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EXPOSURE AND BIOAVAILABILITY OF CERIUM THROUGH THE INGESTION OF SOIL (UGANDA).

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Front cover illustration: Local children and the drying of cassava on the ground, Mukono district, Uganda.

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EXECUTIVE SUMMARY

The UK Department For International Development (DFID) Technical Development and Research (TDR) programme funded a three-year project between 1995 and 1998 entitled "Cerium & Endomyocardial Fibrosis in Tropical Terrains" (Project R6228). The presence of elevated levels of dietary Ce, and deficient levels of dietary Mg in southern India have been assigned as potential environmental cofactors in the aetiology of Endomyocardial Fibrosis, which is also endemic in certain areas of Uganda. One of the aims of the project is to assess the relative importance of various exposure scenarios for the Ugandan population to Ce to define protective measures if required.

Initial assessments of dietary intakes of Ce in Ugandan soils and foodstuffs have indicated that soils are a significant exposure pathway. A review is presented of exposure to soil and its ingestion both world-wide and specifically in Uganda. The deliberate consumption of soil by pregnant women and children (during the crawling stage of their development and between the ages of 4 and 8) is common in Uganda. However, prior to this study no information was available concerning the bioavailability of Ce in ingested soil or soil contaminated food.

A Physiologically Based Extraction Test (PBET) has been used to determine the bioaccessibility of Ce in soil and dust samples from two districts in Uganda. The PBET incorporates gastro-intestinal tract parameters representative of a human for predicting the bioaccessibility of metals from an ingested solid matrix . Results showed there was a large degree of variation in Ce bioaccessibility between the samples, and between the different size fractions of individual samples. There was a marked increase in bioaccessibility when the pH was increased between the stomach and small intestine phases of the PBET for the smaller size fractions between >20 and <1 µm. Variations in mineralogy are considered to have caused the variations in Ce bioacessibility, although it was not possible to accurately determine specific mineral phases due to the small size of the minerals in the finest soil fractions.

The PBET method has been shown to be extremely useful in assessing the mean bioaccessibility of Ce in Ugandan soils which ranged from 0.24 to 24.0 %. In assessing the risk posed by the ingestion of potentially harmful elements it has often been assumed that

they are 100% bioavailable. Results from the PBET are important as they indicate that the actual bioaccessibility is much smaller. These values can therefore be used to make more risk accurate assessments. The potential role of Ce ingestion in the aetiology of Endomyocardial Fibrosis in Uganda requires further investigation.

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1. INTRODUCTION.

1.1 Background

The UK Department For International Development (DFID) Technical Development and Research (TDR) programme funded a three-year project between 1995 and 1998 entitled "Cerium & Endomyocardial Fibrosis in Tropical Terrains" (Project R6228). The project originates from previous research into human exposure to naturally occurring potentially toxic trace elements in the environment, undertaken on behalf of DFID by the British Geological Survey and the Institute for Child Health. However, unlike many potentially toxic trace elements and chemical species such as aluminium, lead, selenium, iodine and arsenic, the effect of chronic long-term exposure to cerium (Ce) on human health has received relatively little attention.

Epidemiological studies by counterparts from Makerere University indicated a high prevalence of EMF in the district of Mukono (ca. 20 km east of Kampala) and this was therefore used as the principal study area for the project. Literature reviews were under taken to assess levels of cerium in tropical and sub-tropical diets and the toxicity of Ce and its role in EMF. The reviews indicated considerable knowledge gaps relating to the abundance of Ce in both tropical and sub-tropical diets and that no additional studies by other workers had been performed to replicate the findings of the Kerala group. Review of the medical literature did however establish that Ce was biologically active and had been shown to toxic at a cellular level.

1.2 Rationale

The ingestion of soil is often overlooked as one of the dominant exposure pathways of humans to geochemical hazards. The ingestion of soil can be inadvertent, particularly amongst children, or advertent, referred to as geophagia. Geophagia is common amongst poor people in tropical countries, and soil materials are sold as mineral supplements in several countries, including Uganda (Abrahams and Parsons, 1997). Soil material and dust can be ingested inadvertently on food, and dust by inhalation. The inhalation of dust is a particularly important exposure pathway in Uganda because surface soils and dusts are dry for the majority of the year leading to the entrainment of dust into the air. In addition, food sold at roadside stalls is often exposed to the air and dust from traffic which leads to increased levels of ingestion when the food is consumed.

