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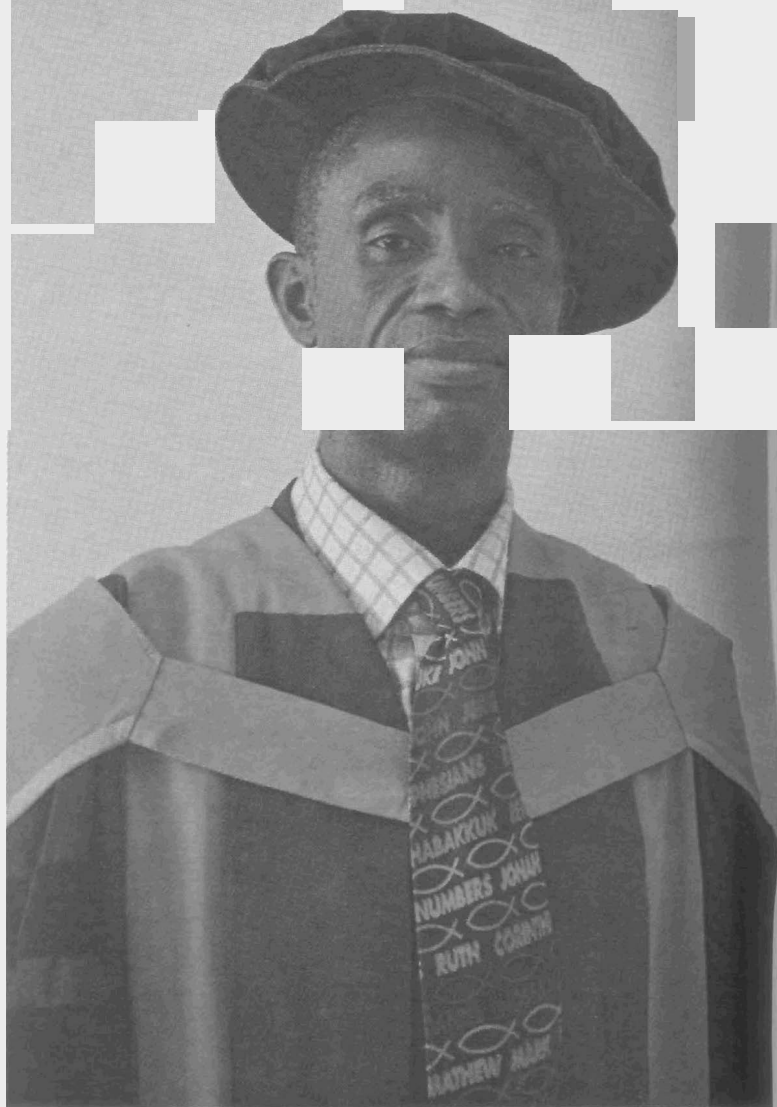
**TRACE MATTERS: Stable and  
Radioactive Elements in Human  
Health and Environment**

**By**

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# **TRACE MATTERS: Stable and Radioactive Elements in Human Health and Environment**

**An Inaugural Lecture Delivered at Oduduwa Hall,  
Obafemi Awolowo University, Ile-Ife, Nigeria  
on Tuesday 9th April, 2013**

By

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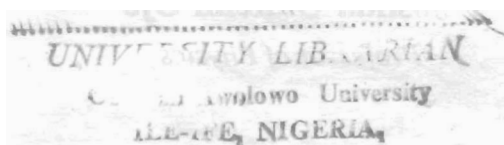
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## **Preamble**

Mr. Vice Chancellor, distinguished guests, ladies and gentlemen. Nineteen years ago this week, on April 8 1994 to be precise, I presented for oral defence, the first PhD thesis in Engineering Physics from this great University. Seventeen years later, this same week in the year 2011, it was the turn of my first PhD student to present his own thesis in Engineering Physics. And now two years on, here I am again today, on the exact same day as my student, and clearly by Divine Providence, presenting an inaugural lecture on the same subject matter. This two hundred and fifty-fifth Inaugural Lecture is the seventh from the Physics Department, and the first by an alumnus of the Department. This is obviously a great privileged and honour. I give all the glory to the Almighty God, and I thank you for being here to listen to my story.

## **Engineering Physics – Science without Borders**

I have always wanted to be a scientist. From childhood, comics about the “eccentric scientist” and fictions of cosmic travels into “other worlds” glorify the world of science and make it very appealing. However, at the point of completing my JAMB form for University admission in 1981, I suddenly realized I needed to put in for a ‘realistic course’ as the course “Science” did not appear anywhere on the brochure!

So, with considerable sorrow, I bid Biology bye, as I filled in Physics as my first choice course. What I did not realize then was that in Great Ife (my first-choice University), the worlds of Biology and Physics - the REAL world, are merged in the field of Medical and Health Physics. This field was one of the options available under the Engineering Physics programme, a programme designed primarily to midwife Nigeria’s entry into the nuclear world. After completing my first degree under the Physics programme, I promptly enrolled for postgraduate studies in Medical and Health Physics. At the present time, Medical Physics

now stands alone as an Option in our Engineering Physics programme, while a new Option, Health Physics and Environment has been created. The remaining two Options in the programme are Nuclear Science and Engineering, and Material Science.

As far as I am aware, even up till today nearly 40 years after the Engineering Physics programme took off at the then University of Ife, we are still the only University offering such a programme in Nigeria; whose goal is to bring the benefits of Quantum Mechanics down to the common man, and the holistic development of the Nigerian nuclear industry in particular. The founding fathers of the programme, under the visionary leadership of Prof A.F. Oluwole, understood, ahead of their time, that no Nuclear Energy programme can be sustainable without a firm Health and Environmental framework. This position is vindicated today by the increasing number of developed countries who are beginning to phase-out their extensive nuclear energy programmes and embrace other alternatives – strictly based on health and environmental concerns. The list includes Switzerland, Germany, Italy, and yet-to-decide Japan. Of course, such a course of action might not necessarily be the best option for Nigeria, especially if we are able to learn from the mistakes of others. Decisions of this nature are not lightly made, and a whole gamut of factors incorporating science, engineering, health, environment, national security, socio-economics, and law are involved. Students of Engineering Physics are trained to holistically assess such factors and come up with optimized decisions. (See Zehner 2012)

No less critical is the ever-present potential for silent routine abuse of the products of nuclear reactions, which unfortunately does not attract screaming headlines as would major incidences at nuclear facilities. To have an idea of the magnitude of such a problem, consider news report earlier this year, of a radiotherapist who received a three year jail term for having consistently over-exposed his patients to ionizing radiation over several years - simply due to incompetence. The incidents

occurred at a high-brow hospital in France, a country with one of the best-developed nuclear industry in the world (see The Australian News, Jan 31, 2013). One shudders at the thought of what could be happening in countries where the industry is less regulated!

On 30th June, 2011, Charles McMillan, Lab Director of Los Alamos National Laboratory in the United States, had reasons to explain to the Press the research focus at this top nuclear facility; and in so doing, he validates the traditional concept and scope of Engineering Physics:

“The lab works on such topics as renewable energy and particle physics, solar flares, forensics on terrorist attacks, and studying the AIDS virus at the molecular level to help scientists develop strategies for developing vaccines. The lab also did early work on the human genome project.”

Continuing, he asked rhetorically:

"You say to me, `Why in the world would Los Alamos be into biology? The path is an interesting one because it's related to nuclear weapons. Back in the 1950s, nobody understood very well how radiation, particularly from nuclear weapons, affected biological systems, and so the laboratory got involved with helping to do those studies."  
(Mac Millan 2011)

The widespread everyday applications of nuclear science, with direct implications on human health, are not limited to the field of medicine alone, however. In his inaugural lecture (January 1999), Prof Ade Amusa from the Physics Department, showed that “hardly any aspect of life in any advanced country, and increasingly in many developing countries, is not touched by the diverse fields of applied nuclear science”. He referred to this

situation as the “positive Janusian face of nuclear science applications”.

The Centre for Energy Research and Development (CERD), established in 1976 was the nursery where our Engineering Physics programme was nurtured. As the visionary pioneer Director of the CERD, Prof A.F. Oluwole recruited and sent out over a dozen fresh graduates from the Physics Department to a number of selected laboratories in Europe and North America; and most of them came back with their PhDs rightfully acknowledging him as mentor and co-supervisor of their theses. But I had the singular honour of being the first PhD he would directly supervise and graduate under his own name. Profs (then Drs) Muheez Durosinmi from our University here in Ile-Ife, and Nicholas Spyrou at the University of Surrey in Guildford, were co-supervisors of my PhD thesis. I am highly indebted to these distinguished scholars for their contributions to my academic training; as well as to the European Union which provided the bulk of the required funding for the PhD under the EEC-LOME III Agreement.

## **Introducing trace elements**

The class of chemical elements known as “trace elements” must certainly be one of the most ill-defined sets in Science. These elements, irrespective of their physico-chemical properties or biological actions, are so called simply because they occur in such low concentrations in the matrix of interest that their precise determination in years past was extremely difficult or impossible. Their concentrations were then given the rather convenient value “trace” - somehow suggesting that they are “negligible and inconsequential”.

However, developments and spin-offs from the nuclear industry have opened up the world of trace elements for precise scientific studies; and the verdict, as this lecture sets out to show, is that “trace” indeed does matter in many areas, especially in

human health and environment. The accurate quantification of trace elements in biological matrices, study of their interactions among themselves, their biological fate when ingested, and ultimately, implications in health and diseases, form the core of the thriving multidisciplinary field now known as Biological Trace Elements Research (BTER) and which I joined as one of the pioneers in Nigeria in 1990, under the tutelage of the aforementioned distinguished scholars. While BTER traditionally involves only stable elements, the study of radioactive elements (which invariably also always come in “traces”, in environmental and human matrices) is the traditional turf of Health Physics. In this lecture I will be addressing the tremendous roles both stable and radioactive trace elements play in human health and environment, and share some of my contributions to date on the fascinating subject.

## **Trace does matter**

What led to the widely reported death of hundreds of children living in several gold mining villages in Zamfara state here in Nigeria (March 2010 till date) was not witchcraft as was first supposed by the villagers, nor was it anything ingested in bulk amounts – toxicologists vainly ran through a series of suspected candidates ranging from cyanide to microorganisms. Rather it was due to the oral ingestion of relatively “trace” amounts of the heavy metal, lead. In Japan the “*itai itai*” disease was so named because thousands of those afflicted by it kept moaning those Japanese words to express the deep pain they felt in their bones and spines, as a result of the ingestion of “trace” amounts of cadmium in their rice. The rice plantation had been irrigated by water polluted by mining activities. In the United States in the 1800s, an epidemic within the hat industry referred to as “hatters’ shakes” was found to be due to exposure to trace levels of mercury during the production of felt. Indeed, the expression “mad as a hatter” has its root in the loss of memory and

damage to the nervous system of hatters following chronic low level exposure to mercury in those days. According to the Blacksmith Institute, the top four global toxic threats for 2010 were trace elements – lead, mercury, chromium and arsenic; followed by pesticides and radionuclides. Indeed, Nair et al (1994) averred that the annual total toxicity of all metals mobilized by human activities exceeds the combined total toxicity of all the radioactive and organic wastes generated each year. Several examples of the deadly impacts of trace levels of stable and radioactive elements abound – in various aspects of life, ranging from public health to plain crime.

The way out-of-proportion influence of trace elements is felt however, not only with their elevated levels. The deficiencies of several of the so-called essential trace elements have been linked with several disease states and conditions, including, as I will show in the course of this lecture from my own works, Sickle Cell Anaemia, Cancers, and even Affective Disorders. While Wilson's disease develops as a result of accumulation of trace levels of un-metabolized copper in tissues, deficient levels of the same copper is directly linked with Menkes disease. Another classic example is Selenium, which below  $50\mu\text{g/L}$  in blood, is associated with a wide range of negative health outcomes including impairment of thyroid hormone metabolism, cancer, degenerative affections, cardiovascular diseases, viral infections, inflammatory conditions, chronic renal failure, etc (see Klapcinska et al, 2006); but with toxic effects manifesting at blood levels exceeding  $500\mu\text{g/L}$ . This is roughly equivalent to having two drastically different outcomes when we have one red ball among ten million others and in another case where the number of red balls was three, among ten million others. Effects of toxic levels of Se include selenosis, loss of hair, accumulation of fluid in the lungs, bronchitis, pneumonitis, bronchial asthma, nausea, abdominal pain, enlarged liver, problems with reproduction, and even death (Lenntech 1998). All of these imply that for any

scientific study to be possible at all in a field of study like BTER, the determination of the levels of these elements must not only be accurately done, it must also be precise: that is, reliably repeatable.

Indeed, for some well-studied trace elements, threshold concentrations leading to specific biological responses are now recognized. For example in the case of lead, while severe toxic effects such as colic i.e. intermittent severe abdominal cramps, encephalopathy (seizures, reduced consciousness coma) and death are associated with high blood lead levels (BLL) - above 70  $\mu\text{g/dL}$ , symptomatic lead intoxication featuring general fatigue, tremor, diffuse abdominal pain and weight loss begins to manifest at BLL of 35 to 50  $\mu\text{g/dL}$  in children and 40 to 60  $\mu\text{g/dL}$  in adults. Below these, though the subject might appear totally asymptomatic, impairment of speech and hearing functions may occur at BLL as low as 10 to 25  $\mu\text{g/dL}$ . (ATSDR 2005). Even below the "action level" of 10  $\mu\text{g/dL}$  recommended by the US CDC, neurological impairments, reduction of IQ, aggressive behavior, delinquency, and attention disorders are also well established in school-aged children (The LEAD Group, 2011).

