials, and consumer products used inside the home. Finally, additional analysis of the total metal concentration and the bioaccessibility of each metal will assess any potential correlation.

1.5 Scope

This study employs the use of a two phase physiologically based extraction procedure (PBET) on a subset of 33 dust samples collected from houses in Montreal (Levallois *et al.*,2014), two NIST CRM dust samples, two NIST CRM soil samples, and one control field sample run on the ICP-MS to assess bioaccessibility. A one-tailed t test will be performed on the arithmetic means of individual home samples comparing the percent bioaccessibility of the gastric alone in metals Zn, Pb, Cd, Cu, Ni, and Cr to their respective percent bioaccessibility in the gastrointestinal phase to evaluate if there statistical significance between each metals differences in bioaccessibility between the two phases. This same one-tailed t test will also be run on the CRM's and control field sample for each metal. Analytical factors controlling the variability of the bioaccessibility within the triplicates of the home samples and the CRMs will also be assessed through RSD values and blanks along with standards and spikes run on the ICP-MS during sample evaluation.

2. Literature Review

2.1 Importance of metal bioaccessibility to human health risk assessments

As mentioned earlier, the indoor environment is a growing concern in terms of the potential for exposure to harmful contaminants, particularly in Canada, where Canadians spend an average of 90% of their time inside with an increase to 96-98% during the winter months (Leech *et al.,* 2002). Over the years, changes in building designs aimed at improving energy efficiency have resulted in an "airtight" structure that reduces air flow, which has created an indoor environment where contaminants are both readily produced and more likely to build up to higher concentrations than are found outside (US HHS, 2006). Indoor air pollutants range from irritants such as animal dander and biological contaminants from skin shedding, to molds and chemical vapors that may be emitted from building materials and furnishings and trace metals found in settled household dust (Lioy *et al.,* 2002). Trace metals are naturally occurring and can be brought indoors through adhesion to our skin or clothing, through open windows, or from the abrasion of objects within the home. In addition to products currently manufactured with metal content, there are important metal sources in older homes—such as windowsills, lead-based wall paint, and copper wiring—that also contribute a significant amount of metal to household dust.

Household dust is composed of a variety of constituents, with soil being one of the biggest contributors: soil has been found to constitute between 20-95% of household dust composition, depending on site-specific factors as well as sampling methodologies (Trowbridge and Burnmaster, 1997). Household dust is considered one of the most important geo-solids in terms of exposure to and effect on the general population and is a rising concern from a human health perspective (Turner, 2011). Soil and dust are ingested every day, and this unintentional ingestion is a major exposure pathway to trace metals for humans, with the highest risk being posed for children (Smith *et al.,* 2010; Turner, 2011).

The US EPA estimation of adult ingestion is approximately 50 mg of soil and dust per day; children aged 1-21 years old ingest approximately 100 mg per day, with the upper percentile at 400 mg (US EPA, 2008). Children have a higher ingestion rate as they have more intense and regular hand to mouth contact on a daily basis (Calabrese and Stanek, 1998). International standards ISO/TS 15800 (2003) and ISO/TS 17924 (2007) classify the main exposure pathways from soil through: eating and swallowing soil that has adhered to hands skin contact, and ingestion or inhalation of house dust

that contains soil. Dust ingestion rates are often derived from estimated soil ingestion rates, but efforts have been put into developing a modelling approach from the World Trade Centre Working Group and the US Environmental Protection Agency that will include a more encompassing formula and calculation of all the variables affecting dust ingestion rate (Wilson *et al.,* 2013). For example, variables such as surface loading on horizontal surfaces, soft surfaces versus hard surfaces, and differences in exposure time are all being reviewed as important factors for indoor soil/dust ingestion (see Figure 1, Wilson *et al.,* 2013)

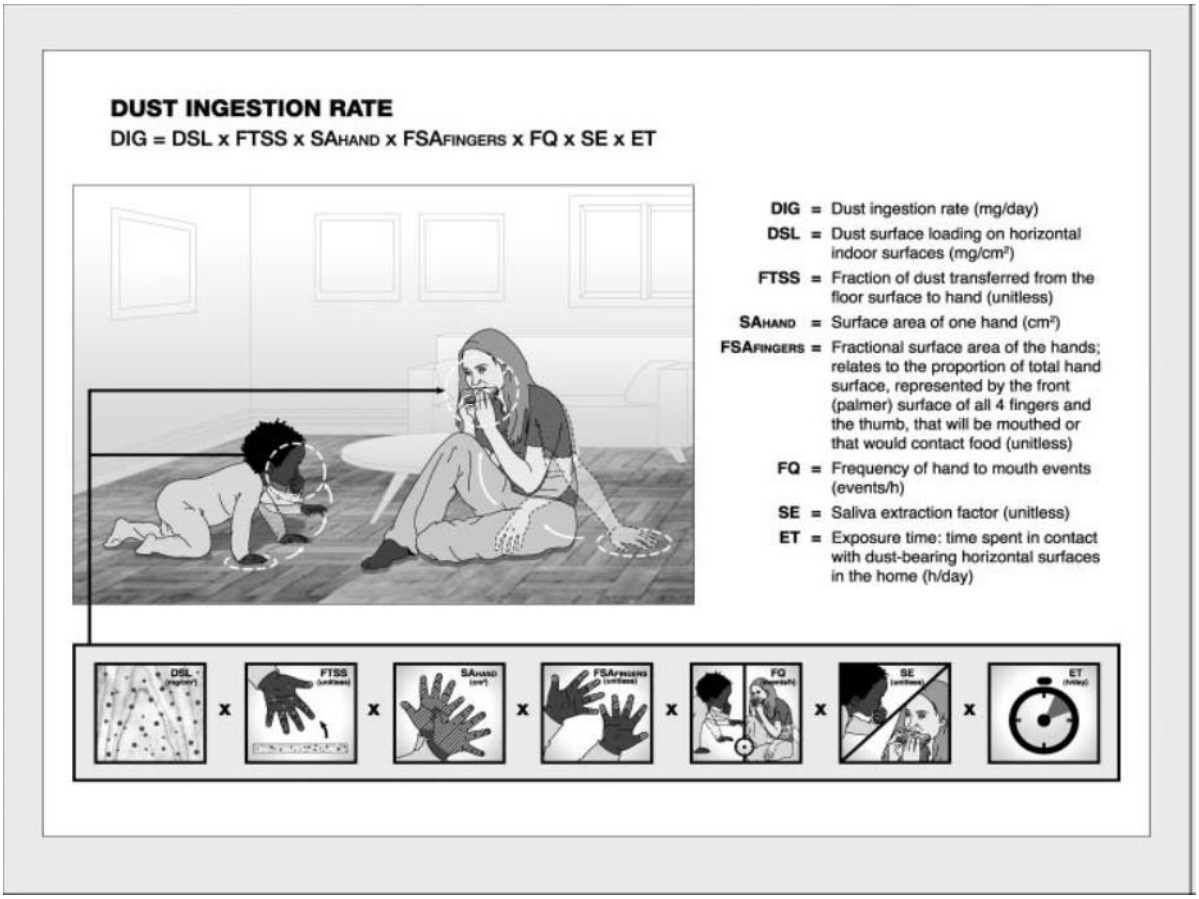


Figure 1: A conceptual diagram showing the estimation of household dust ingestion rates (from Wilson *et al.,* 2013)

When evaluating bioaccessibility at a contaminated site, risk assessment procedures most often assume that all of the trace metals bound to soil or dust are available for absorption by the human gastrointestinal tract after ingestion (Koch *et al.,* 2013). This assumption can overestimate the potential effects and health risks and potentially affect the remediation of such contaminated sites because the binding of inorganic contaminants to solid phases in soils or dust may render the potential absorption capacity of trace metals (Rasmussen *et al.,* 2008, Ljung *et al.,* 2012; Juhasz *et*

al., 2010; Turner and Ip, 2007). Over the years the potential toxic effect and chemical reactivity of the various geochemical forms of trace metals have been reviewed using various sequences of different chemical extractions, including *in vivo* (bioavailability) and *in vitro* (bioaccessibility), that specifically focus on the oral ingestion of soil or dust to show that different geochemical forms of trace metals will have different rates of bioaccessibility (Ruby *et al.,* 1999; Yang *et al.,* 2005). In addition to proper bioaccessibility evaluation over total metal concentration, it is also important to evaluate different environmental matrix for the differences in bioaccessibility in order to avoid over- or underestimating bioaccessibility at a given site, thereby negatively impacting the clean-up goal (Bradham *et al.,* 2014)

2.2 Geochemical Factors Influencing Bioacessibility in a Matrix

When evaluating the various geochemical variables that influence changes in bioaccessibility, trace metals are most often discussed with regards to anions and cations. Each metal behaves differently depending on the environmental matrices in which it is found; this also holds true for the difference in bioaccessibility of a trace metal within the different speciations in which it is found. Bioaccessibility of a metal will change based on the properties of the matrix and the manner in which the metal entered the system. The composition of the matrix itself and its characteristics—such as organic carbon content, pH or mineral content and cation exchange capacity—are some of the more important factors. Changes in the matrix over time and particle size have also been shown to influence bioaccessibility, which will be discussed in detail below. Due to the high contribution of soil to dust composition, soil will be discussed in detail within each subheading as well.

2.2.1 House dust and soil matrices

Household dust is a complex matrix that is a combination of settled or re-suspended particles and is most often classified in accordance with its particle size of≤100μm (Turner, 2011). Since it is composed of different solids settled into one matrix, the precise physiochemical makeup of the dust in a given household depends on a number of factors, including but not limited to the practices of its occupants, the external environmental surroundings of its industrial or urban setting, and importantly, the age and construction of the property (Rasmussen *et al.*, 2004; Lioy *et al.,* 2002). Some trace metals, such as Cd, Cr, and Pb, have more often been linked to external sources such as airborne emissions from industry and vehicles, or soil or street dust that is tracked in through clothing or footwear (Turner, 2011). Internal sources of trace metals also contribute to dust,

including consumer and combustion products such as old batteries (Cd) or the plating in electronic devices (Cr), old construction such as Cu wiring, and paints that may be Pb based (Kim and Fergusson, 1993). Rubber used under carpets and metal alloys used to galvanize roofs have both been identified as major sources for higher internal concentrations of Zn and Cd (Kim and Fergusson, 1993). Identifying indoor sources is important, because while there have been relationships reported between outdoor sources such as local traffic density, distance from mining and smelting activities, and soil geochemistry, household dust metal concentrations tend to exceed those of external sources and therefore have the potential to cause more adverse health effects (Rasmussen *et al.,* 2001; Callan *et al.,* 2012).

Natural soil composition varies depending on the parent rock, climate, and plant and animal interaction. Most often, in remote locations the metal concentration in a soil profile is related to the underlying parent rock. The exception of naturally found trace metals are Pb and Zn, which have been used historically for a variety of purposes and have a higher ubiquitous presence in the environment (Adriano, 2001). In an urban setting, anthropogenic sources such as buildings, infrastructure and human use can increase the trace metal concentration. Some of the major anthropogenic sources of metals Zn, Pb, Cd, and Cu found in soil include building siding and roofs; automobile brakes, tires, and oil leakage; and wet and dry atmospheric deposition (Davis *et al.,* 2001). This study identified important sources to be building siding for Zn, Pb, Cd and Cu and vehicle brake emissions for Cu, tire wear for Zn, and finally atmospheric deposition as an important source for Cd and Pb (Davis *et al.,* 2001).

2.2.2 Ageing

While there are short term fluctuations, soil can undergo long term changes depending on land use or environmental chemical weathering. It has been previously suggested that ageing (weathering) processes on trace metals can have an important effect on the bioaccessibility. Fendorf *et al.* (2004) found induced ion substitution for a matrix ion with the lattice of a mineral, entrainment of the ion and diffusion into pore spaces over time thereby effectively reducing a trace metals bioaccessibility. Copper has recently been reported to have effects with ageing and its changing geochemical speciation, where Cu exhibited patterns of transforming from the more mobile phase (carbonate and Fe-Mn associated) to a refractory phase (residual phase) over time thus reducing its bioaccessibility (Zhong *et al.,* 2012). Other trace metals have also exhibited some effects of age but not with absolute results; Cr for example showed a dramatic decrease in bioaccessibility for the first

50 days but slowed down dramatically between 50 and 200 days with a more pronounced effect on Cr (III) (Stewart *et al.,* 2003). Smolders *et al.* (2009) reported on metal toxicity in soils and ageing and explained similar trends of an increase of toxicity over time proportionally to the rise in the effective the cation exchange capacity of the soil and that the bioaccessibility would likely increase as well. It is important when performing bioaccessibility evaluations of soil to bear in mind that results reflect only the current time period, and that bioaccessibility could easily change over long periods of time.

Recent reports have suggested that the ageing of compounds also affects household dust. In 2011 MacLean *et al.* suggested that for homes >100 years old, Pb carbonates present in house dust can be attributed to primary sources such as paint, whereas in homes <10 years old (given that the use of Pb in paints had since been banned for many years) the possibility of Pb compounds weathering inside the home is possible. More recently MacLean *et al.* (2013) followed this work up by evaluating pure lead compound changes in Pb speciation and bioaccessibility over time in a controlled humidity chamber. The findings of this study showed that over time, with increased humidity, there was an increase in the bioaccessibility of Pb: this was attributed to the growth of Pb carbonate that formed as a result of Pb metal weathering (MacLean *et al.,* 2013). Another study carried out by Rasmussen *et al.* (2014) evaluated changes in bioaccessibility of metals in dust over time and weathering using a controlled humidity chamber. This study revealed that there was an increase in Pb and Zn bioaccessibility, and synchrotron XAS showed that the increase of bioaccessibility was found in the changes of speciation from a less bioaccessible inorganic form to more bioaccessible organic species for both Pb and Zn (Rasmussen *et al.,* 2014). A related study of dust and Zn compounds focused on the effects of humidity on Zn speciation and bioaccessibility, showing similar results, where the bioaccessibility increased due to the transformation of inorganic Zn to organic Zn over the course of four months in a controlled humidity chamber (Beauchemin *et al.,* 2014).

2.2.3 pH

Although the potential for pH changes in household dust is not well understood, understanding the influence of pH on trace metals within a soil matrix is important for understanding bioaccessibility both outdoors and indoors. All soil types contain their own natural buffering systems as a result of their constituents—for example, aluminum helps to maintain soil acidity while calcium carbonate keeps it basic—but the amount of each constituent is unique to each profile (Adriano, 2001). While

there are several variables to take into consideration, such as clay content, organic matter, surrounding plant and animal interaction, and climate and humidity, most soil bodies are within the pH range between 4 and 8 (Adriano, 2001). Generally, when the pH of a soil body increases, the retention capacity for trace metals also increases, with the expected maximum at around circumneutral (Adriano, 2001). The pH has been considered the driving factor for all other geochemical variables in a soil body because it can affect the surface charge of layer silicates found on clays, as well as the charges on organic matter and oxides of Fe and Al (Lou *et al.,* 2012; Ettler *et al.,* 2012; Pelfrene *et al.,* 2011). In addition to the effects on the sorption of cations (which increases with increasing pH), and complexations with organic matter, the precipitation-dissolution reactions and redox conditions are also affected by the pH, which in turn impact the bioaccessibility of trace metals.

Each trace metal will be affected by pH differently. For example, metals with divalent cationic forms are generally less absorbed, and therefore more bioaccessible, in acidic soils than in neutral. There has been a general agreement among studies that Pb, Cd, Zn, and Cu have greater mobility and absorption in low pH soils, but that the maximum bioaccessibility is found in Pb (Pelfrene *et al.,* 2011; Ettler *et al.,* 2012). More recently, Luo *et al.* (2012) reported results wherein the effectively bioavailable Zn and Pb were mostly related to the pH of all the physiochemical properties in soil. In terms of direct human consumption of soil, metals such as Cd are in fact more available in low pH soil, however, at a pH of 6.5 or higher, plants will have a greater affinity for the Cd and thus increase its uptake, making it less available in the soil and therefore placing humans at less risk of absorption through soil ingestion (Wragg *et al.,* 2011). Metals having two different oxidation states, such as Cr, can be affected by pH levels where Cr VI can be rapidly oxidized to Cr III in acidic soils (Stewart *et al.,* 2003). The solubility of Cu in soils is largely dependent on the pH due to the hydrolysis of Cu^{2+} in acidic soils, however, increasing the pH leads to an increase in the degree of complexation of soluble Cu with organic matter so in the case of Cu, organic matter can play a large role as well (Zhong *et al.,* 2012).

2.2.4 Organic Content

The effect of organic matter works in conjunction with the pH, clay content, and the parent rock along with the contamination in question, depending on the metal and scale of contaminant concentration. The organic matter content in a soil profile can range from <1% to >70% and is broken down into two basic categories, non-humic and humic (Cave, 2011). Non-humic contains

unaltered biochemical components which have not been degraded since they entered the soil by means of living organisms, whereas humic is formed by secondary synthesis reactions involving micro-organisms (Cave, 2011). Organic matter itself can degrade over time, but trace metals cannot and will only change in speciation, which is influenced by the surrounding organic matter. Mechanisms that are known to control bioaccessibility in the organic components of soil are: the adsorption of cations on negatively charged sites (ion exchange), the mobility of some metals and their capacity to form chelates, and finally, the retention time of higher molecular weight contaminants in solid forms of humus (Adriano, 2001).

Organic matter also plays a role in competition for sorption of contaminants on oxides and clays for soil and dust. The ability of organic matter to create an environment where Fe-oxides are reduced causes the dissolution of the host and releases the previously absorbed contaminant (Chen *et al.,* 2003; De Miguel *et al.,* 2012). Clay content has a charged surface area that can cause reactions with polar organic compounds. In addition, the small diameter of clays creates micropores and nanopores that can sequester organic chemicals (along with organic matter), and plays a key role in the potential reduction or increase of the bioaccessibility of organic compounds (Adriano, 2001).

Some trace metals show higher affinities for organic matter, such as cobalt, copper, nickel, and lead (Adriano, 2001; Poggio *et al.,* 2003; De Miguel *et al.,* 2012). In other cases, organic matter may in fact be a catalyst for the reduction of a metal's bioaccessibility, as observed in Cr(VI) reduction (Broadway *et al.,* 2012; Stewart *et al.,* 2003). More specifically, the decrease in Cr is a consequence of pH- and soil organic matter mediated reduction in the gastric phase to humic complexes – bioaccessibility values increased from ~10-20% to ~60-70% when the organic carbon was limited and reduction processes were minimal (Broadway *et al.,* 2012; Stewart *et al.,* 2003). Few studies have compared the organic matter content between both soil and dust, but one study identified metals as having a higher affinity for the organic matter inside the home, showing that in conjunction with the release of metals from indoor sources there will be a higher metal concentration than outdoors and also a higher bioaccessibility rate: nearly double for Ni, and three times higher in Zn for dust over soil (Rasmussen *et al.,* 2008). Furthermore, this study demonstrates a significant positive correlation which pointed to the organic phase of the dust as an important control on the accumulation of Cu in dust but did not show a correlation for an increase in bioaccessibility (Rasmussen *et al.,* 2008).

2.2.5 Minerals

Inorganic constituents usually make up most of the mass of a soil profile, and the interactions of the trace metals with these surfaces are an important influence on their bioaccessibility. For example, encapsulation by quartz can trap Pb into an insoluble matrix (Ruby *et al.,* 1999). The formation of insoluble alteration or precipitation rinds also decreases the bioaccessibility of Pb, as well as the formation of iron oxides cements (Ruby *et al.,* 1999). Generally, minerals that form under acidic conditions will tend to be more stable in the acidic environment of the gastric phase, whereas minerals formed in alkaline environments will be less stable and therefore more bioaccessible (Ruby *et al.,* 1999). Phosphate minerals are harder to predict due to the highly variable compositions and the possibility of lead sorbed to clay and other metal oxide mineral surfaces also change the bioaccessibility of each species (Ruby *et al.,* 1999). The effects of mineral and chemical form on Pb bioaccessibility was summarized in a diagram by Ruby *et al.* (1999), and shows the possible physiochemical process governing the bioaccessibility of lead from a contaminated site (Figure 2).

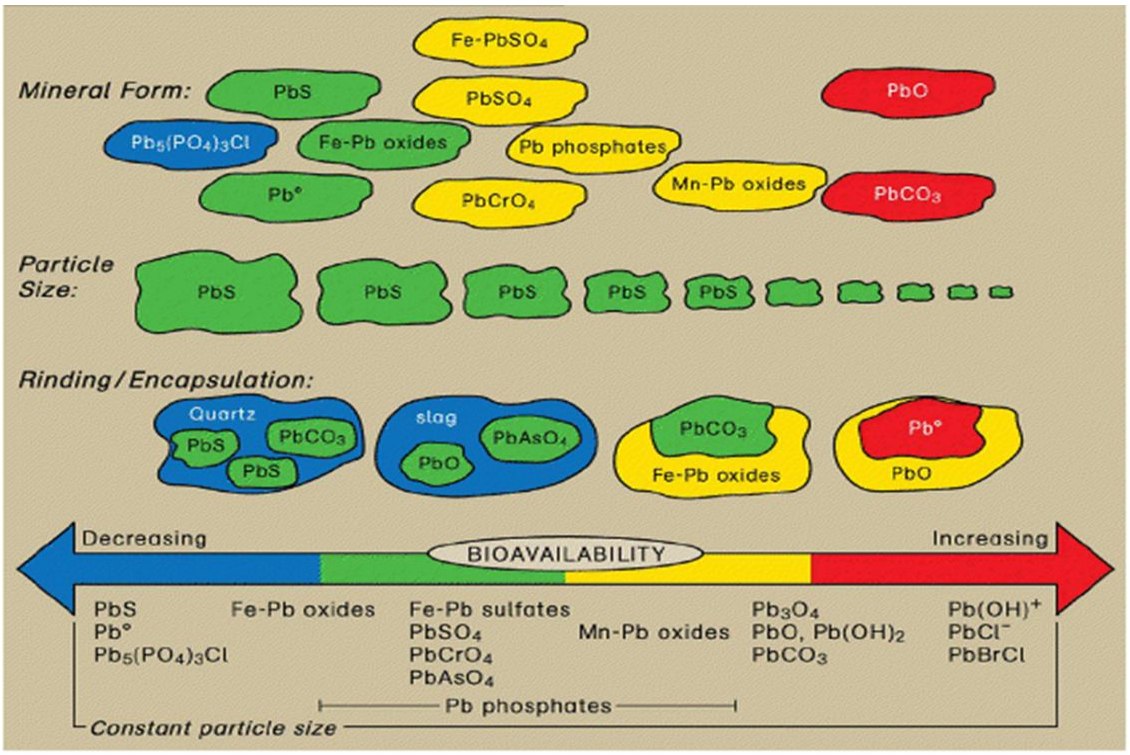


Figure 2: Schematic diagram of different lead species, particle size and morphologies and their differences in bioaccessibility (from Ruby *et al.,* 1999)

Many studies have highlighted the role of clays and oxides of iron, manganese, and aluminum as important mineral classes influencing bioaccessibility. Clay is characterized by its two-dimensional layering sheets containing SiO_4 and AlO_4 with the chemical composition of $(AlSi)_3O_4$ that, when aligned in its natural state, will result in the free oxygen all facing in one direction, thereby creating a charged site. Iron, aluminum, and manganese are also very fine grained (<2um) with large reactive surfaces. Since most trace metals occur in the cationic form in soils, the ability of a soil profile to attract and retain cations is critical to the bioaccessibility and is known as the cation exchange capacity (CEC) (Cave, 2011). As previously mentioned, pH also influences the CEC for both clays and oxides. Clays will be a main contributor to the CEC when the soil is <7 with very little activity from the oxides. However, in low pH environments oxides will have a high rate of anion exchange. Cation exchange is reversible and when the electrostatically bound trace metals are put into very low pH environments, as found in the human stomach, they will be displaced easily.

In 2003 Stewart *et al.* took an in-depth look at the effects of the physiochemical properties of soil and the bioaccessibility of Cr and found that the bioaccessibility decreased as the total inorganic carbon content increased, and that the clay content decreased through the various sorption processes. Pb is another metal that is found to have a reduction in bioaccessibility when transitioning from the gastric phase to the gastrointestinal as a result of complexes formed on charged clay site particles (Ruby *et al.*, 1999). Another study reviewed the clay fraction of a soil profile from the urban area of Sevilla (Spain) and Torino (Italy) and found that metals Pb and Zn accumulate preferentially in the clay portions of the soil and are likely to have a higher bioaccessibility (Madrid *et al.,* 2008)

2.2.6 Solid Phase Speciation and Toxicity

It is useful to measure the physiochemical forms of the contaminants in soil and household dust in order to predict the potential environmental redistribution under different conditions (as described earlier), with the end goal of using the data as another line of evidence to support *in vitro* bioaccessibility assessments. There are different types of spectroscopic methods that can be used, x-ray absorption for fine structure (XAFS), and x-ray absorption near edge structure (XANES). These are extremely sensitive methods that directly measure the oxidation state and chemical bonds holding the contaminant in the soil or dust. Studies have been able to show not only what form and oxidation state a metal may be in, but can also accurately predict where the contaminant came from. For instance, as part of the Canadian House Dust Study multiple tests were run on house dust

samples that confirmed some of the more common Pb species in house dust to be Pb carbonate, Pb hydroxyl carbonate, Pb sulphate, Pb chromate, Pb oxide, Pb citrate, Pb metal, Pb adsorbed to Fe- and Al-oxyhydroxides, and Pb adsorbed to humate (Rasmussen *et al.,* 2011; Beauchemin *et al.,* 2011; MacLean *et al.,* 2011). Walker *et al.* (2011) were able to show not only the solid phase speciation but could source them directly: dust metal concentration in a child's bedroom was linked to the paint after home renovation, and living room dust metal concentration was linked to garden soil (Walker *et al.,* 2011). Other studies have focused on Zn and have found that some of the more common Zn species observed in household dust from previous Canadian studies include Zn carbonate, Zn associated with Fe hydroxide phases, Zn sulphide (sphalerite), and Zn oxide, and they can also be influenced by aging as described earlier (Rasmussen *et al.* 2014).