There is considered to be less of an association between local environmental geochemistry and health in Western societies compared to developing nations. In developing countries, such as Uganda, the poorer in society live 'closer to the ground'. Therefore, relationships between environmental geochemistry and the aetiology of disease may be more apparent. The presence of elevated dietary concentrations of Ce and Mg deficiency have been proposed as potential environmental cofactors in the aetiology of tropical Endomyocardial Fibrosis (EMF; Valiathan et al., 1986, 1989). In Uganda there is a geochemical association between Ce and latosolic soils, carbonatites and granitic rocks containing monazite ((Ce, La, Y, Th) PO_4). An assessment of dietary intakes of Ce in Ugandan soils was undertaken for a range of foodstuffs (Smith et al., 1997). In addition, exposure to elevated loads of Ce was confirmed from the analysis of naturally erupted, or surgically removed, teeth from 50 randomly selected children in the Mukono district which had average Ce concentrations five times that of a UK control group (Smith et al., 1997). To date, the level of exposure to Ce through the advertent or inadvertent consumption of soil has not been assessed. In addition, no data are available to assess the bioavailability of Ce in ingested soil.

The aims of this study are to:

1. review information available on the ingestion of soil to assess its significance as a pathway for dietary intake of geochemical hazards
2. determine the bioavailability of Ce in soils from two districts of Uganda, one of which (Mukono) is an area of endemic EMF

The availability data for the bioaccessibility of Ce in Ugandan soils would enable more accurate risk assessments to be made of exposure through the ingestion of food and contaminated soil.

1.3 Bioavailability and bioaccessibility

The term bioavailability is used to describe the fraction of a total element that is absorbed by the systemic circulation. Bioaccessibility is used to define the total fraction of an element that is available for absorption during transit through the small intestine. In practice, the measurements and interpretation of element bioavailability following ingestion are very complex, as four metabolic stages are involved (Henry, 1996):

i) Nutrient availability in the intestinal lumen for absorption
ii) Absorption of the nutrient from the intestinal lumen
iii) Retention of the nutrient in the body
iv) Final utilisation of the nutrient within the body

There are a number of analytical techniques available to determine the likely bioavailability of metals and metalloids in soil, dust and vegetation following ingestion by humans. These include micro-mineralogical assessments (Tsuji, 1993), use of selective extractants for the removal of specific mineral phases (Tessier et al., 1979) and *in vivo* animal experiments (e.g. Freeman et al., 1996; Groen et al., 1994). *In vivo* animal experiments are ethically questionable and reproducible results are generally only achieved over a prolonged timescale (Henry, 1996). These approaches provide no replication of the conditions prevalent in the human gastro-intestinal tract. This is the principal advantage of the physiologically-based extraction test (PBET; Ruby et al., 1996), which provides an indication of bioaccessibility. Bioavailability can be calculated from PBET tests if the methodology is validated against animal / human experiments. Validation will give the relationship between these two sets of values, which can be applied thereafter to calculate bioavailability from PBET data. The PBET has been validated for Pb and As using animal models based on the premise that data from appropriate animal models can be extrapolated to humans for the purpose of exposure assessment (Ruby et al., 1996).

The PBET was designed to estimate the dissolution of elements in the gastrointestinal environment, in other words, the bioaccessibility of elements. In this sense, bioaccessibility, the fraction of total compound ingested that dissolves in the stomach, is the term commonly referred to in this report.

1.4 Specific objectives

The aim of the experiments undertaken in this study was to assess Ce bioaccessibility in soils from the Nawakakoi district, and soils and dust samples from the Mukono district of Uganda. Bioaccessibility was determined using a laboratory test tube (*in vitro*) method termed the physiologically based extraction test. Bioaccessibility of cerium in four different Ugandan soil and dust was assessed in sub-samples with different size fractions: > 250 µm, > 120 µm, > 63 µm, > 20 µm, > 1µm and <1µm. Replicates of experiments were undertaken to check their reproducibility.

2. REVIEW OF EXPOSURE TO SOIL AND ITS INGESTION

The ingestion of soil is widely recognised as an important pathway of human exposure to potentially toxic trace elements, particularly for children. Two forms of direct soil ingestion can be distinguished: inadvertent (un-intentional) ingestion and advertent ingestion, the deliberate consumption of soil, more accurately termed 'geophagia'. The contamination of food buy soil is the principal indirect pathway of soil ingestion. This is discussed below with particular reference to Uganda.

