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Bioavailability of iron in geophagic earths and clay minerals, and their effect on dietary iron absorption using an *in vitro* digestion/ Caco-2 cell model

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Abstract

Geophagy, the deliberate consumption of earth, is strongly associated with iron (Fe) deficiency. It has been proposed that geophagy may be practiced as a means to improve Fe status by increasing Fe intakes and, conversely, that geophagy may cause Fe deficiency by inhibiting Fe absorption. We tested these hypotheses by measuring Fe concentration and relative bioavailable Fe content of 12 samples of geophagic earth and 4 samples of pure clay minerals. Further, we assessed the impact of these samples on the bioavailability of Fe from an Fe-rich test meal (cooked white beans, WB). Fe concentrations were measured with inductively coupled plasma atomic emission spectroscopy. Fe bioavailability was determined using an *in vitro* digestion/Caco-2 cell model in which ferritin formation was used as an index of Fe bioavailability. Geophagic earth and clay mineral samples were evaluated with this model, both alone and in combination with WB (1:16 ratio, sample:WB). Median Fe concentration of the geophagic earth was 3485 (IQR 2462, 14571) μg/g and mean Fe concentration in the clay minerals was 2791 (± 1782) μg/g. All specimens had Fe concentrations significantly higher ($p \leq 0.005$) than the Fe concentration of WB (77 μg/g). Ferritin formation (i.e. Fe uptake) in cells exposed to geophagic earths and clay minerals was significantly lower than in cells exposed to WB ($p \leq 0.05$) and Fe uptake responses of 11 of the 16 samples were not significantly different from the blank, indicating no bioavailable Fe. When samples were combined with WB, 5 of 16 had mean ferritin levels that were significantly lower ($p \leq 0.05$, one tail) than the WB alone, indicating that the samples inhibited Fe uptake from the WB. None of the ferritin responses of cells exposed to both WB and earth/clay were significantly higher than WB alone. Thus, although geophagic earths and mineral clays are high in total Fe, very little of this Fe is bioavailable. Further, some geophagic earth and clay mineral samples inhibit Fe absorption from foods. *In vivo* research is warranted to confirm these observations and to determine if geophagic earth samples can be a source of Fe and/or inhibit Fe absorption.

Introduction

Geophagy, the deliberate consumption of earth, is widely practiced; it has been observed in hundreds of cultures on all inhabited continents.[1-3] It is frequently associated with both anaemia and pregnancy.[4-11] Three explanations have been posited to explain the association between anaemia and geophagy.[3] In the first, geophagy is hypothesized to be a means of increasing micronutrient intake, particularly of iron (Fe).[12-14] In the second, conversely, geophagy is suggested to cause anaemia by decreasing Fe absorption.[10] This decrease in Fe absorption could occur by adsorption of dietary Fe as the earth particles mix with ingesta or by earth binding to the mucin layer of the intestine, thereby creating a physical barrier to Fe absorption into the body.[15-18] A third possible explanation of the association is that geophagy is an epiphenomenon of Fe deficiency; the mechanisms for this have not been described beyond “key appetite regulating brain enzymes”.[19]

Rigorous testing of the above hypotheses requires data on both Fe absorption, defined as “the movement of Fe from the intestinal lumen across the epithelial cells of the digestive tract into the circulation”[20], and Fe bioavailability, “the proportion of an ingested nutrient that is absorbed and either utilized in a metabolic pathway or sequestered in body stores”.[20] Yet few such data are available for geophagic samples. Instead, data on total elemental composition (TEC) determined using a variety of methods including atomic absorption spectrometry, X-ray fluorescence (XRF), inductively coupled plasma-atomic emission spectroscopy (ICP–AES) and instrumental neutron emission spectroscopy, have been the focus of the majority of the geochemical analyses of geophagic earth performed to date.[11, 14, 21-23] Most of these studies have reported high concentrations of Fe in geophagic earth, leading the authors to conclude that geophagy could be a good source of Fe. Yet TEC does not account for bioavailability, which is known to be highly variable depending on the chemical form of the Fe and other factors.[24]