2.2.7 Particle Size

The United States Environmental Protection Agency created guidelines for soil risk assessment where lead is of concern, suggesting sieve size mesh #60 to give a particulate size of<250 µm (US EPA 2008). This recommendation is founded on a dermal adherence survey taken from literature in previous years. However, an analysis by Ruby and Lowney (2012) on soil adherence to the skin reviewed the results of multiple studies where particulates smaller than the <250 µm adhere to hands. The study further states that 50% of the binding mass is most often under <80 um and 90% of adhering mass is <250 um (Ruby and Lowney 2012). Another study published in the same year found particle sizes as small as <45 µm binding to the skin, regardless of the particle size distribution of the bulk soil (Beamer *et al.,* 2012).This is important not only for soils but for dust as well, because smaller soil particles are more likely to be tracked and settle indoors, where children's exposure is more likely to occur (Beamer *et al.,* 2012). Studies from Rasmussen *et al*. (2008), Beamer *et al.* (2012), and Luo *et al.* (2012) reported a general consensus for an inverse relationship between decreasing particle size and increase in bioaccessibility for both dust and soil. This can be difficult to predict concretely due to the complexity of metal interaction with the matrix and the nature of the matrix itself. Each soil or dust sample has its own site-specific properties bearing organic and inorganic phases, with distinctive physiochemical properties controlling metal distribution and the phase in which it can remain.

2.3 Method Variables Influencing Bioaccessibility

As previously mentioned, there are many different bioaccessibility extraction procedures, and each of which has unique characteristics to mimic the physiological conditions of the human gastrointestinal system. Nevertheless, there are some method variables which consistently have been proven to be important influences on the bioaccessibility of each metal.

2.3.1 pH

With respect to oral bioaccessibility for inorganic elements, multiple reports have shown that the pH is the most influential variable (Turner, 2011; Koch and Reimer, 2012). In 2002 a study of five different bioaccessibility extraction methods was evaluated on three soil types to compare the varied conditions of the RBALP (Relative Bioaccessibility Leaching Procedure) simple gastric only method, the DIN (Deutches Institute for Normung) gastrointestinal, RIVM (National Institute for Public Health and the Environment in Netherlands) complex gastrointestinal, SHIME (Simulated Human Intestinal Microbial Ecosystem) fed and gastrointestinal, and TIM (TNO gastro Intestinal Model) dynamics. The results from this study showed that overall, a higher gastric pH produced lower bioaccessibility, and it was concluded that the gastric pH is the main cause of the different bioaccessibility results (Oomen *et al.,* 2002). Since then, several studies have shown trends of decreasing bioaccessibility with increasing pH where simple gastric extractions were employed and the pH variable was clearly the controlling factor for metals such as Pb, Cd, Rh, Pl and Pt (Koch and Reimer, 2012). More recently, a 2013 study not only compared 17 different methods, but also made inter and intra laboratory comparisons, and likewise found pH to be the main controlling variable for metals Pb, Zn, Cu, and Cd when transitioning from the gastric alone to the gastrointestinal phase (Koch *et al.,* 2013).

Along with the fluctuations seen in the in different initial gastric pH values, the transition from gastric pH (approximately 1-2.5 depending on the method) to the neutral state of the intestinal phase with a pH of approximately 7 can have dramatic effects on some trace metals. Studies have shown that trace metals such as Zn, Pb, Sr, Co and Hg display a large decrease in bioaccessibility when transitioning from gastric to intestinal compartments, whereas for trace metals such as Cu, Ni, Cr, Cs the decrease is not as dramatic, or in some cases is even higher in the intestinal phase (Poggio *et al.,* 2009; Koch *et al.,* 2013; Ruby *et al.,* 1999; Oomen *et al.,* 2002; Stewart *et al.,* 2003). It is worth noting that with each method there are a number of different confounding variables (such as

complexing agents or final pH) that might exert multiple effects on a trace metal, but nevertheless pH has consistently proven to be the most critical.

Food is another variable that works in conjunction with pH. Bioaccessible tests are most often performed by simulating a fasted state because food is believed to reduce the bioaccessibility of metals, depending on the particular complexing agents present in a given food. It is difficult to predict the effects of food on trace metal bioaccessibility because depending on the food ingested, there may be an increase or decrease in pH; each testing method might be affected differently based on the synthetic proteins used to simulate the gastric and intestinal phases. However, in terms of risk assessment and the use of bioaccessibility as a means of protecting human health, it is ideal to work in a fasted state as it will determine the higher bioaccessibility value and therefore the most conservative value (ADEME 2012).

2.3.2 Liquid to Solid Ratios

The liquid to solid ratio (L/S) is dependent on the type of extraction being employed and the quality of the soil or dust being used in the study. With pH playing an influential role on bioaccessibility and the inverse relationship between bioaccessibility and surface area with particle size, the L/S ratio also influences the bioaccessibility. For example, in the early stages of the development of the PBET method, authors noted that liquid to solid ratios between 5:1 and 25:1 can lead to an underestimation of bioaccessibility values but that generally a ratio of 100:1 is apt, whereas methods such as RIVM bioaccessibility increases for elements such as As at a higher ratio of 1000:1 (Ruby *et al.,* 1996; Hamel *et al.*,1998; Van de Wiele *et al.,* 2007). While there are notable differences in the lower ratios, only a small difference in bioaccessibility with ratios ranging between 100:1 to 5000:1 was observed in metals Pb, Ni, Cr, and Cd from the certified reference material NIST 2710 (Hamel *et al.,* 1998). In this same study there were larger differences seen with a field soil sample, however due to the heterogeneity of the sample there was an increase in the variability as well, making it difficult to validate the importance of the differences (Hamel *et al.,* 1998).

Several studies have shown a trend of a small increase of bioaccessibility with an increase of L/S ratios for methods such as the simple gastric solution or the PBET up until a ratio of approximately 1:1000 (Rasmussen *et al.*, 2008; Smith *et al.,* 2010; Koch and Reimer, 2012). The ratio of 100:1 is most commonly used in the PBET method because as previously mentioned, testing showed little

variation with ratios higher than100:1, and also because a determined physiological ratio for children has been found to be 90:1 (9mL of gastric volume, 100 mg/day of ingested soil), making it an accurate representation of what a child would experience physiologically (Oomen *et al.,* 2006).

When working with extremely contaminated samples and metals with lower solubilities, it is important to run background L/S ratio testing as a means of quality control and also to ensure that metal solubility is not dependent on the liquid to solid ratio. Richardson *et al.* (2006) reported that the solubility of a metal compound may in fact be a limiting step for bioaccessibility at low liquid to solid ratios and for highly contaminated samples; it is therefore the saturation that is being observed and not the actual bioaccessibility.

2.3.3 Separation Methods

When performing bioaccessibility screening, the separation of the liquid and the solid occurs after each phase and before the analysis. Methods of extraction most commonly used are centrifugation, filtration and filtration with dialysis. One study observed that centrifugation was the best method of separation followed by a 0.45um filter and finally a 5kDa dialysis filtration (Van de Wiele *et al.,* 2007). More importantly, this study displayed the overall importance of the separation method used and demonstrated the variances between the different approaches not only within a separation method, but also between the various *in vitro* approaches (Van de Wiele *et al.,* 2007). Factors such as fasted state versus non-fasted state have also been shown to have an effect on the bioaccessibility and separation method, in that there was an unexpected finding of higher bioaccessibility with a non-fasted state but a better separation with the use of centrifugation (Van de Wiele *et al.,* 2007). This study showed empirically that the ultrafiltration separation method offers the most accurate method to measure bioaccessible fractions of a contaminant in a human gut and closely approaches the true oral bioavailability value (Van de Wiele *et al.,* 2007).

2.4 Trace metals and Risk Assessments in Canada

Metal selection for this study was based on the presence of these metals in high concentrations in Canadian urban settings, with the understanding that such concentrations suggest a potential health risk. In 2005, the Canadian federal government hired Franz Environmental Inc. to evaluate soils at designated contaminated sites across Canada: they reported that across the 74 residential /parkland areas surveyed, metals were found at the highest concentration of contaminants by 49% (Franz Environmental 2005). Furthermore, when soil depth was taken into consideration, the metal

contamination attributed to 77% of the total contamination from the surface to a depth of 1.5 meters. The ranking for metal occurrence in the soil was Zn>Pb>Cu>Cr>Ni (Franz Environmental Inc. 2005). The inclusion of Cd in this study was due to a general data gap in research, as well as due to its specific identification as a metal to be included in risk assessments by Koch *et al.* (2013). According to the toxicological profiles, all of these metals have the potential for negative impacts on human health – metals Cr, Cd, Ni and Pb pose both non-carcinogenic and carcinogenic risks (WHO 2011).

In Canada the official guidelines and documents for contaminated site and soil remediation are based on the total quantity in different matrices (soil, sediment, and water) but no official documents on household dust (CCME 2014). In Health Canada's Guidance on Human Health Preliminary Quantitative Risk Assessment, the recommendation for contaminated sites states that due to the limited quantity of data on the bioavailability of contaminants, oral ingestion must always assume the relative absorption to be 100% for the most conservative approach. Ontario is the only province that has an official provincial statement in the "Guidance on site specific risk assessment for use at contaminated sites in Ontario," which requires that in order for bioavailability to be used as a measure, the data must be obtained from humans or animals. Other companies have made alternate recommendations to Health Canada; for example, consulting group ENVIRON International Corporation suggest the performance of "gastric" only phase evaluations on the metals Cr and Ni, whereas Cd would require both gastric and gastro-intestinal phases (ENVIRON 2011).

To date, research and methods development efforts as well as regulatory practice have focused on As and Pb; the application of these findings to other metals has been a subject of some debate, and in regulatory settings they have been applied only sporadically at a limited number of contaminated sites. One major concern with having limited data sets for other metals is that the recommendations for risk assessments may not be applicable to each metal, and thus regulations could be set based on non-applicable data.

While there have been efforts in recent years to study metals such as Cd, Ni, and Hg, there are still data gaps that need to be filled in order to build a better understanding of metal behaviour in soil and dust and evaluate risk factors appropriately (Bradham *et al.,* 2014). In spite of previous studies providing information about *in vitro* data validated against *in vivo* data for metals such as cadmium, copper and zinc (Denys *et al.,* 2012, Turner *et al.,* 2008), there is still more work required

3. Materials and Methods

3.1 Samples and controls

Home samples for this study were selected from a previous cross-sectional study in Montreal (Levallois *et al*., 2013). The aim of the Levallois *et al*. study was to simultaneously evaluate the blood lead levels and home indoor environments of young children (1 to 5 years of age) living in older buildings that used or were located close to lead pipe infrastructure in Montreal (Levallois *et al.,* 2013). Sample and data collection was carried out between September 2009 and March 2010, with approval from the ethics committees of the Centre Hospitalier Universitaire de Québec and Health Canada (HC), and included 306 participants. Although there were multiple dust sample types collected from Montreal, only the whole-bag vacuum dust samples were used for this study (n=305).

A total of 33 homes were selected from among the 305 on the basis of their total metal concentration values. Selections were made in order to include varying representations of homes with high, medium, and low total metal concentrations, while still allowing for an extraction of a bioaccessible portion. Selection criteria were designed to capture the true variability of homes in the study area as well as to ensure that the solubility of the metal was not a limiting factor for bioaccessibility calculations. In some cases, pre-selected homes did not have enough remaining dust for the extraction to be done in triplicate as the study required. In such cases, the home with the next-closest ranked value which also contained the required 3-gram minimum was selected.

The certified reference materials (CRMs) were purchased from the National Institute of Standards and Technology (NIST) and included NIST 2583 – Trace Elements in Indoor Dust, NIST 2584 –Trace Elements in Indoor Dust (Nominal 1% Lead), NIST 2710a – Montana Soil I, and NIST 2710 – Montana Soil, where NIST 2710a is an update batch of a similar chemical makeup as NIST 2710. Each NIST comes with a certification of the chemical makeup of the sample to allow for accurate bioaccessibility calculation. A dust control sample (Mosque) collected and prepared by Health Canada according to methods described in Rasmussen *et al.* (2008) was also used as an experimental control for QA/QC. This dust was collected from the city of Ottawa using a purpose-built HVS3 (High Volume Small Surface Sampler) following the ASTM method D 5438-00 and subsequently prepared by air-drying and sieving into multiple size fractions.

3.1.1 Sample Collection

Dust samples were collected with each visit to the home as described by Gauvin et al. (2011). Participants were asked to give the bags of their personal vacuum unit to the field technician; the bags were then stored frozen until sent to HC where samples were later processed. To avoid cross-contamination, the fume hood was vacuumed and wiped down after each sample was processed, and sieves were cleaned using an ultrasonic bath and then oven-dried between each sample processing. Further details of the process can be found in Rasmussen *et al.* (2011). Pet and human hair and large particles were manually removed from the dust samples, which were then sieved to <80 µm in a metal-free fume hood. Previous work from Rasmussen *et al*. (2008) showed a relationship between metal bioaccessibility and particle size, where bioaccessibility is strongly affected by differences in metal speciation among size fractions, hence the need to define a specific fraction when comparing a large number of samples. Each home sample required a minimum of 3 grams of dust as the homes were run in triplicate with 1 gram of dust per sample.

3.1.2 Instrumentation

Instruments used in this study included the Inductively Coupled Plasma Mass Spectrometer (ICP-MS), Perkin Elmer Sciex Elan DRC II Axial Field Technology, which was operated in standard mode equipped with a Meinhard Concentric Quartz Nebuliser, cyclonic spray chamber, and platinum skimmer and sampler cones. Each batch run on the instrument included analytical calibrations and standard mixtures. Standards are matrix matched with samples (same reagent mixture and concentration) and calibration standards of 10, 20, 50, and 100 μg/L for all metals. Equations prescribed by USEPA Method 200.8 were used to correct for interferences on Cd, Pb, and Ni, and additionally in the case of Pb, to correct for variations in isotopic abundances caused by differing sources (USEPA 1994b). The reportable instrumentation detection limits were Cd < 0.1 μg/L, Cr < 0.5 μg/L, Cu < 0.2 μg/L, Pb < 0.5 μg/L, Ni < 0.5 μg/L, and Zn < 0.2 μg/L. Drift over the course of a batch was monitored using quality control standards. No corrections for drift were required within batches, and corrections between the batches over the course of the study are described in Section 3.4.2.

The analytical balance used in this study was the Mettler Toledo XP 205 which was placed on a marble slab that was suspended by hydraulics to control the errors of vibration. Calibrations weights were used before each extraction to ensure the readings were accurate for the day. Finally,

a specially designed tank from Drexler and Brattin (2007) was used for rotating the samples for the extraction (see Figure 3). This tank was built for Health Canada by John Drexler and allowed for 10 samples to be run per batch with full submersion in pre-heated water, in this case at 37.5˚C.

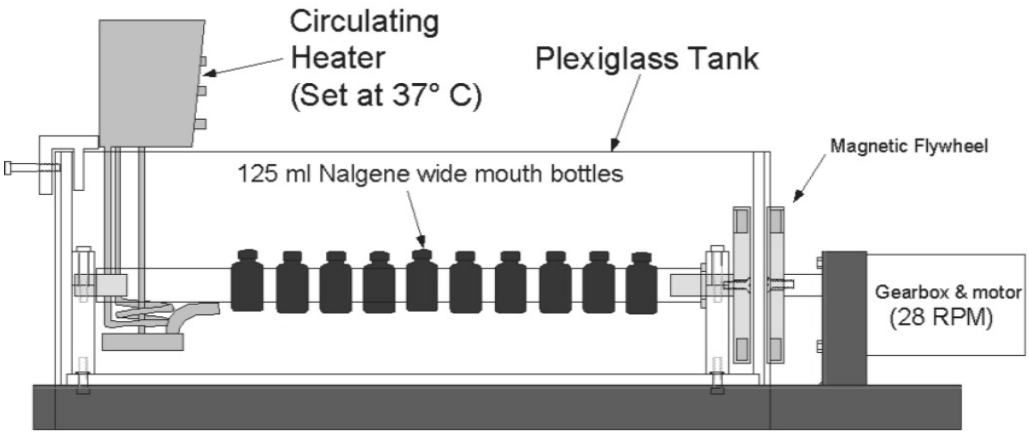


Figure 3: Tank design built for Health Canada by John Drexler (from Drexler and Brattin, 2007).

3.1.3 Reagents

Reagents used in this study to simulate the gastric and gastrointestinal phases were purchased from Sigma Aldrich. These included pepsin (from porcine gastric mucosa), sodium citrate, DL-Malic acid disodium salt, lactic acid solution (85%), acetic acid (99%), sodium bicarbonate, bile salts, and pancreatin. Hydrochloric acid and nitric acid were purchased from Fisher Scientific at reagent grade. All reagents are metal free. All water was deionized with the use of Milli-Q Plus, Millipore at 18.2 M Ω cm. One batch of sodium bicarbonate was purchased from the University of Ottawa Science stores.

3.2 Sample Analysis

3.2.1 Total and Bioaccessible Metal Determination

Total metal concentrations for the Montreal samples were provided by Rasmussen (unpublished data) and were determined by Actlabs Inc. (Ancaster ON) using a 4-acid digestion (HF, $HClO_4$, HNO_3, and HCl) followed by Inductively-Coupled Plasma Optical Emission Spectrometry (ICP-OES) and/or Mass Spectrometry (ICP-MS), as required.

3.2.2 PBET Method for Bioaccessible Metal Determination

PBET is a physiologically based extraction technique that mimics both the gastric alone and the gastrointestinal tract to measure the bioaccessibility of a trace metal as described earlier in Section 1. Using a modified PBET method from Ruby *et al.* (1996) the bioaccessibility of each trace metal selected was determined in house dust samples from 33 homes along with 4 NIST standards and Mosque (control field sample). This test was first developed in 1993 for metallic elements Pb and As found in soil, and was later recognized by the Environmental Protection Agency of the US as a validated procedure for bioaccessibility of Pb in soil (US EPA 2008). Modifications were made later by Drexler and Brattin (2007) who simplified the method, and then correlated the results with *in vivo* measurements of the bioavailability of Pb in soil to validate the method. A maximum of two homes (run in triplicate), two controls (one or two of the four NIST selected for this study), and two blanks were run per batch. A total of 22 batches were run during the course of this study.

3.2.3 Gastric Phase Extraction (G-alone)

To simulate the gastric alone phase (G-alone) a solution was prepared by mixing pepsin, malate, citrate, lactic acid, acetic acid and hydrochloric acid into 1L deionized water (18.2 M Ω cm, Milli-Q Plus, Millipore) that was pre-heated to 37.5˚C. The pH of this solution was adjusted to 1.8 through the subsequent addition of HCl drops. From here 100mL of the mixture was added to 125 mL HPDE plastic bottles with the pre-weighed 1 gram of dust. The samples were then placed for 1 hour in the over-end rotator using a tank design from Dexler and Brattin (2007), where the water temperature was also pre-heated to 37.5˚C to simulate human body temperature and residual time in the stomach while in a fasted state. This rotator minimizes the risk of contamination from blade stirrers and improves the reproducibility of the test through consistent and thorough mixing. Throughout the mixing time, pH adjustments were made at both the 30-minute mark and the 1-hour mark to ensure a consistent pH of 1.8 using HCl. Once the gastric phase was completed, 10 mL would be removed with the use of a 20mL disposable plastic syringe. A disposable 0.45 um cellulose acetate filter was attached to the syringe and 1 mL of the extract was filtered into 9mL of 0.1 HNO_3 and stored until analysis.

3.2.4 Gastrointestinal Phase Extraction (GI)

Following the g-alone phase, the first step for the gastrointestinal phase (GI) was to neutralize the samples to a pH of 7 by adding solid sodium bicarbonate to the solution and then leaving the bottles

un-agitated in a pre-heated water bath of 37.5˚C for half an hour before testing the pH. Once the solutions had been neutralized to a pH of 7 ± 0.2, 10mL of the simulated intestinal solution containing bile extract and pancreatin was added to each sample. The samples were then placed back into the rotator and mixed for another four hours to simulate the time it would take to run through an intestinal system. Again, pH was monitored in two-hour intervals to maintain a pH of approximately 7 throughout this portion of the extraction. When the gastro-intestinal phase was completed, the solution was removed and stored as described before.

3.2.5 Modification to the GI Phase Extraction

As part of the methodological development, a modification to the extraction procedure was made. The original method called for samples to be neutralized using a supersaturated sodium bicarbonate solution, estimated to be approximately 10mL (Ruby *et al.,* 1996). The previous study was working exclusively with a soil matrix and did not include household dust, so it is not unexpected to encounter a different challenge in neutralizing the dust matrix given the significant compositional and bioaccessibility differences observed between soil and dust (Turner, 2011; Dodd *et al.,* 2013).

In early trials of the experiment using control field samples, the supersaturated solution was unable to reach a pH of 7 within a reasonable amount of volume added to the sample. Some samples required an addition of upwards of 60mL to a 100mL solution. These cases would require that a portion of the sample solution be removed, because the 125 mL bottle size would not allow for enough of the supersaturated sodium bicarbonate solution to be added without overflowing before reaching a neutralized state. This was not ideal, particularly when working with a heterogeneous material such as dust, as the composition of the removed portion of solution might differ slightly from the remaining sample, leading to a loss of data. A new approach was therefore devised for the present study wherein solid sodium bicarbonate was added directly into the solution for each bottle and left un-agitated for 30 min in the pre-heated water bath at 37.5˚C. Tests were performed with both a control sample home, NIST samples, and blanks; after a number of trials it was determined that approximately 2.2, 1.75 and 1.75 grams would be added to the G-alone solution respectively in order to reach the required neutralized state for the GI phase.

3.3 Quality Control and Quality Analysis

Initially 4 separate batches of NIST and one with the Mosque control were analysed using the PBET to establish a strong Relative Standard Deviation (RSD). Application of the PBET to the home

samples were done in triplicate (maximum two homes per batch) along with at least one NIST control rotating between NIST 2583 and NIST 2584 –Indoor Dust, NIST 2710a and NIST 2710 - Soil. In addition to the NIST, two blank controls were run per batch. In the beginning stages of the study a dust field sample (Mosque) was also used as a control sample. The dust samples from the 33 homes were completed in 18 batches.

Sample digests were analyzed by Health Canada in their laboratory on the University of Ottawa campus – details of the ICP-MS set up can be found in Section 3.1.2. Standards on the ICP-MS are matrix matched with samples (same reagent mixture and concentration) and calibration standards of 10, 20, 50, and 100 µg/L for all metals were run after ten samples had been processed. All glassware used was acid washed with 0.1 mol nitric acid and rinsed with deionized water (18.2 M Ω cm, Milli-Q Plus, Millipore). Plastic HPDE bottles were rinsed with acid four times, left in an acid bath overnight, then tripled rinse with deionized water and dried in a fume hood with Kim Wipes.

3.4 Data Analysis

3.4.1 Bioaccessibility Calculations

Metal bioaccessibility was expressed as a percentage and calculated as follows: bioaccessible metal (MS) was divided by total metal (MT) to calculate each metal's bioaccessibility, expressed as a percentage ([MS/MT]* 100). Each home sample was run in triplicate; therefore, the arithmetic mean of the bioaccessibility of all three samples from each home is presented rather than the individual value of each sample. When working with a material such as household dust, due to the high heterogeneity of the matrix, one value of the triplicate may carry either a high or low value of a metal and not be the true representation of the sample. If there appeared to be an anomalous value in an individual replicate, the results of other metals from that home sample were reviewed in comparison, with reference to the controls and standards run on the ICP-MS from that batch, in order to ensure that the entire sample or batch was not affected and that it was in fact a true outlier. If all the quality criteria were satisfactory, and the anomalous value was determined to be an outlier, the best two out of three replicates were used to represent that home. Furthermore, if two of the values within the triplicate were not detectable, the home would be classified as below limit of detection (LOD). In all cases means are arithmetic, and all percent relative standard deviations (RSDs) were calculated with respect to the arithmetic mean and its standard deviation.

The data analysis for percent bioaccessibility was carried out differently from the bioaccessible metal concentration with respect to the LOD. More specifically, when presenting the table for the metal concentration of the bioaccessible portion, if a ppm value was below the detection limit, in lieu of a zero value, half of the LOD was used as a representative for the metal. The purpose of this was to present the data set as a whole and show the variability of the metal concentrations within each metal sample.

Once all bioaccessibility calculations were complete, one-tailed t-tests were performed on the percent bioaccessibility. The reason for using a one-tailed t-test instead of a two-tailed test is two-fold: first, the original null hypothesis aims to test if bioaccessibility would be greater in the simulated G-alone phase than in GI phase, with the relationship expected to be established in one direction only; and second, the G-alone is compared to the GI means of the same sample so a one-tailed test is more appropriate. With homes below the LOD a zero was used in place for the home in the t-test. A zero was also used for homes below the LOD when calculating the median of each metal from the home samples.