2.1 Inadvertent soil ingestion

Young children ingest soil material by frequent hand-to-mouth contact. Such contact is less frequent in older children and adults and they will generally ingest less soil via this pathway, although exceptions include smokers and people who bite their nails. Recent research which attempts to quantify inadvertent soil ingestion has focused on mass-balance approaches using non-absorbable tracer elements in soil which pass through the body (Calabrese et al., 1989; Davies et al, 1990; van Wijnen et al., 1990).

Estimates of soil ingestion by children in developed countries were initially based on observations of their behaviour and recording the number of contacts between hand and mouth. However, accurate estimates of soil ingestion cannot be made without an assessment of how much is removed during each mouthing action and the type of soil, as fine particles preferentially adhere to the skin (Sheppard et al., 1992). The more advanced mass-balance approaches were based on the differences between the quantity of ingested non-absorbed elements in soil (e.g. Al, Si and Ti, Binder et al., 1986) and the amount excreted. Detailed studies of soil ingestion by children using tracers have been conducted by Calabrese et al. (1989) and Davies et al. (1990) and point to a mean inadvertent rate of 40 mg day^{-1}. However, a number of criticisms have been levelled against these studies:

- they were conducted over short periods which may have led to a difference between inputs and outputs
- populations were from similar suburban backgrounds
- children were drawn from educated, caring families where soil-eating is less likely to be established as a habit
- children under close supervision during the study are less likely to eat soil

It has been suggested that these tracer studies are likely to underestimate the ingestion of soil by children. The most recent estimate from the U. S. Environmental Protection Agency of inadvertent soil ingestion by a 2-3 year-old child is 0.138 g d^{-1} (Stanek III and Calabrese, 1994). Estimates of inadvertent soil ingestion by adults were not initially supported by empirical data (Hawley, 1985).

The inadvertent ingestion of soil is considered to be far greater in developing than developed countries because in the former household and local environments tend to be more dusty (particularly in Uganda), leading to greater soil and dust contamination during the preparation of food (Smith et al., 1997). However, there is a lack of data which can be used to confirm this theory.

2.2 Advertent soil ingestion (geophagia)

Geophagia has been defined as 'the habit of eating clay or earth', and is considered to be a form of pica, 'a craving for unnatural articles of food' (Weller, 1989). Soil-pica has been defined by Calabrese et al. (1991) as "the ingestion of soil in amounts far exceeding those observed by the average child". The consumption of soil by humans has been recognised since the Ancient Greeks and Romans, documented by Dioscorides in 40 BC (cited in Aufreiter et al., 1997) and in Europe (Black, 1956) where tablets of red earth mixed with goats blood were consumed for medicinal purposes. Geophagy in animals may be a source of supplementary elements, an absorbent of toxins, an adjuster of the pH of the digestive system, or medicinal (Aufreiter et al., 1997). In human societies, the prevalence of geophagy is sometimes linked to religious beliefs and rites (Hunter, 1973). Geophagy is common in Africa, where clays are sold in markets, and eaten for nutritional and medicinal purposes, particularly by child-bearing women. The high clay content is used as a remedy for diarrhoea, intestinal parasites and syphilis (Vermeer, 1966; Henry, 1973; Vermeer and Ferrel, 1985).

The most thorough studies of pica in developed societies were conducted in America (Cooper, 1957) and Britain (Bicknell, 1975). Dirt eating was the most common form of pica reported by Cooper (1975) from a group of 784 urban children in Baltimore. The literature concerning pica is highly fragmented with a large degree of uncertainty associated with the condition (Lacey, 1990). Hence it is difficult to make reliable estimates of soil ingestion via this exposure pathway. No empirical data appear to have been used to estimate the highest published rates of soil ingestion of 5g day^{-1} (USEPA, 1984) and 10 g day^{-1} (USEPA, 1989) and no estimates were given for the duration of ingestion of this magnitude in developed countries.