For babies in the womb, the toxic impact could be far more severe, even at much lower levels (Dorea 2004). Unfortunately, in several developing countries today (Nigeria inclusive), as long as children are not yet dying enmass as a result of their preventable chronic exposure to trace levels of lead, such widespread exposure and established consequences simply appear not to matter.

## **Biological Basis for the actions of trace elements in health and diseases.**

### *Toxic Actions*

That any ingested chemical will exhibit toxic actions when ingested above certain amount, is not altogether surprising. However, the man on the street still finds it incredible that extremely minute quantities of some materials could have such

tremendous deadly impacts. The toxicity of many trace elements derives from their bonding to some biologically important target molecules, thus disrupting their basic functions. This initiates a cascade of effects which may include changes in gene expression causing improper cell division, impaired protein synthesis, changes in internal and external maintenance causing impaired ATP synthesis, or altered membrane function leading to cell injury. Eventually, if the natural repair mechanisms for correcting these effects are overwhelmed, clinical symptoms will result.

Common symptoms of trace elements toxicity include unbalanced homeostasis, bleeding, abnormal neuromuscular activities like tremors, convulsion and paralysis. Arun Shanker (2008) has extensively reviewed the mechanism of action of some specific trace elements toxicity. It is important to note that while the toxicity may be due directly to the action of the trace element in question, in several cases, the toxin may be a metabolite of the parent compound, or it may be one of the numerous free radicals generated during the in vivo transformations of the toxin. It is also possible that the principal toxicant may be an endogenous molecule or compound synthesized as a response to the primary toxin.

### *Essentiality Actions*

The mechanism of action (MOA) of essential trace elements are even more difficult to be intuitively apprehended. They may be classified under four headings: catalytic, electrochemical, structural and miscellaneous. These MOAs often overlap however. For example the catalytic action may be based on some electrochemical effect.

a. Catalytic: According to Green (1941), “enzymic catalysis is the only rational explanation of how a trace of some substance can produce profound biological effects”. Biological catalysts are known as enzymes and without them many essential biochemical

reactions will never proceed at fast enough rates. Impairment of functions and 'dis-ease' is the natural consequence of enzymatic malfunctioning. A good number of enzymes either have highly specific associations with particular metals or at least need some suitable metal to function appropriately. Enzymes in the former class are known as metallo-enzymes while those in the latter group are called metal-activated enzymes. The list of metal-activated enzymes is practically inexhaustible. The commonest enzyme activator is magnesium, which activates the large class of phosphate transferenceases among others.

b. Electrochemical: The electrochemical functions of trace elements are also quite vital to the maintenance of the body in a state of health. Elements which have electrochemical functions exist as free ions, in which form they play a number of significant roles. Primary among these is their acting as a source of free energy for the cell metabolism. With various ions existing at different concentrations within and outside the cell, a potential difference (between 50 – 100 mV) is generated across the plasmalemma. The need for this free energy in such processes as stimulation of nerve cells, tissue respiration etc is obvious. The role of trace quantities of calcium in information transfer has also been well described (Rasmussen, 1989). Elements in free ion forms also play an important role in stabilizing colloidal solutions. Furthermore, the solubility of many proteins is known to depend on the ionic concentrations.

c. Structural: Biological molecules often need to exist in the right structures and configurations in order to function effectively – if at all. The structural functions of the elements N, P and S in proteins, nucleic acids and phospholipids, for example are well known. It is also known that Mg is essential for the stability of ribosome (Bowen 1966a) while Fe plays a vital structural role in the haemoglobin molecule (Stryer 1988, Murayama M and

Nalbandian R.M.. 1973). The role of Ca in cell necrosis has been well studied. The primary mechanism involves damage to the structure of the cell membrane leading to loss of cell contents and inevitably cell death (Trump et al 1981). This has serious implications in a disease like Sick Cell Anaemia for instance, where the anaemia is a direct result of loss of red blood cells whose membranes have been irreversibly damaged as they repeatedly pass through microcirculation. Furthermore, still talking about sickle cell anaemia, low levels of total Mg in sickle cell erythrocytes have been associated with red cell dehydration and consequent increase in sickling following increased formation of polymers in haemoglobin S (de Franceschi et al 1997, de Franceschi et al, 2000). It has been demonstrated that the dehydration is due to abnormally high red cell permeability and loss of potassium ( $K^+$ ). One of the established pathways for K efflux involves the K-Cl co-transport which is known to be abnormally activated by low cell  $Mg^{2+}$  (de Franceschi et al 1997). This causes rapid irreversible loss of  $K^+$  and  $Cl^-$  ions, with water following osmotically.

d. Miscellaneous: Apart from the three principal modes listed above, trace elements also in other manners miscellaneous, play quite important roles in the body's metabolism, even though some of these modes are not quite well elucidated yet. Many compounds are known to contain essential elements apparently playing no important role. It is reasonable to assume that such roles are merely yet to be discovered.

On the other hand, many clinical and pathological disorders in the body have also been traced to trace element deficiency, imbalance or toxicity even though no explanations could yet be given in terms of the above listed modes. It is now well known that metals are also present in nucleic acids (Berg 1990). These metals are believed to play some role in protein synthesis and in the transmission of genetic information. An

example in this regard is the finding by Eichhorn and Clark, (1965) that copper has the ability to unwind and rewind DNA strands. Similarly Vallee et al (1981) reported on the role of zinc in gene expression. Recent findings on the so-called zinc finger domains (Berg 1990) have further confirmed that trace metals may play genetic roles.

### **Challenges of trace elements analyses and methodologies**

As previously mentioned, the primary issue in trace elements matters, (which gave rise to the nomenclature), is the extreme difficulty of accurately quantifying them. But such quantification is a *sine qua non*, if scientific progress is to be made.

In July 2008 I had a number of biological samples to analyse, but the local facilities I intended to use were not working. Since my report to the sponsoring agency (the International Atomic Energy Agency, Vienna) was due, I began a desperate internet search for laboratories abroad that could carry out the analyses, even if it meant paying exorbitant commercial prices. Below is extract from one of the e-mail responses I received. I believe it is a good introduction to the challenges involved in BTER:

*I thank you for your interest in performing analyses in our institution.*

*As a preliminary info, please note than none of our research teams is involved in the study of lead or deuterium in physiological fluids (blood, urine, saliva). If some of your analyses were to be possible in our labs, there would be a potential lack of expertise in the interpretation of results on such samples.....*

*Actually, the person in charge of ICP-MS analyses in our Department of inorganic and analytical chemistry has just retired, and it is not*

*obvious that analyses of Pb in blood and urine could be performed on this instrument without the specialised personnel being present.....*

*I am sorry that I cannot give you more definitive answers to your request*

The above indicates that even when the required analytical facility is available and functional, specialized expertise is required to get quality and reliable outputs in using the facility for different sample matrices. And then when that hurdle is cleared, another set of expertise is required to interpret the outputs so that it is meaningful with respect to the actual biological or environmental problem at hand.

Mr Vice Chancellor sir, even when the facilities are available and in top working conditions, when highly motivated technical teams are on ground and they are backed-up with spare parts and regular maintenance arrangement, and when the scientific teams competent to interpret the results for real life applications are ready for business, there still exist some peculiar challenges that go with BTER work – all deriving from the minute quantities of the elements we seek to reliably quantify. Let me quickly highlight some of these, with emphasis on the need for a holistic, multidisciplinary approach if they are to be successfully overcome.

### 1. Presampling Factors

A lot of initial considerations have to be made even before samples for trace element analyses are collected. The major pre-sampling factor is the decision on what samples to collect and the where and how to collect them. In taking these decisions, consideration has to be made for biological variations associated with subjects' age, sex, genetic composition, existence of unsuspected subclinical conditions, environment, exposure history (either via medication, diet, or occupation), geochemical factors,

seasonal changes including diurnal variations, and long- and short-term physiological influences. This situation clearly demonstrates the need for careful planning and mapping out of research objectives before the work is started. According to Iyengar (1989), the mass of unreliable data from earlier publications in what he called “the Cart-before-the-Horse period” derived from the fact that most of the results were mainly intended to demonstrate the powerful capabilities of the new emerging analytical methodologies rather than solve real-life problems.

## 2. Sampling and Sample Preparation

Sample collection is often a major challenge in trace elements work since in many cases, the level of the trace element of interest might even be higher in the ambient environment than in the biological sample itself. Even after the sample has been collected, the problem of contamination or significant loss of elements remains real as there could be interactions between the sample and the container resulting in leaching or adsorption of trace elements to and from the container. For example in a work involving collection of blood samples by venous puncture (one of the commonest matrices in BTER), the possible sources of contaminations include the needle (and the syringe, if a vacutainer tube is not used directly), the anti-coagulant added to prevent clotting, exchange of materials between the various blood components, etc.

Furthermore, prior to actual analysis, samples have to be taken through a number of processes, depending of the analytical facility to be used. Generally this will include washing, drying/freeze-drying, homogenizing, weighing, making pellets, coating, or digestion of matrices etc. During all these processes, other materials come into contact with the sample; and they may well introduce the element of interest in relatively substantial quantity into the sample, thus totally rubbishing all subsequent analytical efforts or expertise.

In view of the contamination issue, it is therefore absolutely fundamental that all sample preparation must be carried out in a designated Clean Room where first and foremost, the air has been passed through high efficiency particulate air (HEPA) filters. Just a speck of dust landing in an open tube containing the sample could severely distort the results, depending on the composition of that dust and the elements of interest in the analyses. Likewise, because it is often impractical to buy on commercial basis, the volume of required supra pure grade acid needed for routine digestion, it is often necessary to equip the Clean room with acid sub-boiling distillation units that can produce fresh supra pure grade acid as needed. Of course the quality of the water used for routine cleaning must also be assured.

All these foregoing also underscore the tremendously important question of sample representativeness and homogeneity. In other words, how well does the sample we have collected represent the population under study; and how well does that small aliquot eventually presented to the instrument for analysis represent the entire sample? All these issues must be addressed and favourably resolved before moving into the analytical laboratory.

### 3. Analytical Challenges

All said and done, the sample must be presented to the analytical facility and a useful output received. The first problem that arises is that of choosing the appropriate analytical technique for use. This could pose some challenge as often, there would be trade-offs between the various available methods, especially with respect to the types of elements detectable, the detection limits for the elements of interest in the particular matrix, precision and accuracy of the technique, level of difficulty of sample preparation, speed of analyses, sample size available, and invariably, cost of analyses. Often, it might be expedient to use more than one method as complementary techniques to allow for

greater flexibilities and “fine-tuning” of each technique towards particular elements.

Experience shows that many people find it difficult grasping the real implications of the challenges briefly mentioned here. Take sample size for instance. In BTER, there are serious limitations on the quantity of sample available for analysis – consider for instance having to collect blood (and possibly from a sick person). Otherwise, if there were no limitation to the quantity of sample available, it would have been trivial devising protocols to pre-concentrate a sizable quantity of the elements of interest before presenting our aliquot to the analytical facility, thus reducing trace elements analyses to conventional elemental analyses. Protocols that are blind to these basic considerations are not likely to pass the mandatory ethical clearance to start with!

Even after an excellently-executed analytical work, my experience in examining postgraduate theses and other works from within and outside Nigeria, shows that the final step of converting concentrations of elements of interest as obtained from the equipment to the biologically meaningful concentrations in the fresh living sample, is often a challenging hurdle for those not well prepared ahead for the exercise. For instance, if the samples had gone through some form of drying, it would be necessary to have measured the fresh and the dried masses accurately before the analyses. This has to be done for each individual sample - although the density of the fresh sample (another quantity that must be established), might be assumed constant for each sample type.

## **Analytical Methods**

What conventional analytical chemistry waves aside as “trace” matter actually contains a humongous amount of individual atoms. For example, a “trace” amount of Se, say the 50 microgram we referred to earlier, nevertheless contains  $(6.023 \times 10^{23} \times 50 \times 10^{-6} / 34) 1.77 \times 10^{16}$  atoms. Now to have an idea of

how big this number is, suppose we have that many balls and every one of the 6 billion people on earth were recruited to count these numbers of balls. Counting non-stop at an average rate of 1 ball per second, we would all still be on the assignment 4 ½ years after!

So, the gateway to trace element analysis is the ability to stimulate individual atoms in a sample and record the responses of these individual atoms – in short, we turn to atomic or nuclear physics. (The provision of the facilities required for such endeavours constitutes one of the major peaceful applications of the nuclear industry to mankind.) The methods of stimulation and of recording the responses, give the various analytical techniques available today; and the efficiencies for doing these determine the detection limits achievable for individual elements by these techniques.

Broadly speaking, the more physics-like methods target the inner core of the atom (inner shell electrons or even the nucleus) and involve using projectiles such as neutrons, protons, or x-rays for the stimulation – as in Neutron Activation Analysis and X-ray fluorescence spectrometry. The more chemistry-like methods, on the other hand, use thermal energy for atomic stimulation via the valence electrons responsible for chemical bonding – as in Atomic/Optical Emission Spectrometry. Somewhat in between is Atomic Absorption Spectroscopy where photons of specific energies are used to stimulate the valence electrons. In this case however, since specific photons are required for the stimulation of each element, only single elements can be analysed at a time – a serious limitation in BTER. And then there is, in a unique class of its own, Mass Spectrometry, where the atom is stripped of its orbital electrons, for example in a plasma, and the bare nuclei are then detected and identified based on their different masses (and charges).

For radioactive elements however, there is no need for the initial stimulation as the individual atoms of interest in the sample

are already radioactive. All we have to do is determine which of the emerging radiations - gamma or charged particles (usually alpha particles), we wish to use for our analyses. Whereas gamma spectrometry is largely an instrumental method (and thereby located mostly in Physics departments), alpha spectrometry requires more demanding sample preparation and involves much chemistry. However after the isotope of interest has been chemically or electrically separated from the hosting matrix, the determination of radioactivity becomes quite trivial. And the result can be truly astounding!

