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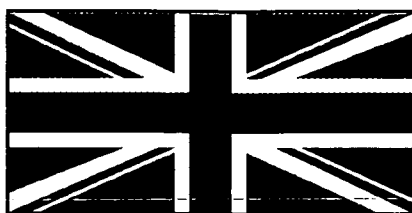
Cerium and Endomyocardial Fibrosis in Tropical Terrain Project Summary Report

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CERIUM AND ENDOMYOCARDIAL FIBROSIS IN TROPICAL TERRAIN

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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 cerium (Ce), and deficient levels of dietary magnesium (Mg) in southern India have been assigned as potential environmental cofactors in the aetiology of Endomyocardial Fibrosis (EMF), which is also endemic in certain areas of Uganda. The principal objectives of the project were to:

- To reproduce the findings of the Kerala, India group in a Ugandan context;
- To investigate whether the hypothesis put forward by workers in Kerala State could explain the occurrence of EMF in Uganda
- To assess the relative importance of various exposure scenarios for the Ugandan population to Ce and to define protective measures if required.

The results of the studies have indicated that whilst Ugandans are exposed to elevated Ce and are likely to be magnesium deficient there is no direct evidence to indicate that exposure to Ce is a significant factor in the development of EMF in Uganda. The hypothesis put forward by workers in Kerala State cannot therefore be used to explain the occurrence of EMF in Uganda. In the course of the study a comparative inventory of cerium and the rare earth elements in the teeth of children from the United Kingdom and Uganda was compiled. This represents a significant breakthrough in the study of the potential relationship between EMF and cerium in that it enables the relative exposure of living EMF patients and members of exposed populations to be determined.

In terms of limiting exposure to Ce and increasing exposure to Mg, the results place particular emphasis on:

- The practice of geophagy (habitual eating of soil), the accurate assessment of its magnitude (especially within the cultural contexts of the developing world) and its potential human health impact in terms of nutrition and toxicity.
- The contamination of cassava by soil during processing at a local level.

Additionally the project has clearly demonstrated the benefits to be gained from multi-disciplinary studies in the fields of environmental geochemistry and health, nutrition and medical studies, and the power of combining modern analytical techniques to the study of trace elements and human health in the developing world.

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Introduction

This report summarises the principal results, applications and developmental benefits of work carried out under Project R6228: "Cerium & Endomyocardial Fibrosis in Tropical Terrains" as part of the Department for International Development (DfID) Technology Development and Research (TDR) programme between 1995 and 1998. It is in the form of an expanded executive summary. As such it is intended to form:

- A reference source for the technically oriented, such as scientists, medical workers and advisers in tropical countries
- A briefing document for administrators who seek to understand the nature and applicability of a scientific methodology without wishing to delve into the underlying detail.

The project originates from previous research into human exposure to naturally occurring potential 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 on human health of chronic long-term exposure to cerium (Ce) has received relatively little attention.

Medical research, principally by workers in Kerala State, India (1986-1993) established an hypothesis linking Endomyocardial Fibrosis (EMF; a fatal form of restrictive heart disease that impairs ventricular filling and valve function), Mg deficiency (common throughout the tropics as a result of the prolonged leaching of Mg from soils and high intake of high phytate foodstuffs) and exposure to Ce. The same researchers, having subsequently corroborated this hypothesis through epidemiological and animal studies, attributed enhanced Ce exposure to the presence of residual monazite minerals in soils, over which the incidence of EMF was enhanced.

EMF is prevalent throughout the tropics and is an acknowledged problem in Uganda where medical workers from Mulago Hospital rank it as a major cause of heart disease amongst the young. Given that the abundance of Ce in lateritic soils is enhanced and that such soils are common throughout Uganda, the principal objectives of this study were:

- To reproduce the findings of the Kerala group in a Ugandan context;
- To investigate whether the hypothesis put forward by workers in Kerala State could explain the occurrence of EMF in Uganda

- To assess the relative importance of various exposure scenarios for the Ugandan population to Ce and to define protective measures if required.

The project initiated and developed collaborative links between: the Geology and Medicine Departments of Makerere University, the Centre for International Child Health, University of London and the British Geological Survey. During the course of the project additional collaboration was developed with: the Nottingham Trent University, the Eastman Dental Institute (London) and Child Health and Development Centre within the Paediatrics Department of Makerere University.

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 undertaken 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 be toxic at a cellular level.

In view of the considerable knowledge gaps relating to the abundance of Ce in foodstuffs preliminary studies were undertaken to develop accurate and precise methods for the determination of Ce and Mg in components of the Ugandan diet. Components studied included staple foodstuffs, occasional foodstuffs, water from sources used for potable supply, dusts associated with the in-house and roadside environment and soils (including those consumed during the habitual eating of soil (geophagy) and in herbal preparations). Developed methods were subsequently used to determine the concentration of Ce and Mg in over 200 separate food samples from throughout Uganda. During the course of Ce and Mg analysis, data were also collected for 21 major, trace and ultra trace components in these samples. These additional data, collected under rigorous quality control, for staple tropical foodstuffs represent a valuable resource for future studies into trace element nutrition and will be made available to other researchers on request.

In addition to their dietary contribution, through accidental and deliberate ingestion, Ce and Mg concentrations were also determined in soils to establish both the range and spatial distribution in their abundance in areas of Uganda in which medical studies had shown EMF to be prevalent. During these investigations Ce and Mg concentrations were determined in over 150 soils collected from sites within the Mukono study area and from over 50 sites associated with different environments throughout Uganda.

A key factor in linking EMF to a geochemical agent was the observation by previous workers in India that heart tissues from EMF cases contained significantly higher levels of Ce compared

to similar tissues collected from control cases. It was therefore considered important to investigate if this observation was also seen in the case of Ugandan EMF cases. To undertake this comparison the Ce concentration in autopsy tissues from EMF, non-cardiac and accidental deaths were determined and compared. Due to the destructive nature of this method of determination, thin sections of each sample were first analysed for the presence of discrete accumulation of Ce bearing phases by Scanning Electron Microprobe-Mapping. If any spatial correlation of accumulated Ce with cellular structures were to be observed it was considered that this would advance our understanding of the toxicological impact of Ce in Endocardial and Myocardial tissues.

Early indications, based on the elevated levels of Ce within the diet of rural Ugandans, appeared to corroborate previous hypotheses formulated by the group from Kerala State (proposing exposure to Ce as an important cofactor in EMF). However, the analysis of Ce in Ugandan heart tissues did not support this hypothesis and cast doubt on the role of Ce in the aetiology of EMF. Given this disparity it was considered important as a precautionary measure to:

- Develop a methodology by which relative exposure of individuals to Ce could be determined without recourse to autopsy specimens; and
- Investigate ways in which human exposure to Ce may be minimised whilst at the same time maximising exposure to Mg

On the basis of the chemistry of Ce and its association with phosphatic minerals, it was decided to investigate the presence of Ce in deciduous teeth (children's "milk teeth"; enamel is predominantly apatite (calcium phosphate)) as a marker for Ce exposure. This had not previously been undertaken and therefore an exercise comparing deciduous teeth from Uganda and the United Kingdom was conducted. As a result of this exercise a method for the determination of Ce in teeth was established and shown to be of considerable value in establishing relative exposure, not only to Ce but also to other biologically essential and/or toxic trace elements.