2.3 The deliberate consumption of soil in Uganda and East Africa

Geophagia is common throughout East Africa (Johns and Duquette, 1991); studies in East Africa have shown that 60-90 % of children within the age 5 to 14 practice geophagy (Geissler et al., 1997). The deliberate consumption of soil by pregnant women and children, during the crawling stage of their development and between the ages of 4 and 8, is common in Uganda (Sserunjogi, 1997). A range of soils are reported to be consumed including that from ant-hills, mud walls of buildings, baked building bricks and clay from traditional medical practitioners. From conducting interviews, Sserunjogi (1997) discovered that pregnant women eat soil on a daily basis and that the stimulus was, in some cases, the aroma of the soil or the smell given off when rain falls on the ground. The same stimulus was given in interviews with children who confirmed they also consumed soil. Some women said they sometimes broke pieces of soil from the walls of their dwellings and baked them in the fire before eating them because this intensifies the flavour.

2.4 Soil contamination of foods in Mukono district, Uganda

Cassava is the largest staple food source in the Mukono district of Uganda (MOFEP, 1994). Cassava flour was considered to be prone to contamination by dust and soil during each stage of its preparation from observations made by Smith et al. (1997) However, the greatest soil contamination was likely to occur when large quantities of cassava tubers are dried on bare ground prior to pounding. Soil contamination of cassava is enhanced relative to other crops such as maize because the surface of the former is considerably more sticky. Soil contamination of cassava in other regions of Uganda is discussed by Hutchins et al. (1998).

2.5 Assessment of soil and food ingestion as a cerium exposure pathway

Elevated concentrations of Ce were reported in the brown, iron rich soils on high-ground in the Mukono and Mawakokie study areas (Smith et al., 1996). Mineralogical studies of these soils have shown that the main Ce-bearing phase is not monazite, but a range of more soluble secondary minerals, which is likely to increase Ce bioavailability. Ce was found to be enriched in the fine fractions of soil and dust, increasing its mobility in the surface environment and its likely absorption through the skin, which was suggested as an important exposure pathway in tropical environments by Price and Henderson (1981).

Simple dietary balances have been performed to establish the relative importance of Ce exposure pathways including water, food and soil ingestion for children and adults using mean, maximum and minimum concentrations (Smith et al., 1997). The results shown in Table 1 indicate that intake of food is the most significant Ce exposure pathway for adults, but that for children practising regular geophagy, exposure through the ingestion of soil is greatest. This highlights the importance of soil ingestion as a potential exposure pathway to Ce for both adults and children in Uganda. However, without more detailed studies concerning soil Ce bioaccessibility, an accurate assessment of exposure cannot be made.

Although Ce concentrations in Ugandan staple food crops were much lower than in some bio-accumulative plants, for example, Robinson and Edginton (1945) reported a Ce concentration of 320 mg kg $^{-1}$ in *Caraya Sp*.), they were greater than those reported for similar staple food groups from the UK (1-51 µg kg^{-1}, Owen, pers. comm.). Median concentrations of Ce were greater in cassava (*Manihot utilissima*; 10 ppb) and sweet potato (*Ipomocea batatas*; 9 ppb) compared to matooke (*Masa spp*.; 1ppb) which is consistent with the two former crops growing as tubers and subject to soil contamination (Smith et al., 1997).

Table 1 - Mean, maximum and minimum calculated annual intake of Ce for an adult diet, expressed in mg per year. Child assumed to be male or female in the age group 5-15 practising geophagy, consuming 28 g of soil per day - median value reported by Geissler et al., 1997 (after Smith et al., 1997).

Source	**Ce (mean)**	**Ce (max)**	**Ce (min)**
Food	64	226	29
Water	0.6	2.2	0.2
Soil (adult)	26	50	12
Soil (child)	2,400	4,670	1,120

3. METHODS AND ANALYSIS

3.1 Physiologically Based Extraction Test (PBET)

The PBET used in this study has been adapted from the procedure presented by Ruby et al. (1996). The test was originally used to estimate lead and arsenic bioavailability in mine waste materials. It consists of an *in vitro* test system that incorporates gastro-intestinal tract parameters representative of a human, for predicting the bioaccessibility of metals from an ingested solid matrix. It should be noted that the PBET does not mimic the entire physiological process controlling uptake of trace elements across the intestinal epithelium. As a result, the test cannot evaluate the dose dependent absorption, or the additional dissolution of the element resulting from the disequilibrium in the small intestine fluid caused by absorption of the elements.