Mr. Vice Chancellor, I recall vividly my excitement in July 2009 when during a Post Doc fellowship at the University of Wisconsin, Milwaukee, in my first alpha spectrometry exercise, I evaluated the activity of Pu-240 in a sediment sample as  $5.32 \pm 1.60$  Bq/kg. This corresponds to the precise determination of the quantity of Pu-240 in the sample as  $640 \pm 190$  femtogram per gram. Femtogram is a billionth part of a microgram! It is such power of trace determination using alpha spectrometry that makes possible ongoing investigations to determine whether or not the late leader of the Palestinian Liberation Organization, Yasser Arafat, might have died due to ingestion of trace amounts of Polonium - eight years after he had died and had been buried! (See for instance [bbc.co.uk/news/world-middle-east-20501945](http://bbc.co.uk/news/world-middle-east-20501945))

A more detailed overview of the more common implementations of the analytical techniques briefly mentioned here is provided in the Appendix to this lecture.

### **Some thoughts on the use of analytical facilities in Nigeria**

Mr. Vice chancellor sir, like every other physical scientist involved in BTER, my entry point was through the analytical door. My research work to date has involved the use of all the facilities mentioned above. At the University of Surrey, Guildford between December 1990 and February 1992, I was involved mainly with Instrumental Neutron Activation Analysis (INAA)

under the tutelage of Nicholas Spyrou using the CONSORT II Reactor located at the University of London Reactor Centre, Ascot. My major stay at a Reactor Centre however was at the Nuclear Radiation Center of the Washington State University, Pullman, where in two short visits during my PhD (one month each, in March and November/December 1991), and a more extensive Post Doc in 1995 I was located at the 1 MW TRIGA Mark III Swimming pool reactor facility. I also used Nuclear Reactors when I paid research visits to the MNSR at the Ghana Atomic Energy Centre, Accra (in 1998) and the LVR-15 experimental reactor of the Nuclear Research Institute, Rez, Czech Republic (in 2002). The 14 MeV neutron generator at our Physics department here at Ile-Ife must have stopped working long before I came into the department as an undergraduate in 1981, but I met the generator at the CERD working, till the tube probably expired in the late 1980s.

While I was in England, together with NAA, Proton-Induced X-ray Emission (PIXE) was used as a complementary analytical method for my PhD work, and I had full access to the 2 MeV Van de Graaf accelerator located at the Department of Electrical Engineering of the University of Surrey. I also did some characterization studies on my samples using the Scanning Electron Microscopy facility at the Chemistry department. It is gratifying to note however that now we have a working PIXE facility here on our Campus at the CERD, one of the only two or three of such facilities on the continent. Through the untiring efforts of Prof A.F. Oluwole we were able to procure both an Energy Dispersive XRF system as well as a TXRF- facility in the Physics Department. I acquired my proficiency with TXRF work during an IAEA sponsored 2-week Scientific Visit to the Stanislaw Staszic University of Mining and Metallurgy (UMM), in Krakow, Poland, working with Prof Marek Lankosz and the amiable Technologist, Beata Ostachowicz. We have recently

replaced the tube of the TXRF in the Physics Department and hope to have it back in active service very soon.

My efforts to get meaningfully involved with the atomic spectrometry facilities are still ongoing. I had an agreement with the Crop Utilization Laboratory of the International Institute for Tropical Agriculture (IITA), Ibadan which allowed my graduate students to take samples there for analyses by ICP-AES. But the charges were rather stiff. However recently, the IITA has come up with a scheme which encourages external users to have full access to their facilities, paying only a modest monthly access fee. This shows the direction analytical services are going, and our University should learn from it. At the present time, most of my major analyses are carried out by ICP-MS and a specialized AAS-based arrangement for mercury determination at the Department of Environmental Sciences of the Jozef Stefan Institute, Ljubljana, Slovenia. The partnership was forged by the International Atomic Energy Agency under an on-going CRP involving about a dozen institutions from different countries.

Of course, I am also involved, (and more keenly interested) with locally-available Atomic Absorption Spectroscopy (AAS) facilities. On this campus, we have at least four of them (two at the Central Science Laboratory and one each at the CERD and Department of Soil Science and Land Resources Management). However, none of them have installed accessories to make them versatile for *BTER*. To be fair, Prof T.A. Olugbade during his tenure as Director of Central Science Laboratory (CSL) did procure a hydride generator accessory unit; but the plain truth is that the suppliers could not get competent personnel to do the installation till now.

Mr. Vice Chancellor sir, the sad reality is that most local suppliers who bid for the supply of scientific equipment for trace elements analyses in Nigeria often under-estimate the challenges involved. Half-way into the supply, they realize the difficulties and decide it better to cut their losses and make away with the

mobilization fees rather than struggle to complete a tasking burden. Such monuments of partially-installed facilities litter several departments and units on campus. I can right away think of the Inductively-Coupled Plasma Atomic Emission Spectrometer (ICP-AES) and Fourier-Transform Infra Red (FTIR) facilities rotting away at the CSL; and the Thermal Ionization Mass Spectrometer in my own laboratory at the Physics department, which installation started in the 1970s!

Perhaps we can still task the pool of our creative but under-utilized technologists on campus, to look through the whole lot and see what can be salvaged, possibly dismantling some units to repair others. The remaining can then be disposed off as junk to allow for fresh initiatives. It should be clear that we are never going to get these machines working no matter how long legal litigations we pursue. The suppliers, despite their own best desires and efforts, simply do not have the expertise to get the facilities working. Not even in the foreign countries of manufacture do we have the experts any more, since most of the models have been phased out, and sometimes, even the companies themselves have folded.

Our efforts at gamma spectrometry have been modest but much more successful. The CERD has just recently commissioned an intrinsic Germanium detector, while the NaI(Tl) based set-up there has been working for well over a decade. In my own laboratory in the Physics Department, I have been able to convert a previously unused counting pile designed for C-14 dating to a station for a CsI-based gamma spectrometry. The CsI detector (and associated multi-channel analyzer) was procured under an initiative coordinated by the CSL, and the set-up has been working excellently since procurement in December 2009.

But getting working facilities, as I have stated earlier on, is solving only half of the problem involved in a Trace elements Laboratory. A robust discussion at one of the recent meetings of the Senate of our University was centred on this very issue. Now

that I have the University's attention, I will like to submit that people will continue to complain of "inaccurate and bad results" from our centralized laboratories as long as we maintain the scientifically naive idea that we can simply drop our samples for some super technologists who will then give us the results of the analyses. This might indeed work for some other chemical analyses, but definitely not for trace elements. Matrices matter and each scientist develops his/her own capabilities and expertise with time and practice. The technologists indeed assist in running the equipment, but this is very far from getting accurate useful results. The University, particularly for the big facilities at the CSL and the CERD, must return to the concept of Users Groups comprising of interested scientists with common research interests meeting regularly and sharing experience on the use of the facilities. The University Research Committee could help facilitate this arrangement by making modest funds available for the Users Groups to meet and present their reports.

I will like to refer to the following announcement, found on the internet, on the use of the ICP facility at the University of California in the USA (accessed Feb 2013):

8-25-2011; ICP-OES Training.

1. There is currently no staff for the ICP facility. For researchers who are prepared to work very independently, training on how to operate the ICP-OES instrument is available. You must be prepared to make up your own samples and standards that are used for every run. **There is no training available for making up samples.** If you are not yet prepared to make up samples, it is premature to learn to run the instrument. ([http://www.mic.ucla.edu/ICP/ICP-OES\\_training.pdf](http://www.mic.ucla.edu/ICP/ICP-OES_training.pdf))

Let me conclude my submission on this point with a vivid illustration. Shown in the figure below is the result of an intercomparison test organized by the IAEA, asking several

interested laboratories to determine the level of the important, yet relatively easy to analyze element, Copper, in a powdered cow's milk reference material prepared by the Agency. The results reported for the same highly homogenized sample varied from 0.08  $\mu\text{g/kg}$  to 72.2  $\mu\text{g/kg}$  – altogether a factor of 880 between the highest and the lowest! (Parr 1983) This should be food for thoughts to those who think trace elements analyses just another little activity they can practice part-time.

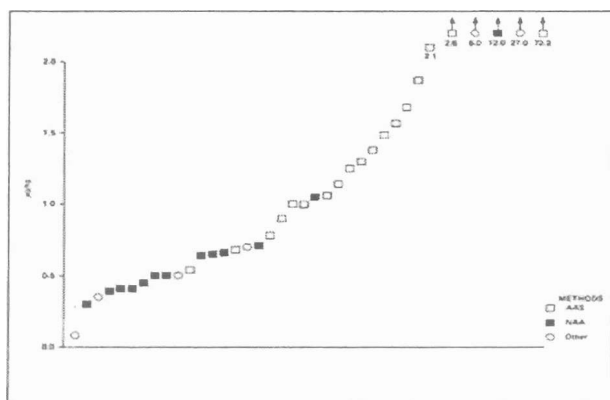


Figure 1: Results of an IAEA interlaboratory comparison for analysis of copper in a reference material made from cow's milk. Each point represents the mean value reported by a single laboratory. The results have been arranged from left to right in order of increasing value of the concentration.  
(Source: Parr 1983)

## **MY CONTRIBUTIONS TO THE PRACTICE OF BTER**

One of my more personally fulfilling accomplishments in BTER so far, was the designing and construction of a functional Clean Room for BTER in the Physics department in 2005. Apart from the clean air provided (with the coarse air particulates - aerodynamic diameter greater than 2 microns - reduced to about 4.3%; and fine air particulates reduced to 16.7% of the values in the laboratory space outside the Clean Room), the facility also provides a hood for acid digestion, a workbench, and other regulars – all locally constructed and at economical cost. For instance, the cost of an imported hood alone would have exceeded the amount expended on the entire project. Also available in the Clean Room is an acid sub-boiling distillation unit, procured as part of an IAEA Project in 2004. I will like to again here acknowledge and thank the then DVC academics, Prof Biodun Adediran who acting on behalf of the University, intervened to help in clearing the replacement unit through the Nigerian Customs, as the first unit got broken in transit from Germany to Ile-Ife. Other necessary accessories procured for our laboratory include single-element and multi-element certified reference standards, as well as Parr digestion bombs which could be used with a commercial microwave oven to speedily digest samples. A number of students and staff, both from within and outside the Department of Physics, have benefited and continue to benefit from these facilities in their trace elements related works.

I should again at this juncture appreciate Prof A.F. Oluwole for his extreme confidence in, and passion for the next generation. During our training, Prof Oluwole would assign us his graduate students, tasks that even regular Professors will balk at. For instance, as a research student under his tutelage, I learnt to write (and on one occasion presented at the relevant governmental forum) multi-million naira research proposals, which were only brought to him for minor corrections and signature. I am glad to mention here that by putting lessons learnt under Professor

Oluwole to use, I have been able to get modest funding – primarily from the International Atomic Energy Agency, Vienna, for my research work, consistently for the past twelve years.

Some of my major contributions to date, including both methodology aspects (particularly optimization of the applications of analytical techniques for various matrices), as well as applications to specific health and environmental problems, are highlighted below.

## **A. CONTRIBUTION TO METHODOLOGY**

Right from my days as a doctoral student, I had been involved in writing computer programmes to simulate various experimental scenarios and their likely outcomes. This was absolutely necessary in dealing with my supervisor at the University of Surrey, Nicholas Spyrou; as he would demand for facts and figures, before considering any new proposal particularly on the existing carefully established INAA protocols for irradiation and counting. My little programmes of those days (such as SAMUEL – Simulated outputs of Activated Multi-Elements) meant for quick demonstration of my ideas eventually developed into GABRIEL which was a predictive programme for INAA patterned after the Advanced Predictive Computer Programme of Prof V. Guinn of University of California, Irvine (which copy I had obtained from one of his students, Prof (then Dr) FOI Asubiojo of our Chemistry department). With such a programme, the analyst can verify ahead of time, the kind of outcome to expect from a particular set of experimental protocols on a particular matrix. This could prove very helpful in optimizing time and economic resources, particularly for people who don't have their own facilities and have to choose which of the commercially-available ones to patronize. Several years later, working with one of my own postgraduate students, we developed the same kind of programme for our own Total X-Ray Fluorescence facility at Ile-Ife (Adejumo and Ojo, 2004, Adejumo and Ojo 2007).

Another notable among my several computer programmes was JOSHWARE, a suite of FORTRAN programmes designed, among other capabilities, to automatically check through the output data of levels of trace elements in a sample and carry out various statistical tests, not only on the levels of individual elements, but on their ratios. Such a rigorous interrogation of the massive amount of data routinely generated in a typical multi-elemental analytical project is vital if we are not to miss out of some hidden treasure of information.

However, my most challenging contribution to methodology would be the development of a software for the implementation of the K-Zero methodology for INAA. To optimize the opportunities in NAA, and quite critically, to make it competitive in the light of emerging new techniques like ICP-MS, it was vital that new methodologies like the K-Zero be implemented at NAA facilities. In traditional INAA, appropriate standards are co-irradiated with the actual samples, and then counted separately in what is known as the multi-elemental comparator methodology. This has considerable negative implications for throughput and economic competitiveness of the technique. The K-Zero method drastically cuts down on the process, but there are not only the challenges of ensuring that the quality of the results remains as high as that of the comparator methodology (by getting appropriate nuclear data for the isotopes, as well as accurate calibration of both the reactor facility and the counting system being used), there are challenges with the logistics of actual implementations.