There were two separate LOD's calculated for this study. This was done because the brand of solid sodium bicarbonate changed halfway through the study – the change in brand was required as the company provider for the first batch had a production delay and were unable to provide the reagent within a short period of time and in order to avoid delays with the extractions a different batch was purchased. Therefore, the LOD is calculated on the basis of pre and post date of the sodium bicarbonate change (August 8th, 2013). Details are given histograms in Appendix 1, which show the bioaccessible metal concentration for all four NIST in both the G-alone and GI phases.

3.4.2 The need for Zn correction

Comparison of the NIST controls included in each batch show a drift over time (between batches) for the Zn values in NIST 2584 and NIST 2710a. Histograms of both controls can be found in Appendix 1a and 1b where the Zn values suddenly decrease over time (over the course of the batches run) and eventually increase back up to the same values as observed in the earlier batches. All blanks were reviewed and consistently demonstrated no outside source to explain changes in Zn, meaning there was no source of contamination accounting for the higher values. Standard solutions run by Health Canada as part of the routine ICP-MS quality monitoring were acceptable and therefore yielded no explanation. In order to report a consistent representation of the actual Zn

values present in each sample, a correction factor was applied to all of the home samples in the study to ensure there was no bias to any of the Zn values calculated. Based on the means of the entire batch of NIST 2584 and NIST 2710a (separately), a unique correction factor was calculated for each batch. The mean of all of the Zn values was the denominator and the value of Zn from a batch on a specific day would be the numerator then this value would be applied to the Zn values for the home samples run on the date of the batch for which the correction factor was calculated. The correction factor was calculated for both the G-alone and the GI phase and depending on which NIST was run with the samples per batch; the bioaccessible metal concentrations for the Zn values for the homes were recalculated.

3.4.3 Analytical Relative Standard Deviation

The analytical reproducibility in this study was acceptable (usually between 5-15% RSD) for all home, control house dust, and NIST dust and soil. The Mosque sample (n=8) is a heterogeneous control field dust sample with a relative standard deviation in bioaccessibility ranging between 12.6% (Zn) at the highest and 2.34% (Pb) at the lowest in the gastric phase and between 12.63% (Cd) at the highest and 6.21% (Zn) at the lowest in the GI phase. NIST soil and dust controls were used because they are produced in a controlled environment where they are ground and milled to give a homogenized product, and are therefore ideal for analytical reproducibility reference. NIST 2583 (n=11) Indoor Dust had a relative standard deviation range of 15.04% (Cd) at the highest to 7.18% (Cu) at the lowest for the G-alone phase and 12.02% (Zn) at the highest to 6.47% (Cu) at the lowest for the GI. NIST 2584 (n=17) Indoor Dust RSD range for the G-alone phase was 16.2% (Zn) and 7.58% (Pb), and 21.06% (Zn) and 12.01% (Cd) for the GI phase. NIST Montana Soil 2710a (n=18) RDS range in the G-alone phase was 17.9% (Zn) and 6.91% (Cu), while the GI phase ranged from 19.3% (Zn) to 10% (Cu). NIST Montana Soil 2710 (n=3) had an RSD range of 2.34% (Zn) to 0.99% (Cu) for the G-alone phase and 8.13% (Ni) to 3% (Pb) for the GI. The RSDs given here do not include the RSDs of controls with samples below the detection limit, which is why none of the Cr RSDs are displayed despite the high and wide range that appears in the tables.

4. Results

4.1 Total Metal Concentrations

Total metal concentrations were quantified by a private lab with a four-acid digestion as described earlier. In order to display the variability within this sample set (n=305) and within this study

(n=33), tables are broken down into two parts, where Table 1a shows the total metal concentration for the homes sampled from the Levallois *et al.* (2013) study and Table 1b displays the selected homes from this study. Although a smaller subset of samples was used from the original study, the sample selection, as described earlier, was done so that the variability between selected homes would be similar to that of the whole set and show the true variability between homes – individual values of home total metal concentration can be found in Appendix 2. Furthermore, since not all samples would necessarily have enough stored dust to allow for an extraction to be run in triplicate and would thus require the use of the subsequent closest number, metal means for this study found in Cr, Zn, and Pb have a slightly higher mean in the subset than for the whole study. The total metal concentration median for the metals in this study is similar to those found in cities around the world such as Sydney, Warsaw, Hong Kong, and Plymouth (Turner, 2011).

Table 1a. Summary of total metal concentration of floor dust from Montreal homes sampled by Levallois *et al.* (2013). Total metal concentrations provided by Rasmussen, unpublished data.

	Total Metal Concentration in ppm					
n = 305	**Zn**	**Pb**	**Cd**	**Cu**	**Ni**	**Cr**
Mean	993	380	4.57	276	79.4	80.6
Median	876	160	3.2	210	62.1	66.6
Max	5190	6585	38.6	5810	2120	868
Min	27.6	6.2	0.3	4.7	2.4	1.4
SD	605	716	4.32	386	134	68.7

Abbreviations: SD standard deviation

Table 1b. Summary of total metal concentration of floor dust in selected samples for this study

	Total Metal Concentration in ppm					
n = 33	**Zn**	**Pb**	**Cd**	**Cu**	**Ni**	**Cr**
Mean	1170	570	6.21	266	78.2	117
Median	929	183	3.05	213	59.5	86.4
High	5190	6585	38.6	1020	313	868
Low	27.6	6.2	0.3	4.7	2.4	1.4
SD	988	834	7.67	201	65.9	150

Abbreviations: SD standard deviation

4.2 Bioaccessible Metal Concentrations in the Montreal dataset

Tables 2a and 2b show the metal concentration after extraction for G-alone and GI phases, respectively. In order to have the best representation of the whole data set of the samples run for homes, if a ppm value was below the detection limit, in lieu of a zero value, half of the LOD was used as a representative for the metal. There are two LOD's in the tables which is explained in Section 3.4.1. Histograms of each metal and individual home bioaccessible metal concentration in ppm can be found in Appendix 3.

Table 2a. Summary of the bioaccessible metal concentrations after a PBET extraction of the G-alone phase from the selected samples with a solid to fluid ratio of 1:100 (g:mL). For samples < LOD .5 LOD was used and the two LOD's shown represent the reagent change and separate LOD calculation

	G-Alone Bioaccessible Metal in ppm					
Whole study (n=33)	**Zn**	**Pb**	**Cd**	**Cu**	**Ni**	**Cr**
Mean	1074	554	2.1	88.6	21.8	<LOD
Median	772	94.4	2.1	69.6	17	<LOD
Max	7250	5586	31.5	313	106	48
Min	331	20.2	0.07	7.39	1.44	8.39
90th percentile	2051	1593	14	189	45.6	34
Homes <LOD	6	8	0	1	1	28
LOD	533/292	23.3/29.9	0.1/0.07	7.6/1.94	0.71/0.96	26.2/27.7

Table 2b. Summary of the bioaccessible metal concentrations after a PBET extraction of the gastrointestinal phase from the selected samples with a solid to fluid ratio of 1:100 (g:mL). For samples < LOD .5 LOD was used and the two LOD's shown represent the reagent change and separate LOD calculation

	Gastrointestinal Bioaccessible Metal in ppm					
Whole study (n=33)	**Zn**	**Pb**	**Cd**	**Cu**	**Ni**	**Cr**
Mean	222	53.7	1.37	80	16.4	<LOD
Median	167	8.68	0.66	12.6	12.6	<LOD
Max	7250	5586	31.5	313	106	48
Min	331	20.2	0.07	7.38	1.43	14
90th percentile	443	152	4.42	167	38.2	27.8
Homes <LOD	6	13	3	1	1	27
LOD	79.6/51.7	8.65/5.65	0.14/0.05	4.2/1.08	0.67/0.6	28.6/27.9

4.3 G-Alone vs. GI for % Bioaccessibility in Home samples

Percent bioaccessibility was calculated for both the G-alone and GI phases for dust analyses from all 33 homes; Figures 4a-1f represent each metal and the homes within the study, only showing results above the LOD (in the numerator) for both the G-alone and GI side by side. Statistical analysis was performed using a one-tailed t-test on each metal for the G-alone percent bioaccessibility compared to the percent bioaccessibility of the GI in order to determine if there was a significant difference between the two phases. Calculated percent bioaccessibility for samples <LOD were not used since there would be too many estimated values used in order to predict the theoretical bioaccessibility and would give uncertainty to statistical analysis. Therefore, only the values calculated from homes above the LOD were used for comparisons using the t-test. The individual bioaccessibility values for each home can be found in a table in Appendix 4. These tables also include a calculated bioaccessibility from the readings below the LOD for the purpose of all data presentation but these values but as previously stated were not used in the t-test statistical analysis. The values for the one-tailed t-test can be found in Appendix 5.

Overall, for the majority of samples the G-alone phase has a higher bioaccessibility than the GI phase. In some cases a bias occurred based on the metals behaviour: for example, both Cd and Pb are metals that are known to have high bioaccessibility in the G-alone phase and low bioaccessibility in the GI phase. In the case of Cd, all three homes below the detection limit were only below the LOD in the GI phase but were well above the LOD in the G-alone phase. For Pb five of the thirteen below detection limit were only below the LOD in the GI phase. In these cases the homes were treated as below the LOD for both phases since it is not possible to make statistical comparisons between the G-alone and GI means with no value for the GI phase. There are some cases where the metal's bioaccessibility approaches or exceeds 100% and some in which the GI is higher than the G-alone; these cases will be reviewed in more detail below.

Zinc had a sample size of 27 homes above LOD, and as discussed in the data analysis, the samples were adjusted using a correction factor calculated based on NIST controls. The t-test of Zn shows a p value of <0.0001 on a one-tailed test: results show a strong statistical significance, with a 99% confidence interval, that bioaccessibility is greater in the G-alone phase than in the GI, with a median G-alone phase of 88% bioaccessibility compared to a median 16% bioaccessibility in the GI phase. With the G-alone phase at a pH of 1.8 compared to the GI with a pH of 7, these results—i.e., a higher bioaccessibility in the G-alone—were expected based on the chemical behaviour of Zn,

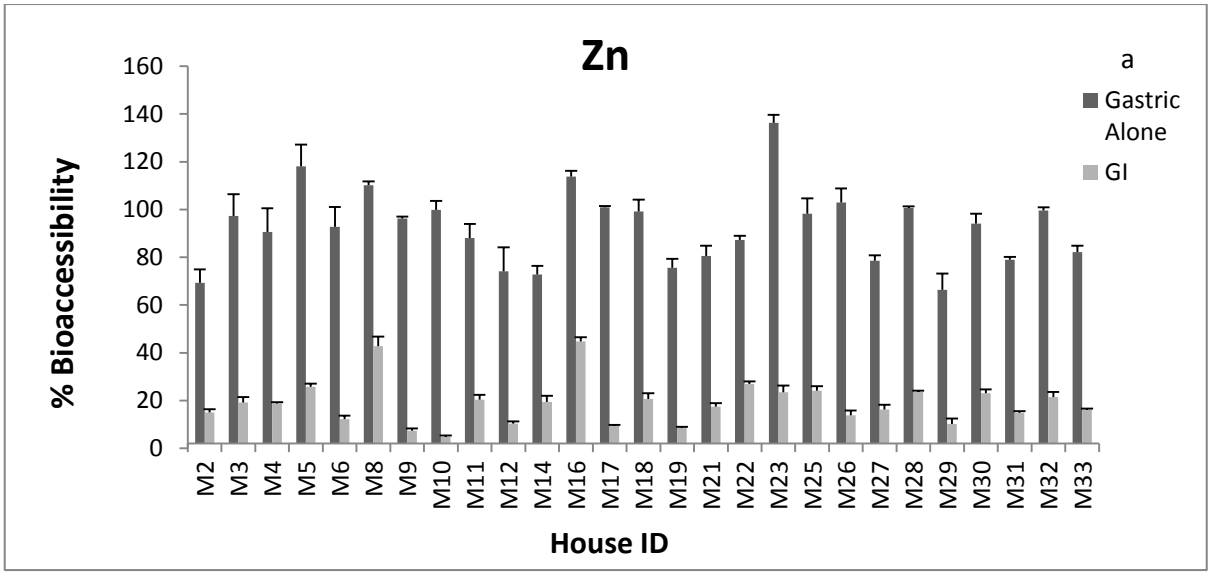
which is more soluble in lower pH environments. It should be noted that in some of the homes for Zn the percent bioaccessibility exceeds 100% for the G-alone phase. These values over 100% are likely the result of analytical uncertainty in the numerator and/or denominator in combination with the heterogeneous distribution of metals and minerals in the dust matrix. As described earlier in the literature review: metals such as Zn have a higher affinity for the organic matter inside the home, showing that in conjunction with the release of metals from indoor sources, such as consumer products and building materials, there will be a higher metal concentration than outdoors and also a higher bioaccessibility rate: nearly three times higher in Zn for dust over soil (Ibanez *et al.,* 2010; Rasmussen *et al.,* 2008). Furthermore it has been reported that calcium-bearing Zn minerals in house dust, which are commonly found in Canadian household dust samples, are readily solubilized in dilute HCl and give higher bioaccessibility in the G-alone phase over the GI (Oomen, *et al*., 2002; Rasmussen *et al*., 2014).

Due to its toxicity and ubiquitous presence in the environment, Pb has been one of the most studied metals in relation to human health risk, particularly in terms of oral bioaccessibility. From the 33 homes selected, 20 were above detection limit with a t-test one-tailed p-value <0.0001 at showing a strong statistical significance with a 99% confidence interval that bioaccessibility is greater in the G-alone phase than in the GI phase, where the median bioaccessibility of the G-alone is 37% versus a median bioaccessibility of 3% in the GI. Similarly, Cd had 29 samples above LOD that gave a t-test one-tailed p-value <0.0001 showing a strong statistical significance with a 99% confidence interval that the bioaccessibility is greater in the G-alone phase than in the GI phase, with a median G-alone bioaccessibility of 72% and a median GI bioaccessibility of 19%. Pb and Cd solubilities are strongly pH-dependent, where solubility increases with the acidic conditions (lower pH) of the G-alone phase; it is therefore expected that the bioaccessibility for these two metals would be significantly higher in the G-alone phase. It has also been suggested that the decrease in Pb and Cd bioaccessibility in the intestinal fluid could be a result of their complexation by biomolecules or chemical precipitation in the higher pH environment (Ellickson K.M *et al*., 2001, Koch *et al*., 2013).

Both Ni and Cu had 32 homes above the LOD and a t-test one-tailed p-value <0.0001 and a 99% confidence interval for Ni and a p-value of <0.005 in the 95% confidence interval for Cu, showing that the bioaccessibility in the G-alone phase is statistically significantly higher than in the GI phase. Ni G-alone %bioaccessibility median was found to be 34% and Cu at 27%, with GI %bioaccessibility of 29% and 21% respectively. As the 23rd ranked metal in the earth's crust, Ni is found in relatively

high abundance in the environment, and its prevalent oxidation state is Ni (II) (Adriano, 2001). Although Cu is less abundant naturally, both Ni and Cu have been used heavily in the automobile and construction industries; the gradual abrasion of buildings and vehicles has therefore led to high concentrations of both in urban areas (Okorie *et al*., 2011). The bioaccessibility levels of Ni and Cu in this study are higher than what has been found in previous soil and urban dust studies (Okorie *et al*., 2011; Poggio *et al*., 2009), but are similar to previous household dust studies (Rasmussen *et al*., 2008; Turner and Ip 2007). Both Ni and Cu have been found to have a high affinity for organic matter and carbon in dust, which could explain the higher bioaccessibility in theG-alone phase, as they will more readily dissolve in the low pH environment (Rasmussen *et al*., 2008; Turner and Ip, 2007; Okorie *et al*., 2011).

The only metal not to show a statistically significant difference between the G-alone and GI %bioaccessibility was Cr, with a p-value of 0.18. It is important to note that of the 33 homes tested, only 4 had bioaccessibility values for Cr above the detection limit and as a result the median values are zero for both G-alone and GI. The lack of samples above the detection limit is to be expected given the chemical nature of Cr, which has a low solubility, particularly in the trivalent state—the most commonly found natural state of Cr in the environment (Stewart *et al*., 2003). The two principal valence states for Cr found in the environment are hexavalent (Cr VI) and trivalent (Cr III) species; the low solubility of Cr III limits its mobility, while Cr VI is more soluble and mobile (Stewart *et al.,* 2003). Organic matter and surface-bound organics, which are often found in higher concentrations in indoor dust, are extremely effective at reducing Cr(VI) to Cr(III) under acidic conditions (Stewart *et al*., 2003). This could potentially explain the high number of samples below detection limit.



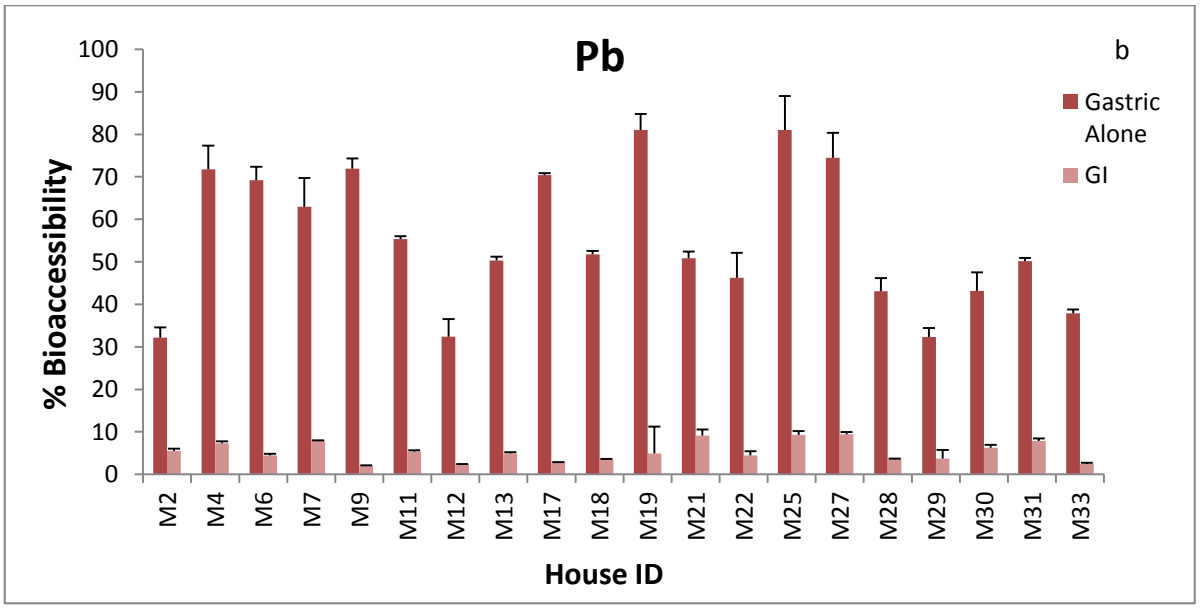
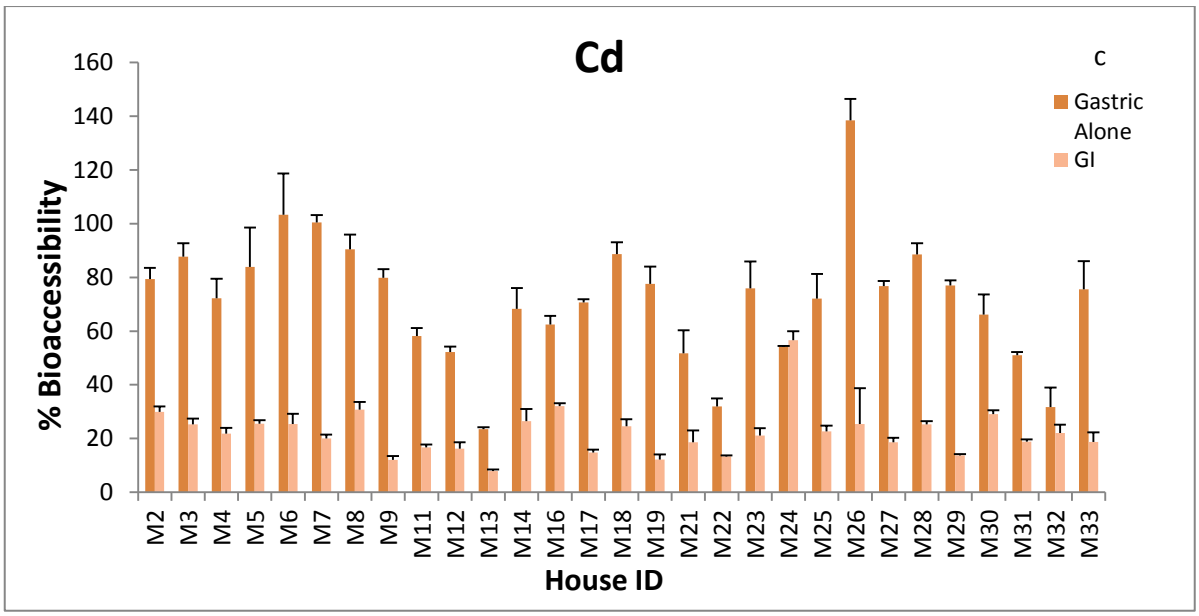


Figure 4a and b: % bioaccessibility of metals Zn(a), Pb(b), Cd(c), Cu(d), Ni(e) and Cr(f) from the PBET extraction with solid to fluid ratio of 1:100 (g:mL) in each home, where M represents each unique home. Bioaccessibility is defined as the concentration of soluble metal in the simulated gastric and/or gastrointestinal phase, expressed as a percentage of the total metal concentration. Error bars represent the standard deviation of the arithmetic mean of 3 replicates. Abbreviation: GI gastrointestinal



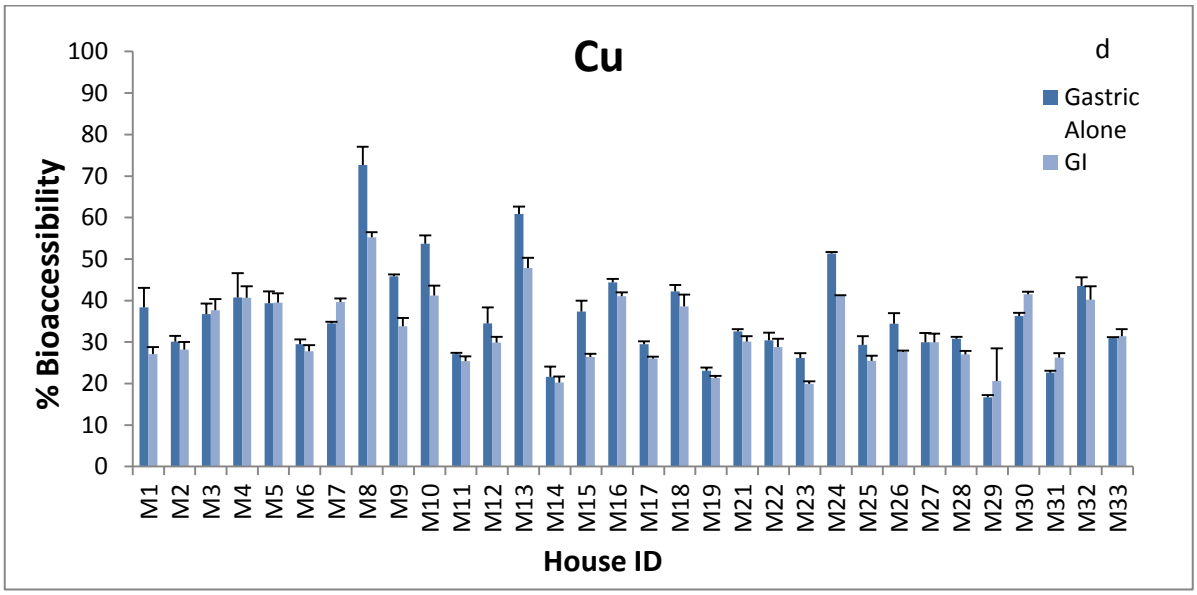
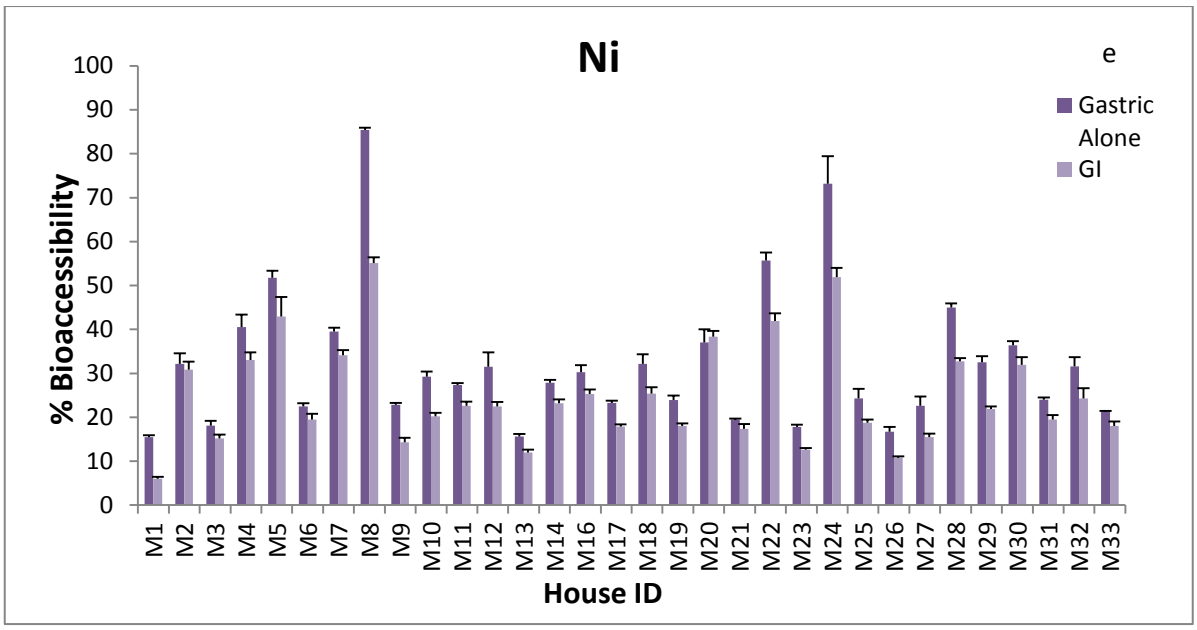