3.2 Selection of PBET parameters

The rationale for selection of stomach and small intestine fluid composition, titration of reaction fluid pH on entering the small intestine phase, and the method for the collection of the *in vitro* extract samples were discussed in Ruby et al. (1993). In cases where only limited information was available to support selection of a test parameter, a conservative value was selected to maintain the overall conservative nature of the test. The PBET was designed around paediatric GI tract parameters for a 2-3 year old child, considered to be at the greatest risk to metal exposure from accidental soil ingestion.

Paediatric gastric pH is quite variable among individuals, and depends on nutritional status. Nevertheless, previous studies have given a mean fasting pH values of between 1 and 4. Following ingestion of food, stomach pH values rise to 4-5 (adult) and returns to fasting values within 2 hours as food is emptied from the stomach. An intermediate paediatric gastric pH value of 2.5 was used in the PBET test. A pH of 7.0 was selected for the small intestine, consistent with previous studies (Ruby et al., 1996).

The mass of soil material used in the reaction vessel (1 g) was selected after considering the U.S. Environmental Protection Agency (EPA) estimate of the mass of soil ingested inadvertently by a 2-3 year-old child (0.138 g d^{-1}; Stanek III and Calabrese, 1994) and the minimum feasible soil sample size (0.4 g; Ruby et al., 1996). Soil-to-fluid ratios in the range of 1:5 to 1:1.25 have been observed to affect dissolution of metals in extraction procedures, most likely due to diffusion-limited dissolution kinetics (Ruby et al., 1996). Insufficient data are available for fasting children to support the selection of a particular solid-to-fluid ratio. The volume of fluid in the reaction vessel was maintained at 100 ml, so the solid-to-fluid volume ratio was 1:167, assuming a soil density of 1.6 g cm^{-3} (Ruby et al., 1996).

Sample bottles were placed on a shaking tray to mix the synthetic stomach fluids in order to simulate the peristaltic mixing of the stomach contents. However, the degree of mixing was not optimised to simulate exactly the stomach and small intestine environments. Stomach emptying occurs in an exponential manner. In an adult stomach, 80% of the contents is emptied in the first hour after ingestion, with complete emptying within 2 hours. A healthy child's stomach is completely empty between 54 and 68 minutes following ingestion (Ruby et al., 1996). In the PBET, the stomach contents retention period was assumed to be 1 hour, despite the fact that this is consistent with the presence of food, while other PBET parameters may be based on a fasting individual. Stomach emptying in the absence of food occurs more quickly.

Small intestinal transit time of a semi-solid meal is considered to last between 3 and 5 hours in adults, 3.5 hours in children, and for a fluid meal ingested by a child, approximately 1 hour. Hence, a 4 hour small intestine transit time was selected for the PBET (Ruby et al., 1996).

3.3 Samples

The mineralogical composition of the Ugandan soil samples are dominated by 'kaoline', a mixture of kaolinite and halloysite, quartz and goethite. Kalonization occurs due to the acid leaching of silicates, and is typical of soils developed on volcanic rocks under tropical conditions. Goethite occurs as a weathering product of iron-bearing minerals such as siderite, magnetite, pyrite, and is indicative of a well drained leaching environment (Prior, 1994). Samples representative of various lithologies encountered in Uganda were selected on the basis of their varied cerium content and proportions of SiO_2 and Al_2O_3 using a Siemens manual XRF and electron microprobe. The samples were separated into specific size fractions: >500 µm, >250 µm, >120µm, >63 µm, >20 µm, >1 µm, <1 µm.

A number of surface soil and dust samples were collected from two districts in Uganda using a clean trowel and gloves respectively, which were cleaned after the collection of each sample. Soil samples 93/51 and 93/52 from Nawakakoi district, soil sample 94/80 from Mukono district and dust sample 95/7/3 (also from Mukono) were selected for use in the PBET because of the high cerium content of their lower size fractions (see Table 1). Further details of the samples and their collection are given in Hutchins (1997). Three replicate PBET tests were conducted for sample 93/51 and 94/80 to assess the reproducibility of the results.

3.4 Procedure

The apparatus used in the PBET is shown in Plate 1. A shaking water bath was used to mix the simulated stomach and maintain the temperature at 37 ^{o}C. Simulated gastric solution for the PBET was prepared by adding 1.25 g of pepsin (activity of 800-2500 units mg^{-1}), 0.5 g of citrate (Fisher Chemical Co.), 0.5 g of malate (Aldrich Chemical Co.), 420 µL of lactic acid (synthetic syrup), and 500 µL of acetic acid (Fisher Chemical Co.) to 1 litre of di-ionised (DI) water which had been adjusted to a pre-determined pH (see above) using 1% HCl.