Analysis of the Uganda diet and data collected in our studies clearly indicated three ways in which exposure to Ce could be minimised:

1. Discouraging the practice of geophagy. However the prevalence of geophagy within a given population is considered to be related to trace element deficiency (particularly with respect to iron and Mg) and further work is required to estimate the likely side effects, or even potential success, of discouraging this practice without more effective dietary supplementation. It should also be noted that Mg deficiency has been observed to increase

the uptake of Ce in animal studies and discouraging this practice may lead to increased uptake of Ce from other dietary sources.

2. Improved food hygiene with particular emphasis on the careful washing of soil from tubers during food preparation, avoiding the drying of prepared foods and especially cassava tubers directly on the soil, and by covering of foods sold at the roadside (particularly murrum roads). Whilst of secondary importance in areas where geophagy is practised, exposure via these routes probably represent the most practicable way of reducing exposure with the added, more widespread, benefits of good food hygiene. It should also be noted that encouraging the drying of cassava tubers on mats, rather than directly on the soil, as is now common practice, would reduce the contamination of cassava flour by other soil bound trace elements (toxic or essential) and biological pathogens.
3. Using water from deeper aquifers. Whilst the concentration of Ce in shallow wells used by the poorest communities is enhanced compared to other sources of water, the relative exposure to Ce through drinking water is low. However, levels of magnesium are considerably enhanced in waters from deeper aquifers. Encouraging the utilisation of these deeper aquifers would therefore offer, not only a reduced vulnerability to contamination by biological pathogens derived from human and animal wastes, but an increase in dietary magnesium.

OBJECTIVES

The project addressed TDR Theme G2 "Identify and ameliorate minerals-related and other geochemical toxic hazards". The objectives of the study were:

- The provision of high quality environmental, technological and geochemical expertise and assistance in support of medical workers investigating a known problem relating to human health: the link between Ce, magnesium and EMF, a tropical coronary disease.
- The integration and development of geochemical and analytical expertise relating to the geochemistry of Ce and Mg in tropical environments, the assimilation of Ce by man through definition of dietary uptake, and its presence in diseased tissue through micro-probe analysis of tissue specimens over a period of 3 years.
- The establishment of an extensive, detailed data and knowledge base relating to the environmental mobility and abundance of Ce and associated trace elements.
- The development of a validated model describing the environmental and dietary uptake of Ce.

- The establishment of a knowledge base for formulation and optimisation of remedial measures, if appropriate, developed over a three year period.
- The development of collaborative links between geoscientists, medical and health scientists from the Geology Department of Makerere University, medical staff of Mulago Hospital, Kampala and the BGS, relating to the aetiology of EMF in Uganda.

WORK PROGRAMME AND KEY RESULTS

As detailed in the previous sections, the project set out to improve our knowledge of the geochemical variation, availability and dietary uptake of Ce, a rare earth element identified as being a possible contributory factor in EMF. The work-plan was therefore designed to define the environmental exposure to Ce under tropical conditions, to test the hypothesis that Ce, in conjunction with a low Mg diet, contributes to the development of EMF and to suggest simple remedial measures if appropriate. The scientific results of the study are detailed in supporting reports, numbers 2, 13, 15, 16 and 17 of the project outputs listed in the Appendix to this summary overview. They are available on request to those with a specialist interest in the topics covered. Methodologies employed and key results associated with each task are outlined in detail in the following sections.

1. Regional Baseline Studies and Literature Reviews

a) Occurrence and geochemical distribution of Ce in tropical environments.

Cerium (Ce) is a member of a group of elements known as the rare earth elements (REE) the mobilisation of which depends upon factors related to the mineralogy of the parent rock, the distribution of the REE between them and the stability of these minerals during weathering. Once released from the crystal structure, Ce and associated REE can be adsorbed onto clay surfaces, incorporated within secondary minerals or leached into porewaters and thence groundwater depending on the prevailing conditions. Investigation of Ce in lateritic profiles developed over a gneissic basement represent an analogue to conditions in much of central and northern Uganda and indicated the presence of secondary enrichment of Ce as a result of weathering and the occurrence of detrital grains of monazite. An extensive literature search in both commercially available abstract databases and in the records of the Uganda Department of Geological Survey and Mines has established that no published work has been undertaken to establish the distribution and environmental mobility of REE in the Ugandan environment. However, the association of Ce with igneous rocks, its generally low solubility and mobility and the aggressive long-term weathering environment of the majority of central Uganda indicates that Ce is likely to exceed its average crustal abundance (83 mg/kg) in Ugandan soils.

b) Ce Toxicity

A review of published studies of exposure to Ce was undertaken. Literature covering immunological studies, neonatal effects, cardiac tissue and liver function was identified. This supported a steadily increasing body of knowledge confirming that Ce, administered *in vitro* or to experimental animals, has quite profound effects on a range of tissues. However, the mechanisms by which Ce is thought to damage cardiac muscle have not yet been elucidated.

2. Chemical and Mineralogical Analysis

Preliminary investigations and data were obtained on soil and water samples collected in the Mukono region of Uganda during previous investigations into the occurrence of Al in tropical environments. This particular area was also considered to be appropriate for the study of EMF because of its similarity to the majority of central and northern Uganda, being developed on undifferentiated granitic gneiss. Additionally, observations by staff from Mulago Hospital had indicated that EMF was more prevalent in Mukono district than in other areas of central and southern Uganda. Subsequent campaigns in 1996 and 1997 collected samples of foodstuffs to establish baseline exposure to Ce within the Mukono region and further afield in Uganda. Samples were also collected from the households of EMF patients, with the long-term aim of establishing if levels of Ce were abnormally high in these particular environments.

a) Sample Collection, Collection of dietary material, foodstuffs and local vegetation from representative regions, chemical analysis for total Ce and Mg to estimate dietary intake of Ce.

Soils: Soil samples were collected at each site in which either a water or food sample was collected. Samples of heavy dust from the household environment were collected by sweeping floors and hard-standings. Samples of fresh rock were obtained in the limited number of areas where accessible exposures allowed.

Ninety percent of the soils sampled were collected in the Mukono and Nawakokie field areas. Soils within these areas overlie undifferentiated Precambrian granitic gneiss of the Basement Complex and shales, quartzites and phyllites of the Toro System (Geological Survey Department, 1965). Concentrations of Ce in freshly exposed country rocks from these areas lay within the range 14 to 140 ppm and 58 to 112 ppm respectively. Concentrations of Mg in similar samples ranged from 200 to 28,000 and from 60 to 10,000 ppm respectively. The soils may be grossly divided into reducing, grey, quartz-rich soils that are found within the lower valley floors and swamps, and oxidising, red, iron-rich loams that constitute the mid slopes. Both types of soil yielded pH values between 5 and 6 on saturation with distilled H₂O. The higher ground on which the majority of settlements are found exhibit only sporadic occurrences of laterite (partially as a result of land improvement). In addition, samples of soil were

collected from the central and southern regions of Uganda that overlie lithological units containing granites of post basement age; members of the Mityana Series, the Karagwe-Ankolean System and the Bunyoro Series; mafic volcanics of Mesozoic to Tertiary age; and Pleistocene volcanic and sedimentary rocks.