Figure 4c and d: % bioaccessibility of metals Zn(a), Pb(b), Cd(c), Cu(d), Ni(e) and Cr(f) from the PBET extraction with solid to fluid ratio of 1:100 (g:mL) in each home, where M represents each unique home. Bioaccessibility is defined as the concentration of soluble metal in the simulated gastric and/or gastrointestinal phase, expressed as a percentage of the total metal concentration. Error bars represent the standard deviation of the arithmetic mean of 3 replicates. Abbreviation: GI gastrointestinal



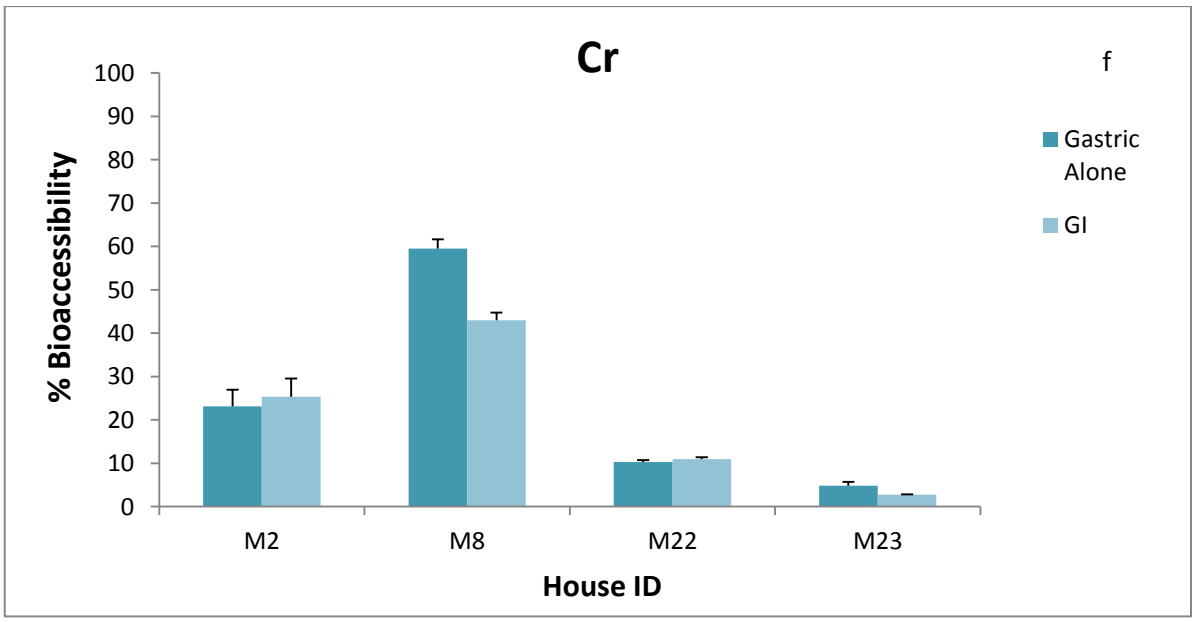


Figure 4e and f: % bioaccessibility of metals Zn(a), Pb(b), Cd(c), Cu(d), Ni(e) and Cr(f) from the PBET extraction with solid to fluid ratio of 1:100 (g:mL) in each home, where M represents each unique home. Bioaccessibility is defined as the concentration of soluble metal in the simulated gastric and/or gastrointestinal phase, expressed as a percentage of the total metal concentration. Error bars represent the standard deviation of the arithmetic mean of 3 replicates. Abbreviation: GI gastrointestinal

Figures 4a-f clearly display the variability of the G-alone and GI phases among the various homes, however it is also important to note the differences in bioaccessibility between the individual metals. Figure 5 displays the median of each metal in a side-by-side bar graph of the G-alone and the GI. In the case of homes below the LOD, a zero was used as a representative for that home when calculating the median. Differences between the metals' bioaccessibility is most likely due to the different chemical forms to which the metals are bound in the dust matrix; bioaccessibility variations are also an interesting indicator of the spatial variations between homes, which will be reviewed later in the discussion. Furthermore, it has been suggested that metals from anthropogenic sources will have higher rates of bioaccessibility, which could explain the high rates of bioaccessibility found in the home samples (Ljung *et al.*, 2007).

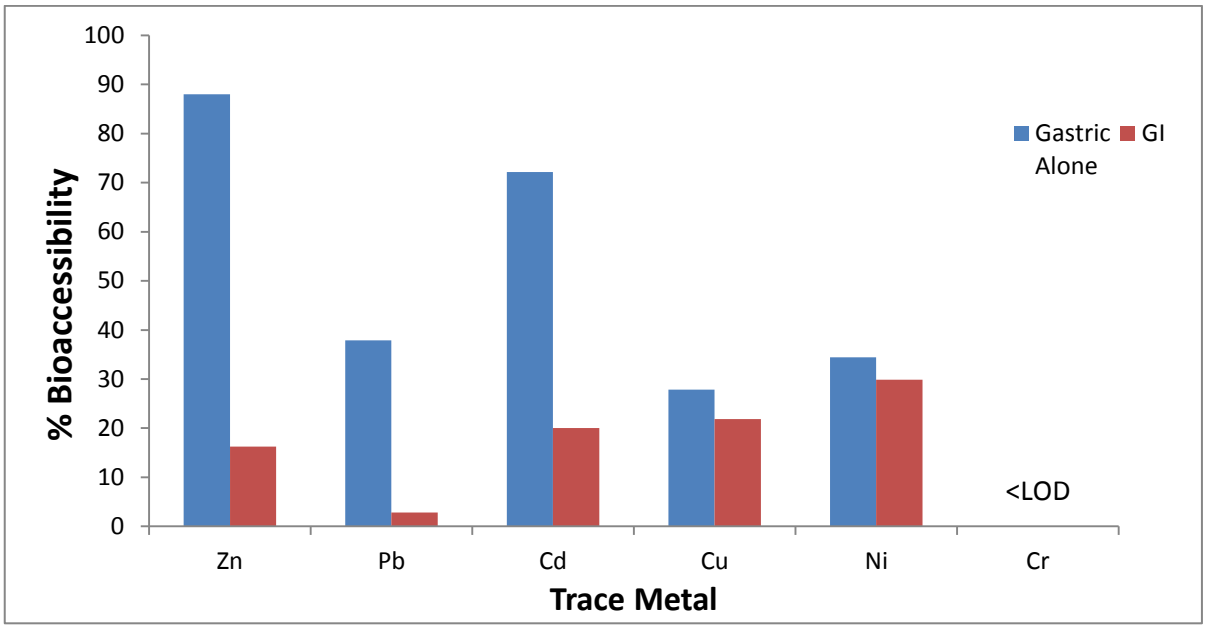


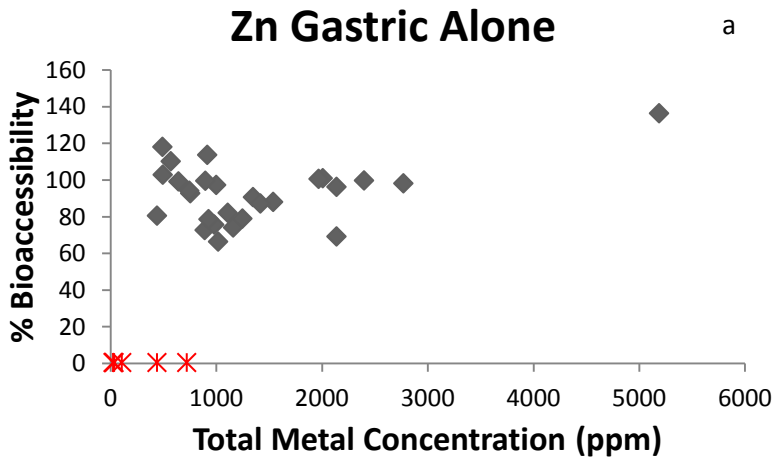
Figure 5: The median of each metal percent bioaccessibility from the home samples (n=33) at a solid to fluid ratio of 1:100 (g:mL) using the PBET method. Error bars represent the standard deviation for each sample. Abbreviation: GI gastrointestinal

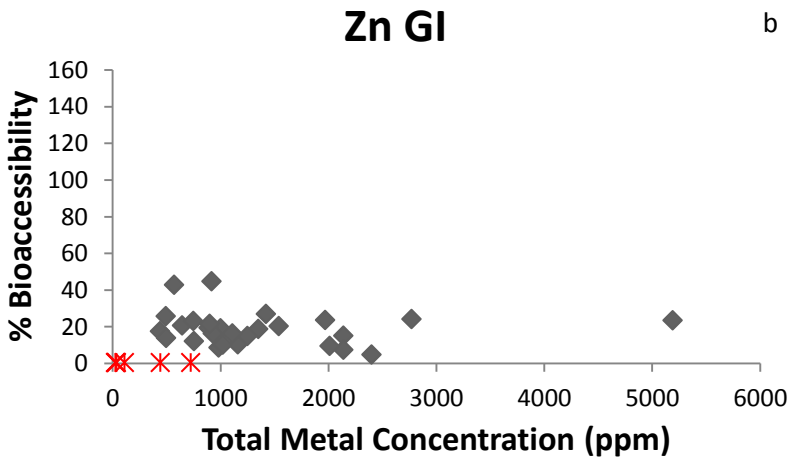
A Spearman rank coefficient (r) was calculated for each metal with the samples that were above the detection limit. The Spearman rank is a nonparametric analysis that measures the statistical dependence of two variables: in this case, G- alone and GI. For each metal the coefficient was calculated to be $-1 < r < 1$, where r is the observed value, meaning there was no correlation between the variables. For example: if the bioaccessible metal concentration increases in the G-alone phase,

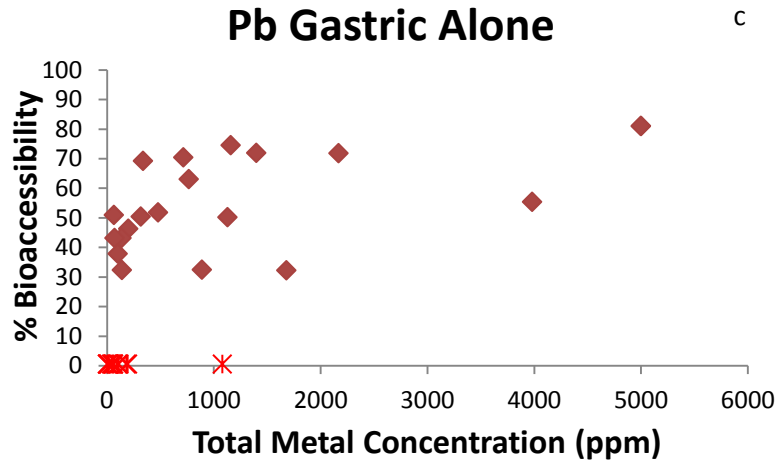
GI bioaccessible concentration is not expected to increase or decrease, as the variables are independent of one another.

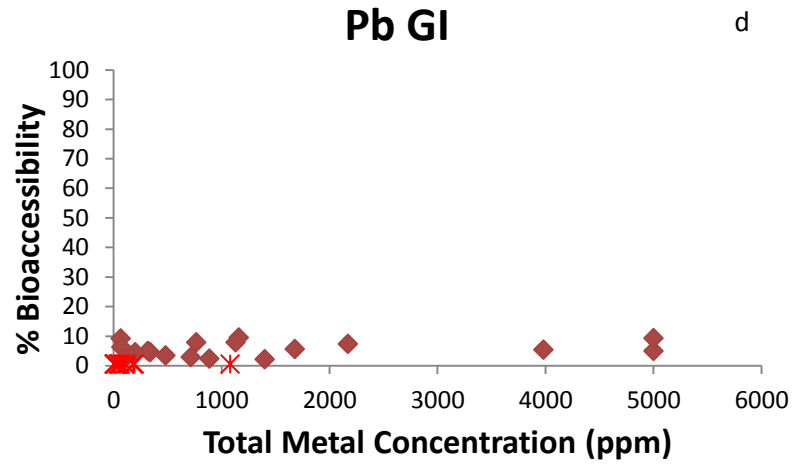
4.4 Bioaccessibility versus Total Metal Concentration

Total metal concentration was compared to % bioaccessibility in both the gastric and gastrointestinal phases for each metal, for all home samples above and below the LOD. Scatter plots for total metal concentration versus bioaccessibility in Figures 6 a-l below display the relationship between the two factors for both G-alone and GI. R values were calculated including homes above and below the LOD – all R^2 values for each metal in the home samples no trend could be observed. R^2values were also calculated only using homes above the LOD. This was done just to see if there are any possible trends in a metal. For R^2 values calculated only using bioaccessible portions above the LOD Pb, Ni, Cu, Cd, and Zn, there was no correlation to be seen with the R^2 value range between R^2=0.265 being the highest (seen in Pb for G-alone) and as low as R^2=0.015 (seen in Zn GI). The only metal to show a trend for the total metal concentration and the bioaccessibility above the LOD was Cr with R^2=0.865 for the gastric alone and R^2=0.987 in the gastrointestinal. These will results will be reviewed in more detail in the discussion.


Zn Gastric Alone
a
% Bioaccessibility
Total Metal Concentration (ppm)
0
20
40
60
80
100
120
140
160
0
1000
2000
3000
4000
5000
6000


Zn GI
b
% Bioaccessibility
Total Metal Concentration (ppm)
0
20
40
60
80
100
120
140
160
0
1000
2000
3000
4000
5000
6000


Pb Gastric Alone
c
% Bioaccessibility
Total Metal Concentration (ppm)
0
10
20
30
40
50
60
70
80
90
100
0
1000
2000
3000
4000
5000
6000


Pb GI
d
% Bioaccessibility
Total Metal Concentration (ppm)
0
10
20
30
40
50
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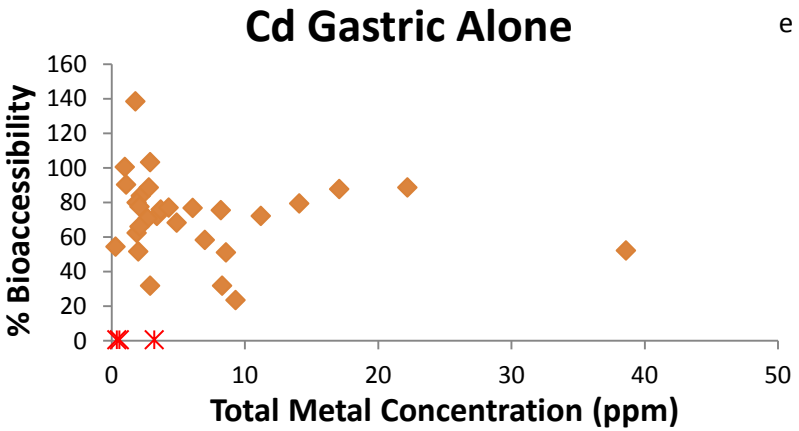
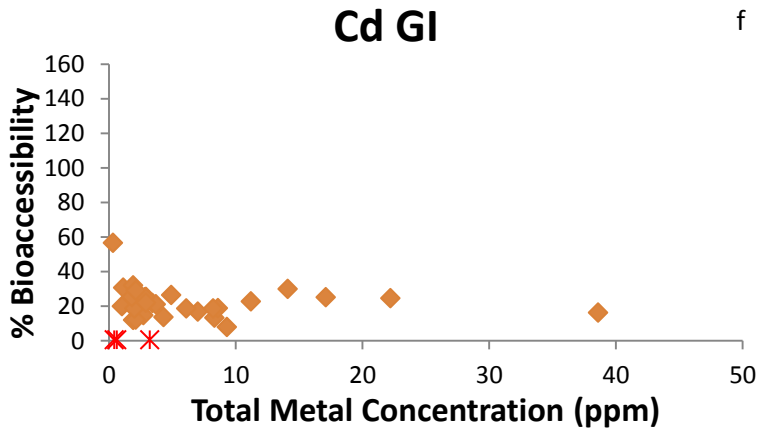
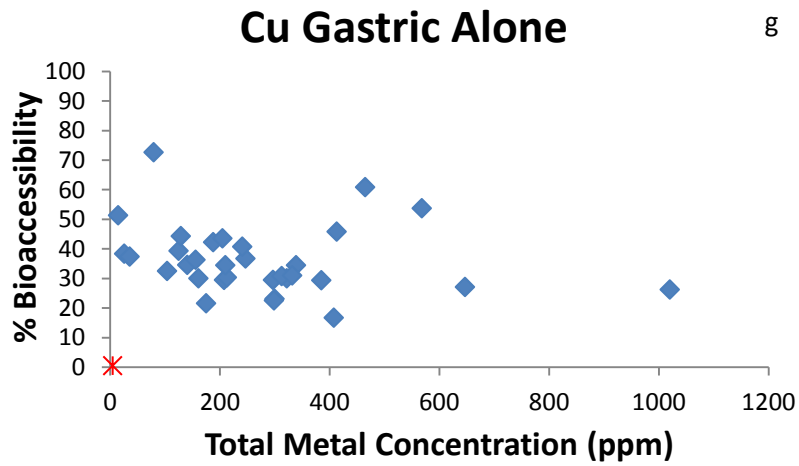
70
80
90
100
0
1000
2000
3000
4000
5000
6000


Cd Gastric Alone
e
% Bioaccessibility
Total Metal Concentration (ppm)
0
20
40
60
80
100
120
140
160
0
10
20
30
40
50


Cd GI
f
% Bioaccessibility
Total Metal Concentration (ppm)
0
20
40
60
80
100
120
140
160
0
10
20
30
40
50


Cu Gastric Alone
g
% Bioaccessibility
Total Metal Concentration (ppm)
0
10
20
30
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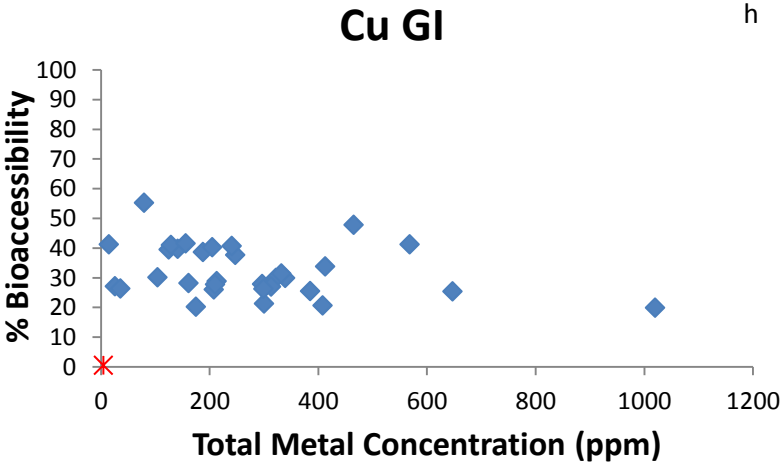
50
60
70
80
90
100
0
200
400
600
800
1000
1200


Cu GI
h
% Bioaccessibility
Total Metal Concentration (ppm)
0
10
20
30
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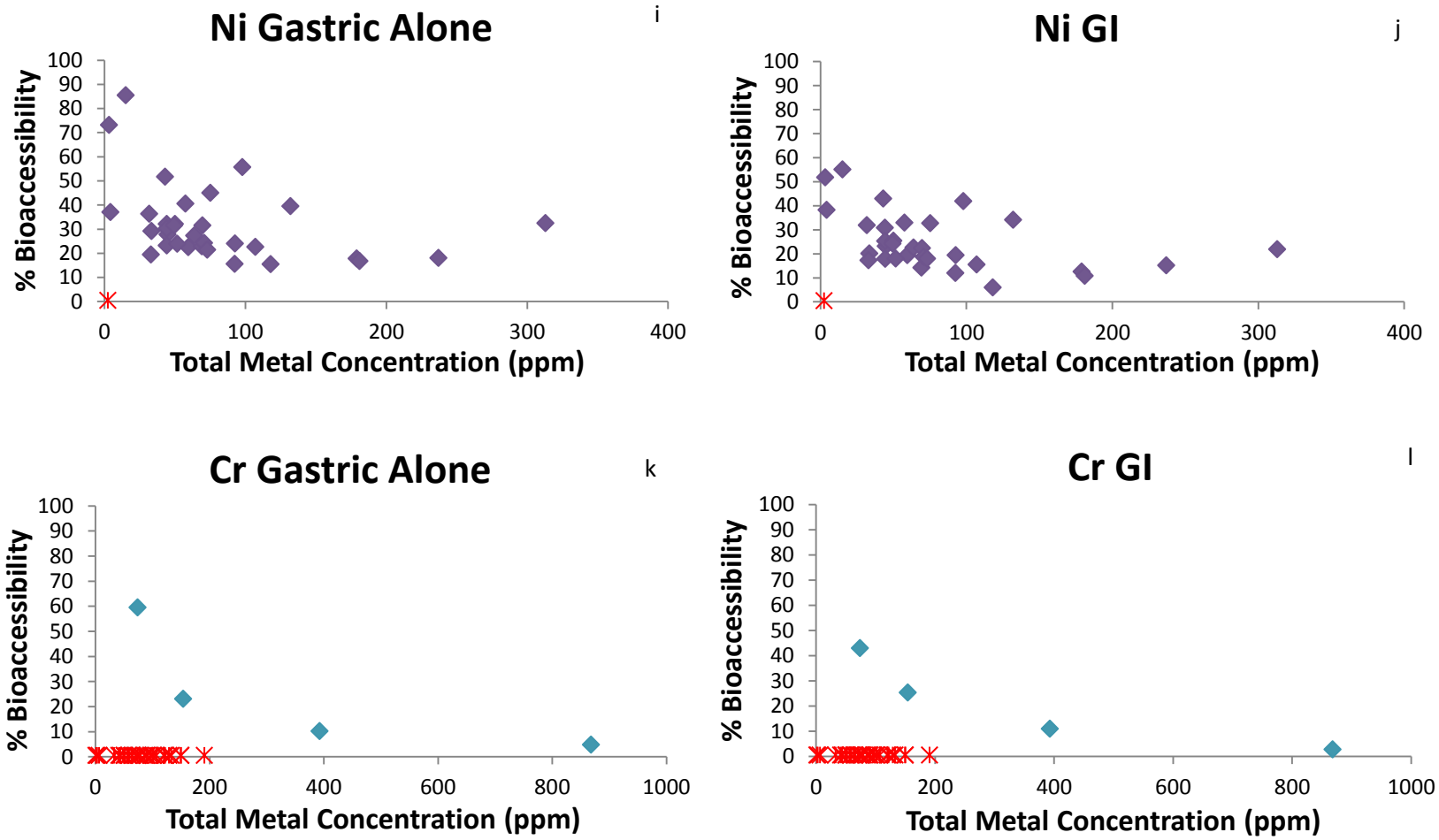
60
70
80
90
100
0
200
400
600
800
1000
1200



Figures 6a-l: scatter plots of the calculated % bioaccessibility against the total metal concentration. Inserted asterisk represents the metal concentration of home samples that were below detection limit. Abbreviation: GI gastrointestinal

4.5 NIST and Mosque Bioaccessibility

The bioaccessibility of the various NIST controls tested for G-alone and GI can be found in Tables 3a and 3b, respectively. Statistical analysis shows that for metals Zn, Cd and Pb, the G-alone phase is more bioaccessible than the GI. In the case of Cu, however, there was no statistically significant difference in NIST 2584, an indoor dust, and it is observed that the bioaccessibility is higher in the GI than in the G-alone. Cu having higher bioaccessibility in the GI phase than in the G-alone phase has been observed before in household dust (Poggio *et al.,* 2009; De Miguel *et al*., 2012; Li *et al*., 2013). However, there are no studies that I am aware of that have used NIST 2584 with this method and included Cu for comparison. In the case of Cr, it was below detection limit for all of the NIST samples in both the G-alone and GI phases. In addition to the Cr, some of the Ni samples were below LOD in the NIST 2710a and a few in Pb in NIST 2583. Although it was not all of the samples there were the more than 20% of the samples below the detection limit and Ni and Pb were therefore reported as below LOD for the respective NIST.