Studies in humans have investigated the effect of geophagy on Fe absorption using Turkish,[16, 18, 25] Texan,[26] and South African[17] geophagic earth. Although they have a number of limitations, including outdated methods, very small sample sizes, and inadequate statistical analyses, these studies represent the only available *in vivo* data.[10] Their findings suggest that geophagy does not enhance Fe status and may even lower it, but the mechanisms and degree to which geophagic substances adsorb or inhibit Fe absorption remain unclear.

In the last decade, more sophisticated studies of the Fe content of geophagic earth have been conducted in which bioaccessibility, i.e. the fraction of a mineral that is soluble in the gastrointestinal environment and available for absorption,[27] has been considered.[15, 28-31] These studies have used *in vitro* methods that take into account the gastrointestinal environment to varying degrees. Most[15, 29-31] but not all[28] found little bioaccessible Fe. The one study that examined the potential for adsorption of Fe by geophagic earth found that it adsorbed bioaccessible Fe (and other minerals) in solution, but did not test the capacity of geophagic earth to bind Fe in a food matrix.[15] Because bioavailability depends upon bioaccessibility, these findings suggest that bioavailability would be limited.

Given the potential public health implications of geophagy and the limited data on Fe bioavailability, the objectives of the current study were to assess the *in vitro* Fe bioavailability and the capacity of geophagic earths and clay minerals to inhibit dietary Fe absorption using methods that better approximate human physiology. Specifically we used the *in vitro* digestion/Caco-2 model, which has been demonstrated to be a sensitive yet robust model, capable of handling a wide range of foods with varying Fe bioavailability.[32-39]

Materials & Methods

Sample selection

Analyses were conducted on 11 geophagic earths consumed in Zanzibar, Tanzania, 1 geophagic earth consumed in Tororo, Uganda and 4 samples of pure clay minerals (kaolinite, smectite, and halloysite) (Table 1). The Tanzanian samples were selected because they have been well characterized, with extensive mineralogical, biochemical and ethnographic data.[40] The Ugandan specimen was chosen because data collected within an ongoing clinical study (NCT00993031, http://clinicaltrials.gov) suggested that nearly half of the pregnant participants ate earth. The clay minerals were included because these three clay minerals are those most commonly found in high proportion in geophagic earths[41] such that their role in predicting Fe bioavailability or ability to inhibit Fe absorption from dietary sources would be highly relevant (Table 1).

To assess Fe bioavailability of earth/clay samples, 0.1 g of geophagic earth or clay mineral specimen was run in the Caco-2 model. Cellular ferritin responses were compared to a digest of an Fe-rich test meal of white bean (WB, 0.5g). WB (Goya brand) was obtained from a grocery store in Ithaca, NY, was cooked by autoclaving, and then cooled and freeze-dried. Earth samples were ground as collected (without autoclaving), using a porcelain mortar and pestle. To test the capacity of samples to inhibit dietary Fe absorption, 0.03g of geophagic soils and mineral clays were digested in combination with WB (0.47g) and compared to WB only (0.47g). The ratio of WB to geophagic earth or clay minerals (16:1, respectively) was selected to represent a typical ratio of food to soil consumed by people who practice geophagy. A number of studies have described the daily intake of geophagic material to be approximately 30 g[41]; we estimated that an average adult ingests 500 g (dry weight) of food per day, resulting in a ratio of approximately 16:1.