The highest Mg and Ce concentrations are associated with the Mesozoic to Tertiary and Pleistocene volcanic (carbonatitic) soils from the vicinity of Fort Portal, the rift valley and Tororo. In these areas the incidence of EMF is considered to be low. Mg and Ce concentrations within soils developed on the other sampled soils lay within the range observed in soils overlying the Basement Complex gneisses that cover the majority of central and northern Uganda. Interestingly, no significant difference could be observed in soils overlying the Toro System and the Basement Complex gneisses. It is therefore difficult to attribute the spatial distribution in EMF cases to the similar spatial distribution of these units with the Mukono-Luwero-Kampla triangle.

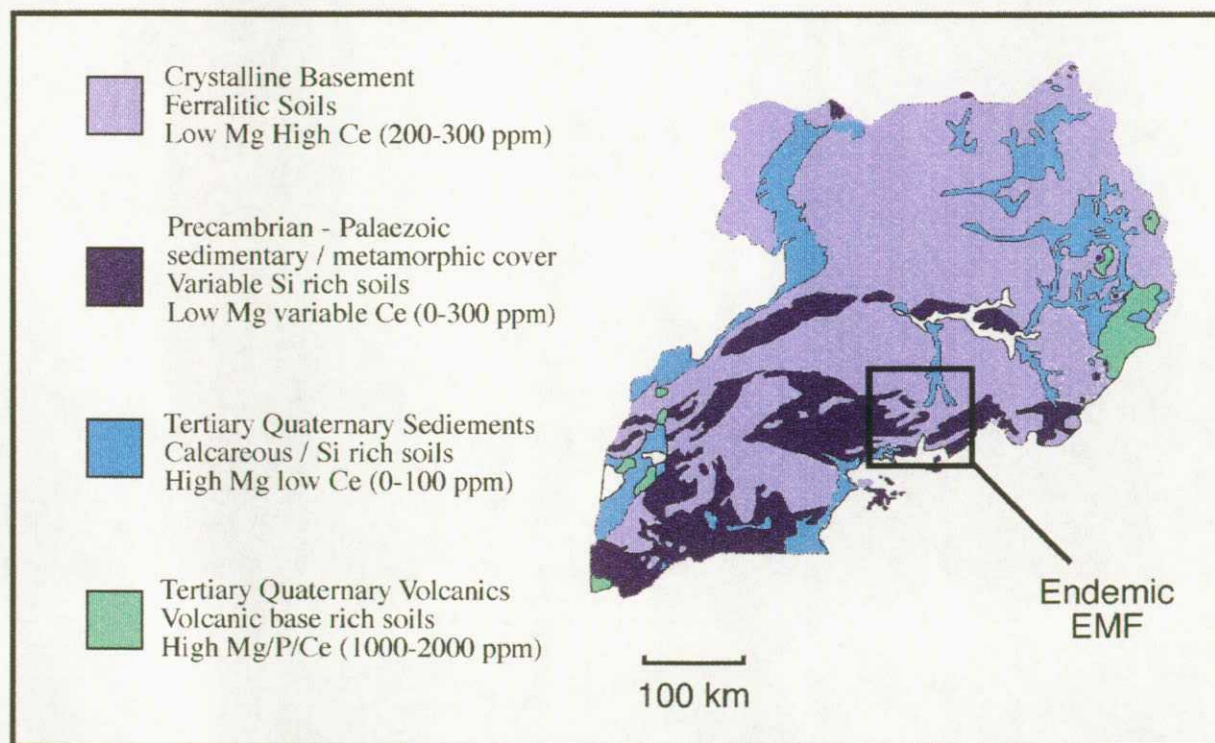


Figure 1. Simplified geological map of Uganda and observed ranges of Ce and Mg found in soils developed over each unit. Note the widespread occurrence of crystalline basement and precambrian – palaeozoic rocks throughout Uganda, that underlie the region of endemic EMF.

Water: Water samples were collected from a wide range of water sources (springs, hand dug pits and wells, boreholes and streams) that were used for the supply of potable water. Where prior sampling had indicated an elevated level of Ce, ultra-filtration was used to establish if Ce was present as colloidal material or in true solution.

Water supplies in Uganda have been undergoing gradual improvement in line with developmental policy since the middle of this century. The range of wells sampled in this study included the most basic of hand dug pits serving the poorest, most dispersed communities, to private boreholes within commercial homesteads and schools. In Mukono the most common water supplies were hand-dug wells, complete with concrete rings and hand operated pumps, and natural springs. These tended to be sited on the lower valley sides and, whilst the hand dug wells were productive, they often failed during dry periods. Elsewhere, such as in Nawakokie, boreholes tapping fractured aquifers within the crystalline basement were more common. The maximum observed Mg and Ce concentrations in filtered waters were 85 mg/l and 6 $\mu\text{g/l}$ with median observed concentrations of 1.9 mg/l and 0.29 $\mu\text{g/l}$ respectively.

The median value for Mg is considerably lower than Mg levels typically encountered in a wide range of UK aquifers whilst that for Ce is comparable to those encountered in granitic groundwaters from the UK but greater than those observed in aquifers hosted in sedimentary

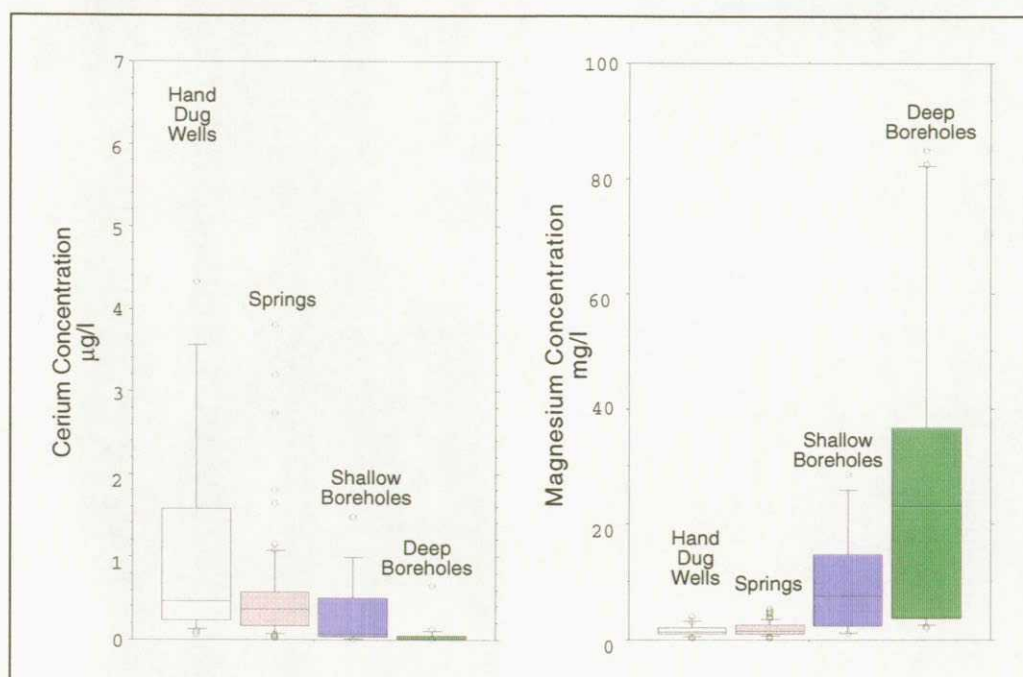


Figure 2. Box plots comparing percentile distributions of Ce and Mg determined in water supplies from the Mukono and Nawaikoke field study area. Horizontal bars correspond to the 10th, 25th, 50th, 90th and 95th percentiles.