So as I arrived for a World Bank sponsored postdoctoral fellowship at the Nuclear Radiation Center (NRC) of the Washington State University in February 1995, my supervisor, Roy Filby (a radiochemist) made it clear that he would greatly appreciate it if I, having a Physics background, could develop the K-Zero technique for INAA use at the Center. There was already a commercial software available at this time (costing several

thousands of US dollars), but it was appropriate only for INAA conducted at reactor channels with stable fluxes – which can therefore be calibrated maybe once a year. For a TRIGA reactor, such a condition exists only at the fringes, away from the core, where the constant movement of the control rods implies ever changing reactor parameters. The NRC however operates a relatively small TRIGA and could only get the required high flux needed for meaningful, competitive INAA from the reactor core. I felt quite fulfilled that I was able to come up with the software which I named ZAKI, in the six months I was at the Center (Ojo and Filby 2001). ZAKI, the Hausa name for Lion, which I adapted for the WSU mascot, Cougar, was also a play on the word K-Zero as well as my grandfather's name - Zacheus. It was written in Visual basic – a language that was just coming out in those days and which I learnt at the Center.

## **B. CONTRIBUTIONS TO SPECIFIC HEALTH AND ENVIRONMENTAL ISSUES**

To present an overview of my contributions to specific issues in BTER, I will run through the thrust of my works under five headings:

### **1. Establishment of Baseline values**

As the first major student recruit into BTER in the country, my work provided the first serious and systematic study of trace elements in biological and environmental matrices, with direct application to human health. In that privileged circumstance, results largely from my PhD, provided reliable and extensive baseline values for several trace elements in a number of physiologically important tissues from Nigerian subjects.

These, first of all, allowed comparisons to be made between Nigerian subjects and subjects from other countries; as well as provided the basis for later works on various specific populations of interest in Nigeria (e.g. diseased, occupationally-

exposed, subjects on some particular diets, etc). It also provides the basis to check temporal trends. For instance, I personally got alerted to the fact that Nigeria had changed from leaded petrol to unleaded through the surprising lowered levels of blood lead I obtained for women at Ile-Ife in 2007 (Ojo et al, 2010), when compared with our baseline results published sixteen years earlier (Ojo et al, 1994). In a similar manner, other events, say some nuclear or industrial incident even in some far away country can be monitored in Nigeria based on the already accurately established baselines. My works provided baseline data for 17 trace and minor elements in whole blood, erythrocytes and plasma of the same subjects (Ojo et al, 1994); and also for 30 trace and minor elements in head hair and finger nails of some Nigerian subjects (Oluwole et al 1994).

Apart from the intrinsic values of the baseline data in ways enumerated above, by the rigorous interrogations of my high quality multi-elemental data using specially-written computer programmes, I was able to provide some new basic scientific information in BTER. For instance, in 1994, I was able to establish for the first time, to the best of my knowledge, the unexpected positive correlations between zinc levels and also between chlorine levels in erythrocytes and head hairs in human subjects. (Ojo et al 1994a). Not only is this interesting in terms of understanding the mechanisms for loading of these important elements into these two body compartments, it offered the possibility of replacing blood analysis with the atraumatic and relatively easier hair analysis in some situations. In a similar work involving nails and head hairs of human subjects, I was able to contribute to the discussion of the mode of incorporation of Cu into head hair (Ojo et al, 1994b)

In one of my first post doctoral initiatives, I set out to establish the range of normal values for the two important essential elements, selenium and iron, in the erythrocytes of two healthy adult Nigerian subjects under different conditions (Ojo

and Kucera, 2004). The short study involved the painstaking collection of 36 blood samples from the two healthy Nigerian adult volunteer subjects (myself and a friend!) at various times and after different dietary loading, over a three-week period. The erythrocytes, after extraction and subsequent lyophilization at Ile-Ife, were analyzed by INAA technique at the Light Water LVR-15 experimental reactor of the Nuclear Research Institute, Rez, in Czech Republic, using several different protocols which were then evaluated and exhaustively discussed. The three-week trip to Czech was at the kind invitation and part-sponsorship of Jan Kucera after an IAEA CRP meeting we had in Vienna in 2002.

## **2. Trace Elements in Health and Diseases**

### ***Sickle Cell Anaemia***

The main thrust of my PhD thesis was Sickle Cell Anaemia – frequently referred to as the “black man’s disease”. Working with 170 sickle cell patients and controls from the University College Hospital, University of Ibadan and the Obafemi Awolowo University Teaching Hospital Ile-Ife, under the direct supervision of Prof M.A. Durosinmi, we investigated the levels of several essential elements in hair, whole blood, erythrocytes and plasma of the same subjects. Earlier before the commencement of my PhD, Prof Oluwole with some co-workers had analysed 12 elements in scalp hair and nails of paediatric Sickle Cell patients (Oluwole et al, 1990). It became my lot to look at all the results together and draw conclusions. The works clearly demonstrate that trace elements are important in Sickle Cell Anaemia (SCA). Our results published in a number of international journals were the first systematic and extensive work on Nigerian subjects (Oluwole et al 1990, Durosinmi et al, 1993).

My analyses involved not just comparing levels of elements in three categories of subjects (those with SCA; those with the trait, i.e. haemoglobin HbA+S or C; and those with HbAA), elemental distribution and ratios in the various blood

compartments were studied and compared (Ojo et al 1993). In 2006, I did a holistic review of the results (obtained with both PIXE and INAA) in an effort to determine which of them have significant promise for clinical applications. The results published in the African Journal of Medicine and Medical Sciences showed that the elements Na, K, Rb and Br are clearly playing key roles in maintaining homeostasis in the steady-state SCA patients. It also indicated there may be gender-related differences in the utilization of K, Br and Fe in sickle cell anaemia (Ojo et al, 2006).

We also confirmed the well-reported influence of zinc in alleviating several of the complications in SCA. Indeed, zinc is so strongly implicated that, at the Seventh Ann Arbor Conference on The Red Cell (1989), Ananda Prasad had suggested that any clinician who fails to administer Zn supplement to their SCA patients who are confirmed to be zinc-deficient ought to be charged for malpractice! (Prasad et al 1989). The snag however is that even establishing the zinc status of the patient may not be trivial in an environment where BTER is not routinely practiced. Patients with normal zinc status will not benefit from zinc supplements. My results show that Nigerian SCA patients, unlike subjects from Saudi Arabia for instance, are generally deficient in zinc. (Alayash AI. 1989; Perrine et al 1972)

Unfortunately, we have not been able to probe this work further due to non-availability of required medical facilities and collaborations. Elsewhere, trace elements are actually forming the basis of therapy in SCA, The kinetics of the formation of polymers by the haemoglobin S molecules in each erythrocyte showed an astronomical concentration dependence - anywhere (depending on other factors) between 15th - 50th-power! (Maugh 1981, Mozzarelli et al, 1987) This fact implies that small reductions in the concentration of haemoglobin S might translate to considerable clinical benefits and possibly provide considerable improvement in quality of life for SCA patients. While Hydroxyurea is providing considerable benefit by providing such

action (via the stimulation of HbF production to dilute the HbS – Eaton and Hofrichter, 1995), a simpler and more elegant approach to achieving similar dilution of the concentrations of HbS, would be to simply prevent cell dehydration. ( Mueller and Brugnara 2001). In HbSS, dehydrated sickle red cells are particularly prone to haemolysis, contributing to many of the clinical complications such as stroke, leg ulcers and general poor nutrition. Preventing cellular dehydration would help to reduce complications by blocking pathways leading to red cell K<sup>+</sup> permeability and replacing nutrient losses. Considerable efforts have therefore gone into the development of membrane-active drugs that could stop or even reverse cell dehydration. As already mentioned in the discussion on the biological bases of the essentiality of trace elements, magnesium is a major candidate for such action and a number of magnesium-based drugs have already been developed. (Steinberg 1999, Okpala 2005, Hyacinth et al, 2010). Unfortunately, my extensive studies on Nigerian subjects did not include magnesium.

Apart from seeking to delay the formation of Hb polymers as described above, another possibility for the incorporation of trace elements in the management of SCA could be to hasten the flow of the red blood cells through microcirculation, such that the erythrocyte is already returned to the arterial side for re-oxygenation before the catastrophic phase of polymer formation. This could be achieved in a number of ways including decreasing the viscosity of blood, or reducing the binding of HbS to membranes, for which zinc, for instance, has shown some promise. (Prasad 1976)

### *Blood Cancers and Affective Disorders*

Primarily to confirm the general applicability of the protocols (analytical and data analysis) that I established during my PhD for Sickle Cell Anaemia to other specific disease conditions, similar trace elements profiling studies comparing

diseased and healthy subjects were carried out for some blood cancers (Burkitt's lymphoma, chronic granulocyte leukaemia, chronic lymphocytic leukaemia, Multiple Myeloma, and Hodgkin's disease). (Durosinmi et al 1994) and also for affective disorders – both manic and depressive phases (Durosinmi et al 1994, Ojo et al, 1996). Our results confirm that trace elements indeed have roles to play in these diseases and provide basis for further in-depth works by interested medical professionals. Bromine, potassium and zinc were significantly elevated in the erythrocytes of the cancer patients compared to the controls, whereas iron was significantly depressed. Copper showed no significant difference in the erythrocytes for the two groups, but was significantly elevated in the plasma of cancer patients compared with the controls (Durosinmi et al 1994).

The importance of elemental ratios in the interpretations of such studies was underscored by our findings on Affective Disorders showing that the elemental ratios are generally more closely regulated (lower relative dispersion) in the patients with affective disorders than in the control subjects. The only exception to this out of the nine elements we studied was Cu. The ratios of plasma levels to erythrocyte levels for K, Ca, Cu, and Zn were found to be statistically elevated (at  $p < 0.005$  level) in patients with affective disorders relative to the normal controls (Ojo et al, 1996).

### **3. Nutritional health**

Faced with the several significant hurdles standing in the way of any serious investigation of therapeutic applications of trace elements in diseases through medicine, I found myself moving towards nutrition therapy, since conditions for the use of foods and natural products are far less stringent than the medical case. Moreover nutrition therapy offers the potential opportunity to deal with problems at the grass root, public health level - before they become medical problems at all.

### *Uptake studies*

Following the implication of zinc as a major trace element playing crucial roles in Sickle Cell Anaemia in Nigerian subjects; and following suggestions to that effect by workers in the USA and India (Brewer et al 1977, Hider et al, 1990), I sought to investigate the uptake of zinc into blood following the ingestion of some typical local diets to assess the possibilities of obtaining the needed zinc from dietary means. It is known that food components like phytates block zinc absorption while others such as picolinic acid, histidine, cysteine and cystine (frequently found in food) could enhance it. In the study, which was funded by the University Research Council of our University, some zinc-enriched multivitamin tablet was co-ingested with the various diets. No significant uptake of zinc was observed in the erythrocytes. (Ojo et al 2006). However, at least one valuable spin-off from the study was the accurate determination of the levels of the essential trace elements zinc, iron, selenium, rubidium and cobalt in seven food items from Ile-Ife; as well as the study of the variations in the plasma levels of these elements within 6 hours after oral ingestions. My invaluable collaborators in the study were Drs Omolaja Osoniyi and Aaron Aboderin of Biochemistry and Medical Microbiology departments respectively of this University.

### *Essential Elements in foods and diets. Recommended Dietary Intakes and Bioavailability*

I was later able to carry out a more extensive work on trace elements contents of local food through an IAEA sponsored work on the Jos plateau in 2001-2005 (Research Contract NIR 11925). and simulate their bioavailability *in vitro*. Together with my colleagues in Nutrition and Dietetics departments of Jos University Teaching Hospital (Mrs Hauwa Owolawase), Obafemi Awolowo University Teaching Hospital Complex (Mr Obinna Ogbonna), Department of Home Economics and Family Nutrition (Dr (Mrs) Beatrice Ogunba)) and the University Health Centre (Dr

Biodun Hassan), we did a food basket survey on the plateau, collected 43 food items representing over 95% of the food consumed on the Jos plateau, and analysed them for five trace elements using TXRF both at our facility at Ile-Ife and the UMM Krakow, Poland.

Furthermore, by administering about 150 questionnaires soliciting information on food frequencies from both children and adult subjects, we were able to come up with estimates for the daily intakes of the elements in the subjects. Apart from copper, the daily dietary intakes were well above the usual tolerable levels recommended by the various responsible organizations. However, our work assumed that all the foods consumed by the subjects come from their immediate contaminated environment. In reality, a good fraction of the food would come from outside the contaminated tin mining region.

To determine the fractions of the trace elements content of food that are actually available for uptake into the blood stream, I collaborated with Prof Steve Adewusi and Dr Olumuyiwa Falade from the Chemistry department of this University, to simulate *in vitro* the digestive process in the human stomach and gut. Eight of the major food and diet items were investigated. There were some limitations, with the analyses of Cr in all the eight food items, but we were able to determine that dried Dankali (sweet potato) has the lowest bioavailability for Pb and Cd (below detection limit, and 2.2% respectively). Maximum bioavailability for Pb was found in Groundnut with 73.3% and that for Cd was found in Maize with 38.5%. Maximum bioavailability for Zn was 14.3% in Acha and that for Cu was 6.9% in Maize. (Ojo et al 2003)

My study of essential trace elements in foods and diets; and the determination of their daily intakes naturally led to a study of levels of toxic elements in those same items – a subject for Environmental Health.

#### **4. Environmental Health**

One of the recent research results I personally found alarming was that of Alatisé and Schrauzer, showing a definite link between the sharply rising incidences of breast cancer recorded here in Ile-Ife with lead pollution (Alatisé and Schrauzer, 2010). This is definitely an unwelcome addition to the myriads of carcinogenic substances already identified in both our foods and environment. However, it is also known that, often, some essential elements do ameliorate the effects of their toxic counterparts. Well-known examples include the Hg-Se, Pb-Se and Cu-Zn antagonisms. It is therefore necessary to include such elements as Se, Cu and Zn in a study of the toxic elements. I have studied toxic and essential trace elements arising from the two major environmental pollution sources in Nigeria - the transportation sector and the mining/allied industry sector.