Laboratory Analyses

Total Fe concentration in the samples was determined using ICP-AES following nitric-perchloride digestion and wet ashing (Thermal Jarrell Ash Trace Analyzer, Jarrell Ash Co., Franklin, MA). Cation exchange capacity (CEC) was analysed using a modified ammonium acetate methodology.[42] Briefly, air-dried and ground earth and clay samples were saturated with neutral ammonium acetate. Samples were then heated and sodium hydroxide was added to convert displaced ammonium ions to gas. The application of 4% boric acid solution then converted ammonia gas back to ammonium and this solution was then titrated with 0.1N hydrochloric acid to determine the cation exchange capacity (CEC, mmol/100 g).

Fe bioavailability was assessed with an *in vitro* digestion/Caco-2 cell culture model.[33] The *in vitro* Fe bioavailability model is a two-stage assay employing a simulated gastric and intestinal digestion of food, coupled with a culture of human intestinal epithelial cells, specifically the Caco-2 cell line. In this model, ferritin formation in the Caco-2 cells is used as a measure of Fe bioavailability. The procedure has been described previously.[33] Briefly, samples were brought through simulated digestion, applied to a Caco-2 cell monolayer and ferritin formation by the cells was then measured. To simulate gastric digestion, samples were mixed with saline buffer (140 mMNaCl, 5 mMKCl), brought to a pH of 2 and then incubated on a rocker at 37°C for 1 hour in the presence of pepsin. Intestinal digestion was simulated by adjusting the contents to pH 7 and incubating at 37°C for 2 hours with pancreatic enzymes and bile salts. The simulated intestinal digestion was carried out in the upper chamber of a two-chamber system, created by fitting the bottom of Transwell insert ring with a 15000 Da molecular weight cut-off membrane (Spectra/Por 2.1, Spectrum Medical, Gardena, CA). This system allows Fe from the digested samples to diffuse into the lower chamber containing the Caco-2 cells. The cells are then able to take up the soluble Fe in proportion to its bioavailability. The Caco-2 cells were seeded at a density of 50000 cells/cm^2 and the experiment was conducted 13 d post seeding.

After intestinal digestion, the inserts were removed and the cells were further incubated at 37°C for 24 h to allow ferritin to form. The cell monolayer was then harvested. Ferritin concentration of the cell suspension, an indicator of Fe uptake, was measured using an immunoassay (FER-IRON II Ferritin assay, Ramco laboratories, Houston, TX). Cell protein concentration was determined using a colorimetric assay (Bio-Rad DC Protein assay, Bio-Rad, Hercules, CA). The index of Fe bioavailability used was the ratio of cell ferritin to total protein. All measurements were performed in triplicate. All experiments were conducted with quality controls including a “blank digest” that included only the digestive enzymes with the saline buffer; an $FeCl_3$ digest containing 1 umol of Fe; and the $FeCl_3$ digest plus the addition of ascorbic acid (20 umol). These quality controls were routinely used to ensure that contamination Fe was not present (i.e. baseline cell ferritin < 8 ng/mg cell protein), and that the assay was responsive to bioavailable Fe. The values for these quality controls all fell within the acceptable ranges indicating valid runs of this bioassay.

Statistical Analyses

Mean Fe concentrations (n=3) and ferritin responses (n=3) were compared between earth samples and WB, and between all samples and the blanks in Microsoft Excel using paired t-tests. Significance was defined as $p \leq 0.05$.

In order to investigate the ability of CEC, clay mineral composition and total Fe (μg/g) to predict Fe bioavailability and inhibition, simple linear regression analyses were performed in JMP® Pro 10.0.0 software. Mean ferritin responses of earth samples (bioavailability) and the mean ferritin responses of the samples when combined with WB (inhibition) were normalized by expressing as percentage of the mean ferritin response of the WB standard in their respective assays. These normalized values were fit against the samples’ mean CEC, Fe concentration, total clay content, and specific clay mineral contents (kaolinite, halloysite, mixed layer (kaolin/smectite)). Pure clay mineral samples were excluded from these

regression analyses, to isolate the potential predictors of Fe bioavailability and inhibition for geophagic samples only. ANOVA of these bivariate fits were performed; significance was defined as $p \leq 0.05$.