environments. Unfiltered water samples (as used by the local population) exhibited higher levels of Ce. Results of ultra-filtration indicate that Ce is present as colloidal material, with a typical cut off size equivalent to 100,000 daltons (the size of a large protein). The gradual decrease in Ce from $> 0.45 \mu\text{m}$ to 300,000 daltons is consistent with the presence of poorly soluble Ce rich phases observed in the $>63\mu\text{m}$, $>20\mu\text{m}$ and $>1\mu\text{m}$ soil size fractions.

Foodstuffs: Levels of Ce and Mg determined on all foods sampled in this work yield maximum concentrations of 1.725 and 3640 mg/kg (Mg = 0.36 %) (fresh weight) respectively. Observed Ce levels are between two and four orders of magnitude lower than those for contemporary soils. Whilst Ce values are much lower than those observed in some bio-accumulative plants (e.g. 320 mg/kg in Caraya Sp., they are greater than those reported for similar staple foods groups from the United Kingdom (1 to 51 µg/kg). A similar comparison for Mg indicates that Mg concentrations are analogous to those observed in UK staple food groups (70 to 2100 mg/kg).

Comparison of data for individual staple food groups from Uganda (cassava (*Manihot utilissima*), sweet potato (*Ipomoea batatas*) and matooke (*Musa spp.*)) indicate broadly similar abundance of Mg within the foods. Cerium concentrations were elevated in sweet potato and cassava compared to Matooke, consistent with both being tubers and subject to contamination from soil. There was no evidence to suggest that staple foods from environs with elevated Ce and Mg levels (e.g. from the rift valley) showed enhanced levels of these two elements.

The highest Ce levels were observed in beans and green leafed plants, including tea. However, within the Ugandan diet these are generally only consumed within a relish and have a much smaller portion size than staples such as cassava and sweet potato. Levels of Ce in processed cassava and maize flour were observed to be an order of magnitude higher than that in carefully peeled and processed cassava roots and maize. This is consistent with contamination by soil during drying and/or the grinding process.

b) Mineralogical analysis of representative subsets to define mineralogical forms of Ce and estimation of Ce availability.

Mineralogical analysis was performed to identify the presence of Ce-rich minerals within soil samples. The samples were analysed for the presence of Ce and size fractions containing the greatest proportion of Ce were then subjected to more detailed analysis by electron microprobe (Cameca SX50). The sequential analysis of size fractions for Ce undertaken on a representative subset of soils (n=7) from Mukono and Nawakokie areas indicated that the majority of Ce lay within the finer size fraction (< 63 µm) of these soils. Electron microprobe mapping of the <20 µm sized fractions was undertaken for Ce, La, P. This identified Ce-bearing phases, a few of which also had high P and high La indicating sub 20 µm grains of monazite. However, the Ce:P ratios of most grains were high and therefore more consistent with a secondary hydrated phosphate than monazite. Examination of a combination of La, Ce and P maps showed that the highest Ce intensities occurred in grains with no significant La or P, indicating the presence of a Ce-bearing, non-phosphate mineral, possibly carbonate, oxide or hydroxide. The occurrence of Ce without La and other REE is generally an indicator of a mineral formed at low temperatures in the near-surface environment. Analysis of dusts obtained

from the floors and surfaces within the household environment, and samples of soil used in the practice of geophagy gave a similar range of Ce concentration to those from soil samples.

c) Baseline Dietary Uptake:

Two approaches were used to investigate the relative importance of different food groups, accidental and habitual practices on total dietary exposure. Initially a simple non-probabilistic dietary model (Model 1) was constructed to calculate annual exposure (as intakes i.e. assuming 100% uptake) of Ce and Mg using the mean, maximum and minimum concentrations found in samples of soil, food and water. At a later stage, as more information relating to dietary patterns became available, a second probabilistic model (Model 2) was constructed to investigate the sensitivity of various exposure scenarios to variations in dietary habits such as geophagy.

Dietary Model 1. Scenario: annual intake of Ce and Mg by an average adult and child living in the Mukono District of Uganda. Assumed daily diet comprised 0.5 kg matooke, 1 kg cassava, 0.5 kg sweet potato, 0.1 kg beans, 0.05 groundnuts, 0.2 kg cassava flour, 0.1 kg maize flour and 0.2 kg green vegetables. Daily water consumption was assumed to be 2 litres per day (WHO, 1993), consisting of 1.5 litres from hand dug wells and 0.5 litre from springs. In addition it was assumed that 300 mg of soil and 100 mg of dust were inadvertently ingested daily.

The results clearly showed that the soil is a significant source of Ce compared to other sources such as food and water, even for adults who have been assumed to ingest a very small quantity compared to children. In children practising geophagy, using the mean values for adult non-soil intake, the Ce intake from the soil represents 94% of all the Ce entering the body. In the case of children, water and food represent a negligible consumption of Ce especially when adjusted to account for body weight and diet. It should also be noted that the relatively large magnesium intake attributable to food most probably represents a gross overestimate as its bioavailability in cassava based diets is known to be particularly low. In the case of Mg, soil contributed < 5% of the total dietary intake, water < 1% and food > 95%. However, it is well established that in foods such as cassava the proportion of available Mg is limited due to the presence of phytic acid. In such cases the importance of Mg from soil ingestion and water will be enhanced.

Dietary Model 2: The second stage in dietary intake modelling involved a more refined risk assessment coupled with more sophisticated computational procedures and the use of data from the experimental determination of bioaccessibility. The same four major pathways of hazard are represented (water, food, soil and dust). However, uncertainty in the daily quantity and cerium content in each of the pathways has been accounted for by fitting statistical distributions to the observed available data.

Cassava preparation was investigated in three districts to allow analysis of the geographical variability in dietary habits. These districts, Arua, Hoima and Mukono, have all been assessed in terms of the typical percentage contribution to daily calorific requirement which the various staple foods found in Uganda (matooke, cassava and sweet potato) provide. Furthermore, the quantity of each foodstuff needed alone to satisfy the daily calorific requirement for each of (i) a 65 kg man, (ii) a 50 kg woman, (iii) a 20 kg child (7 years) and (iv) a 10 kg child (2 years) has been determined.