##### *Transportation Sector*

My first work along this line was the landmark study under Profs A.F. Oluwolé and Roy Filby where thirty-nine trace elements were determined in forty crude oil samples obtained from ten different locations on the Niger Delta. Apart from providing data on the trace elements that could potentially be released into the environment during the use of products of these crude oils in the transportation sector, the data were also critical for the crude oil refinery process; and were eventually used by our collaborators in Geology Department (Prof J.N. Nwachukwu in particular) in a pioneering effort at using trace elements for geo-exploration of oil in Nigeria (Oluwolé et al 1993)

Thirteen years later, taking into cognizance that pollution from the transport sector (combustion of fuel, wears of tyres and engines, etc) represents a major source of toxic elements into people in urban locations, I initiated a study to check ambient levels of toxic elements in the air on the main streets of Ile-Ife, and correlated these with elemental levels in the blood of exposed

subjects. The study was in context of an IAEA Research Contract (NIR 13248), and because babies in the womb, followed by women, are the more vulnerable subjects to the impact of toxic trace elements, the study was based on women of childbearing age. The subjects included street traders, petrol station attendants and traffic wardens all from Ile-Ife. Previous workers like Ndiokwere (Ndiokwere et al 1984) and Sridhar (Sridhar et al, 2000) had established the fact that air-borne lead is disproportionately deposited near the emission sources – along the streets.

The results, as I already mentioned earlier, showed that the mean blood lead level of Nigerians had considerably reduced following the Nation's transition from leaded petrol to unleaded around 2004. However, we found that 6.5% of subjects with normal nutrition still exceeded the current US CDC "No Action" blood lead level of 10  $\mu\text{g}/\text{dl}$ . Compared with the corresponding figures of 10% and 35.4% respectively for subjects in the underweight and overweight/obese categories who exceeded this critical level, our results suggest a clear association between blood lead levels and nutritional status in these women. Furthermore, Analysis of Variance results show that the blood levels of As and Cu were significantly different ( $p < 0.05$ ) in subjects with normal nutritional status and those with various categories of abnormal nutritional status. I will be referring to these results and provide more details later on in this lecture.

### *Mining and allied industries*

Most of my environmental work has focused on the mining industry, from the SW, Jos Plateau and Zamfara regions of Nigeria.

### SW Nigeria: Ile-Ife, Igun, Iperindo

My first work along this theme was initiated in 2004. In the study, we sought to determine whether artisanal/small scale

gold mining, which is very common around Ile-Ife amount to any significant pollution of the environment. Contamination to the soil, flora and the water bodies were studied. Using our TXRF in the Physics Department, we determined 16 elements in soil samples and 15 in plant samples taken from 3 mining sites and a non-mining site. Furthermore, taking gold as a typical heavy metal, the distribution of gold between the fronds and stalk of a fern ubiquitously associated with the mining environment was determined using Instrumental Neutron Activation Analysis technique at the Nuclear Research Institute, Rez in the Czech Republic. Samples from the mining sites were significantly enriched in As, Mn, and Pb compared with identical samples from the non-mining site. Comparative analysis also indicated that heavy metals are better absorbed and translocated by vegetables than other crops like raphia palm. Gold (as a typical heavy metal) was found to be stored more in the leaves than in the stalk, calling for caution in using such fronds in applications that can impact the food chain, such as their use as animal feed (Ojo and Oketayo 2006).

In the fluvial component of the same study, twenty elements were determined by TXRF analytical technique in 23 water samples collected from the same sites. For samples from rivers in the 3 mining sites, the elements As, Fe, Mn and Pb were found at levels that were not only significantly higher than those from the non-mining site, but were also above limits recommended by regulatory bodies. A general decrease in the concentrations of these elements with increasing downstream distance from the sieving areas further confirm mining as the source of these unexpected elevated levels. (Oketayo and Ojo 2006).

In a later study at Iperindo, we collected blood and breast milk samples from 34 nursing mothers to evaluate the trace elements levels and see if there is any correlation with levels in soils and water samples taken from abandoned and current mining

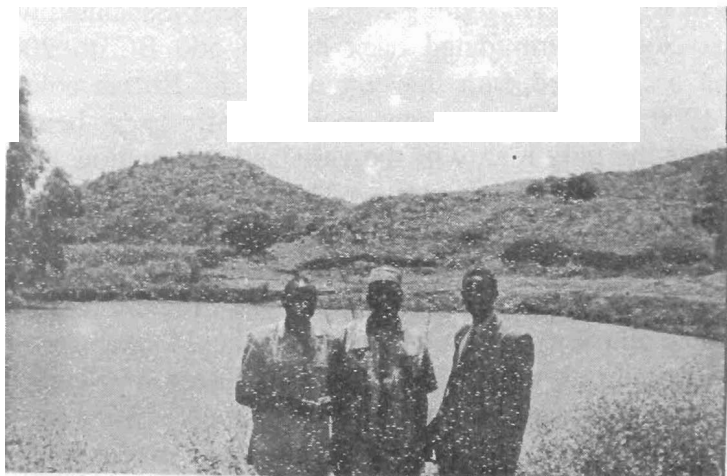
sites. This research is still on-going at the moment with analyses being carried out at IITA, Ibadan (using ICP-AES) and JSI, Ljubljana (Using ICP-MS and DMA).

### Tin Mining and Smelting on the Jos Plateau

The tin mining industry on the Jos Plateau is one of the oldest in Nigeria. Tin mining which has been associated with the famous Nok culture (dated back around 500 BC to 200 AD) became accentuated with the coming of the British colonialists about 1900. It is only in recent years that efforts are being made to address the ugly reality of the vast land devastation associated with this extensive activity. The reclaimed mining sites invariably, are being converted to farmlands on a very large scale; and because of the peculiar temperate climate on the plateau, several agricultural products from the region are widely distributed all over the country. In short, should toxic trace matter be transferred into these foods, several millions of Nigerians, all over the country, would be potentially at risk. (*see Plate 1*)

As previously mentioned, in collaboration with government agencies including the School of Mining, and JUTH Dietetics department I investigated levels of essential trace elements, in foods and diets from the Jos Plateau. At the same time, I also investigated levels of toxic elements and radionuclides in soil, food crops and diets mainly from Barkin Ladi Local Government where the practice of reclaiming abandoned tin mine fields for agricultural purposes in what is known as the *Lambu* system of farming (dry/irrigation farming) was most extensive. For the human health impact assessment of these studies, levels of the same toxic elements were investigated in blood and hair samples of over 100 adult residents in the villages of Tenti and Werh in Bokokos and Barkin Ladi Local Government Areas, some 75 km from Jos. Tenti hosts an extensive farm settlement, including a Farm Project sponsored by Plateau Foods/AG Leventis. Both Total Reflection X-Ray Fluorescence and Flame Atomic

Absorption Spectrometry were used for elemental analyses, while both an intrinsic Ge detector and a NaI(Tl) detector were used for assay of radioactivity in food and soil samples respectively. Medical personnel on the team looked out for symptoms that could be related to toxic elements and radioactivity.



*Plate 1: Reclaimed Mining site on the Jos Plateau. The 'hill' in the background is tailings dug out from the ground, with the pit now covered by water – the small 'lake' in the foreground. Food crops planted on the hill, as well as the fishes from the lake could be exposed to heavy metals and radionuclides that would ordinarily have remained deep in the ground together with the tin ores, were it not for the mining activities. Photo: Joshua Ojo*

The research showed that the practice of using reclaimed mines land for agriculture does introduce elevated toxic metals from the soil into the food chain; but does not lead to significant health hazard in the general population, except for individuals who subsist mainly on foods grown from the contaminated farmlands. There is cause for concern however, on the Cd levels in Maize

(0.86 – 2.08 ppm), especially with a relatively high bioavailability of 38.5%. Fresh maize had much lowered levels, but the dried ones seemed to have concentrated Cd. Interestingly, the same dried maize samples also had elevated levels of radioactivity (Ojo 2009). The study was funded by the IAEA NIR/11925.

### Zamfara Gold mines

I heard about the lead poisoning on-going at Zamfara gold mines on the newspapers, at a time I was having serious problem (for about a year) trying to convince the people at Iperindo that exactly such a danger exists. I thereafter quickly found on the internet the telephone number of Dr Henry Akpan, the country's then chief epidemiologist, and called him to offer my perspectives. He kindly invited me to the next stakeholders meeting at Abuja where I was able to appraise the nation's response, which was largely being driven by foreign organizations including the Centers for Disease Control and Prevention - Atlanta, Medecins Sans Frontiers, Zurich, and the World Health Organization, Paris.

While I appreciated the efforts to contain what was turning out to be a national emergency and disaster, I had some concerns about the approach which comprised of the bulk remediation of contaminated soil (to prevent further exposure of people to lead), and chelation therapy (to attempt to remove lead from the blood of children and mothers with extremely high levels). Unfortunately, as it was clear to all, these interventions though scientifically correct, are prohibitively expensive and are not sustainable. (some reports indicate up to a million naira is budgeted for remediating an average compound of mud houses – see *Daily Trust*, 19<sup>th</sup> June, 2012) Worse still, they only address the incidents of acute exogenous lead poisoning and not the co-existing chronic endogenous exposure.

Mr. Vice Chancellor sir, let me quickly explain this dicey situation. It is well known that the main storage for lead in the human body is the bones, where the half life is about 22 years for

trabecular bones and 34 years for cortical bones (Pounds and Leggett, 1998). It is also well known that lead could be remobilized from the long-term storage sites and transferred into blood. Indeed, such mobilization of lead from bones is known to occur mainly in women during periods of high need for Calcium such as during pregnancy and lactation. (Gulson et al. 1997 and 1998). At such times, Pb is released together with Ca with which it shares similar chemistry and is passed on to the foetus or baby through either placental exchange or breastfeeding as the case may be. Thus, endogenous exposure of mother and baby is guaranteed to continue for several years to come, even if all exogenous exposure is entirely eliminated today. In plain terms, the very expensive remediation efforts of the external environment are only of limited benefit for people who have already been massively exposed over the years. This is particularly important as babies in the womb are the most vulnerable group to the toxic effects of lead (Dorea 2004). Unfortunately, since it is either these babies are born with some impaired mental status, or aborted right away in the womb, they don't attract newspapers' headlines, and they tend to be overlooked!

I was therefore convinced of the need to seek realistic, home-grown, evidence-based measures to address the gaps in the current measures. Since funds is the limiting factor here, it is obvious to me that, especially where no medical emergencies are involved, alternatives to bulk soil remediation and chelation therapy should be sought, principally by seeking to alter the vulnerability of exposed people to lead toxicity. At the stakeholders' meeting at Abuja, I mentioned the possibility of seeking nutritional intervention to modify lead toxicity, but I dare say, a meeting comprising of largely bureaucrats from government ministries is not exactly a scientific conference; and one should not feel too bad if some ideas are not as enthusiastically received as one would have hoped for. I decided to pursue the matter a little

on my own and hopefully get some results that might compel attention from the bureaucracy.

Since my participation at the National Stakeholders meetings was being sponsored from my own research funds, I persuaded the IAEA to allow me use part of the budget approved for my studies in SW Nigeria to investigate the lead poisoning in Zamfara. Again, with the kind collaboration of Dr Henry Akpan, I (and my team of two postgraduate students) was allowed to tag along a team of Federal Ministry of health officials scheduled to visit Zamfara state – thus providing critical access to the location and the subjects. I later forged a fruitful research partnership with Dr Mohammed Kanoma, the Chief Medical Director at the Zamfara State General Hospital who provided the four-wheel drive without which the locations could not have been accessed.

Based on my focus on obtaining a long-term solution, (and need to clarify the possible synergistic involvement of other toxic elements associated with the gold mining industry, such as Arsenic and Mercury), I decided to determine the following parameters:

- i. blood levels of Pb and other toxic and essential elements in females of child-bearing age – this would determine levels transferred to foetus via placental exchange
- ii. Milk levels of the same elements in lactating mothers – determining how much elements are transferred to babies via breastfeeding
- iii. Quantities of breast milk ingested by babies at various ages
- iii. Relationships/Correlations between trace elements in blood and milk, and
- iv. The influence of time on the levels of these elements and their interactions with one another.

My team spent a month (July 2010) shuttling between Gusau, Yargalma and Dareta in Zamfara state. We surveyed the local environment (including mine ores and tailings) for radioactivity using a survey meter (radiation levels were normal

and comparable to those at Ile-Ife). We also collected other environmental samples including soil, mining effluent water, drinking water as well as blood and breast milk samples from lactating mothers. Our attempt to evaluate breast milk intake in the children using the deuterium dose-to-mother technique was not successful due to the very difficult field conditions that did not allow careful measurements. Although we managed to collect the difficult saliva samples needed from each baby and mother (over a two week period), problems with basic anthropometric measurement (heights, weights) proved insurmountable as our hired staff of local female health assistants (necessary, due to cultural restrictions) could not take accurate readings with the analog equipments we had to use, since there was no electricity to use our digital equipments. We painfully had to discontinue the breast milk intake aspect of the study for Zamfara.

Nevertheless, the results of the trace elements in environmental samples, blood and milk were a treasure of data.