Results

Median Fe concentration of the geophagic earth was 3485 (interquartile range (IQR) 2462, 14571) μg/g. Mean Fe concentration of the clay minerals was 2791 ± 1782 μg/g. Fe concentrations in all specimens were significantly higher ($p \leq 0.005$) than the Fe concentration of WB (76.8 μg/g) (Table 1). Median CEC was 8.47 (IQR 5.2, 28.8) mmol/100 g soil (Table 1).

In bioavailability experiments, ferritin formation in cells exposed to geophagic earths and clay minerals was significantly lower than in cells exposed to WB ($p < 0.05$, one tail) (Figure 1). Ferritin responses of 11 of the 16 samples were not significantly different from the blank, indicating no bioavailable Fe (Figure 1). Five samples had mean ferritin responses that were significantly higher than the blank [PS-11, PS-18, PS-19, PS-33, PS-36, (3 geophagic earths, 2 clay minerals) $p \leq 0.05$, two tail], indicating some bioavailable Fe in these samples.

In experiments in which geophagic and clay mineral samples were combined with WB, none produced significantly higher ferritin responses than with WB alone (one tail, Figure 2). Five of 16 (PS-18, PS-20, PS-29, PS-31, PS-36, Figure 2) samples had mean ferritin levels that were significantly lower ($p \leq 0.05$, one tail) than WB alone (4 geophagic earth, 1 clay mineral), indicating an inhibition of Fe bioavailability by the geophagic earth or clay minerals (Figure 2).

Regression analyses elucidated no significant correlations between earth ferritin response or WB + earth ferritin response and CEC, clay mineral content and total Fe concentration (Figure 3).

Discussion

Our primary objectives were to assess the *in vitro* Fe bioavailability of geophagic earths and clay minerals and their effect on dietary Fe absorption. This assessment was done by using an *in vitro* digestion/Caco 2 cell model.[33] We found that despite extremely high levels of elemental Fe, it was not bioavailable in the majority (11/16) of samples and very little was bioavailable in the rest (Figure 1). Secondly, the addition of geophagic earths and clay minerals to an Fe rich test meal, WB, did not produce significantly higher ferritin responses than WB by itself, suggesting that when consumed together in this ratio these geophagic materials do not have the ability to significantly contribute bioavailable Fe. Indeed, there was evidence that some geophagic earths and clay minerals may inhibit Fe absorption; ferritin responses were significantly lower when geophagic earth or clay materials were combined with WB than for WB by itself in 5 of 16 samples (Figure 2).

The ICP-AES analysis of the geophagic earth and clay mineral samples revealed Fe concentrations that were lower than those reported in other studies. For example, Hooda et

al. performed ICP-OES on five samples from Uganda, Tanzania, Turkey and India, and reported a Fe concentration range of 14825-94007 μg/g.[43] Similarly high concentrations were found using XRF analysis on Ugandan geophagic soils and herbal soil/remedies (15800-81600 μg/g)[31] and AAS and flame emission spectroscopy on soils form south Asia (median 96000 μg/g)[28]. In contrast, we observed a range of only 940-26432 μg/g Fe with a median of 3485 μg/g. Though we observed lower Fe concentrations in our clay mineral and earth samples than other studies, the concentrations observed were still orders of magnitude higher than foods considered to be Fe-rich. For example, ground beef and cooked lentils contain approximately 30 μg/g and 33 μg/g of Fe respectively (http://ndb.nal.usda.gov/).The lower Fe concentrations we observed may be attributable to real differences in Fe content due to geographical variation, or due to the nature of different analytical techniques.