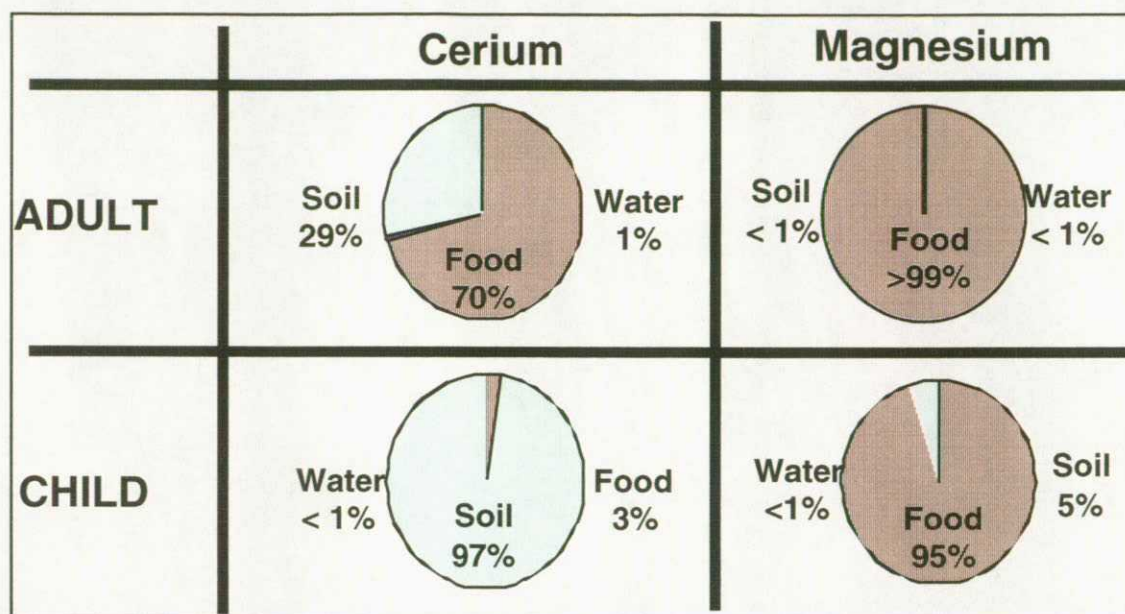


Figure 3. Dietary mass balance based on calculated mean annual intakes (Dietary Model 1). Child assumed to be male or female in the age group 5-15 practising geophagy. Note the importance of soil in controlling exposure to Ce and food in controlling exposure to Mg in both children and adults.

These staple foods are consumed with a relish, typically consisting groundnuts, beans or other green vegetables and this component has also been accounted for in terms of the daily calorific requirement. Green leaf vegetables are often unpalatable for young children.

Simulations also considered differences in methods of cassava preparation, which may involve different levels of contamination and hence a wide variability in the resultant elevation of cerium content.

In terms of water consumption, it is unlikely that individuals will take water from more than one source per day, although it is possible that seasonal variations in both source and quality may occur, as sources dwindle during the dry season, for example. In Model 2 it has been assumed that hand dug wells provide the most likely source of water with a small contribution to daily intake being provided by springs. Some analysis from shallow and deep boreholes has

been carried out and these sources have also been considered in the analysis, as access to different sources of water will probably vary between social groups. The poorer classes are less likely to have access to borehole supplies. Hence, as the Ce concentration in borehole water is lower than in springs or hand dug wells, the problems of ill-balance and limited variability of diet encountered by the lower social groups are exacerbated by the difficulty of access to good quality (i.e. low Ce) water supplies.

Results have shown that there is no appreciable difference in the Ce content of anthill soils and herbal medicinal preparations to that determined from distribution fitting of samples of profile soils from the region.

Hence the distributions used to determine inadvertent and geophagic ingestion of soil were identical. The amount of soil ingested by Kenyan children practising geophagy has been reported to range from 8 g to as high as 108 g per day. It seems unlikely that many individuals will consume much more than the median value of 28 g as constipation is likely to result. Hence, a probability density function (beta distribution) with an expected value (mean) of 28 g per day for geophagic soil ingestion has been used here in this study. Geophagic behaviour has been assessed in the cases of women and children within the risk assessment. The inadvertent consumption of soil and dust has been treated in the same way as in Model 1 with added representation of uncertainty. In both pathways (geophagy and inadvertent exposure to soils and dusts) a bioaccessibility factor was also included based on the experimental determination of bioaccessibility of Ce in Ugandan soils (approximately 10%)

Twenty two different scenarios of mean daily intake by men, women and children from the three districts have undergone analysis using Model 2. These results confirm that geophagic behaviour will always result in cerium ingestion above a level of 1.34 mg per day, whereas this figure is unlikely to be reached in any of the non-geophagic cases. The results illustrate the over-riding importance of the presence or absence of geophagic behaviour on individual daily Ce intake. This feature is consistent across all ages and all regions and contributes more than 1 mg per day Ce. The inadvertent consumption of soil and dust is generally of very little significance (c. 1% daily intake), whereas the consumption of water appears to have negligible impact on the dose (c. 0.1% daily intake).

Given a geophagic individual, soil consumption is the dominant contributor to daily Ce intake (c. 70% daily intake), whereas for non-geophagic behaviour the food pathway is by far the most important contributor to Ce exposure (>80% daily intake) and contamination of cassava flour by soil during preparation is the most significant factor controlling the magnitude of exposure. A change to preparation in a controlled environment will cause a very significant reduction in the intake (a 10-fold reduction in soil contamination would result in lowering Ce

intakes by approximately 0.25 mg per day). The preparation of cassava as chunks rather than as flour results in similar differences.

The study also demonstrates the importance of the Ce content in maize flour when estimating total Ce ingestion. In most of the 22 scenarios simulated, maize flour Ce content proved to be the most sensitive parameter. This is despite the greater importance of deliberate soil ingestion and of cassava flour consumption in determining total Ce intake. The Ce content of maize flour is very variable, possibly resulting in it being a very significant ingestion pathway. As in the case of cassava, there is potential for maize to become contaminated with soil during the drying process. More samples of maize flour are needed for further definition of its chemistry and investigations are necessary regarding patterns of its consumption. The importance of the maize flour pathway is particularly apparent in the cases of non-geophagic individuals living in an area where cassava is not the dominant staple food, such as Hoima.

In terms of regional differences, simulations for the Arua region show the highest Ce intake whereas those from Hoima show the lowest. These trends are consistent with the relative differences in dependence on cassava flour as a staple food. Cassava dependence increases in the order Hoima<Mukono<Arua. However, medical data and investigative studies have demonstrated that EMF is far more prevalent in Mukono district than in Arua. Variation in dependence on cassava results in a difference of between 0.15 and 0.50 mg per day Ce intake from Hoima to Arua, with the smallest differences being observed in those eating the smallest quantities (i.e. 2 year olds). Differences are considerable and are of similar significance to those introduced by changing the method of cassava flour preparation. (For example, if a man in Arua were to eat cassava chunks rather than cassava flour, his Ce intake would be similar to that of a man eating his cassava as flour in Hoima.)