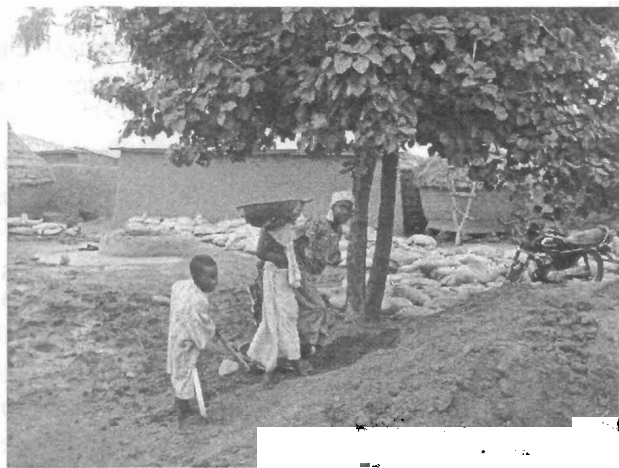


Plate 2,  
*Yargalma, Zamfara State. Children are seen engaged in packing contaminated soils prior to encasement, thus increasing their exposure to lead contamination.* Photo by J.O. Ojo (July 2010)

The significant contamination of the environment leading to the reported cases of Pb-poisoning in Zamfara gold mines was easily confirmed by elevated levels of Pb measured in samples of soil and effluent water. But happily enough the drinking (well) water was unpolluted confirming that the pathway for pollution was mainly through soil and dusts. The clinical symptoms observed were also confirmed by very high levels in blood and human milk. The levels of Pb in blood ranged from 7.9 – 86.8  $\mu\text{g/dL}$ , with geometric mean of 50.7  $\mu\text{g/dL}$  (compared with a mean value of  $6.81 \pm 2.61 \mu\text{g/dL}$  reported by us for Ile-Ife women about the same time); while levels of this toxic element in human milk ranged from 2 – 140 ng/ml, with geometric mean of 30 ng/mL (compared with the mean value of 4.1 ng/mL cited by the WHO for a previous study on Nigerians). Levels of the other elements measured however, As, Se, Hg, Cu and Zn were normal.

A number of interesting correlations between elemental levels in milk and blood of mothers, and the age of mother and period after birth were demonstrated, offering the possibilities we seek to alter the vulnerability of the exposed people to the toxic effects of lead. Details of our findings (see figure 2) will be presented at a coming international conference in the United States (Ojo et al 2013). Central to our goal however is the interesting correlation between Se and Pb. The results showed that higher levels of Se in blood are associated with lower levels of Pb in both blood and milk. It would therefore suggest to us that Selenium supplementation (possibly through natural foods and including vitamin E) for women, before pregnancy, during pregnancy, and during the lactation period could reduce their baby's exposure to lead. Literature review shows that the very same idea has met with considerable success with respect to calcium supplements. Not only did daily calcium supplementation during lactation significantly reduce blood and milk levels of lead, up to 31% reduction in lead blood level during pregnancy was also recorded in subjects from Mexico city who received daily 1,200

mg doses of calcium (Potera 2009). In our follow up study at Iperindo, we have therefore incorporated Ca into the elements of interest, and we hope to be able see what a combination of Se and Ca supplements could do to blood lead levels of lactating mothers, especially as there are some issues with taking high doses of calcium supplements alone. We are quite hopeful of coming up with scientifically sound counsel that would help mitigate the adverse impact of lead poisoning, not only in Zamfara state, but wherever it occurs on the planet.

It is pertinent to add here that, as high as the levels of lead in milk from the subjects from Zamfara are, even higher (median value of 67 ng/ml) levels were previously measured in nomadic semi-pastoralist Nigerian women in Jos, with no known specific exposure to lead. The study was carried out by VanderJagf and co-workers in the United States (VanderJagf et al, 2001) and it shows that the problem of lead poisoning in Nigeria is extensive, and could be silently causing severe havoc in many places without their being reported. Collaborating with me on this project are Dr (Mrs) Perpetua Obiajunwa of Department of Paediatric and Child Health; and Dr Olaniyi Komolafe of Zoology Department of our University.

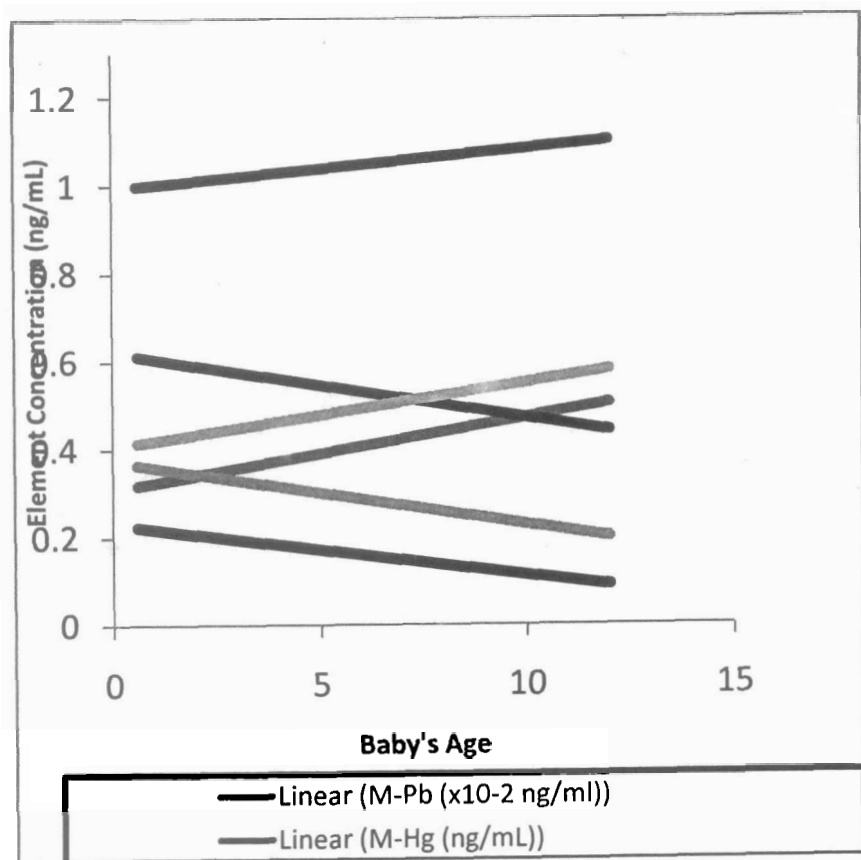


Figure 2: Correlations between levels of the elements Pb, As, Cu, Hg, Se and Zn in milk and Time Elapsed since delivery (Baby's Age)

### Environmental Radioactivity

During my M.Sc research work, I had been part of a study that studied the radioactivity in vegetables at dry season farmlands where phosphatic fertilizers were being heavily used around the Opa River and its tributaries in Ile-Ife. We not only found an elevated radioactivity level in these farmlands relative to other

places in Ile-Ife, we also found traces of Cs-137 no doubt associated with French nuclear tests in the Sahara desert (Fasasi et al 1999). I have later extended this line of study to food and soil samples from the Jos tin mines as well as the Zamfara gold mines. Similar work is going on right now on soil samples from some mines in SW Nigeria including Ijero (Ekiti State), Ageti (Oyo State), and Awe (Osun State) as well as Ile-Ife.

## **5. Modifying Toxicity**

As discussed under the Zamfara lead poisoning case, the solution to such widespread chronic pollution cases, especially with inevitable endogenous exposure, might lie more in seeking to modify the toxicity of the elements concerned, than conventional engineering of the external environment or medical treatment of symptoms. I mentioned earlier the apparent influence of Nutritional Status in levels of blood lead in some women exposed to traffic pollution in Ile-Ife. The concepts of Nutritional Status as well as Physiologically-Based Kinetic Modeling are inexorably connected with the modification of toxicity; and these are areas I am currently seeking to make some contributions.

### **Nutritional Status: Body fat/water measurements**

Several works by nutritionists have clearly shown that nutritional status of a subject has a vital role to play in determining the body's vulnerability to xenobiotics.

The little problem is to define what exactly is "nutritional status". One of the most commonly used indices for the assessment of nutritional status is the Body Mass Index (BMI) which is simply the ratio of body mass to square of height. Although a fair and useful association has been made between BMI and several diseases including diabetes and cardiovascular diseases (Arora et al 2007, Chandha et al, 2006), it is realized that a more useful assessment of Nutritional Status would include total

body fat and its distribution in the body. As discussed by Prof Onajobi in her inaugural lecture titled *Fats of Life* (Onajobi 1999), measurement of body fat is not exactly trivial. Working with colleagues in nutrition and dietetics, I have been able to make contributions which will facilitate accurate and routine measurements of body fat in Nigerian subjects.

Accurate absolute measurement of body fat would require rigorous and expensive techniques such as the underwater weighing technique or isotope dilution technique but there are several other relative methods such as those based on bioimpedance analysis or anthropometry, which can be calibrated against one of the absolute methods and then used routinely.

The usual practice is to use one of the absolute techniques to determine the body fat in a group of subjects representative of a given population. The distribution of fats around the body of the subjects is also simultaneously determined by measurement of skinfolds and body circumferences. Some mathematical equations are then generated fitting these measured anthropometric parameters to the experimentally measured body fat. All that is needed to subsequently determine the body fat in other subjects from that population is use a flexible tape (and sometimes maybe calipers) to determine the anthropometric parameters which are then plugged into the equation. Depending on the goodness of the initial mathematical fitting, this can provide a very convenient and sufficiently accurate measurement of body fat.

The only problem with this practice is that distribution of body fat in people depends not just on their gender and age, but on their races as well. By the time I got involved in determination of nutritional status, the equations still predominantly used in Nigeria were those of Durnin and Womersley (1974) and that of Vogel et al (1984) established for Caucasian populations. Furthermore the techniques used for the generation of these equations are not as sensitive as the isotope dilution method, which is now recognized as the "gold standard". Now isotope dilution is a quite straight-

forward technique, but the reason it has not been used in Nigeria before now is the difficulty in accurately quantifying trace amounts of deuterium in whatever body water (saliva, urine, plasma) that is to be used.

Until about a decade ago, the accurate assay of trace amounts of deuterium requires Isotope Ratio Mass Spectrometry (IRMS), a high-tech facility available only in advanced laboratories in the developed countries. However, recent and now well-proven studies have shown that Nuclear Magnetic Resonance (NMR) as well as Fourier-Transform Infra Red (FTIR) spectrometry could be used, equally as accurate as IRMS, to determine deuterium in body fluids (Getahun et al 1999, Kim et al 2004). The FTIR method is based on the detection of the vibrational band produced by deuterated water between 2400 and 2600 $\text{cm}^{-1}$ , in the infrared region of the spectrum. Under a research project supported in part both by the IAEA and the URC of our University, and with the active encouragement of Prof T.A. Olugbade, then Director of the CSL, my research group has acquired considerable expertise in the determination of body fats using isotope dilution technique. We had to change our initial plans to use the NMR facility at the CSL for the assay of deuterium as that would require disrupting the default settings, which could be difficult to re-set. We then shifted to the use of FTIR. Even though the FTIR at the CSL was discovered to be unserviceable, Prof Olugbade arranged for the procurement of the needed special  $\text{CaF}_2$  cell with which I was able to shop around other Centres in the country for a suitable FTIR facility.

Today, as far as I am aware, we are the only group using this technique in Nigeria, and we are already getting good results with it. In a University of Ibadan PhD thesis in human nutrition which I co-supervised, and which will be presented any moment from now, we have used the isotope dilution method to determine the body fat in Nigerian women from the Hausa, Ibo and Yoruba

tribes. We have thereafter come up with anthropometric equations (with excellent regression parameters) that can be used for the determination of body fat not only in Nigerian women generally, but also specifically for these three major tribes within the country. Nutritionists should therefore be able to get more accurate routine determination of nutritional status in Nigerian women as from now on. Some of our results are already published in local and international journals (Ojo et al, 2011, Oketayo and Ojo 2011).

Apart from these equations, I have also been able to contribute to the discussion of nutritional status in more fundamental ways. In one study done in collaboration with Prof SRA Adewusi (Chemistry), Dr A.O. Aboderin (Medical Microbiology) and Dr A.A.Salau (Chemical Pathology), we carried out a random survey of 299 women of child-bearing age in Ile-Ife and established that the incidence of underweight ( $BMI < 18.5 \text{ kgm}^{-2}$ ) was 7.3% while that of Obesity ( $BMI > 30 \text{ kgm}^{-2}$ ) was 8.7%.

Furthermore, we determined in 63 of these subjects, over 30 anthropometric, biochemical and biophysical parameters, together with blood levels of 7 toxic and essential trace elements, viz As, Pb, Hg Se, Cu and Zn. By checking for correlations between these parameters (which include Body fat, Total Cholesterol, Low-density lipids, high-density lipids, Triglycerides, Uric acid, Plasma Creatinine, Urinary Creatinine, Creatinine Clearance, Packed Cell Volume, Systolic and Diastolic Blood Pressure, seven skinfolds, six body circumference), and Body Mass Index (BMI), the traditional definition for nutritional status, we were able comment on the possible value of incorporating some of these other parameters in the assessment of nutritional status – especially with respect to trace elements toxicity. One particularly interesting finding was the unexpectedly very high correlations between hip circumference and %body fat in these subjects ( $r$  value of 0.9440,  $n=62$ ,  $p<0.0001$  see figure 3). With

such correlation, hip circumference alone could very well be used to define nutritional status in Yoruba women of low economic status (within child-bearing age), who were the subjects used in this particular study. (Oketayo and Ojo 2011)

In this same study, quite importantly, we were able to demonstrate that the interactions of the elements As, Cu, and Hg (and potentially their impacts) were modified by nutritional status.