There are some noteworthy differences between the NIST soil and NIST dust bioaccessibility for both the G-alone and GI phase. For example, in the G-alone phase of Cd in NIST 2710a Montana Soil, there is a bioaccessibility of 44%, which differs from the NIST 2584 Indoor dust bioaccessibility rate of 79%; likewise, Zn bioaccessibility in the NIST 2710a soil is at 40% versus the NIST 2584 dust at 84%. Ni also exhibits this trend of higher bioaccessibility in dust over soil, with a bioaccessibility of 11% in soil and 22% in dust. These trends are seen in the GI phases as well. For Zn, Cd and Ni, this study confirms previous reports showing that the bioaccessibility of metals is higher in dust than in soil (Rasmussen *et al*., 2008; Dodd *et al*., 2013).

Table 3a. Percent bioaccessibility for the G-alone phase of metals from reference materials for soil and dust determined using the PBET extraction method with a solid to fluid ratio of 1:100 (g:mL)

		Metals bioaccessibility (%) - Gastric alone					
Sample		**Zn**	**Pb**	**Cd**	**Cu**	**Ni**	**Cr**
NIST 2710a	**Mean**	40.5	45.4	44.6	52.6	N/A	N/A
(n=18)	**SD**	7.26	3.55	3.98	3.64	-	-
	% RSD	17.9	7.83	8.93	6.91	-	-
NIST 2710	**Mean**	29.7	65	74.6	63.3	16.6	N/A
(n = 3)	**SD**	0.69	1.29	1.7	0.63	0.21	-
	% RSD	2.34	1.98	2.28	0.99	1.24	-
NIST 2584	**Mean**	84.6	56.8	79.1	38.9	22.4	N/A
(n = 17)	**SD**	13.7	4.31	8.5	3.16	1.81	-
	% RSD	16.2	7.58	10.7	8.14	8.09	-
NIST 2583	**Mean**	99.8	N/A	72.5	33.5	26.9	N/A
(n = 11)	**SD**	11.3	-	10.9	2.41	3.11	-
	% RSD	11.3	-	15	7.18	11.6	-
Mosque	**Mean**	178	46.5	88.2	49	36.3	N/A
(n = 8)	**SD**	0.24	0.01	0.04	0.02	0.01	-
	RSD	12.6	2.34	4.1	3.7	3.87	-

Abbreviations: SD standard deviation, %RSD relative standard deviation. N/A signifies metals that below the LOD.

Results of this study illustrate an overall trend whereby percent bioaccessibility is significantly lower in the GI phase than in the G-alone phase; this is as expected, given previous studies (Turner, 2011). Some metals demonstrate a more dramatic decrease than others: for example, Ni decreases less than 4% between the two phases, whereas Pb decreases by as much as 40% (from 45% to 5% in 2710a). Overall, the percent bioaccessibility of each metal from the different NIST controls is within range in comparison to other studies (Koch *et al.*, 2013; Turner, 2011; Ibanez *et al.,* 2010) which employed several different extraction procedures.

Table 3b. Percent bioaccessibility for the gastrointestinal phase of metals from soil and dust samples determined using the PBET extraction method with a solid to fluid ratio of 1:100 (g:mL)

		Metals bioaccessibility (%) - Gastrointestinal					
Sample		**Zn**	**Pb**	**Cd**	**Cu**	***Ni**	**Cr**
NIST 2710a	**Mean**	12.8	5.07	23.3	38.1	N/A	N/A
(n = 18)	**SD**	2.48	0.72	2.75	3.84	-	-
	% RSD	19.34	14.2	11.8	10.1	-	-
NIST 2710	**Mean**	11.5	15.7	39.4	44.6	12.5	N/A
(n = 3)	**SD**	0.75	0.47	2.02	1.6	1.01	-
	% RSD	6.47	3	5.14	3.59	8.13	-
NIST 2584	**Mean**	22.9	8.33	29.5	39.6	17.7	N/A
(n = 17)	**SD**	4.83	1.75	4.14	5.15	2.64	-
	% RSD	21.1	21	14	13	14.9	-
NIST 2583	**Mean**	43.3	N/A	39.8	31.3	21.4	N/A
(n = 11)	**SD**	5.21	-	4.74	2.02	2	-
	% RSD	12	-	11.9	6.47	9.35	-
Mosque	**Mean**	41.2	3.91	29.6	44.5	27.3	N/A
(n = 8)	**SD**	0.02	0	0.04	0.05	0.03	-
	% RSD	6.21	9.34	12.6	10.1	11.3	-

Abbreviations: SD standard deviation, %RSD relative standard deviation. N/A signifies metals that below the LOD.

4.6 Bioaccessible Metal Concentrations for NISTs and Mosque

Tables 4a and 4b show the metal concentration after PBET extraction. Values for Cr and Ni are displayed though in the case of Cr, all were below detection limit and some were below detection limit for Ni in NIST 2710a and for Pb in 2583. In these cases half of the LOD was used in place of a zero.

Table 4a. Summary of the bioaccessible metal concentrations after a PBET extraction of the G-alone phase from the NIST samples

		PPM Gastric Alone in ppm					
Sample		**Zn**	**Pb**	**Cd**	**Cu**	**Ni**	**Cr**
NIST 2710a	**Mean**	1691	2504	1050	1801	0.8	0.45
(n=18)	**SD**	303	196	1165	124	0.43	8.03
	RSD	17.9	7.83	110	6.91	50.7	1783
	Median	1834	2528	5.67	1823	0.9	5.7
NIST 2710	**Mean**	2066	3595	16.3	1867	2.37	6.67
(n = 3)	**SD**	48.3	71.3	0.37	18.5	0.03	0.18
	RSD	2.34	1.98	2.28	0.99	1.24	2.69
NIST 2584	**Mean**	2182	5544	7.91	124	20.1	11.4
(n = 17)	**SD**	354	420	0.85	10.1	1.63	8.08
	RSD	16.2	7.58	10.7	8.14	8.09	70.8
	Median	2273	5393	8.2	125	20.1	15.2
NIST 2583	**Mean**	894	18.8	5.29	78.1	25.2	14.4
(n = 11)	**SD**	101	7.71	0.8	5.6	2.92	4.17
	RSD	11.3	41	15	7.18	11.6	29.1
	Median	930	14.8	4.92	78.4	24.8	15.4
Mosque	**Mean**	953	42.7	1.32	64.5	11.7	15
(n = 8)	**SD**	139	4.8	0.15	4.49	1.32	1.47
	RSD	14.6	11.3	11.5	6.95	11.2	9.8
	Median	897	43.9	1.32	64.8	12	15.4

Table 4b. Summary of the total metal concentrations after a PBET extraction of the GI phase from the NIST samples

		PPM Gastro Intestinal in ppm					
Sample		**Zn**	**Pb**	**Cd**	**Cu**	**Ni**	**Cr**
NIST 2710a	**Mean**	535	278	2.86	1303	0.6	0.82
(n=18)	**SD**	104	39.7	0.34	131	0.32	8.27
	RSD	19.3	14.2	11.8	10.1	55.6	1006
	Median	538	277	2.79	1266	0.7	5.54
NIST 2710	**Mean**	802	868	8.58	1315	1.78	8.19
(n = 3)	**SD**	51.9	26	0.44	47.2	0.14	1.05
	RSD	6.47	3	5.14	3.59	8.13	12.8
NIST 2584	**Mean**	591	814	2.95	127	16	12
(n = 17)	**SD**	125	170	0.41	16.5	2.38	9.06
	RSD	21.1	21	14	13	14.9	75.4
	Median	601	745	2.95	119	16.2	15.6
NIST 2583	**Mean**	388	7.4	2.87	72.9	20.1	15.1
(n = 11)	**SD**	46.7	3.72	0.35	4.72	1.88	4.6
	RSD	12	50.3	12.2	6.47	9.35	30.6
	Median	398	4.3	2.89	74.6	20.7	16.6
Mosque	**Mean**	219	3.56	0.44	58.6	8.84	16.1
(n = 8)	**SD**	20.2	0.39	0.05	6.2	1.44	1.69
	RSD	9.2	10.9	12.5	10.6	16.3	10.5
	Median	217	3.53	0.41	59.3	8.45	16.3

5. Discussion

5.1 Gastric alone versus Gastrointestinal Bioaccessibility

The main objective of this study was to evaluate physiologically-based extraction techniques (PBET) for determining the bioaccessibility of metals in house dust. The overarching concern for risk assessors is whether to simulate passage of the particle-bound metal through the G-alone phase (pH 1.8), or to simulate passage through both the G-alone and GI (pH 7.0) phases. The goal of this experimental work was to determine which phase would yield the most conservative (protective) value when evaluating human health risk.

As pH is a key controlling parameter for metal solubility, it is expected that the selection of a G-alone or GI simulation will depend on the metal being considered, as different metals respond differently to changing pH conditions. Based on extensive comparisons with animal models, the US-EPA has determined that gastric alone is appropriate for testing Pb bioaccessibility in soil and dust at contaminated sites (US EPA 2008). Cd and Zn are similarly highly bioaccessible in the G-alone phase (Dodd *et al*., 2014; Turner and Ip, 2007; Rasmussen *et al*., 2004). However, several previous studies reported that Ni and Cr in dust and soil matrices demonstrated higher bioaccessibilities in the GI phase than in G-alone (Turner and Ip 2007; Poggio *et al.,* 2009; Karadas *and Kara* 2011). A review from 2011 also showed reports of Cu having higher bioaccessibility in the GI phase in household dust from the UK as well as Saudi Arabia (Turner, 2011).

Based on these previous studies, it was hypothesized at the outset of this thesis that G-alone would yield the most conservative (highest) bioaccessibility value for Pb, Zn and Cd, while in contrast GI would yield the most conservative bioaccessibility value for Ni, Cr and Cu. In fact, the experimental results show that G-alone is conservative for all 6 metals studied, in both soil and dust, as will be discussed in detail in the following section. These findings are in keeping with an unpublished 2011 report by the consulting group ENVIRON International Corporation: this report not only recommended that the type of research conducted in this thesis was necessary, but also predicted that further evaluation might demonstrate that G-alone is appropriate for Cr and Ni (ENVIRON 2011).

5.2 Bioaccessibility in the Gastric Alone Phase

Figure 5 compares the medians (with standard deviations) of metal bioaccessibility in the G-alone and the GI phases from the analysis of all home samples. The high degree of variation for a given metal as well as the lack of correlations among metals is consistent with the heterogeneous distribution of source materials. In comparing the medians, the order of bioaccessibility for the home samples was: Zn (88%) > Cd (72%) > Pb (37%) > Ni (34%) > Cu (27%) and Cr <LOD. The expected result would be an observably higher bioaccessible fraction in the G-alone phase, since metals are expected to hydrolyse at low pH and precipitate at higher pH (Poggio *et al*., 2009). The rate of diffusion into particles decreases with increasing ionic diameter: Pb > Cd > Zn > Cu > Ni > Cr also supports the results of the low bioaccessibility of Cu, Ni and Cr (Ljung *et al*., 2007). The high rates of bioaccessibility found in Zn and Cd will be discussed below.

The results from this study for both the home samples and the NIST samples are within the range of bioaccessibility found in previous studies (Rasmussen *et al*., 2008; Turner and Ip, 2007; Koch *et al*., 2013). Discrepancies between earlier studies and the results presented here are most likely related to differences in experimental pH, which was 1.8 in the stomach phase of the present PBET (representative of an "average" gastric state), but can range from 2.5 to 1 in the literature depending on the method of extraction. Differences in pH of this order can significantly affect the degree of metal mobilization from a variety of contaminated solids (Oomen, G. A. *et al*., 2002). Discrepancies between studies are common, and thus it is difficult to make sound comparisons between countries and periods given the lack of data on household dust and bioaccessibility (Ibanez *et al*., 2010).

In a few cases, the bioaccessibility of Cd and Zn was close to or in excess of 100%, suggesting that the simulated gastric solution was at least as efficient as the total extraction in solubilizing these metals, and also that the metal compounds were not evenly distributed due to the heterogeneity of the samples (i.e., the samples were not homogenized through crushing). It is characteristic of household dust to have high variability of metal concentration and bioaccessibility typically within the range of ±20-25% (Rasmussen *et al.*, 2013). Both Cd and Zn are found in high abundances naturally (in both soil and rocks), so in addition to the high concentrations that could be brought into the home, indoor source contributions could make for exceptionally high total metal concentrations. These indoor sources could also explain the high bioaccessibility as it has been suggested that metals from anthropogenic sources will have higher rates of bioaccessibility, which could explain the high rates of bioaccessibility found in the home samples (Okorie *et al*., 2007). Factors identified as influencing Cd and Zn metal concentrations in settled house dust samples include carpeting, roof type (galvanized), road type (if located near a busy street), rubber underlay, less ventilated homes, heating and air conditioning systems and balconies in the home (Ibanez *et al.,* 2010; Kim and Fergusson, 2003). As previously mentioned, Zn is found in such naturally high concentrations, and is so often associated with organic matter and calcium-bearing minerals in house dust—which are readily solubilized in acidic environments—that these high bioaccessibility values are to be expected (Rasmussen *et al*., 2008; Oomen, G. A. *et al*., 2002). In the case of Cd, some studies have suggested that the G-alone phase can sometimes over-predict bioaccessibility due to the high solubility of Cd in the acidic solution, and that high bioaccessibility values are therefore to be expected (Juhasz *et al*., 2010; Oomen, G. A. *et al*., 2002). Speciation also plays an important role with Cd, in that Cd is found to be highly soluble in carbonate species but relatively

low in solubility compared to its sulfides. As previously mentioned, household dust has a high organic and carbon content, so it likely that the Cd in the home samples case were associated with this organic and carbon portion resulting in high bioaccessibility (Juhasz *et al*., 2010; Rasmussen *et al*., 2008).

5.3 Bioaccessibility in the Gastrointestinal Phase

Overall, the metals in this study display a trend of reduction in bioaccessibility from stomach to intestine. Figure 5 illustrates that the bioaccessibility of the metals in the GI phase is also varied among the different metals: Cu (32%) > Ni (24%) > Cd (22%) > Zn (18%) > Pb (5%) and Cr <LOD. Compared with the G-alone phase, median bioaccessibility in the intestine is either lower (Zn, Pb, Cd, Ni) or similar – there are some exceptions wherein some individual homes it is higher (Cu, Cr). One of the main parameters in the GI environment that affects the bioaccessibility of trace metals is the pH, which is pronounced in the change from the G-alone to the GI phase (Ruby *et al*., 1999). In addition, speciation is important because the higher pH of the intestinal phase will dissolve organic matter, and contaminants bound to that matter will be released (Ljung *et al*., 2007). Cationic metals are solubilised through complexation with bile acid, and some metals may precipitate due to the high pH or phosphate present in the GI (Ljung *et al.,* 2007). There is also the possibility of re-adsorption of solubilised ions on charged surfaces, such as clay minerals and organic matter, in the high pH environment (Ruby *et al*., 1999).

The observed decrease in bioaccessibility in the GI phase has been previously reported for Pb in both soil and house dust (Koch *et al.,* 2013; Ruby *et al*., 1996), and is attributed either to re-adsorption or to precipitation as lead phosphates, such as chloropyromorphite [$Pb_5(PO_4)3Cl(s)$], in the intestine. Although phosphate was not a component of the present PBET, it is likely that quantities sufficient to effect precipitation were released from the dust samples. Cu did show statistical significance between G-alone and GI phases for the home samples where the bioaccessibility was higher in the G-alone phase; however, there are some cases in the home samples where the bioaccessibility is higher in the GI phase than in the G-alone phase. In addition, there was NIST 2583 (indoor dust) that did not show statistical significance between the G-alone and the GI phases. Cu having a higher bioaccessibility in the GI phase has been observed before (Turner and Ip 2007; Sialelli *et al.,* 2010; Li *et al.,* 2013). It has been suggested that Cu can be stabilized in solution to a greater extent by complexation with available organic ligands as the pH rises (Turner and IP, 2007). Furthermore, it has also been suggested that complexing agents can be

derived from the reagents used in the extraction procedure. For example, malic acid (which is used as a surrogate for digested sugars) includes the malate ion as well as anions of bile acids (such as chenodeoxycholate and hyodeoxycholate), and components of organic matter digested from the dust matrix all have the potential for complexation with metals (Turner and Ip, 2007). With respect to bile acids, polarographic measurements indicate that Cu complexes and aggregates are more stable than those formed by other heavy metals like Cd, Pb and Zn (Feroci *et al*., 1995).

There were also some cases for Cr where samples bioaccessibility was higher in the GI than in the G-alone phase. This may be attributed to the existence of negatively charged, polyatomic forms of these metals, such as CrO_4^{2-}/ $HCrO_4^-$ (Villalobos *et al*., 2001). The adsorption of Cr to dust particles is predicted to be promoted in a low pH environment and enhanced as the pH rises; the latter effect is particularly favorable in the intestine because of the abundance of carbonate, which acts as a competing ligand and/or sorbate (Turner and Ip, 2007). Application of a PBET to dust and soil samples from a former mining district of the UK has shown enhanced mobility of As in the intestine relative to the stomach (Wragg *et al*. 2011, 2000; Rieuwerts *et al*., 2006), consistent with the oxyanionic nature (hence anion-like adsorption) of Cr. The bioaccessibility of Cr is also largely dependent on the initial solid phase concentration of Cr before the simulated digestion extraction procedure. The oxidation state and soil or dust properties also have a strong influence on Cr's bioaccessibility. Cr(III) can be significantly reduced in bioaccessibility by its ability to strongly bind to organic matter as well as to Cr-hydroxides precipitates on the soil, whereas Cr(VI) can be quickly reduced to Cr(III) in organic-rich and Fe(II)-bearing minerals: this is important as Cr(VI) is considered more toxic, and Cr(III) will be more strongly absorbed and less bioaccessible (Stewart *et al.,* 2003).

Accurate information on intestinal uptake is lacking, but measurements of Cu and Pb in model solutions, with or without contaminated soil particles, suggest that complexation by specific ligands of dietary and non-dietary origin may be highly important for metals (Oomen G. A. *et al*., 2002). Accordingly, it has been suggested that free metal may be liberated from complexes in contact with the cell surface, or from oxidized minerals reduced within the bacterial mucilage covering the mucous membranes (Marschner *et al*., 2006). It is also possible that relatively small complexes may themselves be transported across the intestinal membrane via a passive, hydrophobic mechanism (Oomen *et al*., 2006).

Ultimately, the precise bioaccessibility of a metal depends on the mineralogical, biogenic and artificial phases in which the metal exists or is encapsulated, as well as the kinetics of release from

such under acidic and enzymatic conditions and in the presence of potential inorganic and organic complexing agents. In the higher-pH, carbonate-rich environment of the intestine, metals may be stabilized in solution by complexation, undergo re-adsorption to pre-existent or altered sites at the particle surface, or precipitate as relatively insoluble compounds. Results observed in the bioaccessibility between the G-alone and GI phase in Cu and Cr where in some cases the bioaccessibility in the GI is higher, should not be interpreted as a need for the intestinal phase, since pronounced differences have been seen for other samples, and results are sample-dependent both within this study and in others (Meuneir *et al*., 2011). A follow-up study measuring speciation and changes of speciation of Cu and Cr in house dust would help to clarify bioaccessibility trends; such research activities, however, are beyond the scope of this study.

5.4 Total Metal Concentration versus Bioaccessibility

It has been suggested that when using a smaller volume-to-mass ratio, there is the potential to exceed the saturation point for the contaminant investigated, particularly if the substance has low solubility (Richardson *et al*., 2006). Previous work done for the Ontario Ministry of Environment reported that with increased soil concentrations of Ni, there was also a decrease in the metal's bioaccessibility: high-concentration samples and low liquid-to-solid ratios could therefore be a limiting variable in bioaccessibility, and potentially lead to underestimations of bioaccessibility. From this report, Richardson *et al.* go on to suggest that in order to ensure the validity of the bioaccessible results, a range of contaminant concentrations should be tested as a means of confirming that bioaccessibility is independent of solubility limitations.

As described earlier, the selection of home samples was done in consideration of the total metal concentration for all metals to ensure variability of each metal's concentration range was represented. Since Ni and Cr both have low solubility and could potentially exceed the saturation points, Ni was reviewed as well. The total metal concentration versus %bioaccessibility in this study clearly showed that the liquid-to-solid ratio was not a limiting factor for Ni since no trend line was established. Comparisons between the total metal concentrations and the %bioaccessibility suggest that it is the low solubility of Cr itself that prevents the metal from going into solution in bioaccessibility extraction procedures, and not the liquid-to-solid ratio (there are samples below the LOD, for instance, that have either higher or similar concentrations to the four samples above the LOD).

Other studies that had similar results with higher bioaccessibility of Cr at lower concentrations have suggested that it is the adsorption process of Cr that is the dominant process and therefore the limiting step (Stewart *et al*., 2003). It was suggested that at low surface coverage (< 20%), Cr (III) adsorption processes began forming inner-sphere complexes with the soil, while at higher surface coverage (> 20%) there was surface precipitation (Stewart *et al.*,2003; Fendorf *et al.,* 1994). The same study concluded that the mechanism of Cr sequestration has a higher proportion of inner-sphere bonds related to precipitated phases, which could cause a higher percent of Cr(III) that is bioaccessible at lower solid phase concentration. However, the study in question was performed on a soil matrix that was spiked with Cr solution in a controlled environment, and it is difficult to predict if that is the same mechanism that took place in this study—particularly since there were so many samples within the range of bioaccessible readings but still below the detection limit.

Multiple studies that have evaluated Cr and Ni bioaccessibility in both soil and dust could not find a correlation between total metal concentration and bioaccessibility without taking other properties into account, such as clay content, pH, organic matter and oxides (Cox *et al*., 2013; Ljung *et al*., 2007; Tuner and Ip, 2007).

5.5 Analytical Reproducibility versus Spatial Variation

Analytical reproducibility in this study was very good, as evidenced by consistently low RSD values for both the home and control samples (Tables 3a-b, Appendix 4). Of all the metals, Zn typically shows the highest RSD in the home samples. The Mosque control dust and all four NIST reference materials displayed RSDs that were typically below 15%, with the highest RSDs associated with Zn (12.4% RSD in Mosque and 17.9% in NIST 2710a; Tables 3a-b). The low RSD values contribute to reducing uncertainty in the comparison of bioaccessibility results between the G-alone and GI phases for an individual home: analytical error is low, and the reproducibility and consistency of the extraction procedure was very good throughout the batches.

The RSD for each individual home sample was calculated based on mean and standard deviation for triplicate analytical runs, with 89% of samples below 10% RSD for each metal and the remainder of the samples having RSD values below 20%. There were two exceptions: the RSD for Cd in one sample was 25% and the RSD for Pb in another sample was 28%, both in the GI phase. The RSD calculated for each metal based on the mean and standard deviation in all 33 home samples provide an indication of the spatial variability. Depending on the metal the RSD varies greatly: in the G-

alone phase, Pb has the highest RSD in the G-alone phase with 95% followed by Ni with 52%, Cu and Zn at 50%, and Cu with the lowest at 35%. The RSD remains fairly consistent in the GI phase with only minor changes compared to the G-alone RSD; RSDs for Ni and Cu all displayed a small decrease compared to the G-alone, while RSDs for Zn, Pb and Cd all displayed an increase compared to the respective G-alone RSD. It is interesting to note that the metals with the more pronounced decreases in bioaccessibility from G-alone to GI are also the metals to observe an increase in the arithmetic RSD.

As previously discussed, total metal concentration is not a good predictor for the bioaccessibility of metals in ingested house dust, and the RSD values within a metal dataset further demonstrate the true differences in sources, pathways and speciation amongst the metals. Sources of bioaccessible metal within the home are of great concern in terms of human health risk assessment; it is also interesting to note that the age of a home may play an important role as well, due to the presence of older substances such as lead-based paints or exposed copper wiring (Ibanez *et al,.* 2010). Furthermore, in new homes, due to a general lack of air flow there is the potential to increase the humidity and depending on the season there is the potential for speciation change over time, which could also lead to an increase in bioaccessibility (Rasmussen *et al*., 2014; Beauchemin *et al*., 2014)

6. Conclusion

The results of this study have the potential to suggest a significantly more cost- and time-effective means of bioaccessibility evaluation for human health risk assessment. Our data show with statistical significance that for the metals Zn, Pb, Cd, Cu and Ni, G-alone is the more conservative evaluation for bioaccessibility in household dust. These experimental results, in accordance with previous studies, show that pH is one of the most important factors controlling bioaccessibility, where the higher the initial pH starting point, the higher the bioaccessibility (Turner, 2011; Koch *et al*., 2013). Our conclusions indicate that the G-alone component, with a pH 1.8 is the most important evaluation tool, and that GI is not in fact required.

The implications of this finding for human health risk assessment are important not only in terms of a potential reduction in the time required for extraction procedures, but also in terms of overall cost reduction. Taking into consideration the cost of reagents and standards for the extraction procedure, the use and maintenance of sensitive equipment (pH probes, balance, ICP-MS), the use of standards for sample evaluation, the extension of the extraction procedure by an additional 5

hours, the necessity of running additional samples on the machine, the use of more technicians and the cost of their salaries, and finally, the time spent analyzing the data, the finding that a simple gastric-phase extraction yields a conservative estimate of bioaccessibility should generate significant financial and temporal savings for future researchers.