Each soil sample (1.000 g) was mixed with 100 ml of stomach fluid in volumetric glass flasks. The flasks were placed in a shaking water bath maintained at a constant 37 ^{o} C. Nitrogen (1 l min^{-1}) was supplied for 5 minutes each time the flasks were opened in order to provide an anoxic atmosphere. This recreates the characteristic reducing conditions of the stomach and small intestine. The PBET was divided into three stages, defined by three sets of sampling. Stage 1 corresponds to the period spent in the stomach environment, and stage two and three that spent in the small intestine. Two samples were taken during the small intestine phase to ensure that equilibrium had been established between the fluid and solid phases.

Plate 1 - Apparatus used in the Physiologically Based Extraction Test.

Stage 1: An aliquot of 5 ml was removed from each flask one hour from the beginning of the experiment. In order to maintain the 100 ml volume in the reaction flask throughout the experiment, each time sample aliquots were taken, their volume was replaced immediately with the same amount of stomach solution.

Stage 2: All solutions were neutralised by introducing a dialysis tubing 8000 Molecular Weight Cut Off (Spectra/Por cellulose ester tubing) containing $NaHCO_3$ (sufficient to increase solution pH from 2.5 to 7) and 5 ml DI water into each flask. When the gastric solution had reached pH 7.0, 175 mg of bile salt and 50 mg of pancreatin were added to each flask. A 5 ml aliquot was taken from each flask after 2 hours at pH 7.0.

Stage 3: Another 5 ml aliquot was taken after a further two hours.

Cerium concentrations in sample aliquots were measured by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES(detection limit 0.5 ppm)) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS (detection limit 0.5 ppb)). In addition, blank aliquots were analysed from replicate flasks in which no soil sample was added to the gastric solution.

4. RESULTS

The percentage bioaccessibility of Ce in samples from Uganda was calculated as the fraction of the element present in the fluid phase (determined by ICP-MS) divided by the total Ce content of the samples, determined in on-going studies at BGS. Raw data from the PBET tests, including the concentration of Ce in solution and % bioaccessibility at each stage of the PBET tests for the replicate samples is shown in Annex A. A summary of the data is provided Table 1 showing the variability of Ce bioaccessibility in different size fractions in triplicates of the PBET, mean and standard deviation of the replicates (samples 95/7/3 and 94/80).

Table 2 - Results from PBET test of Ce bioaccessibility in soils and dust samples from the Mukono and Nawakakoi districts of Uganda.

Extract	Sample and District	**95/7/3 DUST MUKONO**				**94/80 SOIL MUKONO**				
	Size fraction (μm)	>63	>20	>1	<1	>120	>63	>20	>1	<1
	% of total soil	15	9	10	11	12	8	21	13	12

	Soil Ce (ppm)	60	120	550	360	60	70	240	490	280
1	Mean conc. (ppm)	0.77	2.61	4.01	2.44	1.00	1.51	37.20	35.33	18.97
	Mean Bioacc. (%(	1.29	2.17	0.73	0.68	1.66	2.15	15.50	7.21	6.77
	1 S. D. Bioaccces.	0.83	0.19	0.03	0.06	0.56	0.07	3.29	2.61	2.92
2	Mean conc.	1.20	5.46	8.62	5.16	0.76	0.57	69.93	116.10	49.70
	Mean Bioacc. (%)	2.01	4.55	1.57	1.43	1.26	0.81	29.14	23.69	17.75
	1 S. D. Bioaccces.	0.26	0.19	0.22	0.42	0.17	0.52	3.60	8.46	3.63
3	Mean conc.(ppm)	1.07	5.30	8.66	4.23	0.34	0.93	67.30	117.90	51.80
	Mean Bioacc. (%)	1.78	4.42	1.57	1.18	0.56	1.33	28.04	24.06	18.50
	1 S. D. Bioaccces.	0.29	0.23	0.06	0.35	0.30	0.23	0.79	7.59	3.33
	Mean Bioacces (%)	**1.76**	**3.73**	**1.29**	**1.17**	**1.08**	**1.48**	**24.0**	**15.5**	**14.6**