Despite these high quantities of total elemental Fe, all geophagic earth and clay mineral samples had ferritin responses significantly lower than the WB samples, which had a far lower Fe concentration (76.8 μg/g, Figure 1). Further, 11 of 16 had mean ferritin responses that were not significantly different than the blank, indicating no bioavailable Fe. The five samples (PS-11, PS-18, PS-19, PS-33, PS-36) that did have significantly higher ferritin responses suggest that some samples may be able to provide Fe to the consumer if digested without other foods. No apparent commonalities were observed among these five samples. The known characteristics of these samples were not able to singly predict their ability to provide bioavailable Fe. It is possible that characteristics that were not investigated in this study or an interaction of its properties could help explain the availability of Fe in these earths.

Though these samples exhibited the ability to provide some bioavailable Fe when digested alone, it is not clear that geophagic substances are ever consumed well before or after a meal, such that they are able to be digested in isolation.[3] Further, there is evidence from animal studies to indicate that earth may remain bound to the mucosal layer in the intestine for many hours, even days, after it has been ingested,[44] which suggests that bioavailability of Fe in these geophagic samples cannot be studied in isolation and its interactions with ingestae must be considered. It should also be noted that the preparation of the samples by grinding in a mortar and pestle is likely to increase the ability of leaching to access and remove elements; therefore the measurements of Fe bioavailability in these soils is likely to be an overestimate of bioavailability when consumed as is.

None of the investigated characteristics of the soil samples (Fe content, clay mineral content, CEC) were found to be statistically significantly correlated with ferritin response. This could be due to the small sample size analysed, which does not permit sufficient statistical power to detect very small differences, or these characteristics simply are not adequate single predictors of Fe bioavailability. Specifically, it is worth noting that elemental Fe content was not predictive of ferritin response (Figure 3).

No experimental runs in which geophagic samples and WB were combined had significantly higher ferritin response levels than the WB alone; this suggests that if the geophagic earth is consumed in conjunction with food in typical ratios (1:16) it does not have the ability to provide supplemental Fe to the diet. Five of the 16 samples (PS-18, PS-20, PS-29, PS-31,

PS-36) had mean ferritin levels that were significantly lower than the WB alone, indicating that some, but not all, may have the potential to inhibit Fe uptake from food (Figure 2). In Hooda et al.'s experiments,[15] all geophagic samples were able to adsorb bioavailable mineral Fe from solution; differences observed in our study are potentially due to differences in the form of Fe being tested, as the Fe we used was within a food matrix.

There are currently two proposed non-exclusive mechanisms by which earth might inhibit the absorption of dietary Fe[10]: 1) binding of dietary Fe by the earth particles as they mix with the food[15-18] and/or 2) creating a physical barrier to Fe absorption by either binding to the mucus layer of the intestinal wall[44, 45] or stimulating increased mucin production,[44] both of which could interfere with Fe absorption.

With this model we could explore the first mechanism, the binding capacity of geophagic earth. None of the investigated characteristics of the geophagic samples (specific clay mineral content, total clay content, CEC) were found to be significantly correlated with inhibition of dietary Fe absorption in the current study (Figure 3). As mentioned above, this could be due to the small sample size analysed, which does not permit sufficient statistical power, or that these characteristics are not adequate single predictors of Fe inhibition. Other characteristics that could better predict inhibition (or bioavailability) worth investigating in future studies include the detailed mineral forms of Fe and factors such as surface area and clay dispersion characteristics under conditions in the gastrointestinal tract.

We had expected clay content to be an important determinant of dietary Fe absorption inhibition. Clays have the ability to adsorb a wide variety of molecules and elements; their structure consists of multiple layers. Many clays such as smectites are negatively charged and able to attract cations such as Fe^{3+} or Fe^{2+} others like kaolinite carry little if any charge.[46] However, significant correlations between total clay content or specific clay mineral content and inhibition were not observed, and the R^2 values for the regressions were quite low, suggesting minimal power of clay mineral content to predict Fe inhibition.