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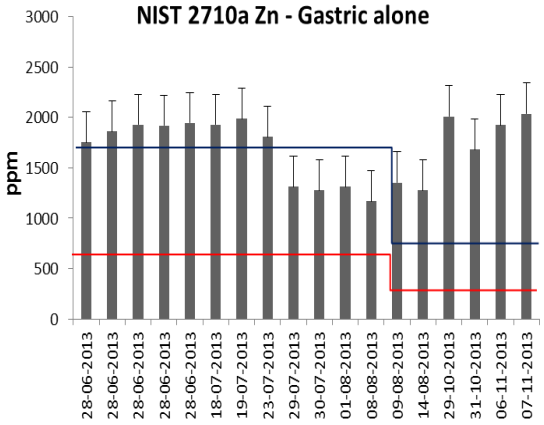
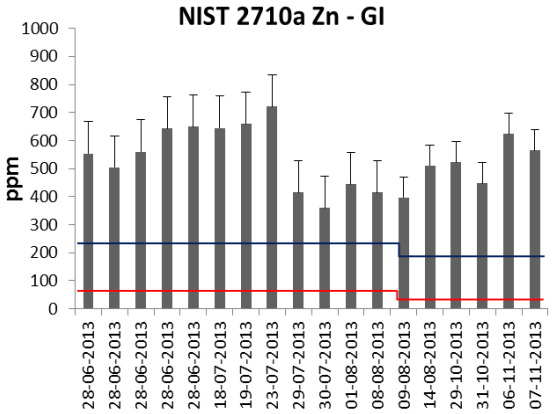
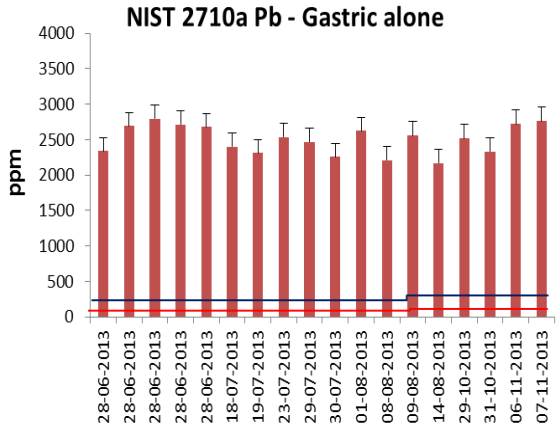
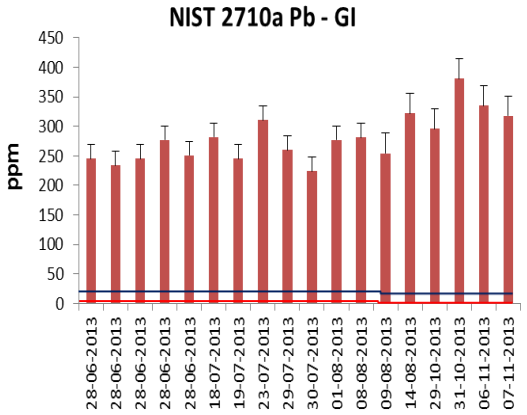
8. Appendices

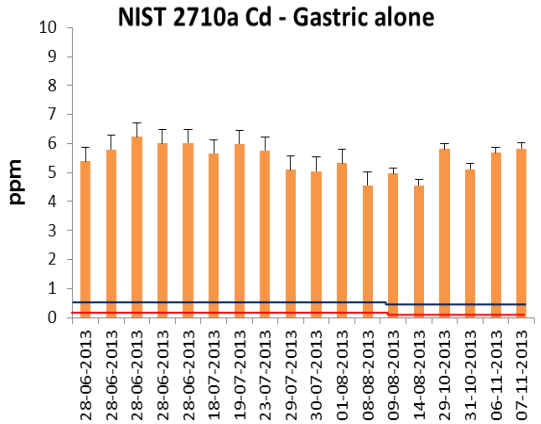
Appendix 1. Bioaccessible metal concentrations in ppm from the NIST samples.

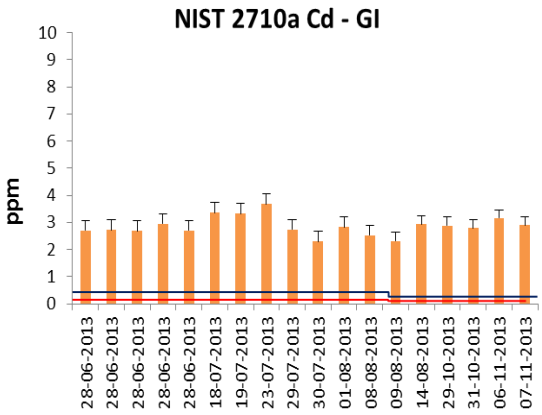






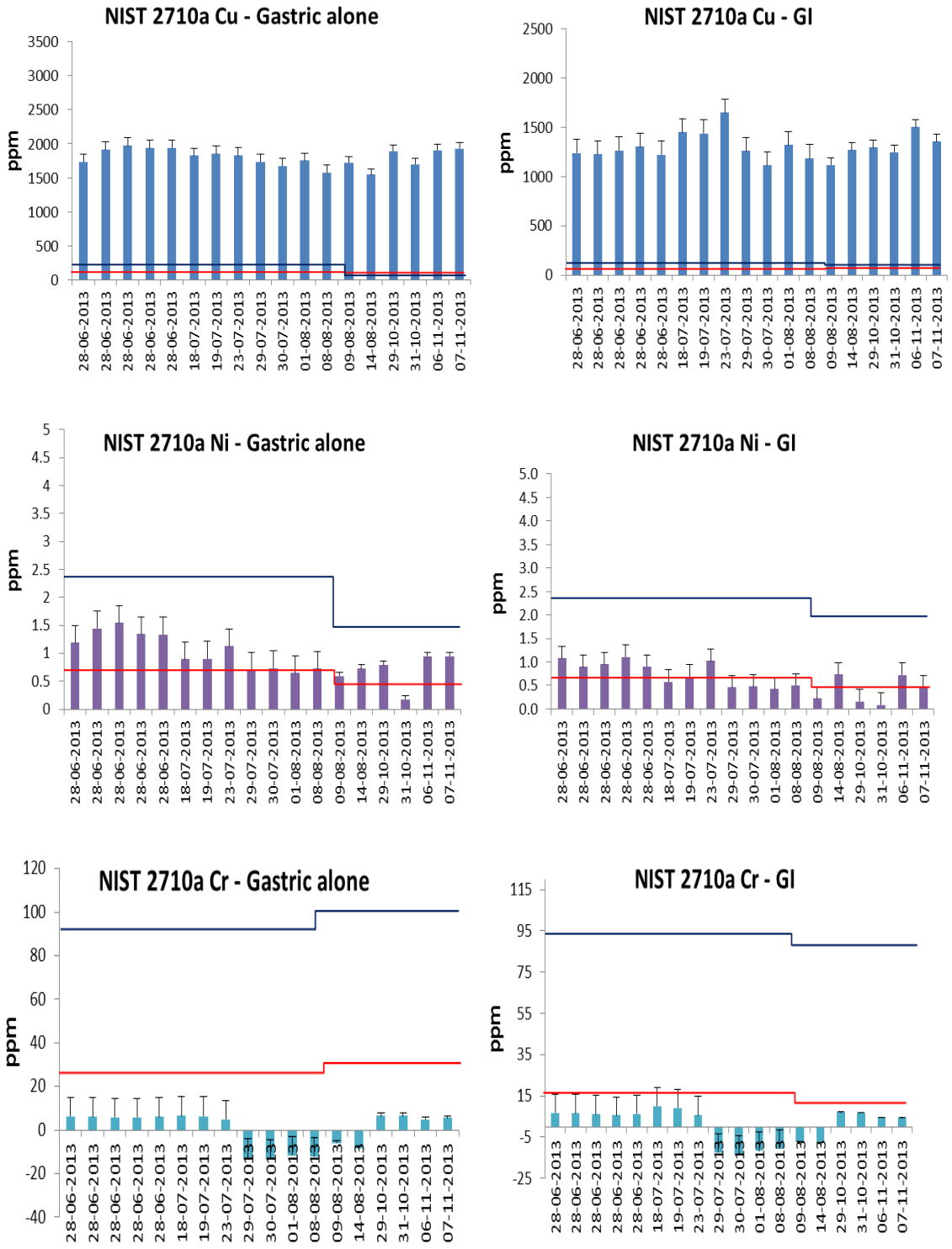






Limit of Detetection (LOD)

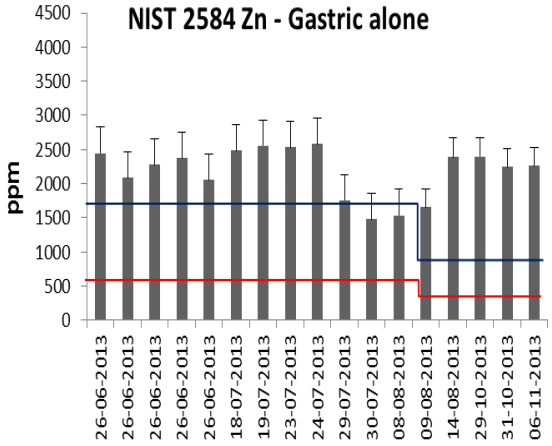
Limit of Quantification (LOQ)

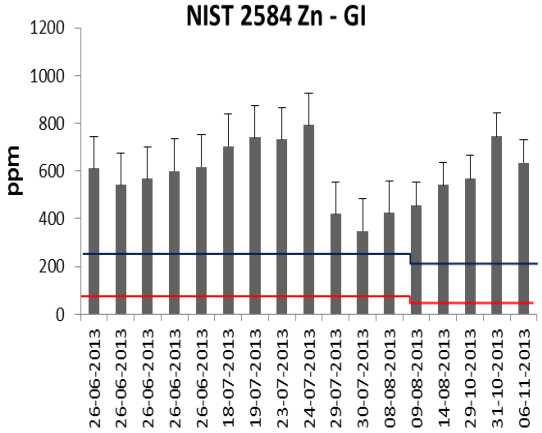


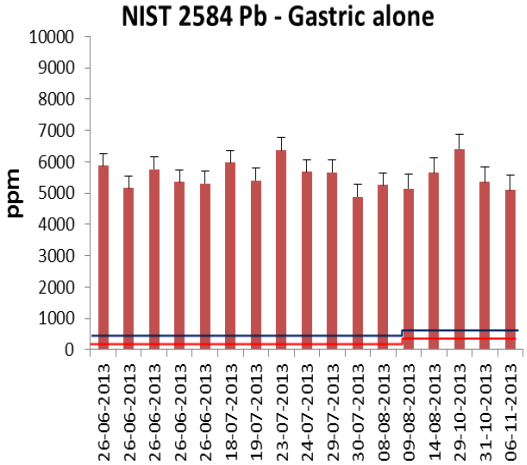
Appendix 1a. Bioaccessible metal concentration after PBET extraction for NIST 2710a Abbreviation: GI gastrointestinal. Error bars represent the standard deviation of the replicates within each metal.

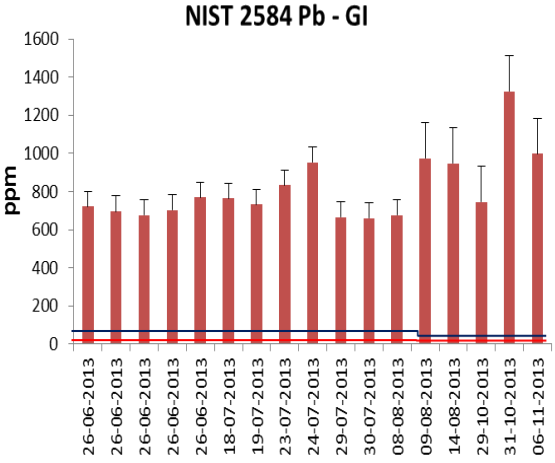
Limit of Detection (LOD)

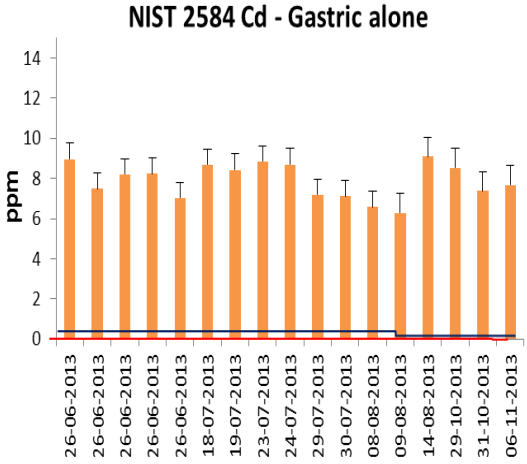
Limit of Quantification (LOQ)

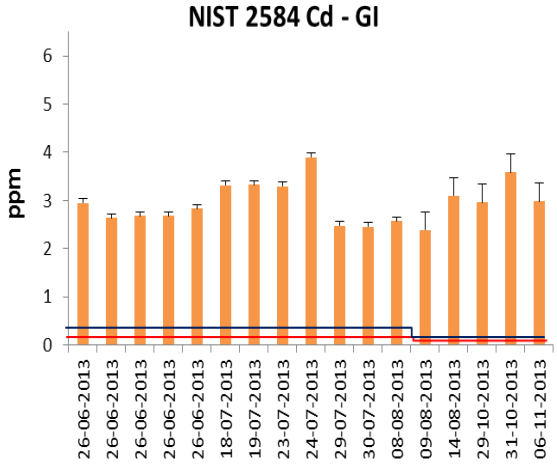












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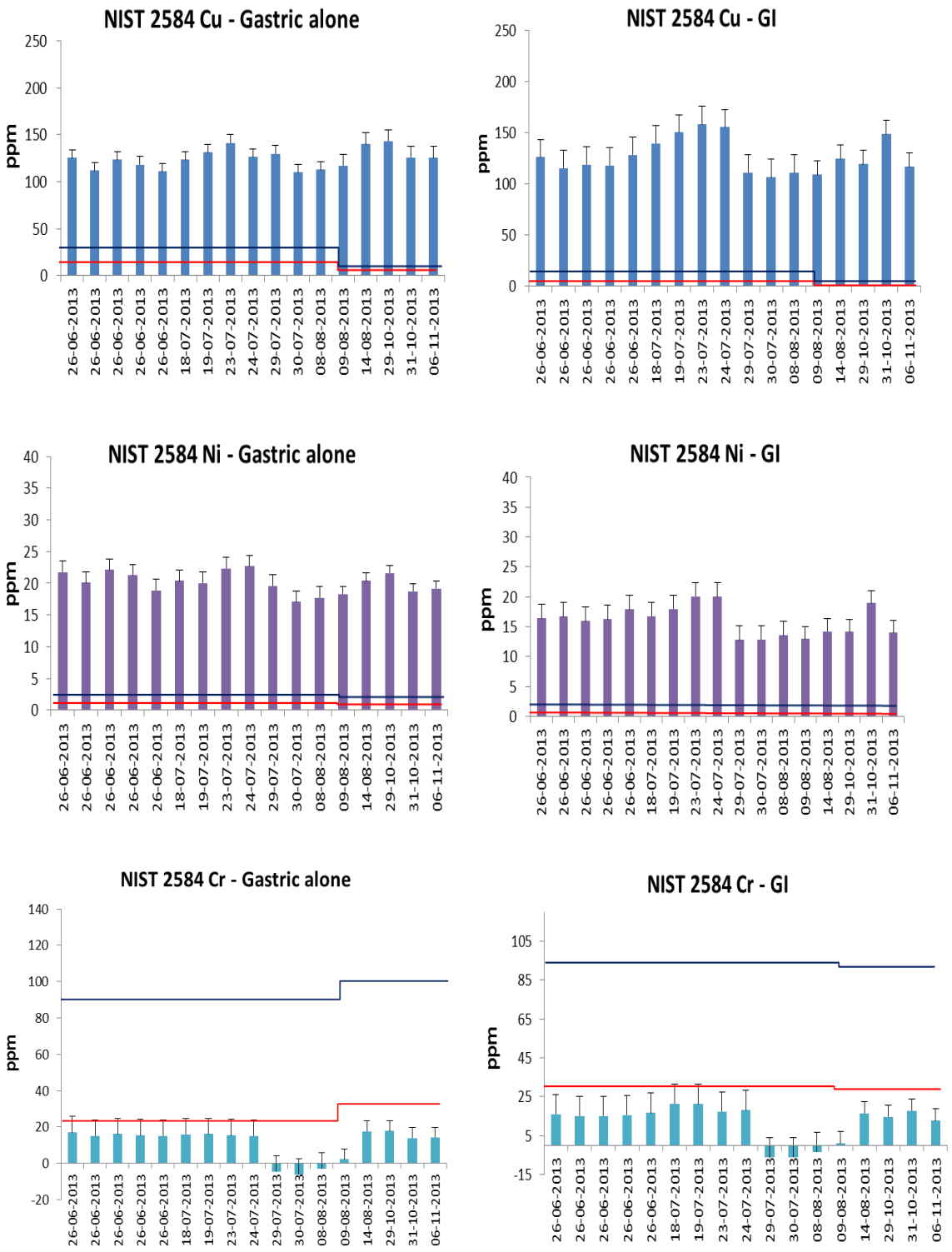
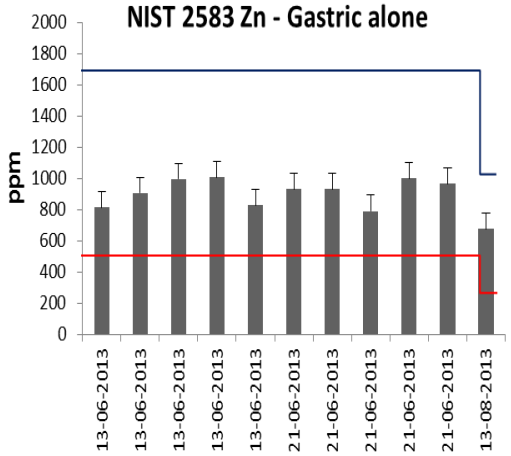
Limit of Quantification (LOQ)

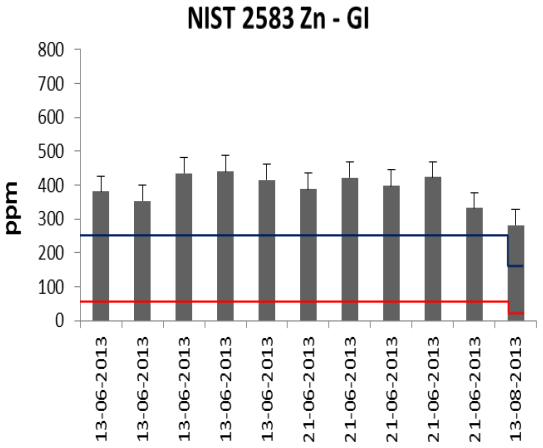


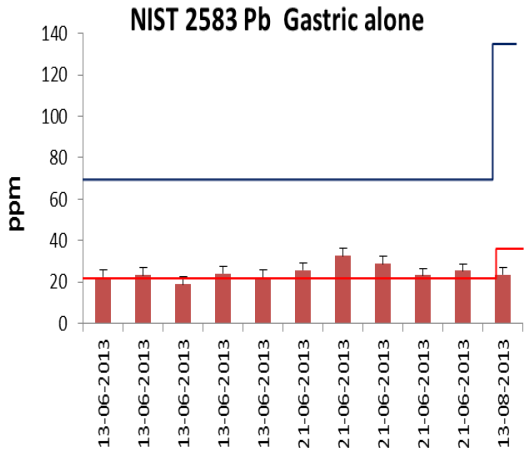
Appendix 1b. Bioaccessible metal concentration after PBET extraction for NIST 2584 Abbreviation: GI gastrointestinal. Error bars represent the standard deviation of the replicates within each metal.

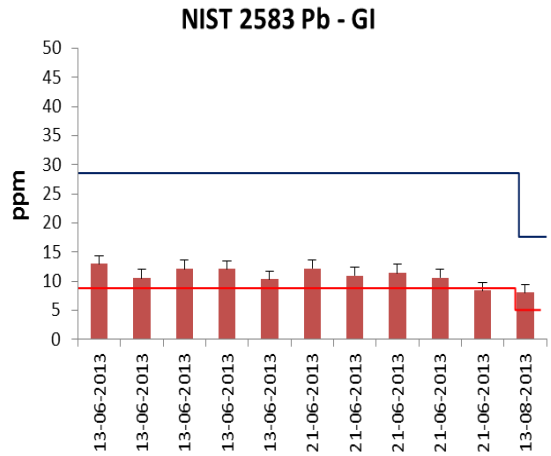
Limit of Detection (LOD)

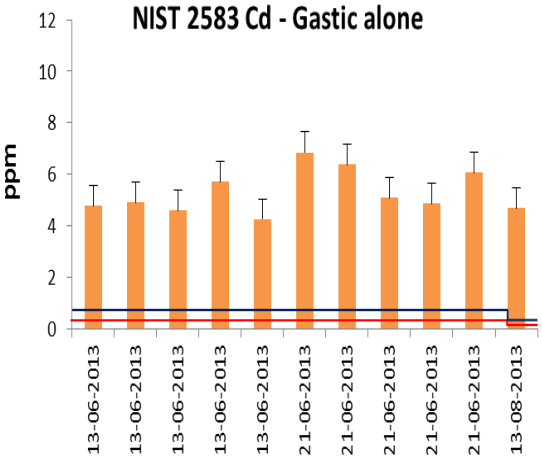
Limit of Quantification (LOQ)

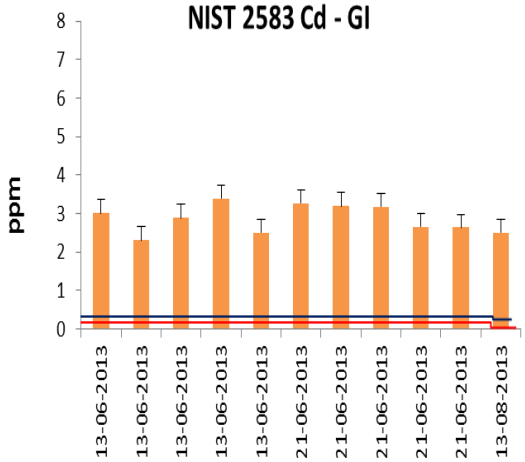






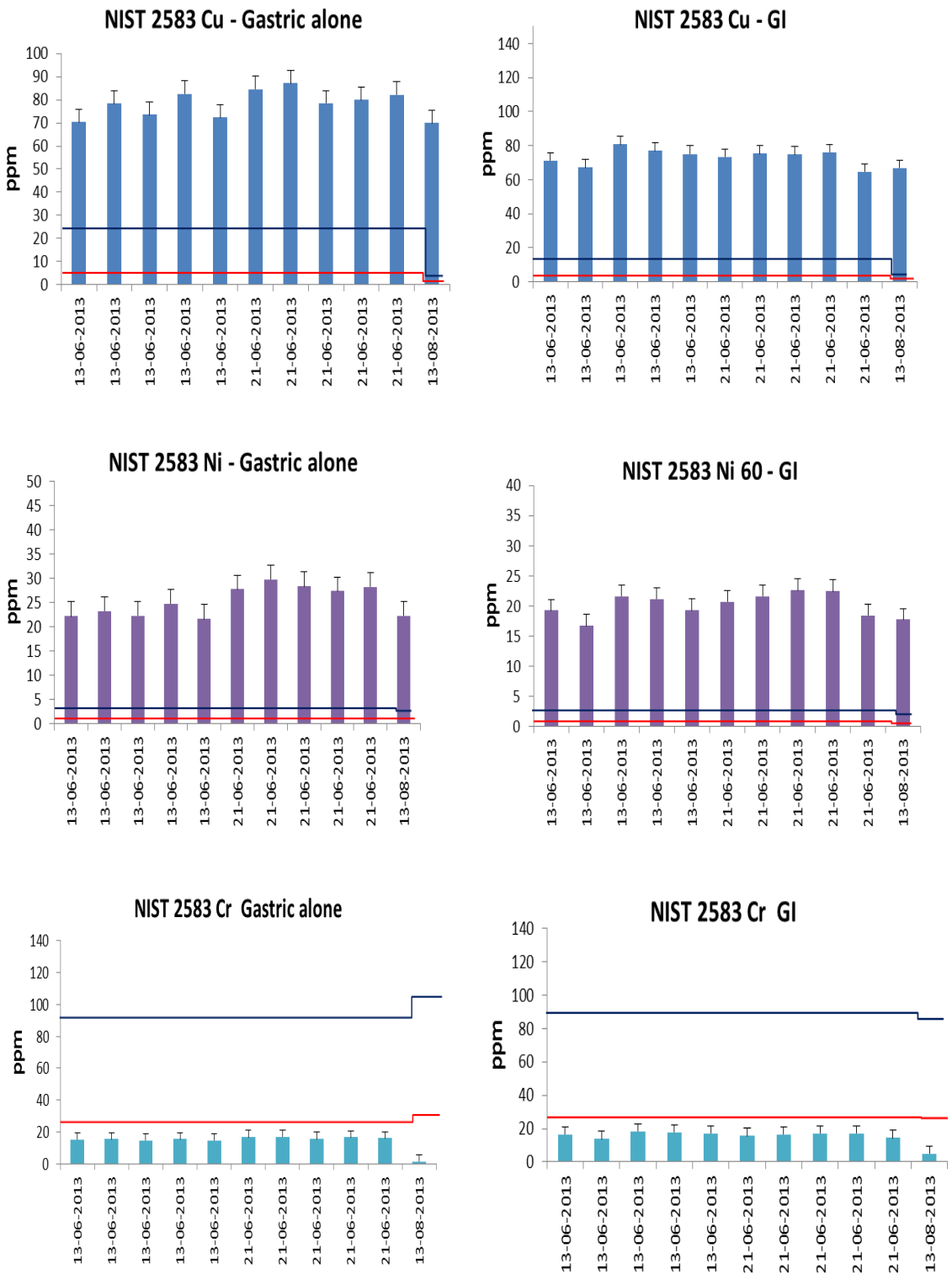






Limit of Detection (LOD)

Limit of Quantification (LOQ)



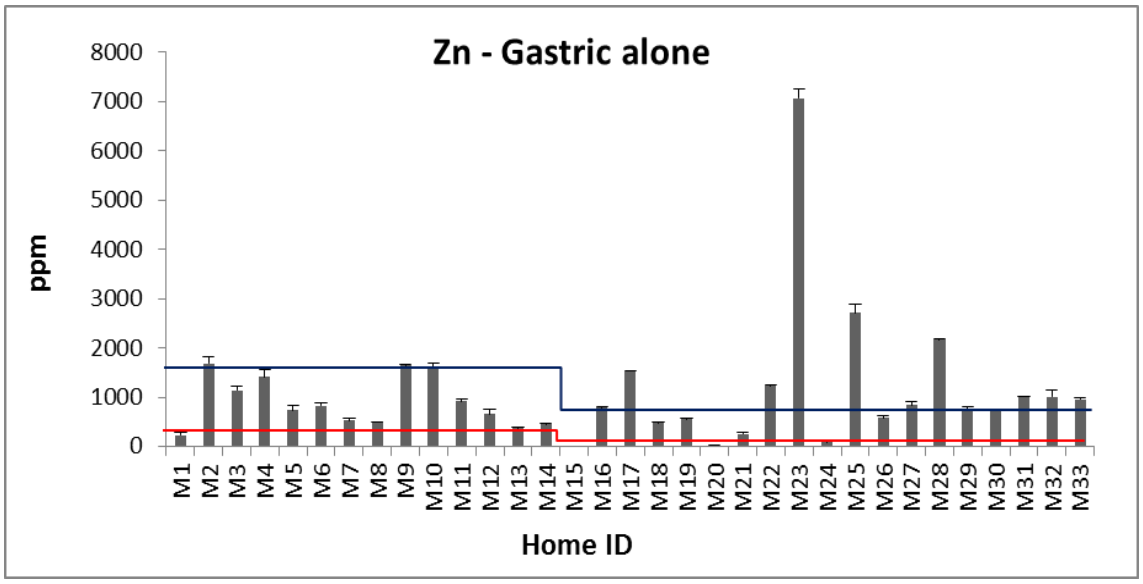
Appendix 1c. Bioaccessible metal concentration after PBET extraction for NIST 2583 Abbreviation: GI gastrointestinal. Error bars represent the standard deviation of the replicates within each metal.