Extract	Sample and District	**93/51 SOIL NAWAKAKOI**				**93/52 SOIL NAWAKAKOI**				
	Size fraction (μm)	>120	<15	<1		>250	>120	>63	>20	<1
	% of total soil	15	20	17		15	17	11	7	5
	Soil Ce (ppm)	60	220	140		130	70	100	230	120
1	Solut. conc. (ppm)	2.20	2.09	2.58		0.06	0.27	0.01	3.98	2.95
	Bioaccess. (%)	3.67	0.95	1.84		0.05	0.39	0.01	1.73	2.46
2	Solut. conc. (ppm)	0.59	7.61	4.14		0.81	0.55	0.35	12.47	6.61
	Bioaccess. (%)	0.98	3.46	2.96		0.62	0.79	0.35	5.42	5.51
3	Solut. conc. (ppm)	0.10	6.84	3.36		0.42	0.72	0.37	12.84	6.75
	Bioaccess. (%)	0.17	3.11	2.40		0.33	1.03	0.37	5.58	5.62
	Mean bioaccess (%)	**1.60**	**2.50**	**2.40**		**0.33**	**0.74**	**0.24**	**4.24**	**4.53**

Figure 1

Figure 2

Figure 3 and 4

5. DISCUSSION

There was a large degree of variation in Ce bioaccessibility between the samples (Figure 1) and between the different size fractions of individual samples, particularly sample 94/80 (Figure 2). The greatest Ce bioaccessibility of 24% was observed in the >20 µm size fraction in soil sample 94/80 from the Mukono district. The dust sample from Mukono district had a lower range of mean Ce bioaccessibility between 1.17 and 3.73%. The finer fractions of the Nawakokoi samples (93/51 and 93/52) generally had greater Ce bioaccessibilities than the more coarse fractions The mean bioaccessibility of all the size fractions for this sample was 8.8%, calculated by multiplying the relative quantities of each soil size fraction by the mean bioaccessibilities of the replicates (Table 1). The largest size fractions generally had lower mean bioaccessibilities than the smaller fractions in each sample (Table 1 and Figure 1). There was a marked increase in bioaccessibility when the pH was increased between the stomach and small intestine phases of the PBET for the smaller size fractions between >20 and <1 µm for each of the samples, most notably in sample 94/80 (Figure 2).

Results from the replicate PBET tests for sample 94/80 (shown in Figure 3) indicate that although there is some degree of variability for replicate tests, they generally show good reproducibility. If all other factors, such as mineralogy, had been equal, Ce bioaccessibility would have been expected to be greater in the smaller size fractions because of their greater surface area. However, there was no clear relationship between total soil Ce content and mean Ce bioaccessibility (Figure 4), although with only 17 samples, firm conclusions cannot be drawn. The absence of a clear relationship suggests that variation in the solubility of the Ce-bearing mineral phases in the different size fractions may be a significant factor controlling Ce bioaccessibility. However, specific mineral phases could not be identified in the finest fractions with the highest Ce bioaccessibilities due to their small size.

6. CONCLUSIONS AND RECOMMENDATIONS

The ingestion of soil, or food contaminated with soil and dust, is known to be a significant pathway of exposure to Ce in certain districts of Uganda, which may be implicated in the aetiology of EMF. The advertent ingestion of soil (geophagia), particularly by children and pregnant women, is a practice which may significantly enhance exposure to Ce. However, prior to this study no information was available concerning the bioaccessibility of Ce in soils and dust in Uganda. Mean bioaccessibilities in the different size soil fractions ranged from 0.24 to 24.0 %. In assessing the risk posed by the ingestion of potentially harmful elements it has often been assumed that they are 100% bioavailable. The results presented in this report are significant as they indicate that the actual bioaccessibility values are much smaller than would be assumed in risk assessment calculations.

Results from the PBET test indicate on four soil and dust samples indicate there is a large degree of variation in Ce bioaccessibility. The availability of this data has assisted in more accurate risk assessments for exposure to Ce (Hutchins et al., 1998) which would otherwise have been based on assumptions of 100% bioaccessibility. Further investigation is required to determine the mineralogical composition of the Ce-bearing phases in the different size fractions, and their importance in controlling Ce bioaccessibility. Further studies are also necessary to validate the PBET bioaccessibility values using an *in vivo* method to determine bioavailability. The PBET has proved to be a useful technique for providing relative values for bioaccessibility and therefore improving risk assessments for exposure to potentially harmful elements.

7. REFERENCES

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