The results further emphasise that there are considerable benefits to be gained from improvements in the storage and processing of foods. However, the study has increasingly served to focus attention on the issue of geophagia. It is evident from field studies that Ugandans perceive that considerable nutritional and physiological benefit can be made from this practice and such habits are deep-rooted in the local culture. On the other hand, apart from the specific concern of enhanced cerium intake, other hazards are introduced, such as a greater exposure to intestinal worms if the preparations are not carefully baked. The key difficulty lies in weighing up the balance between these perceived benefits and the associated hazards.

In summary, these observations are consistent with the results of epidemiological studies by co-workers in Uganda that indicate particular risk factors in Ugandan EMF to include low socio-economic status and a cassava based diet. However, the high risk attributed in epidemiological studies to the use of poor quality water supplies is not reflected in the relative contribution of Ce and or Mg from water. Such discordant observations are well documented

in other studies investigating the relative importance of exposure via drinking water to total dietary exposure.

3. Model Development and Case Studies.

In order to develop the models a number of topic specific case studies were carried out and reported separately.

a) Identification of areas of high EMF prevalence and correlation of likely Ce uptake.

i) High EMF prevalence: A case control study of risk factors for EMF in Uganda, and its association with dietary risk factors and/or eosinophilia was undertaken by staff of Mulago Hospital. The study involved 61 case studies of EMF together with two control groups, one with other types of heart disease (n=59) and one comprising patients hospitalised for trauma and/or elective surgery (n=61). Each eligible and consenting patient completed a questionnaire asking for details of occupation, home address, education, proxy measures of socio-economic status, usual diet, and exposure to water and soil before the patient became ill. It should be noted, however, that the questionnaire was prepared and the survey undertaken before our research had implicated geophagy as a potential exposure route and that the section of the questionnaire related to water supply did not allow for differentiation between springs, hand dug wells and deeper boreholes.

Results of this study confirm the preponderance of EMF among immigrants from Rwanda/Burundi, although the proportion of indigenous Baganda with EMF was observed to be rising. An endemic area of EMF was identified in the Luwero-Mukono districts and these results are consistent with previous data suggesting a role for an environmental factor such as Ce. Consumption of cassava as a staple food was observed to be a very high risk factor for EMF. It is worthy of note that cassava varieties used in Mukono are principally of the low cyanide (non-bitter) variety. However, cassava consumption is common world-wide and forms part of the staple diet in regions where EMF is extremely rare. It is therefore unlikely to be a primary cause of EMF. The results of the study also indicated additional support for the eosinophilia hypothesis and no correlation between EMF and the quality of the water resource.

ii) Low EMF prevalence (control areas).

From the above survey by staff from Mulago Hospital and from investigations performed during the course of our studies it was clear that:

- consumption of cassava was a key risk factor in EMF (even though cassava is consumed in areas in which EMF is rare).
- cassava flour contained relatively high levels of Ce due in part to contamination by soil.

Arua and Hoima were identified as suitable control areas as they were areas of high cassava consumption and a negligible level of EMF (according to the case control study reported above). During two visits to these areas subsets of the local population were screened by echocardiography. In both cases the incidence of EMF was established to be considerably lower than that of Mukono. Analysis of cassava and cassava flour from these regions indicated a broadly similar range in observed Ce and Mg concentrations when dried on local soil. No significant differences were observed in the Ce and Mg contents of bitter cassava as preferred in the North of Uganda to those varieties used in Mukono.

b) Collection and analysis of biological samples to define human Ce uptake and micro distribution; Modelling of Ce mobility and uptake

i) Heart Tissue samples: Seventy five post-mortem samples of endocardial and myocardial tissue from patients whose cause of death was attributed to EMF, cardiac failure non-EMF and non cardiac failure, non-EMF (accidental death) were obtained as specimens embedded in wax blocks (courtesy of the M. Odida, Pathology Department, Mulago Hospital). These samples of tissue were analysed for the presence of rare earth rich zones using scanning electron microscopy - electron probe micro analysis (SEM-EPMA) of thin sections and for total rare earth content by inductively coupled plasma mass spectrometric (ICP-MS) analysis of dissolved tissue.

The determination of Ce gave concentrations of 8 to 1150 $\mu\text{g/kg}$ Ce (expressed as dry weight). This concentration range is similar to that reported by the workers from Kerala Province, India when data is normalised to dry weight on the basis of a moisture content of 75 to 85 %. However, in the case of our Ugandan studies there was no statistically significant difference between tissues from the two control groups and those of the EMF patients. Indeed, tissues from the non-cardiac, non-EMF cases contained a statistically significant higher concentration of Ce than either the EMF tissues and/or those from cardiac, non-EMF cases. This is the opposite trend to that observed by workers from Kerala Province.

The concentrations of other REE elements in the tissue samples (La, Nd, Sm and Pr) show a high degree of correlation with Ce and are dissimilar to that observed in Ugandan soils and dusts that may have been a source of contamination during preparation.

Examination of tissue sections for zones of enhanced rare earth abundance was carried out on nine EMF hearts, four hearts from cardiac non-EMF cases and two hearts from non-cardiac, non-EMF cases using an automated rare phase searching technique. Ce-rich particles were only found in two sections, one EMF heart and one with other heart disease. The particles were extremely small (approximately 2 μm) and consequently very difficult to identify. The particle in the EMF heart consisted of Ce, iron, oxygen and an unidentified element, possibly hydrogen or carbon. This composition does not match any known naturally occurring mineral and is not

thought to be contamination. The other particle was located in a heart with non-EMF disease and consisted of Ce with other REE but no other detected elements. This suggests it might be an oxide, hydroxide or carbonate of some kind, which could possibly be contamination from dust.

ii) Dental analysis:

Children's deciduous teeth from a matched subset (age, sex, type) of Ugandan (n = 50 from Mukono) and British children (n = 50) was obtained by either collecting naturally erupted teeth

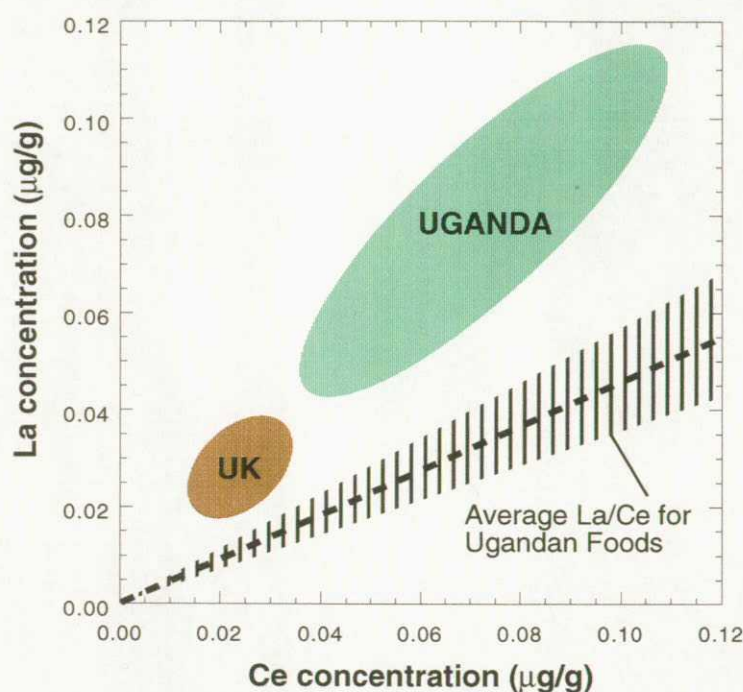


Figure 4. Comparison of the concentrations of Ce and La in childrens teeth from Uganda and the UK. For comparison average La:Ce ratios are indicated for Uganda soils, foods

or those removed during surgical procedures. The teeth were either (i) ground and digested for whole tooth analysis; (ii) ground and separated on the basis of density for dentine and enamel analysis; (iii) cut and thin sectioned for analysis by laser ablation microprobe - ICP-MS.