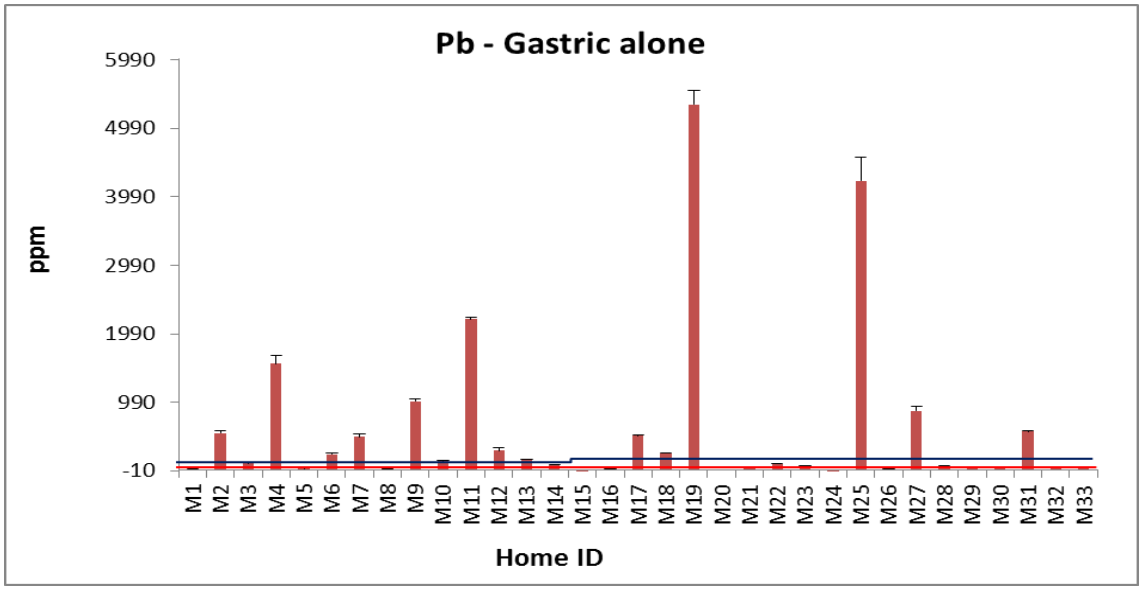
Appendix 2. Table for the total metal concentration of the selected samples

House ID	Sample ID	Zn	Pb	Cd	Cu	Ni	Cr
M1	**1300-04411A**	109	13.2	0.6	26.1	118	133
M2	**1300-04292**	2140	1680	14.1	161	44.2	154
M3	**1300-01244**	1000	183	17.1	247	237	50.9
M4	**1300-02863**	1350	2170	3.4	241	57.5	86.4
M5	**1300-04570**	493	61.3	2.2	125	42.9	41.6
M6	**1300-03846**	755	338	2.9	297	59.5	64.6
M7	**1300-04339**	442	767	1	141	132	120
M8	**1300-01150**	571	43.4	1.1	79.5	15.1	73.9
M9	**1300-03226**	2140	1400	1.9	413	69.1	43.8
M10	**1300-03948**	2400	1080	3.2	568	33.4	78.7
M11	**1300-03949**	1540	3980	7	647	63.7	91.1
M12	**1300-03023**	1160	888	38.6	339	69.5	89.9
M13	**1300-04168**	724	318	9.3	465	92.5	102
M14	**1300-02609A**	891	195	4.9	175	44.8	50.1
M15	**1300-03779B**	34.7	6.7	0.4	36	2.4	6.9
M16	**1300-01250**	917	52.3	1.9	129	44.3	71.3
M17	**1300-04071**	2010	716	2.7	208	44.3	58.6
M18	**1300-02412**	643	480	22.2	188	50	64.8
M19	**1300-03371**	982	6585	2.1	300	51.6	57.6
M20	**1300-04113**	27.6	16.3	< 0.1	4.7	4.3	1.4
M21	**1300-04472**	440	65.4	2	104	32.9	33.1
M22	**1300-03495**	1420	199	8.3	213	97.9	393
M23	**1300-01581**	5190	102	3.7	1020	179	868
M24	**1300-02893**	30.1	6.2	0.3	14.5	3.2	3.1
M25	**1300-04214**	2770	5216	11.2	385	70.7	74.5
M26	**1300-4172**	496	63.1	1.8	210	181	150
M27	**1300-4611**	929	1160	6.1	322	107	191
M28	**1300-2127**	1970	136	2.8	313	75.2	107
M29	**1300-3800B**	1020	140	4.3	408	313	122
M30	**1300-1552**	748	72.9	2.1	156	31.7	129
M31	**1300-4647**	1250	1130	8.6	299	92.6	150
M32	**1300-1211**	899	109	2.9	205	49.4	101
M33	**1300-2298**	1110	102	8.2	332	73	97

Appendix 3

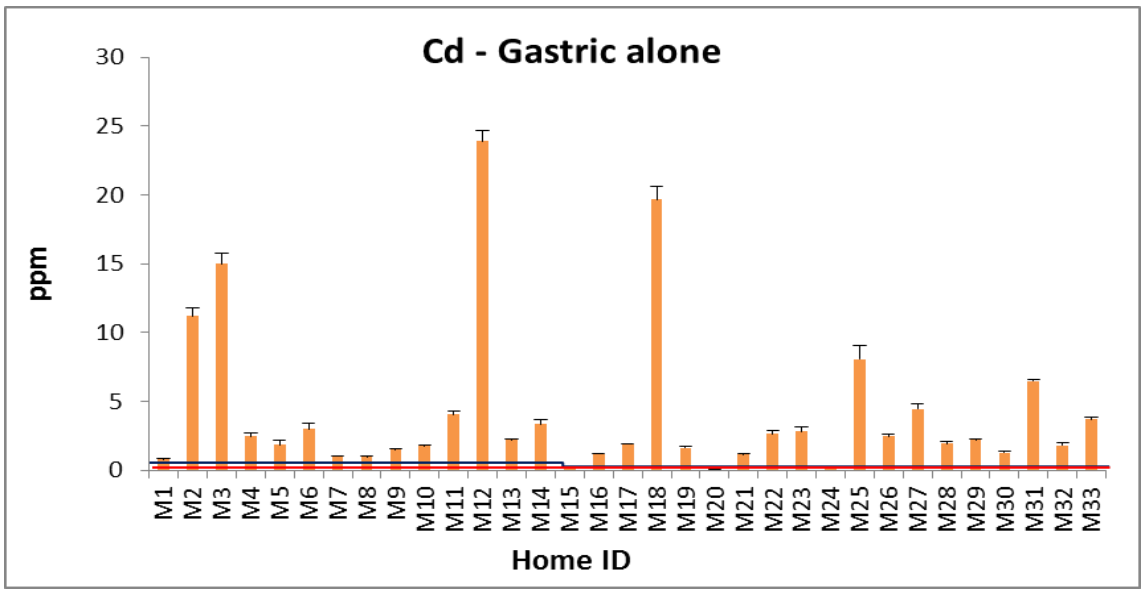
Appendix 3a. Bioaccessible metal concentrations in ppm after a PBET extraction of the gastric alone phase from the individual home samples.

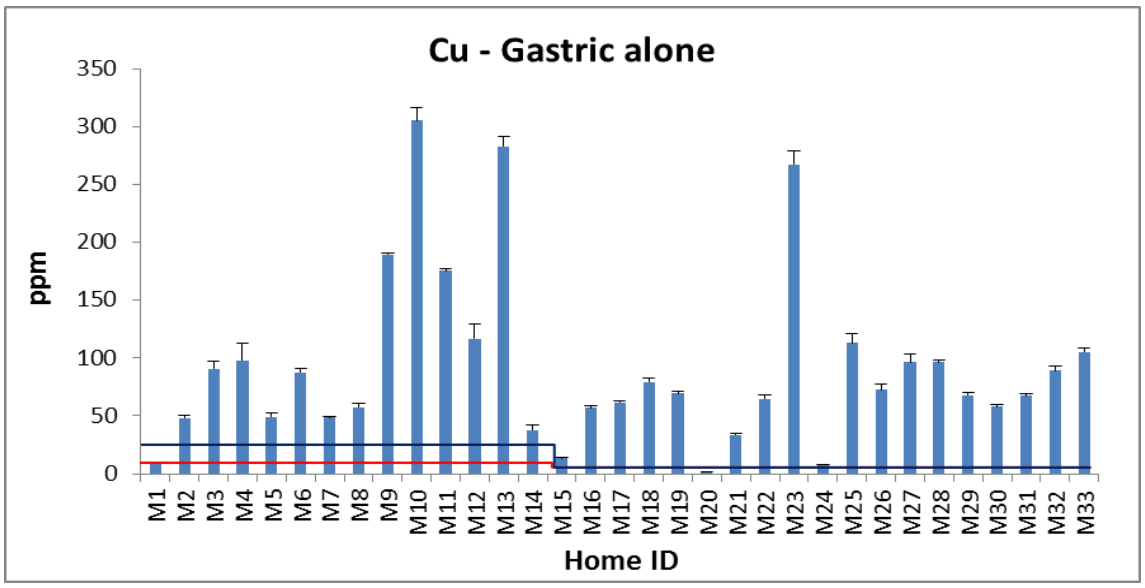




Limit of Detection (LOD)

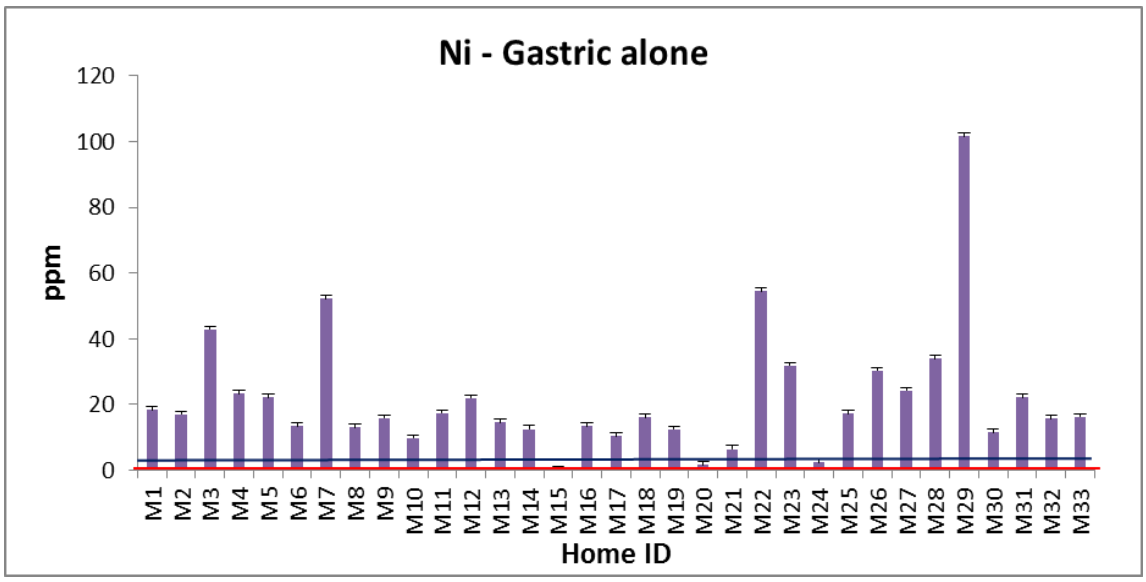
Limit of Quantification (LOQ)

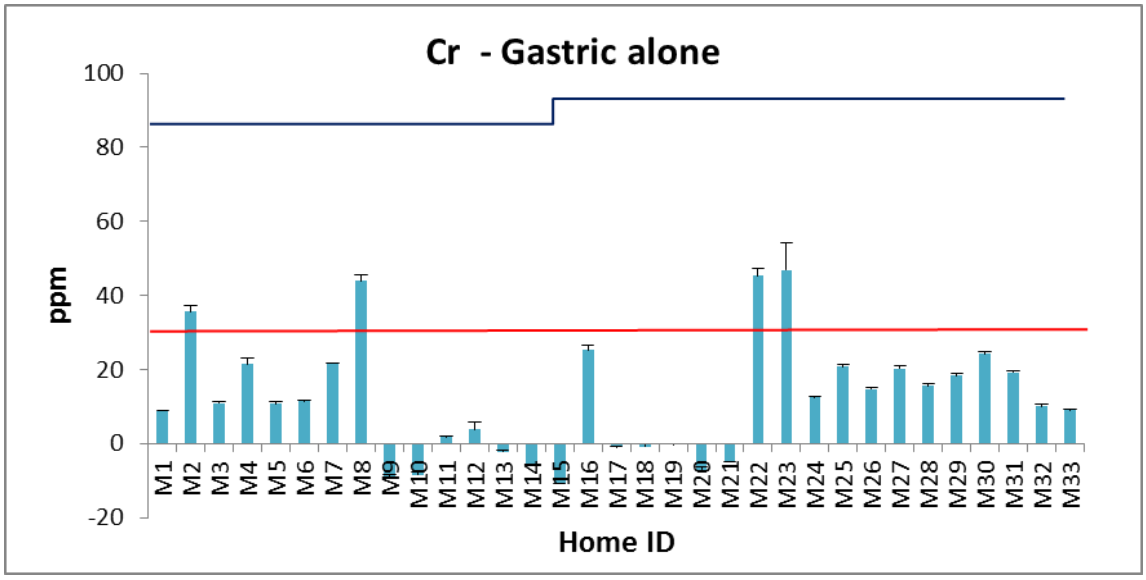




Limit of Detection (LOD)

Limit of Quantification (LOQ)



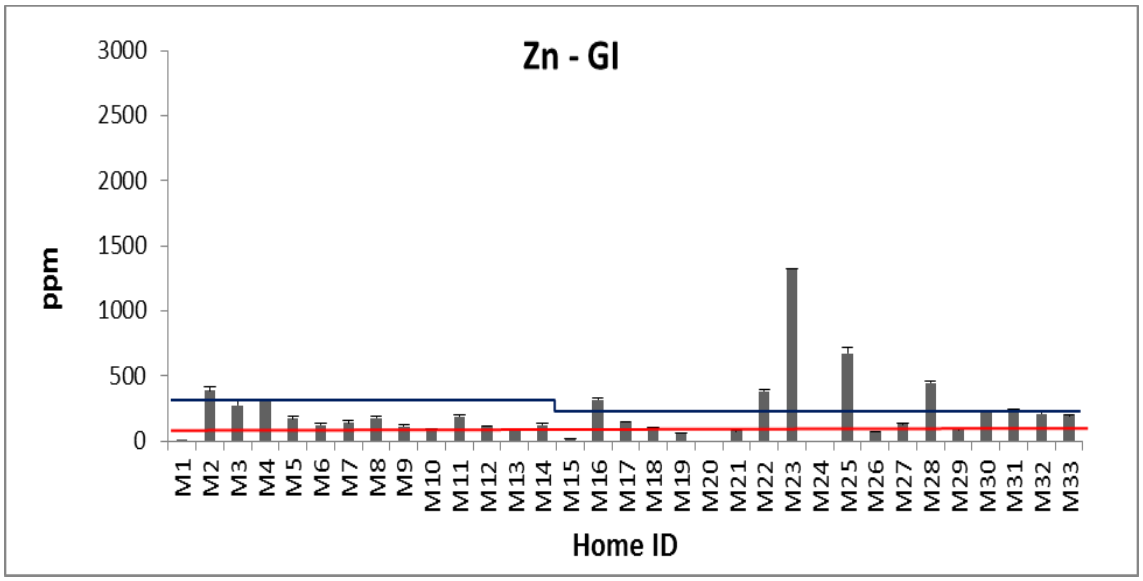


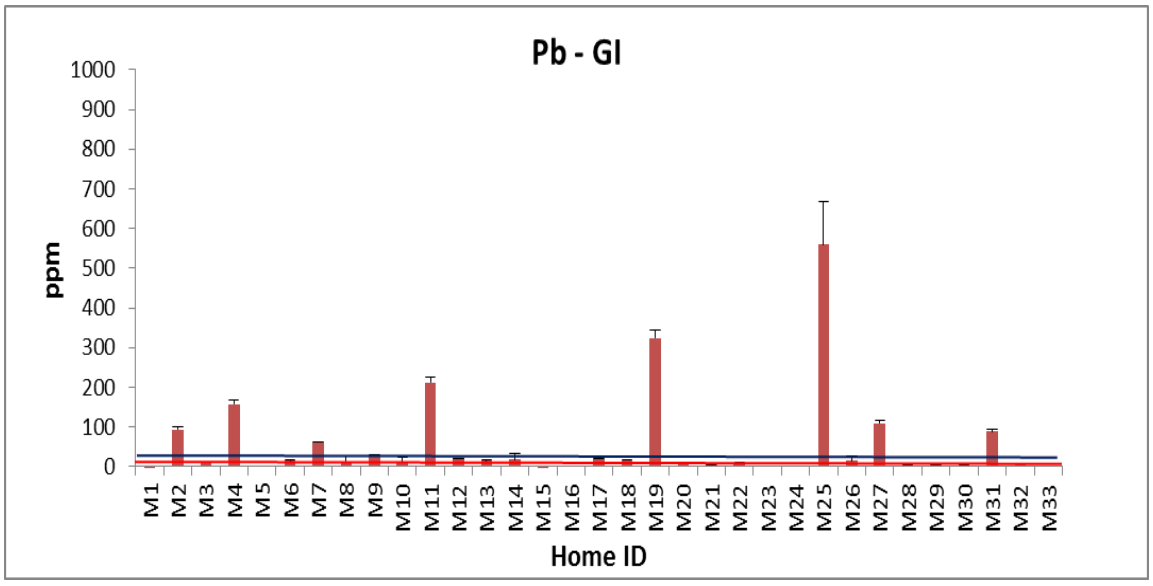
Appendix 3a. Bioaccessible metal concentration after PBET extraction for house dust samples. Error bars represent the standard deviation of the replicates within each house sample.

Limit of Detection (LOD)

Limit of Quantification (LOQ)

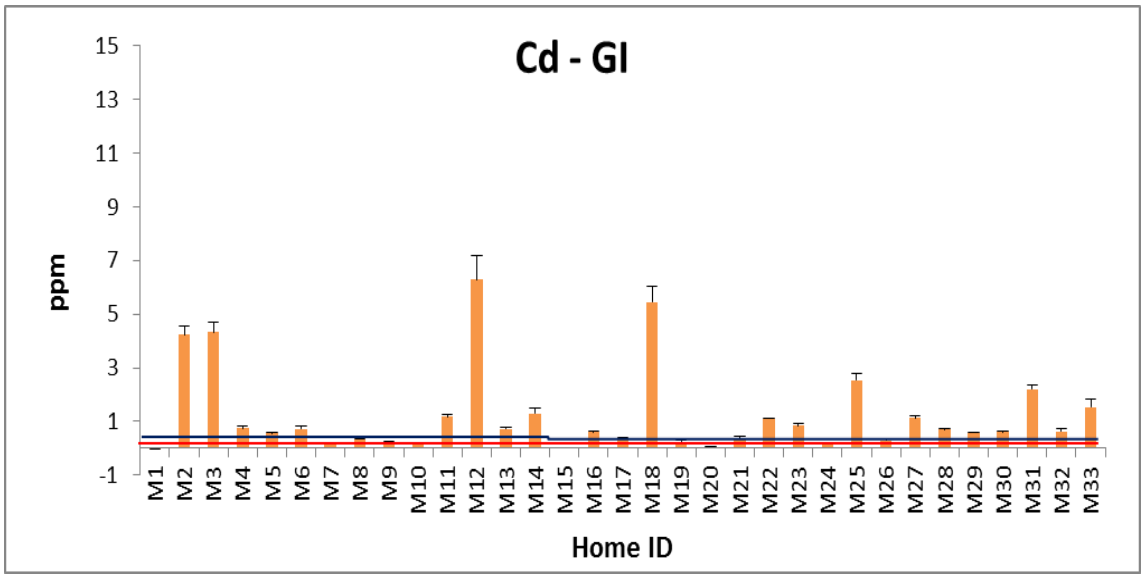
Appendix 3b. Bioaccessible metal concentrations in ppm after a PBET extraction of the gastrointestinal phase from the individual home samples.