A soil or clay's ability to bind to some substances may be explained in part by its layer charge and consequent CEC[15, 41]; we expected that a higher CEC would reduce the bioaccessibility of Fe from the WB thereby inhibiting its absorption. We investigated the relationship between CEC and ability to inhibit Fe uptake from dietary Fe sources, but the bivariate analyses did not reveal a significant correlation, although the fit was in the predicted direction (Figure 3).

As for clay mineral types, we expected smectite to have the greatest inhibitory effect. Smectite clays have been found to bind well to a number of substances (e.g. herbicides,[47] tannic acid,[48] T-2 toxin[49] and aflatoxins[50]). Of all three pure clay minerals tested, smectite had the largest Fe bioavailability inhibition. PS-36, one of the two pure smectite samples, significantly reduced the ferritin response from WB. The other pure smectite (PS-13) sample gave a similar response but it was not significant (perhaps because mean was calculated from 2 replicates instead of 3).

Future experiments should address the effects of geophagy on other Fe-rich foods and use varying quantities. The proportion of WB to earth (16:1) used in this study did not capture

the range of daily earth consumption and daily total food intake by geophagists; there are a number of reports of daily consumption of earth > 30 g. In addition, a typical human diet does not consist of just one food item; those who engage in geophagy consume a multitude of foods which may be differently affected by geophagic earth than WB. Other sources of Fe, especially heme-Fe might interact very differently with the geophagic earths. Furthermore, in our model, WB was digested at the same time as the earth/clay samples. This assumes that the two are consumed simultaneously, which is not always the case. Lagged effects of geophagy also need to be investigated further.

For these reasons *in vivo* studies are warranted to explore observations made in this study. They offer the possibility of assessing whether the effects that were observed in this study are biologically relevant. Furthermore, the presence of a mucin layer will permit a more accurate picture of the ability of geophagic earth to impact Fe absorption. Because the *in vitro* model used in this experiment does not have a mucin layer component, the inhibitory effects that we observed were likely due only to the physical binding of the Fe by the earth particles. An *in vivo* study will likely be the best means to determine if the earth samples interact with the intestinal mucus layer and if so, the impact of that interaction.

These experiments have established that although geophagic earths have high Fe concentrations compared to Fe-rich foods, this Fe is not bioavailable, and that some geophagic earths inhibit dietary Fe absorption. Our findings also make it clear that more research is needed to elucidate the underlying mechanisms that explain the relationships between Fe content, Fe bioavailability, and inhibition of dietary Fe absorption by geophagic earth samples.

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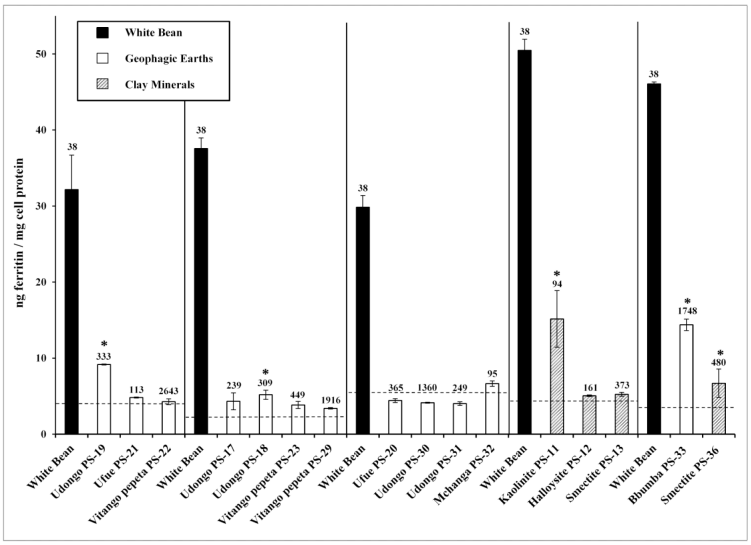

Figure 1. Bioavailability of iron in geophagic earths and clay minerals

Caco-2 cell ferritin formation in wells containing geophagic earth, clay mineral samples (0.1g) and white bean (0.5g). Blank (-----) refers to mean ferritin levels in cells without added Fe, WB or soil. Ferritin formation values are means ± SEM, n=3. Values above bars indicate the mean amount of iron (Fe μg) in the well calculated from ICP data (n=3). An asterisk (*) indicates those soil/earth samples whose means are significantly different ($p < 0.05$, two-tailed) than the mean of the blank. Partitions indicate separate experimental runs.