Conclusions from the study showed conclusively that the concentration of trace elements in teeth from UK and Ugandan children were significantly different. Ugandan teeth were shown to contain higher concentrations of Ce than UK teeth. During analysis by laser ablation microprobe - ICP-MS Ce was observed to be present in the outer layers of the enamel suggesting sorption from the oral cavity as the main mode of incorporation. No evidence was found to indicate that Ce was present due to the physical incorporation of particulate Ce in the outer layers of the enamel.

These results indicate that teeth are a good marker for exposure to Ce and may be also used for monitoring exposure to a wide range of other trace elements including zinc, lead and uranium.

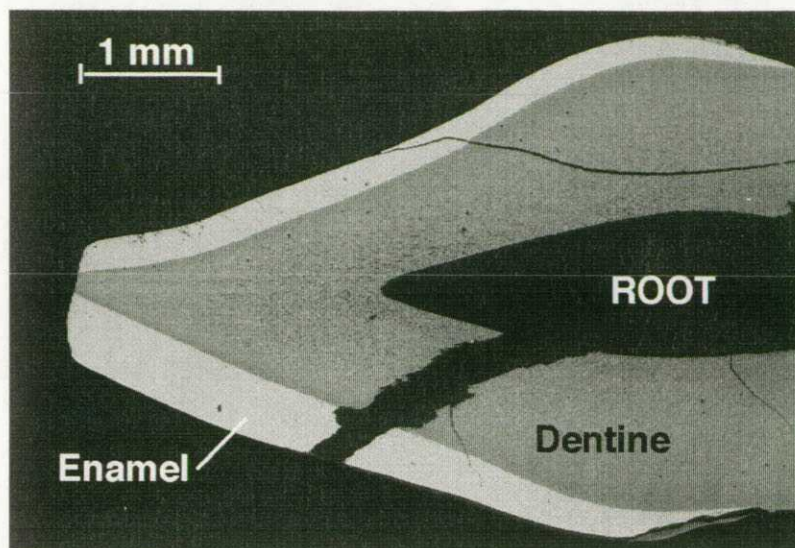


Figure 5. Cross section of Ugandan tooth. Ce was concentrated in the outer 25 μm of the enamel at concentrations at least 25 times higher than those observed in either the inner enamel and/or dentine.

4. Summary of Results and Conclusions

This project has shown that the populations of the greater part of Uganda (including Mukono, Nawakokie) are exposed to similar concentrations of Ce through ingestion of staple foods and/or soil. Exposure is likely to be significantly increased by contamination of dried and processed foods through drying them on local soil.

Analysis of dietary factors and their impact on the magnitude of exposure throws particular emphasis on the practice of geophagy, the need for accurate assessment of its magnitude (especially within the cultural contexts of the developing world) and its potential human health impact in terms of nutrition and toxicity

It has been established that elevated levels of Ce in water supplies are due to the presence of particulate and semi-colloidal Ce bearing phases, most probably derived from the surrounding soil and/or regolith. Despite these elevated levels, a simple mass balance indicates that Ce in drinking water contributes only a small proportion of the total exposure. Levels of Ce and Mg were directly linked to the type of sampled water: shallow sources having higher Ce (a potentially detrimental factor) and deeper sources having greater Mg (a potentially beneficial factor).

Determination of Ce in heart tissues from EMF patients and controls show no statistically significant correlation between EMF and elevated levels of Ce in heart tissue. This clearly

conflicts with the hypothesis linking EMF and Ce put forward by the group studying EMF in Kerala Province. However, it is still possible that the wax blocked samples from the Ugandan post-mortem archive may have either lost or gained excess Ce as a result of the blocking process. Unfortunately this uncertainty will remain until either:

(a) the collection of fresh post-mortem tissue (or teeth) and the performance of post-mortem studies on EMF patients becomes a routine procedure in Mulago Hospital.

(b) Mulago Hospital is able to perform cardiac biopsy.

Studies on the use of Ce in children's teeth as a marker of Ce exposure have proven to be successful, and a clear trend indicating enhanced exposure in the case of Ugandan children has been established. Obviously this technique has the benefit of not requiring a post-mortem to obtain samples for study and could be extended by investigating the concentration of Ce in EMF patients teeth, via a case control study in which free dental work was undertaken in return for the donation of extracted teeth.

In the context of remedial measures aimed via a cautionary approach at reducing exposure to Ce, there is clearly benefit to be gained from improving procedures used in the domestic drying and storage of foods. In addition to the specific concerns of the present study this has also direct relevance to minimising exposure to other potentially toxic trace elements that are derived from the soil zone.

Medical survey work undertaken by the staff of Mulago Hospital has clearly demonstrated the occurrence of EMF amongst populations exposed to similar dietary levels of Ce and Mg in Arua district and elsewhere in Toro district. However, levels of EMF within Mukono district are clearly well above these background levels and this cannot be explained simply by the distribution of Ce and Mg in the diet of this district. This, and the lack of enhanced concentrations of Ce in diseased heart tissues, suggest that exposure to Ce, in a Ugandan context, is not a significant factor in the development of EMF.

This finding when set in the context of other international studies of EMF (and especially those performed in Kerala Province which indicate such a strong association between EMF and Ce) is consistent with observations in the literature that suggest that EMF is a common endpoint of different causal factors or a combination of various causal factors.

CONCLUSIONS, RECOMMENDATIONS AND DEVELOPMENTAL BENEFITS

Conclusions.