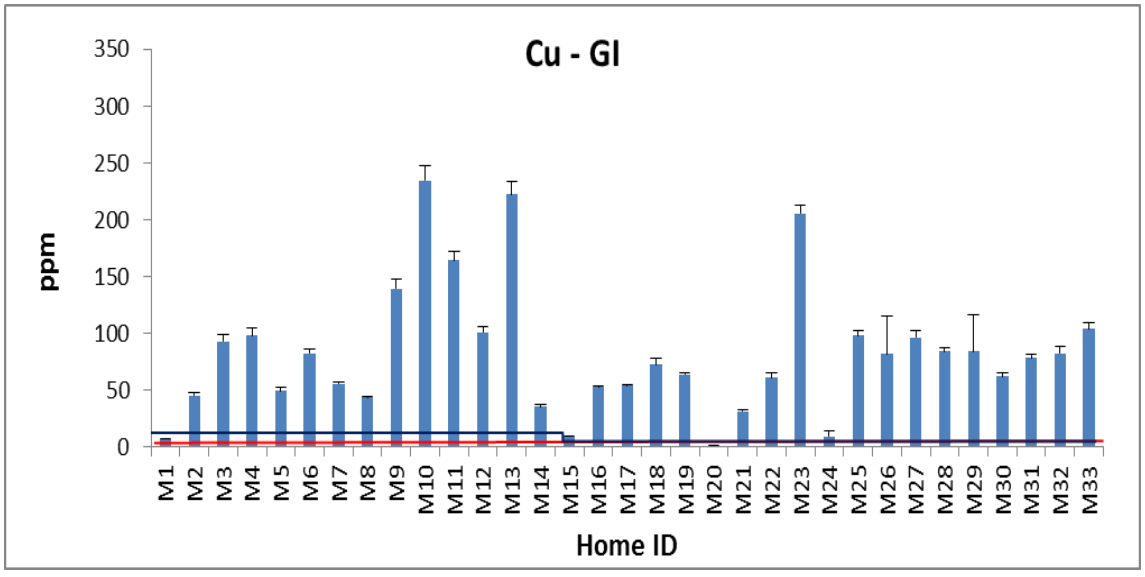




Limit of Detection (LOD)

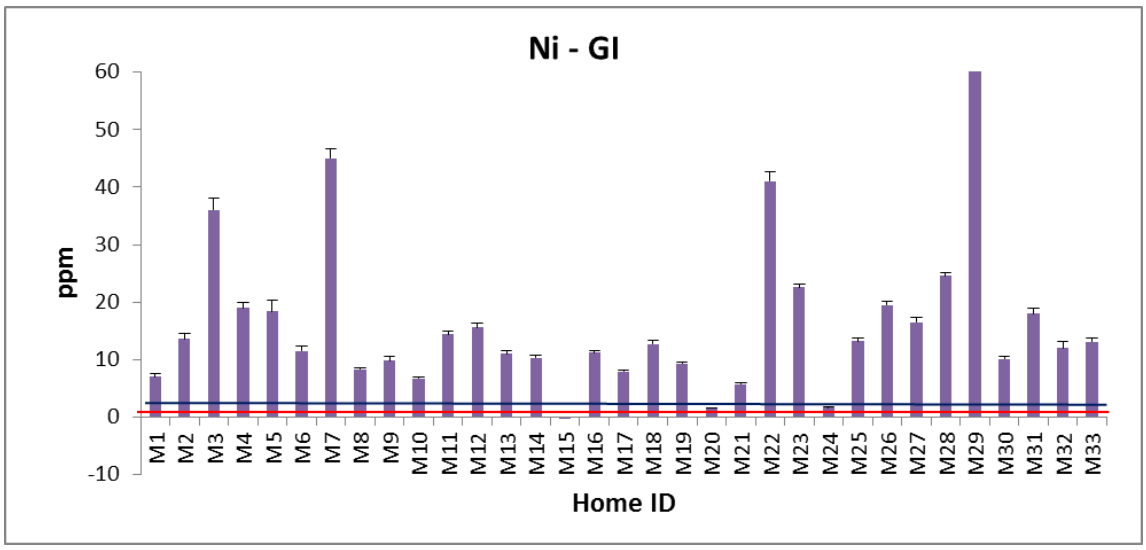
Limit of Quantification (LOQ)

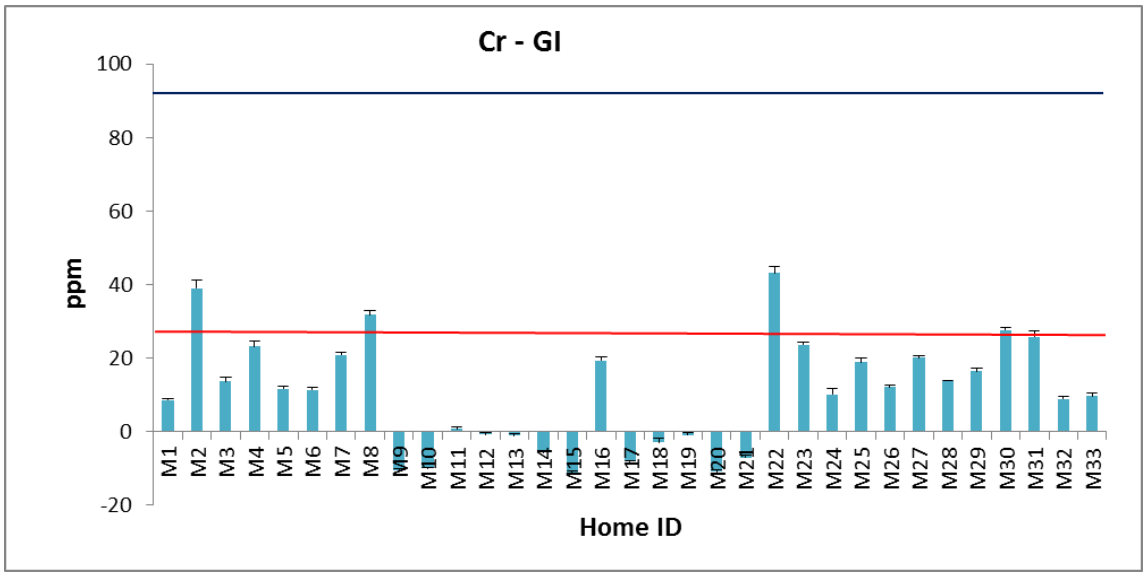




Limit of Detection (LOD)

Limit of Quantification (LOQ)





Appendix 3b. Bioaccessible metal concentration after PBET extraction for house dust samples Abbreviation: GI gastrointestinal. Error bars represent the standard deviation of the replicates within each house sample.

Limit of Detection (LOD)

Limit of Quantification (LOQ)

Appendix 4

Appendix 4a. Table of percent bioaccessibility for the gastric alone phase of metals from each house dust sample. Abbreviations: StDev standard deviation, %RSD percent relative standard deviation

		Metals Bioaccessibility (%)					
Sample		**Zn**	**Pb**	**Cd**	**Cu**	**Ni**	**Cr**
1300-04411A	*Mean*	198.10	57.37	134.78	38.39	15.44	6.57
House ID M1	*StDev*	40.86	56.11	13.26	4.66	0.48	0.17
(n=5)	*%RSD*	20.63	97.79	9.84	12.15	3.13	2.51
1300-04292	*Mean*	69.24	32.15	79.37	30.07	38.23	23.12
House ID M2	*StDev*	5.64	2.44	4.19	1.40	2.39	3.81
(n=5)	*%RSD*	8.15	7.58	5.28	4.65	6.25	16.50
1300-01244	*Mean*	97.34	54.95	87.74	36.71	18.09	21.52
House ID M3	*StDev*	9.09	3.23	4.92	2.57	1.07	0.92
(n=5)	*%RSD*	9.34	5.87	5.61	7.00	5.89	4.28
1300-02863	*Mean*	90.63	71.78	72.18	40.72	40.50	24.73
House ID M4	*StDev*	9.86	5.55	7.32	5.90	2.88	1.86
(n=3)	*%RSD*	10.88	7.73	10.14	14.49	7.11	7.52
1300-04570	*Mean*	118.00	34.94	74.50	39.37	51.74	25.71
House ID M5	*StDev*	9.19	1.93	7.31	2.88	1.59	1.36
(n=3)	*%RSD*	7.79	5.53	9.81	7.31	3.07	5.27
1300-03846	*Mean*	92.71	69.23	92.90	29.47	22.45	17.70
House ID M6	*StDev*	8.39	3.13	5.54	1.20	0.76	0.47
(n=3)	*%RSD*	9.05	4.53	5.96	4.07	3.39	2.63
1300-04339	*Mean*	103.45	62.98	100.43	34.51	39.52	18.03
House ID M7	*StDev*	9.93	6.73	2.82	0.40	0.89	0.16
(n=3)	*%RSD*	9.60	10.68	2.81	1.16	2.26	0.89
1300-01150	*Mean*	110.14	49.47	90.41	72.66	85.44	59.49
House ID M8	*StDev*	1.68	1.98	5.47	4.37	0.48	2.16
(n=3)	*%RSD*	1.53	4.01	6.05	6.01	0.56	3.64

1300-03226	*Mean*	96.17	71.92	79.88	45.80	22.82	-20.57
House ID M9	*StDev*	0.80	2.41	3.13	0.47	0.47	1.08
(n=3)	*%RSD*	0.83	3.35	3.92	1.02	2.07	-5.23
1300-03948	*Mean*	99.79	12.37	55.73	34.48	29.24	-10.75
House ID M10	*StDev*	3.82	0.20	0.31	3.85	1.13	0.79
(n=3)	*%RSD*	3.83	1.60	0.56	11.16	3.87	-7.37
1300-03949	*Mean*	88.02	55.38	58.20	27.10	27.32	1.98
House ID M11	*StDev*	5.89	0.67	2.95	0.30	0.46	0.37
(n=3)	*%RSD*	6.69	1.22	5.06	1.12	1.69	18.80
1300-03023	*Mean*	74.09	32.39	52.16	34.48	31.47	4.17
House ID M12	*StDev*	10.09	4.15	2.02	3.85	3.28	2.10
(n=3)	*%RSD*	13.62	12.82	3.88	11.16	10.42	50.42
1300-04168	*Mean*	67.13	50.33	23.52	60.84	15.59	-2.21
House ID M13	*StDev*	3.83	0.86	0.68	1.79	0.64	0.28
(n=3)	*%RSD*	5.70	1.72	2.88	2.94	4.10	-12.84
1300-02609A	*Mean*	72.77	39.80	68.34	21.62	27.83	-11.81
House ID M14	*StDev*	3.66	0.68	7.71	2.47	0.70	0.43
(n=3)	*%RSD*	5.04	1.72	11.29	11.44	2.50	-3.66
1300-03779	*Mean*	12.69	-35.14	39.57	37.36	4.73	-159.13
House ID M15	*StDev*	58.50	8.01	0.48	2.65	5.10	4.14
(n=3)	*%RSD*	461.10	-22.80	1.22	7.09	107.71	-2.60
1300-01250	*Mean*	113.74	28.79	62.43	44.33	30.29	28.52
House ID M16	*StDev*	2.39	3.39	3.22	0.86	1.55	1.73
(n=3)	*%RSD*	2.11	11.76	5.16	1.93	5.11	6.07
1300-04071	*Mean*	100.92	70.38	70.63	29.52	23.24	-10.07
House ID M17	*StDev*	0.55	0.45	1.21	0.69	0.51	0.60
(n=3)	*%RSD*	0.54	0.64	1.71	2.33	2.21	-5.92
1300-02412	*Mean*	99.20	51.78	88.64	42.23	32.16	-8.93
House ID M18	*StDev*	4.97	0.81	4.42	1.50	2.14	0.91
(n=3)	*%RSD*	5.01	1.57	4.98	3.54	6.67	-10.19
1300-03371	*Mean*	75.60	81.00	77.56	23.11	23.92	-9.06
House ID M19	*StDev*	3.70	3.09	6.51	0.73	1.03	0.48
(n=3)	*%RSD*	4.89	3.81	8.40	3.15	4.30	-5.33

1300-04113	*Mean*	22.14	58.28	78.78	37.96	37.07	-854.74
House ID M20	*StDev*	102.16	4.55	7.77	0.22	2.92	55.04
(n=3)	*%RSD*	461.46	7.80	9.86	0.59	7.87	-6.44
1300-04472	*Mean*	80.52	50.86	51.70	32.53	19.49	-29.80
House ID M21	*StDev*	4.30	1.56	8.58	0.61	0.21	0.67
(n=3)	*%RSD*	5.34	3.07	16.59	1.89	1.07	-2.25
1300-03495	*Mean*	87.20	46.21	31.90	30.39	55.69	10.26
House ID M22	*StDev*	1.75	5.93	2.97	1.84	1.83	0.46
(n=3)	*%RSD*	2.01	12.83	9.30	6.04	3.29	4.51
1300-01581	*Mean*	136.31	51.98	75.94	26.20	17.78	4.82
House ID M23	*StDev*	3.38	10.49	9.99	1.10	0.55	0.87
(n=3)	*%RSD*	2.48	20.18	13.16	4.21	3.07	18.05
1300-02893	*Mean*	208.24	-4.58	54.40	51.31	73.15	238.92
House ID M24	*StDev*	37.27	45.79	0.00	0.35	6.27	6.74
(n=3)	*%RSD*	17.90	-998.80	0.00	0.68	8.57	2.82
1300-04214	*Mean*	98.18	81.00	72.15	29.36	24.29	21.23
House ID M25	*StDev*	6.48	6.51	9.17	2.07	2.15	0.67
(n=3)	*%RSD*	6.60	8.03	12.71	7.06	8.87	3.14
1300-4172	*Mean*	102.94	37.28	138.49	34.44	16.71	9.78
House ID M26	*StDev*	5.85	0.59	7.96	2.50	1.09	0.36
(n=3)	*%RSD*	5.68	1.59	5.75	7.26	6.53	3.68
1300-4611	*Mean*	78.55	74.46	76.78	29.99	22.64	10.56
House ID M27	*StDev*	2.28	5.92	1.90	2.21	2.10	0.50
(n=3)	*%RSD*	2.90	7.95	2.47	7.36	9.26	4.73
1300-2127	*Mean*	100.68	43.10	69.75	30.77	44.98	14.61
House ID M28	*StDev*	0.70	3.08	4.10	0.50	0.92	0.64
(n=3)	*%RSD*	0.70	7.15	5.87	1.61	2.05	4.39
1300-3800B	*Mean*	66.38	32.29	51.20	16.66	32.49	15.05
House ID M29	*StDev*	6.82	2.16	1.98	0.60	1.39	0.49
(n=3)	*%RSD*	10.27	6.70	3.87	3.63	4.29	3.26

1300-1552	*Mean*	94.08	43.14	59.90	36.29	36.39	18.78
House ID M30	*StDev*	4.17	4.35	7.52	0.74	0.93	0.59
(n=3)	*%RSD*	4.43	10.08	12.56	2.03	2.55	3.15
1300-4647	*Mean*	78.91	50.17	75.40	22.60	23.98	12.82
House ID M31	*StDev*	1.19	0.76	1.16	0.50	0.54	0.29
(n=3)	*%RSD*	1.51	1.51	1.54	2.23	2.23	2.23
1300-1211	*Mean*	99.64	41.60	62.40	43.49	31.57	9.96
House ID M32	*StDev*	1.26	5.72	7.19	2.12	2.11	0.41
(n=3)	*%RSD*	1.27	13.74	11.52	4.88	6.67	4.11
1300-2298	*Mean*	82.16	37.85	45.29	30.95	21.40	9.28
House ID M33	*StDev*	2.64	0.90	1.56	0.22	0.01	0.37
(n=3)	*%RSD*	3.21	2.38	3.44	0.71	0.07	4.00

Appendix 4b. Table of percent bioaccessibility for the gastrointestinal phase of metals from each house dust sample. Abbreviations: StDev standard deviation, %RSD percent relative standard deviation

		Metals Bioaccessibility (%)					
Sample		**Zn**	**Pb**	**Cd**	**Cu**	**Ni**	**Cr**
1300-04411A	*Mean*	-0.96	-14.98	-4.54	27.12	5.96	6.47
House ID M1	*StDev*	4.90	0.24	2.25	1.70	0.48	0.49
(n=5)	*%RSD*	-508.40	-1.58	-49.53	6.25	8.14	7.53
1300-04292	*Mean*	15.08	5.52	29.97	28.19	30.85	25.32
House ID M2	*StDev*	1.28	0.48	2.00	1.83	1.79	4.21
(n=5)	*%RSD*	8.51	8.63	6.69	6.48	5.80	16.63
1300-01244	*Mean*	19.12	2.61	25.24	37.66	15.20	26.79
House ID M3	*StDev*	2.28	0.33	2.21	2.70	0.83	1.97
(n=5)	*%RSD*	11.93	12.75	8.77	7.16	5.47	7.34
1300-02863	*Mean*	18.84	7.28	21.84	40.65	32.99	26.93
House ID M4	*StDev*	0.47	0.48	2.13	2.80	1.78	1.44
(n=3)	*%RSD*	2.52	6.65	9.73	6.89	5.41	5.35
1300-04570	*Mean*	25.77	2.33	21.56	39.48	42.94	28.16
House ID M5	*StDev*	1.35	0.78	1.27	2.30	4.44	1.33
(n=3)	*%RSD*	5.23	33.66	5.90	5.82	10.34	4.71
1300-03846	*Mean*	12.16	4.46	25.39	27.81	19.46	17.65
House ID M6	*StDev*	1.48	0.35	3.84	1.44	1.33	0.81
(n=3)	*%RSD*	12.15	7.78	15.11	5.18	6.82	4.61
1300-04339	*Mean*	23.74	7.89	19.99	39.65	34.10	17.44
House ID M7	*StDev*	3.55	0.08	1.42	0.89	1.19	0.45
(n=3)	*%RSD*	14.94	0.97	7.10	2.23	3.48	2.57
1300-01150	*Mean*	42.78	9.43	30.70	55.20	55.07	43.01
House ID M8	*StDev*	4.06	1.45	2.89	1.25	1.35	1.74
(n=3)	*%RSD*	9.50	15.35	9.40	2.27	2.46	4.05
1300-03226	*Mean*	7.43	2.06	12.01	33.81	14.27	-23.67
House ID M9	*StDev*	0.95	0.05	1.41	2.04	1.03	0.94
(n=3)	*%RSD*	12.78	2.25	11.72	6.02	7.23	-3.98

1300-03948	*Mean*	4.80	1.04	3.94	34.48	31.47	4.17
House ID M10	*StDev*	0.56	1.21	0.32	3.85	3.28	2.10
(n=3)	*%RSD*	11.69	116.92	8.20	11.16	10.42	50.42
1300-03949	*Mean*	20.38	5.33	16.74	60.84	15.59	-2.21
House ID M11	*StDev*	2.04	0.31	1.04	1.79	0.64	0.28
(n=3)	*%RSD*	10.01	5.74	6.22	2.94	4.10	-12.84
1300-03023	*Mean*	10.38	1.83	16.27	29.88	22.43	-0.77
House ID M12	*StDev*	0.94	0.08	2.31	1.36	1.05	0.53
(n=3)	*%RSD*	9.08	4.44	14.20	4.56	4.67	-68.57
1300-04168	*Mean*	12.41	4.90	7.93	47.81	11.96	-1.01
House ID M13	*StDev*	0.48	0.33	0.58	2.52	0.66	0.51
(n=3)	*%RSD*	3.88	6.78	7.32	5.27	5.50	-50.95
1300-02609A	*Mean*	19.41	2.03	23.30	20.22	23.16	-11.36
House ID M14	*StDev*	2.56	1.60	1.58	1.48	0.87	1.38
(n=3)	*%RSD*	13.20	78.62	6.76	7.30	3.77	-12.15
1300-03779	*Mean*	9.14	-0.04	4.07	26.38	-1.16	-164.58
House ID M15	*StDev*	53.99	0.00	0.04	0.79	0.97	9.74
(n=3)	*%RSD*	591.03	-11.88	0.98	3.01	-83.28	-5.92
1300-01250	*Mean*	44.76	3.87	32.09	41.03	25.33	27.08
House ID M16	*StDev*	1.77	0.02	1.01	0.91	0.97	1.40
(n=3)	*%RSD*	3.95	0.55	3.16	2.22	3.82	5.16
1300-04071	*Mean*	9.44	2.81	14.74	26.00	17.90	-13.78
House ID M17	*StDev*	0.32	0.06	1.07	0.47	0.47	0.09
(n=3)	*%RSD*	3.44	2.05	7.28	1.81	2.65	-0.62
1300-02412	*Mean*	20.71	3.37	24.58	38.59	25.40	-4.60
House ID M18	*StDev*	2.43	0.26	2.65	2.83	1.43	1.99
(n=3)	*%RSD*	11.72	7.81	10.78	7.35	5.62	-43.28
1300-03371	*Mean*	8.55	4.92	12.22	21.31	18.05	-1.72
House ID M19	*StDev*	0.46	0.31	1.81	0.54	0.55	1.22
(n=3)	*%RSD*	5.35	6.34	14.85	2.52	3.07	-71.06

1300-04113	*Mean*	-22.24	50.91	63.25	28.49	38.32	-77.72
House ID M20	*StDev*	22.27	2.38	5.14	0.88	1.32	4.13
(n=3)	*%RSD*	-100.11	4.67	8.13	3.08	3.45	-5.32
1300-04472	*Mean*	17.49	8.13	18.65	30.07	17.36	-21.39
House ID M21	*StDev*	1.47	0.33	4.38	1.32	1.09	2.27
(n=3)	*%RSD*	8.39	4.11	23.47	4.41	6.27	-10.59
1300-03495	*Mean*	26.95	4.46	13.35	28.82	41.90	10.97
House ID M22	*StDev*	1.03	1.00	0.41	1.96	1.76	0.43
(n=3)	*%RSD*	3.82	22.47	3.10	6.81	4.19	3.90
1300-01581	*Mean*	23.52	1.25	21.14	19.89	12.61	2.73
House ID M23	*StDev*	2.77	0.30	2.76	0.69	0.37	0.07
(n=3)	*%RSD*	11.77	23.87	13.04	3.45	2.94	2.62
1300-02893	*Mean*	-4.40	14.28	56.62	41.20	51.87	329.37
House ID M24	*StDev*	9.05	12.49	3.33	0.07	2.12	47.11
(n=3)	*%RSD*	-205.96	87.46	5.88	0.18	4.09	14.30
1300-04214	*Mean*	24.19	9.28	22.69	25.50	18.72	25.36
House ID M25	*StDev*	1.83	0.09	2.12	1.20	0.79	1.57
(n=3)	*%RSD*	7.56	0.92	9.36	4.72	4.22	6.18
1300-4172	*Mean*	13.86	7.78	16.29	27.69	10.79	8.05
House ID M26	*StDev*	2.00	6.31	4.14	0.24	0.34	0.34
(n=3)	*%RSD*	14.42	81.14	25.43	0.86	3.15	4.18
1300-4611	*Mean*	16.29	9.45	18.59	29.93	15.48	10.52
House ID M27	*StDev*	2.00	0.52	1.63	2.10	0.77	0.24
(n=3)	*%RSD*	12.30	5.48	8.75	7.02	4.99	2.26
1300-2127	*Mean*	23.63	3.51	25.22	27.02	32.75	12.99
House ID M28	*StDev*	0.51	0.18	1.25	0.85	0.68	0.12
(n=3)	*%RSD*	2.14	5.04	4.97	3.14	2.07	0.95
1300-3800B	*Mean*	10.25	2.29	13.70	15.06	21.88	13.53
House ID M29	*StDev*	2.19	0.79	0.47	0.46	0.62	0.59
(n=3)	*%RSD*	21.33	34.62	3.43	3.04	2.81	4.37
1300-1552	*Mean*	23.08	6.23	29.04	41.52	31.93	21.40
House ID M30	*StDev*	1.59	0.74	1.54	0.63	1.75	0.57
(n=3)	*%RSD*	6.87	11.82	5.29	1.53	5.47	2.65

1300-4647	*Mean*	14.89	7.83	18.78	26.27	19.46	17.25
House ID M31	*StDev*	0.69	0.62	0.86	1.04	1.05	1.08
(n=3)	*%RSD*	4.60	7.96	4.60	3.96	5.37	6.27
1300-1211	*Mean*	21.53	5.53	19.81	40.19	24.28	8.84
House ID M32	*StDev*	2.07	0.28	0.64	3.26	2.34	0.70
(n=3)	*%RSD*	9.60	4.99	3.25	8.11	9.63	7.89
1300-2298	*Mean*	16.27	2.47	16.30	31.42	18.04	9.99
House ID M33	*StDev*	0.34	0.23	0.77	1.67	0.97	0.83
(n=3)	*%RSD*	2.12	9.46	4.75	5.31	5.38	8.26

Appendix 5. One-tailed t tests for home samples

t-Test: Paired Two Sample for Means
Zn

	Gastric Alone	*GI*
Mean	92.73766	18.94638
Variance	258.3004	86.3246
Observations	27	27
Pearson Correlation	0.48913	
Hypothesized Mean Difference	0	
df	26	
t Stat	27.21165	
P(T<=t) one-tail	6.2E-21	
t Critical one-tail	1.705618	
P(T<=t) two-tail	1.24E-20	
t Critical two-tail	2.055529	

t-Test: Paired Two Sample for Means
Pb

	Gastric Alone	*GI*
Mean	55.41971	5.345294
Variance	266.9614	5.811464
Observations	20	20
Pearson Correlation	0.358107	
Hypothesized Mean Difference	0	
df	19	
t Stat	14.31977	
P(T<=t) one-tail	6.21E-12	
t Critical one-tail	1.729133	
P(T<=t) two-tail	1.24E-11	
t Critical two-tail	2.093024	

t-Test: Paired Two Sample for Means

Cd

	Gastric Alone	*GI*
Mean	72.0728351	22.26229827
Variance	542.006852	81.04388345
Observations	29	29
Pearson Correlation	0.18314416	
Hypothesized Mean Difference	0	
df	28	
t Stat	11.4765634	
P(T<=t) one-tail	2.1173E-12	
t Critical one-tail	1.70113093	
P(T<=t) two-tail	4.2346E-12	
t Critical two-tail	2.04840714	

t-Test: Paired Two Sample for Means

Cu

	Gastric Alone	*GI*
Mean	36.15620598	32.42393959
Variance	136.0256947	74.10323094
Observations	32	32
Pearson Correlation	0.890821191	
Hypothesized Mean Difference	0	
df	31	
t Stat	3.776549263	
P(T<=t) one-tail	0.000338494	
t Critical one-tail	1.695518783	
P(T<=t) two-tail	0.000676989	
t Critical two-tail	2.039513446	

t-Test: Paired Two Sample for Means

Ni

	Gastric Alone	*GI*
Mean	32.0881969	24.78911232
Variance	253.697397	136.9322558
Observations	32	32
Pearson Correlation	0.95471342	
Hypothesized Mean Difference	0	
df	31	
t Stat	7.00520833	
P(T<=t) one-tail	3.6488E-08	
t Critical one-tail	1.69551878	
P(T<=t) two-tail	7.2977E-08	
t Critical two-tail	2.03951345	

t-Test: Paired Two Sample for Means

Cr

	Gastric Alone	*GI*
Mean	25.24326719	20.88985916
Variance	571.7556249	300.1592803
Observations	4	4
Pearson Correlation	0.968786714	
Hypothesized Mean Difference	0	
df	3	
t Stat	1.046353648	
P(T<=t) one-tail	0.186137912	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.372275825	
t Critical two-tail	3.182446305	