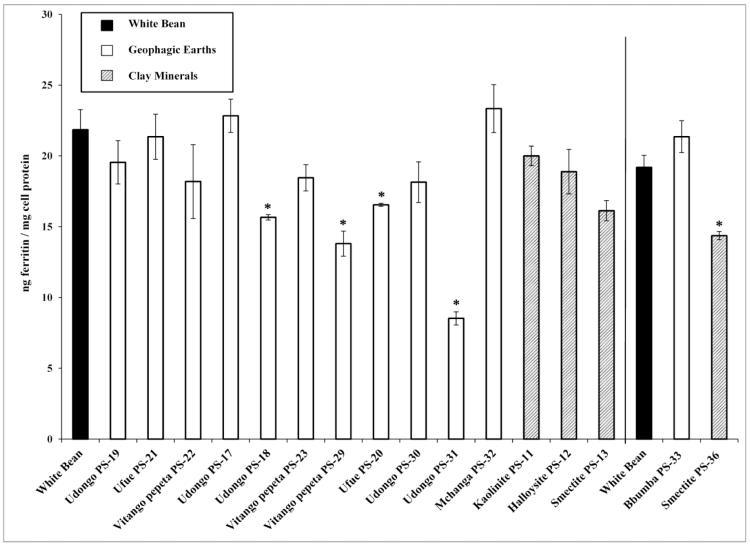

Figure 2. The impact of geophagic and clay mineral samples on iron uptake from white beans

Caco-2 cell ferritin formation for experiments with earth and white bean in 1:16 ratio. White bean amount is constant in all dilutions and in white bean control (0.47g). Ferritin formation values are means ± SEM, n=3 (exception is PS-13, n=2). An asterisk (*) indicates those samples whose means are significantly lower than the white bean sample ($p < 0.05$, one tailed).

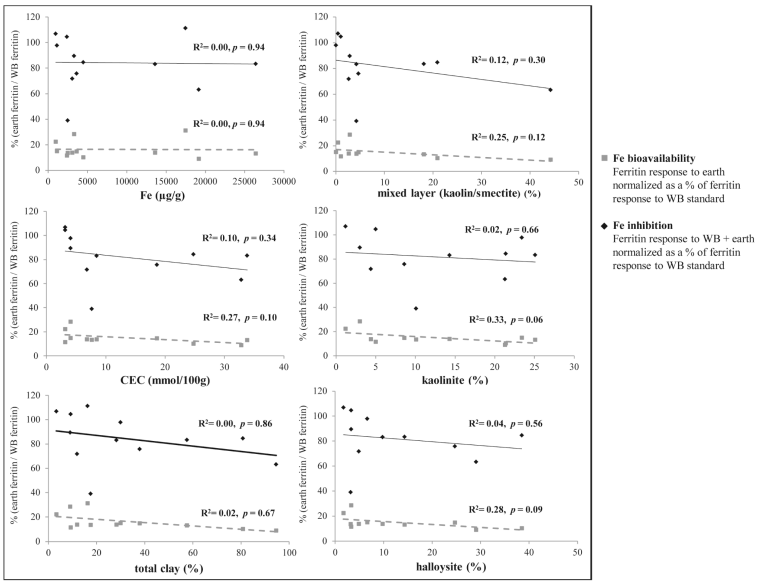

Figure 3.