1. Work undertaken in this study clearly demonstrates the benefits to be gained from multi-disciplinary studies in the fields of environmental geochemistry and health, nutrition and medical studies. And the “broad brush” approach needed in the quantitative assessment of potential exposure scenarios for a given range of trace elements. In addition, the study indicates the power of linking modern analytical techniques to the study of trace elements and human health in the developing world.
2. Whilst there is strong evidence to suggest that the Uganda population is exposed to increased Ce and Mg there is no direct evidence (such as geographical relationship between environmental concentrations of Ce/Mg and the prevalence of EMF, and/or elevated concentrations within diseased heart tissue) to suggest that exposure to Ce, in a Ugandan context, is a significant factor in the development of EMF.
3. In the context of exposure to Ce and Mg, the study throws particular emphasis on the practice of geophagy, the accurate assessment of its magnitude (especially within the cultural contexts of the developing world) and its potential human health impact in terms of nutrition and toxicity.
4. Ce was found at low concentrations in a wide variety of staple foods and water supplies. In the context of cassava (the consumption of which is in itself an implicated factor in the aetiology of EMF), levels in fresh tuber were negligible compared to that observed in cassava dried on the soil at a local level, prior to milling into flour. This increase was attributed to the cassava becoming contaminated with Ce from the soil surface upon which it was dried.
5. The project has produced for the first time a comparative inventory of cerium and the rare earth elements in the teeth of children from the United Kingdom and Uganda. This represents a significant breakthrough in the study of the potential relationship between EMF and cerium in that it enables the relative exposure of living EMF patients and members of exposed populations to be determined. Previously only post-mortem tissue has been used.

Recommendations

1. In the context of remedial measures, there is clearly benefit to be gained from the improvements in procedures used in the drying and storage of foods that have been traditionally dried at the household level. In addition to the present study this is of direct relevance to minimising exposure to other potentially toxic trace elements or biological pathogens that are derived from the soil zone.
2. Whilst the relative importance of exposure to Ce and Mg from drinking water is considered to be low compared to that derived from the practice of geophagy, there is clear evidence to suggest that the use of water abstracted from deeper aquifers increases exposure to essential trace nutrients such as Mg and minimises exposure to potentially toxic components derived from the near surface. This therefore represents an additional beneficial factor to be taken into account when balancing the costs and benefits of developing water resources derived from deeper basement aquifers.
3. In respect of geophagy it is doubtful whether traditional soil eating habits can and should be altered. However, given its potential health impact, the importance and prevalence of this apparently “normal” human activity, including its beneficial and detrimental aspects must clearly be the subject of further research.
4. The use of teeth as a bio-marker of exposure to Ce should be exploited to study the observed relationship between EMF and Ce in Southern India or, alternatively, to the study of exposure to other potential toxic and/or essential trace elements such as arsenic, lead, zinc and uranium in areas of known excess (mine workings) and deficiency (refugee camps).
5. Whilst the use of geochemical techniques and methodologies can be used to study the impact of trace element excess and deficiency on human health, the study has highlighted the need for the provision and incorporation of the geochemical and environmental data produced into integrated, well managed, national data banks. The continued development of these should therefore be seen and actively promoted as important infra-structural markers, particularly during the development of national geological and industrial resources.
6. Whilst exposure through geophagy and inadvertent ingestion of soil are clearly important, the bioavailability of Ce (or other potential toxic or essential elements) in Ugandan foods requires investigation. In this respect it is also important to assess the bioavailability of Mg (foods and soils) and the influence that deficiency in Mg in the diet may have on Ce uptake.

Developmental Benefits.

1. The project has facilitated contact and scientific discussion between medical staff of Mulago Hospital, the Child Development Department of Makerere University Medical School, Makerere University Geology Department the International Centre for Child Health (London) and the British Geological Survey (Nottingham). Such interchange between scientific disciplines at both an applied and theoretical level clearly has developmental benefits and has led to the publication and presentation of over 18 scientific reports and publications. Many of these were wholly or jointly authored by members of staff from counterpart organisations.
2. Data collected during the study will be of direct benefit to ongoing research at Mulago Hospital and the International Centre for Child Health in the fields of Mg and Fe deficiency.
3. Quality assured analytical data collected for over 300 separate comprehensive soil, water and food samples (for Ce, Mg and other trace elements such as Fe, Cd, Zn and Pb) are available for circulation to appropriate national and international bodies for inclusion in baseline databases. As such the data will form a resource against which contextually to set and compare national and international drinking water, soil and food standards. This is especially true of the food analysis undertaken in this work, which represents a major contribution in respect of the trace element content of tropical foods such as cassava, sweet potato and matooke.
4. Expertise in the use of integrated chemical, hydrological, and geochemical studies has been exchanged and transferred to counterpart organisations, particularly to medical and health practitioners, enabling a greater understanding of the need for interdisciplinary studies to underpin the formulation and implementation of future national and international policy.

ACKNOWLEDGEMENTS

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APPENDIX

5. Project Reports and Publications

(a) Publications, Posters and Oral Presentations

1. SMITH, B., TIBERINDWA, J.V., FREERS, J., TOMKINS, A., BROWN, C.J., CHENERY, S.R.N., COOK, J., HAMPTON, C., RUTAKINGIRWA, M., and SSERUNJOGI, L. and STYLES, M. (1997) Cerium & Endomyocardial Fibrosis in Tropical Terrain, Interim Technical Report. British Geological Survey Technical Report Number: WE/98/25.
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3. BULLOCK, S (1996) Unpublished MSc Thesis, Nottingham Trent University.
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6. RUTAKINGIRWA, M., (1996) Unpublished MSc Thesis, Makerere University
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8. FREERS, J., MANANJA-KIZZA, H., ZIEGLER., J.L. and RUTAKINGIRWA, M (1996) Echocardiographic diagnosis of heart disease in Uganda., Tropical Doctor, July, 125-128.
9. FREERS, J., AMANDUA, J and MUGERWA., R (1993) Endomyocardial Fibrosis and Eosinophilia. Lancet, 342, 1233.
10. FREERS, J., MANANJA-KIZZA, H., RUTAKINGIRWA, M. and GERWING, E (1996) Endomyocardial Fibrosis: why is there marked ascites with little or no peripheral edema. Lancet, 347, 197.
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12. SMITH, B., TIBERINDWA, J.V., FREERS, J., TOMKINS, A., BROWN, C.J., CHENERY, S.R.N., COOK, J., HUTCHINS, M.G., RUTAKINGIRWA, M., and SSERUNJOGI, L. and STYLES, M. (1998) Cerium and its implications to the aetiology of Endomyocardial Fibrosis in Uganda. Presented at Geoscience 1998, Keele, United Kingdom.

13. RAWLINS, B.G., CORDEIRO, M.J.A.R., SMITH, B (1998). Exposure and bioavailability of cerium through the ingestion of soil (UGANDA). British Geological Survey, Technical Report WC/98/12
14. CORDEIRO, M., HUTCHINS, M.G., RAWLINS, B.G. and SMITH, B (1998) Bioavailability factors for cerium, uranium, arsenic and antimony determined using an extended PBET methodology. 16th European Conference, Society for Environmental Geochemistry and Health, Derby, UK.
15. HUTCHINS, M.G., SMITH, B and SSERUNJOGI, L (1998) Pathways and factors defining enhanced cerium hazards in Uganda. British Geological Survey, Technical Report WC/98/22
16. STYLES, M., CHENERY, S.R.N, and SMITH, B (1998). A study of the distribution of cerium and other trace element concentrations in heart tissues from Uganda. British Geological Survey, Technical Report WC/98/20
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18. SMITH, B., COOK., J.M. and CHENERY., S.R.N (1997) The association of cerium and heart disease in Uganda. *Geoanalysis 97*, 3rd International Conference on the Analysis of Geological and Environmental Materials, Vail, Colorado.