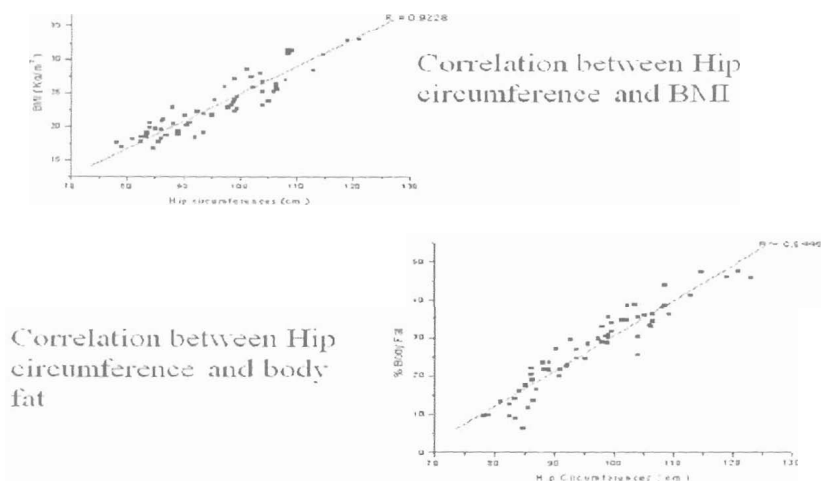


Figure 3: Correlation between Hip circumference and Nutritional Status indices in Female Yoruba subjects

### Physiologically-based kinetic modeling

In his inaugural lecture in July 2009, Prof Oluseye Bolaji of the Faculty of Pharmacy, discussed what he termed “apartheid” in drugs utilization by different individuals. He in particular cited an annual figure of over two million cases of Adverse Drug Reactions (ADRs) in the United States alone, where drugs not only fail to work as expected, but end up causing more problems for the patient. Each year, some 100,000 of these ADR cases in

the US eventually result in fatalities! The same apartheid situation applies to toxic elements and it would be highly helpful if the fate of ingested toxic elements could be modelled in individuals using several of the available well-tested models based on actual volumes of the physiologically relevant body compartments for those individuals (see figure 4). The specified rates of exchange of the elements of interest between the compartments can also be improved upon by generating and using local data in the available models. In our current efforts along this line, we are particularly interested in modeling the absorption, distribution, metabolism and elimination of lead in humans. A Masters student from this University will be presenting our initial models for her thesis very shortly.

There are several other factors that are known to be capable of altering the vulnerability of people to toxic elements – including both ionizing radiation (e.g. x-rays) and microwave radiations (e.g. in GSM phones and base stations). In the oxidative stress theory of carcinogenesis popularized by authors as Ray D Strand (Strand 2002) and George Malkmus (Malkmus 2006), the ultimate precursor for most chronic diseases including cancer is the presence of free radical generators, of which toxic elements are a type; and anti-oxidants, such as essential elements, are possible antidotes. Part of my pioneering work on microwave radiation in our environment is the measurement of radiation from GSM phones as well as base stations in Ile-Ife metropolis and development of a microwave radiation map for Ile-Ife. Details of this is outside the scope of the current lecture.

## **RECOMMENDATIONS**

Mr. Vice chancellor sir, kindly permit me to make the following recommendations, based on my experience so far in the exciting field of BTER, in addition to those already mentioned in the body of this lecture.

1. While we must appreciate the excellent efforts of surgery and medicine, including medical physics, in dealing with the scourge of cancer and other chronic diseases, I believe that, with the abundant evidences available, it is much better the science community puts in far more efforts at tackling the issue at the public health level before they become medical or surgical cases at all. At least this will produce much needed reliefs to our medical facilities which can then deal more carefully with the reduced number of inevitable cases. This point is better appreciated if one considers that even in the United States with one of the best medical facilities and tradition, iatrogenic fatalities is the third largest cause of death (some 225,000 per annum) - following heart disease and cancer! (Starfield 2000). The term iatrogenic fatalities, by the way, stands for deaths caused inadvertently by a physician or surgeon or by medical treatment or diagnostic procedures. It is doubtful if anyone keeps such figures in Nigeria, but clearly the less the pressure on the medical facilities and personnel, the better for all of us.

We should take the battle to the gates – on the minefields be it, at Jos, Werek, Zamfara, Iperindo, Ijero, etc where simple common-sense procedures can keep much of the toxic matter away from humans in the first place. This should go hand in hand of course, with helping the human body use its natural defence mechanisms – essentially through good nutrition and healthy lifestyle - to deal with these toxins and prevent dangerous buildups.

This perspective is not only true for dealing with toxic trace elements but also holds in the cases of those diseases which could be amenable to intervention by essential trace elements - such as sickle cell anaemia. Consider, for example, this incisive opinion by Martin H. Steinberg (1999) on the issue of SCA:

“A better and longer life for patients with sickle cell disease in developed countries has resulted from basic and clinical investigation. Yet the chief burden of this disease lies in Africa, where minimal standards of care are often lacking. In parts of Africa....., about 120,000 babies with sickle cell disease are born yearly (as compared with 1000 in the United States), but less than 2 percent survive to the age of five. High-technology treatment will benefit the fortunate few, but to have an important effect, any treatment must be translated into a form that can be applied in the less developed and poorer countries of the world.”

We certainly need a locally-driven, multidisciplinary, thinking-out-of-the-box approach if we are to solve our most serious peculiar public health challenges - whether it is lead poisoning at Zamfara or getting an effective solution to Sickle Cell Anaemia.

2. Research funding should be sourced from Government mainly on a Client - Service provider basis, rather than just as basic grants to keep the labs and equipment running. In this part of the world, with so many pressing needs that nobody is going to help us solve, we have to attack real needs of government and society; and steady funding will surely flow in. A sizable portion of government budget for health, engineering, defence, etc could thereby be ploughed back into the University in a win-win scenario.

3. Fellowships in foreign labs are crucial as they provide opportunities to see how things run, and in particular for standardizing our own set-ups. But, for scientists involved with laboratory work, it is criminal ignoring the setting up of local facilities and depending *ad nauseum* on foreign facilities. For

local expertise to be developed, more and more research needs to be initiated and implemented locally. It will be useful if the University can incorporate appropriate rewards for people starting up demonstrably functional laboratories locally; rather than put all the emphasis on number of publications - many of which are jointly published with foreign laboratories and which cannot be replicated here. Last year, I met a European editor of an international peer-reviewed journal who sneeringly described Nigerian scientists as people who are very good [only] at writing papers. But we are only responding to the values set for us by our employers!

## **IN CONCLUSION: A PINCH OF NATURAL PHILOSOPHY**

The original name for Physics was Natural Philosophy, and often, physicists draw inspiration from the physical world into other aspects of living. Indeed, one of the basic axioms of the scientific method (attributed to Francis Bacon) is that nature is unified, implying that paradigms can be transferred across different areas of nature. I crave the indulgence of Mr. Vice Chancellor and this august audience to share one major insight I have gained from my studies in Biological Trace Elements Research.

This is the two-sided composite worldview that trace indeed does matter, and that we must nevertheless be holistic in our outlook. In laboratory terms, this means that we must decide *a priori* the quality of output we desire, and apply our efforts uniformly to all the processes involved. For instance, it would amount to absolute waste, all the laborious months-long effort in producing results from a typical BTER project if one pays all the efforts to getting the analytical facilities in top condition but fails to take commensurate efforts to ensure the integrity of the sample during long-term storage (for instance taking care of the freezer during the inevitable power outages!). As the saying goes, not straining out gnats while swallowing camels!

Everyday illustrations of this concept abound. Explaining why he was not going to invite some notable stars with character flaws into his team for the 2013 African Cup of Nations, Stephen Okechukwu Keshi, coach of the Super Eagles, the eventual winners of the Cup explained, and I paraphrase, “you don’t need all the best individual players to get the best team”. That’s vintage holistic reasoning! Similarly, the Lord Jesus Christ in the Bible says: “I have come that they might have life, and that they might have it more abundantly” (John 10:10b). Life more abundantly speaks of life in all its ramifications. A holistically well-balanced life, rather than some narrow-minded pursuit of one-sided “success”.

In human health, environment, and all of life, we must learn to keep an eye on the whole; while noting the tremendous influence (positive or negative) of some minor/trace issues. For instance, as has been clearly expounded in this same hallowed hall by several past inaugural lecturers from different disciplines (ranging from Olusi, 1983; to Adelekan, 2007; Ojofeintimi, 2008; Durosinmi, 2008; and more recently Olasode, 2012), cancer, diabetes, cardiovascular diseases and several such very weighty issues, might as well be outrightly preventable by such relatively light efforts as regular exercise and consumption of diets free of excessive fats but enriched with anti-oxidants. However there is little value in going to extremes in one area (say diet) while we remain sloppy with another (say, unnecessary exposure to microwave radiation through continued abuse of the GSM phone). These are very weighty issues, but we can extrapolate this worldview to even weightier matters in the society.

Inductively applied to the weightier socio-political issues currently topical in our Society, my twin-postulate will look like this: The majority must recognize and respect minority rights, yet it must be done in a holistic manner. Take for instance the hot issue of homosexuality. In my opinion the big issue with homosexuality is not so much the act itself, but the vociferous

advocacy for it. The miniscule minority of gay advocates insist that homosexuality is no longer a private matter between individuals, but the public (particularly our children and vulnerable youths) must be taught about it; and recruited, even coerced into it. However they, at the same time, insist on denying this same basic right of advocacy to the other minority in the gay issue - ex-homosexuals! The response of the gay advocacy group to anybody who would claim to have found the homosexual lifestyle horrific and has decided to come out of it is predictable. In Europe and North America ex-gays are endlessly and crudely pilloried, harassed and gagged. Any support for them is labeled homophobic and could attract stiff jail terms! One lesson from this lecture is that while we should embrace minorities, it must be done holistically!

The same analysis can of course be applied to other pressing issues in our country today including Boko Haram and Niger Delta insurgencies.

Mr. Vice chancellor sir, apart from my main sponsors and collaborators already cited in the body of this lecture, there are several others who have gone out of their ways one time or the other to assist me in my works. I really don't know how to express my deep appreciation of their kind efforts other than mention them in a lecture like this. These include facilitators like Prof A.A. Onayade of Community Health Department of this University, Dr John Awosise and Mrs Okebiorun of the Comprehensive Health Centres, Enuwa and Iperindo respectively; Mr. L. Adebesein, Principal, School of Mines Jos; Mr. Agbo, Head, Geological Survey, Jos; and Mr. Sam Pam of JUTH Blood Bank and of course several others who worked with these fine Nigerians. I also like to appreciate the personnel at both OAU Bursary and Audit departments as well as at the IAEA's Section of Nutritional and Health-Related Environmental Studies, Division of Human Health for various logistics supports.

The foundation for my career in research was carefully laid by Prof J.B. Aladekomo in Physics Department (assisted by Prof - then Dr - Craig Obafemi of the Department of Chemistry) who taught me the basics of fluorescence spectroscopy and measurements of life-times of excited states of molecular species, during my undergraduate Final Year Project in 1985. My adult research teeth cut under the mentorship of Prof A.F. Oluwole was actually in a group setting, under the direct supervision of his lieutenants including Profs (then Drs) F.O. I. Asubiojo, P.O. Aina, H.B. Olaniyi, F.A. Akeredolu and F.A. Balogun. Any of these could rightly claim I was their student, and I am proud to flaunt my association with them. I also gladly appreciate Prof E.E. Balogun, who during the financially trying days of my M.Sc, arranged, in his capacity as the Head of Physics Department, for me to receive a University Postgraduate Fellowship. I probably wouldn't be here today were it not for that Fellowship at a very critical juncture of my career. I also gladly acknowledge the currently serving Professors in the Physics Department and CERD, who have continued to encourage me on in my academic career, taking me as a colleague even though many of them were my teachers.

To my wife, sister, and friend, Olusola; and to our children (Oluwasegun, Oluwatobiloba, DideOluwakusidede, Enibaletikogbo, Oore-Ofe and Grace-Mercy), I say thank you for all your loving supports and kind understanding.

Mr. Vice Chancellor sir, Principal officers of the University, erudite scholars – including mentors, colleagues, friends, and classmates from the 1981-85 set at Great Ife, distinguished guests, patriotic members of the Press, my dear students, ladies and gentlemen, let me conclude this lecture with a prayer that we all experience and enjoy life more abundantly, even as we pay holistic attention to the trace matters of life.

Thank you for listening.

## **APPENDIX: A brief Description of major Analytical Methods used in Biological Trace Elements Analysis**

### **Neutron Activation Analysis (NAA)**

This is clearly the king of analytical techniques. Here, neutrons are employed to stimulate (technically, activate) the atoms of the sample, rendering them radioactive. The resulting emissions are then studied to provide both a qualitative and quantitative characterization of the parent atoms.

When NAA was first described in 1936 by George Hevesy and Hilde Levi, substantial radiochemistry was involved. The radionuclide of interest is separated by chemical means and the beta emission is then easily measured by any crude radiation detector – even a Geiger counter. However, with developments in radiation and solid state physics, and the availability of sophisticated semi-conductor-based detectors, neutron activation analysis shifted from the realm of chemistry to physics, and the activated sample could be assayed directly on high resolution gamma-ray detectors without the need for any laborious and risky radio-chemical separation.

The superiority of NAA over most other analytical methods lies in the fact that it is a nuclear method as the neutron interacts with the nucleus of the atom. Now, for the same element, there could be a number of different isotopes, meaning different nuclei, with which the neutrons could possibly interact. Thus we can have simultaneously, several independent assays of the element of interest. This helps to spot and correct for possible systematic errors. And with radiochemistry now excluded, various different nuclear reactions could be very easily utilized simply by playing with such parameters as the neutron profile (e.g. fast, epithermal or thermal); chosen irradiation and counting protocols (very short, short, intermediate, or long - reflecting the half-lives of the nuclide of interest - in continuous, intermittent, or cyclic modes); the decay time between irradiation and counting;

etc. Furthermore, each resulting radionuclide in turn could yield several gamma lines at various energies. All these fantastic opportunities are a physicist's delight, and have led to what is known as purely Instrumental Neutron Activation Analysis (INAA). To maximize the benefits available in INAA however, it became imperative to develop various smart algorithms for peak area determination, making corrections for several effects contributing to the background, and ingenious methodologies for computing the amount of the trace elements of interest, such as kzero or monostandard comparator methodologies, etc.