Linear regression analysis of Fe bioavailability (ferritin response to geophagic earth normalized as % of ferritin response towhite bean (WB) standard) and WB Fe absorption inhibition capacity (ferritin response to WB + geophagic earth normalized as % of ferritin response to WBstandard) against total Fe, CEC, total clay, mixed layer (kaolin/smectite) kaolinite, and halloysite content. ANOVA of bivariate fits were performed; R^2 and *p* values are reported for each regression analysis. Clay mineral samples were excluded from these analyses, such that n=11 for Fe and total clay regressions and n=10 for CEC, mixed layer (kaolin/smectite), kaolinite and halloysite regressions; data for PS-33 were not available.

Table 1

Sample Characteristics

Sample ID	Secondary ID	Description[d]	Mineral analysis (wt%) of bulk geophagic soils[e]					Iron Content (μg/g)[h]	Cation Exchange Capacity (CEC)[i]
			Mixed Layer (Kaolin/smectite)[f]	Kaolinite	Halloysite	Smectite	Total-Clay[g]		
PS-11	Kao-74[a]	Kaolinite	-	97.0	-	-	97.0	940.1*	6.2
PS-12	Hal 969192[a]	Halloysite	-	-	100.0	-	100.0	1695.7*	11.4
PS-13	Mont2[a]/STx-1[b]	Smectite	-	-	-	67.0	67.0	3732.7*	75.8
PS-36	STx-1b[b]	Smectite	-	-	-	67.0	67.0	4795.4*	84.4
PS-17	818[c]	Udongo (Tnz)	1.0	5.0	3.3	-	9.3	2392.5*	3.2
PS-18	832[c]	Udongo (Tnz)	2.6	4.4	4.9	-	11.9	3086.6*	6.8
PS-19	839[c]	Udongo (Tnz)	2.8	3.0	3.3	-	9.1	3325.7*	4.1
PS-20	833[c]	Ufue (Tnz)	4.6	8.6	24.7	-	37.9	3644.9*	18.6
PS-21	845[c]	Ufue (Tnz)	0.0	23.4	6.6	-	30.0	1130.3*	4.1
PS-22	834[c]	Vitango pepeta (Tnz)	18.1	25.1	14.3	-	57.5	26432.1*	33.8
PS-23	842[c]	Vitango pepeta (Tnz)	20.9	21.4	38.5	-	80.8	4479.1*	24.8
PS-29	812[c]	Vitengo pepeta (Tnz)	44.2	21.3	29.1	-	94.6	19161.3*	32.8
PS-30	849[c]	Udongo (Tnz)	4.2	14.3	9.8	-	28.3	13603.5*	8.5
PS-31	835[c]	Udongo (Tnz)	4.2	10.1	3.2	-	17.5	2485.6*	7.7
PS-32	838[c]	Mchanga (Tnz)	0.4	1.2	1.7	-	3.3	958.1*	3.2
PS-33	-	Bbumba (Ugn)	22.4	58.1	-	-	80.5	17474.9*	-
White Bean	WB	Cooked, small	-	-	-	-	-	76.8	-

[a] Samples from the The James Hutton Institute collection

[b] Mineralogical *(Clay Minerals Society)* IDs http://www.clays.org/SOURCE%20CLAYS/SCdata.html. PS-13 and PS-33 specimens are different samplings of the same source.

[c] IDs used inYoung et al.[40]

[d] Swahili (Tanzania, Tnz) and Luganda (Uganda, Ugn) names of samples

[e] Data from X-ray diffraction full pattern fitting analysis method described in Young et al.[40] except STx1 and STx1b which are based on analyses reported in Chipera and Bish[43].

[f] Mixed layer clay, consisting of an unknown ratio of kaolin and smectite.

[g] Total-clay is sumof kaolin/smectite, kaolinite and halloysite

[h] Data from from ICP-AES analysis of samples, values are means (n=3)

[i] Values are means (n=3)

* Significantly higher than WB, $p \leq 0.005$