There are drawbacks nonetheless in NAA, chief of which is the very high cost required to start off and maintain a nuclear reactor which is the only way to obtain a high enough flux of thermal neutrons with adequate interaction cross-sections for most trace elements of interest. Another source of concern with the operation of reactors is the potential for nuclear incidents, including possible terrorism. To compromise on cost and remove the concern about terrorism, a new breed of low-flux ( $\sim 5 \times 10^{-11}$  n/s.cm<sup>2</sup>) reactors, with extreme safety nets, have been designed. Canada pioneered this as the SLOWPOKE reactors, while the Chinese version (available at GAEC Accra and CERT, Zaria) are simply called Miniature Neutron Source Reactors (MNSR). Other sources of neutrons which can be used for NAA include isotopic and photo-neutronic sources, 14 MeV neutron generators, as well as charged particle accelerators; but none is as effective as nuclear reactors.

It should also be noted that, despite all the versatility afforded, it still is not every element of interest that has suitable isotopes that can be activated (and therefore analysed) by INAA. From health and environment perspective, perhaps the most important of these is lead.

## X-Ray Fluorescence spectrometry

Unlike thermal neutrons which are able to interact with the nuclei of atoms, the other projectiles available for stimulating the atoms have most of their interactions with the orbital electrons. Such interactions are called 'atomic' as opposed to the 'nuclear' interactions involving nuclei. There are principally two different responses that can be used for characterizing and quantifying the interacting atoms. The first is the characteristic x-ray photons (keV range) emitted from inner shell electrons (X-ray fluorescence); while the other are the characteristic optical photons (in eV range) from excitation of outer electrons (Atomic Absorption Spectroscopy).

Inner shell electrons can be stimulated by a number of projectiles. In (Scanning) Electron Microprobe, electrons in the 10 – 50 keV energy region are used, while in conventional x-ray fluorescence, x-rays from an x-ray tube or some isotopic source are used for the excitation. Other (positively) charged particles could also be used to induce x-ray emission in a sample. If the particles are produced from linear accelerators (in the MeV region) the technique is referred to as Particle-induced X-ray Emission (PIXE) and when it is from radioisotope sources such as the alpha-emitters  $^{210}\text{Po}$  and  $^{244}\text{Cm}$ , it is called (RIXE).

Positively charged particles could penetrate the orbital electrons much further than electrons or x-ray probes. From elemental analysis point of view, the most effective among them is protons in the 2 – 3 MeV range. Even though the interaction cross section is increased with the use of heavier particles (e.g. Helium and Oxygen ions) or use of higher energies for the protons, a number of other nuclear reactions also become prominent in those cases. The gamma radiations associated with these reactions significantly degrade the background in the resulting spectra via Compton interaction in the Si(Li) detector, leading to poor detection limits. Hence, today, the letter "P" in PIXE is taken, in most occasions, to stand for "proton in the 2 -3 MeV range".

Proton beams in particle accelerators are typically  $> 10^{12}$  particles per second, which is up to a million times the flux commonly obtained via radioisotope sources.

PIXE therefore is the foremost x-ray method for trace elements analyses. Nevertheless, the high background mentioned above is a serious issue. Even when the background resulting from nuclear interactions have been minimized by using 2-3 MeV protons as mentioned, there is still considerable generation of bremsstrahlung (braking radiation) as the projectile protons and ejected atomic electrons are slowed down as they move through the sample matrix. The extent of the degradation of the resulting detection limits depends on the particular elements of interest and the sample matrix. Unfortunately, this is mostly significant in biological and environmental samples, especially for light elements (atomic number less than Calcium).

In this regard, a variant of Conventional X-ray Fluorescence, called the Total Reflection X-Ray Fluorescence (TXRF) could actually rival or even surpass the analytical capability of PIXE for biological and environmental samples. In this mode, the matrix is first removed entirely by acid digestion and the sample is then presented in liquid form. By setting the angle of incidence of the stimulating x-ray beam such as to produce total internal reflection within the sample, the flux is doubled. This doubled flux and total elimination of matrix (and therefore competing backgrounds) makes the TXRF a very versatile but simple technique indeed – especially for biological samples.

## **Atomic Absorption and Atomic Emission Spectrometry methods**

Atomic Absorption and Atomic Emission methods are like two sides of the same coin, but there are significant differences in their implementation. In the absorption case, the outer electrons are stimulated by an incident beam of photons. According to

quantum theory, the portion of the beam that the atoms of a particular element will absorb, is just that portion which can promote them from the ground state to some excited state. Since the energy gaps between the various states are unique for each element, knowledge about the absorbed energy can provide a qualitative identification of the absorbing atoms. Furthermore, by comparing outputs obtained in the analysis of standard samples (where the levels of the various elements have been previously ascertained), levels of the same elements in an unknown sample can also be accurately determined.

In the case of atomic emission however, it is the photons emitted by the atom as it returns to the ground state following its prior stimulation that is measured. Again, these photons, where considered holistically, are characteristic for each atom and could be used for both qualitative and quantitative assay of the element involved.

There are significant differences in the implementation of these two related methodologies, however. In AAS the sample is stimulated by light from hollow-cathode lamps, which are discharge lamps producing narrow emission from atomic species. Usually, a different lamp is required for each element, which means only a single element can be determined at a time. This is a significant drawback in a field like Biological Trace Elements Research, where as previously mentioned, it is not just the levels of individual elements that matter, but their interactions with one another. In order to provide some sort of simultaneous multielemental capabilities, some AAS spectrometers now use excitation lamps comprising of two elements (but the emission efficiencies are invariably compromised). In some more modern spectrometers, up to 5 different lamps can be activated at the same time to permit stimulation of the atoms by as many photon groups.

In AES on the other hand, the excitation of the atoms is thermal, and all elements in the sample are simultaneously stimulated. This is actually the basis for flame photometers in O'

level chemistry where metals are identified by the 'flame test'. Here a sample is presented into a flame from the Bunsen burner. The heat from the flame creates free atoms by breaking chemical bonds as well as promotes the atoms into excited electronic states. The light emitted as the atoms return to the ground electronic state are in the optical visible range and can be used for identification of the sample. For this reason the technique is also referred to as Optical Emission Spectrometry.

Of course, a modern AES/OES facility is far more sophisticated than a flame photometer. Here, using appropriate light dispersive facility and an array detector, emitted intensities at the various wavelengths representing different elements are scanned, and all the elements are simultaneously determined. The bigger excitement however is in the excitation mechanism.

Although the number of the atoms in a "trace" amount of an element in any sample is humongous, the detection limits achievable with a particular method would only depend on the relative fraction of these total atoms present that are stimulated; and the efficiency with which the response to the stimulation is captured and recorded. According to the Boltzmann distribution, which is applicable to low density matter at elevated temperature as we often are dealing within BTER, the relative number of atoms that are excited to those that are left unstimulated is highly dependent on the temperature of the excitation mechanism.

One mechanism frequently used for excitation in Atomic Emission Spectrometry therefore is the electric spark (or arc). This leads to what is called Spark or arc atomic emission spectroscopy which is used mainly for solid samples. For non-conductive materials, the sample is first ground with graphite powder to make it conductive. The state-of-the-art method however for AES involves the use of inductively-coupled plasmas (ICP) for the excitation, giving rise to the names ICP -AES or ICP-OES. The commonly used argon plasma typically generates temperature of about 8000°C. Samples are introduced into the

plasma in a process that desolvates, ionises, and excites them. With the high number of atoms participating in the analyses, ICP-AES gives detection limits that are far superior to those ordinarily obtainable by AAS.

Detection limits of AAS could however be dramatically improved (to well within the parts-per-billion range) with the use of some accessories. For instance, in the so-called Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS) the samples are atomized in an electrically heated furnace (also called electrothermal vaporization) before being presented to the AAS unit.

Also, in the Hydride Generation Atomic Absorption Spectroscopy (HG-AAS), the elements of interest are first separated from the sample matrix by formation of volatile hydrides. The hydrides are then presented to the conventional AAS unit now pre-concentrated and relatively free of possible interferences, thereby considerably enhancing the detection limits. Elements for which useful hydrides could be formed include antimony, arsenic, bismuth, germanium, lead, selenium, and tellurium. The technique could in turn be combined with ICP-AES to further improve the sensitivity. AAS without any accessory is referred to as F-AAS (the "F" standing for Flame), and is of limited use in BTER.

### **Inductively Coupled Plasma Mass Spectrometry (ICP-MS)**

If INAA is the "king" of analytical techniques, then Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) could be described as the "queen". Since its debut in the early 1980s, ICP-MS now poses not only the biggest challenge, but "threat" to NAA. On account of these relative easy-to-use facilities, several INAA facilities are suddenly being considered for possible decommissioning. ICP-MS combines the easy sample introduction and quick analysis of ICP technology with the accurate and low detection limits of a mass spectrometer. The resulting instrument

is capable of trace multielemental analysis, often at the part per trillion levels. Furthermore, like NAA, elemental determination is carried out directly on radioisotopes, thus allowing systematic errors to stick out for easy recognition.

ICP-MS involves the decomposition of neutral elements in a high temperature argon plasma and their subsequent analysis based on their mass-to-charge ratios. This is accomplished by passing the decomposed samples through a mass analyzer which allows only particles (ions) with a specific mass/charge ratio to wiggle through some electric obstruction, such as a quadrupole arrangement. A suitable ion detector, such as the channeltron electron multiplier, located at the other end of the channel thereafter receives the ion and generates as many of  $10^8$  electrons which could give measurable reading after passing through appropriate electronic circuitry.

Problems often encountered in Inductively Coupled Plasma Mass Spectrometry (ICP-MS) include various types of interferences (mainly isobaric, polyatomic, and double charged ion interferences), matrix differences between standards and samples, mass-discrimination effects, detector dead-time, drift due to nebulizer plugging, changes in sample argon, power supply and room temperature instability.

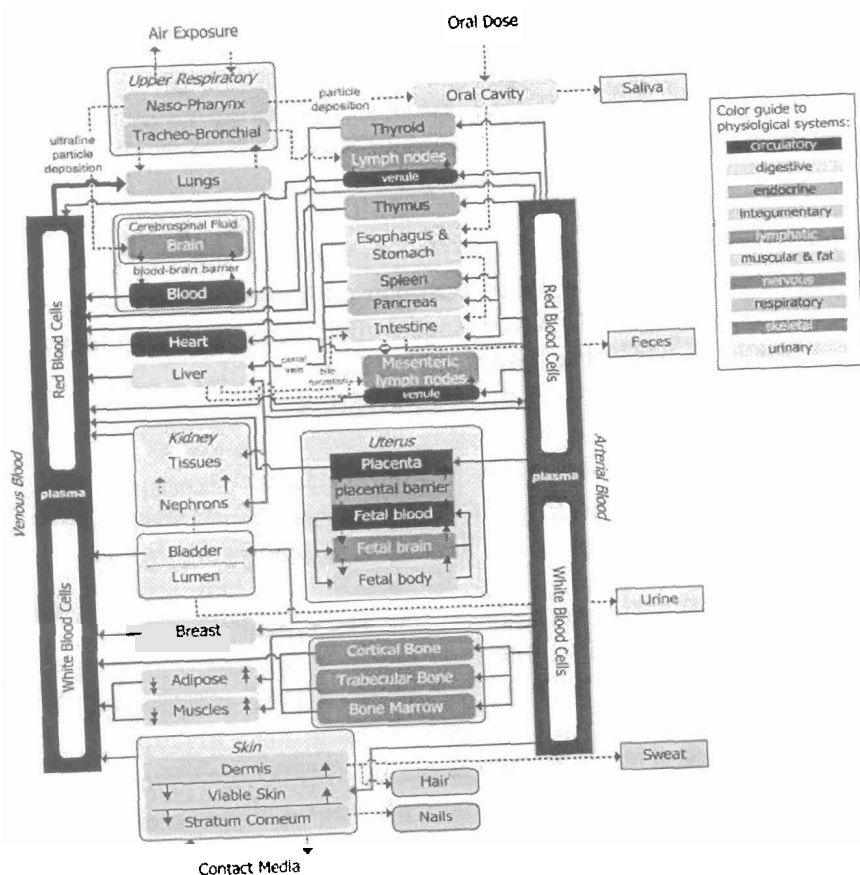
### **Trace Naturally-occurring radionuclides**

As already discussed, in NAA, the sample is first rendered radioactive by activation with thermalized neutrons. However naturally-occurring radionuclides are already radioactive and all that is needed is to present the radiation being emitted to appropriate detectors for the assay of the radionuclides involved. The challenge here is to distinguish the low-level radiation coming from our sample of interest from similar radiations from neighbouring materials, including from the measuring facility itself! The principal radiations coming from a natural sample are the charged particles alpha and beta radiations, and the high

energy gamma rays. Because of their specificity, alpha particles and gamma rays can be used to identify and quantify trace levels of radioactive matter.

In gamma ray spectrometry, after the installation of an appropriate shield made up of high Z materials (usually lead) specially quarried from regions of the world with very low naturally-occurring radiation, radioactivity from the sample is counted at optimized geometry on an appropriate detector such as a scintillator (NaI or CsI, both activated with Tl) or better still semi-conductor based ones such as hyperpure Ge with their excellent resolutions. However the challenge with semi-conductor detectors, apart from the high acquisition cost (approximately N20 million) is the need to provide liquid nitrogen about every 10 days to keep the facility in operation.

Whereas gamma spectrometry is largely an instrumental method (and thereby located mostly in Physics departments), alpha spectrometry requires more demanding sample preparation and involves much chemistry. However after the isotope of interest has been chemically separated from the hosting matrix, usually by electroplating, the determination of radioactivity becomes quite trivial. And the result can be truly astounding!



**Figure 4. Human Exposure Pathways and Distribution of Heavy Metals in Organs and Tissues (PBTK Modeling)**  
Adapted from Georgopoulos, 2008.

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