



Contents lists available at BioMedSciDirect Publications

## International Journal of Biological & Medical Research

Journal homepage: [www.biomedscidirect.com](http://www.biomedscidirect.com)

### Original Article

## DETERMINATION OF ELEMENTAL COMPOSITION AND MEDIAN LETHAL DOSE OF CALABASH CHALK

M. B. EKONG<sup>†\*</sup>, A. I. PETER<sup>†</sup>, T. B. EKANEM<sup>‡</sup>, E. E. OSIM<sup>#</sup>

<sup>†</sup>Department of Anatomy, University of Uyo, Uyo, Nigeria

<sup>‡</sup>Department of Anatomy, University of Calabar, Calabar, Nigeria

<sup>#</sup>Department of Physiology, University of Calabar, Calabar, Nigeria

#### ARTICLE INFO

##### Keywords:

Calabash chalk

Elements

Median lethal dose

Mice

#### ABSTRACT

Calabash chalk is a geophagic material consumed mostly for its emetic function, though other reasons are not rule out. A report of a sample analysis revealed toxic metals/metalloids, as well as persistent organic pollutants. This led to this study to investigate the elemental composition of a sample from southern Nigeria, and to determine its median lethal dose. Non-salted calabash chalk was analyzed for the presence of some major and trace elements using inductively coupled plasma-mass spectrometry, and their concentrations was determined by inductively coupled plasma-quadrupole mass spectrometry. The 'limit test' in the 'up and down' procedure was used to determine the median lethal dose (LD50) with up to 5000 mg/kg body weight of calabash chalk suspension on mice. Analysis results revealed magnesium (1100±100 mg/kg±SD), aluminium (16000±15000 mg/kg±SD), potassium (5500±500 mg/kg±SD), calcium (160±14 mg/kg±SD), titanium (11000±1000 mg/kg±SD), vanadium (125±10 mg/kg±SD), chromium (130±10 mg/kg±SD), manganese (40±5 mg/kg±SD), iron (15000±1500 mg/kg±SD), cobalt (4.1±0.2 mg/kg±SD), nickel (25.5±1.3 mg/kg±SD), copper (15.5±0.7 mg/kg±SD), arsenic (11.5±0.8 mg/kg±SD), silver (0.50±0.03 mg/kg±SD), cadmium (0.76±0.04 mg/kg±SD), antimony (0.42±0.02 mg/kg±SD), barium (200±10 mg/kg±SD), thallium (0.33±0.02 mg/kg±SD), lead (57±3 mg/kg±SD) and zinc (<100mg/kg). No mortality and/or toxicity was recorded with up to 5000 mg/kg body weight of the single dose treatment of calabash chalk. In conclusion, calabash chalk contains many biological beneficial, as well as adverse elements depending on their concentrations in the chalk, as well as their bioavailability. Though this chalk may be relatively less toxic in acute usage, the opposite may apply in chronic usage; hence, its use is discouraged.

©Copyright 2010 BioMedSciDirect Publications IJBMR - ISSN: 0976:6685. All rights reserved.

### 1. Introduction

Calabash chalk is also known as poto in English, la craie in French, and mabele in Lingala of Congo. In Nigerian languages, it is called nzu in Igbo and ndom in Efik/Ibibio. The chalk is commercially available, and may be sold in blocks, as large pellets, and in powder forms [1]. It may be packaged in a clear plastic bag with or without labelling, or sold without any packaging, and in a variety of forms. Reports show that the ones commonly ingested in Africa contains phosphorus, potassium, magnesium, copper, zinc, manganese, and iron [2,3]. The chalk could be naturally occurring or artificially formulated. The naturally occurring chalk is chiefly made up of fossilized seashells, while the artificial form may be prepared from clay and mud which may be mixed with other ingredient including sand, wood ash and sometimes salt. The resulting product is moulded and then heated to produce the final product [1].

Calabash chalk is generally made up of aluminium silicate hydroxide, which is a known member of the kaolin clay group, with

the formula: Al<sub>2</sub> Si<sub>2</sub> O<sub>5</sub> OH<sub>4</sub> [4]. Several other contaminants which could be poisonous to the body have also been reported [4, 5, 6, 7]. These includes; metals, metalloids and persistent organic pollutants [4]. The metals include; iron, aluminium, potassium, titanium, barium, chromium, zinc, manganese, nickel, rubidium, copper, tin and lead as shown in Table 1 [4], and the metalloid being arsenic [4, 5, 6, 7]. The organic pollutants as reported include alpha lindane, endrin, endosulphan II and p, p'-dichloro diphenyl dichloroethane (DDD) [4].

Not much researches have reported the effect of calabash chalk on the biological system. Reports on animal models revealed sinusoidal enlargements and fragmented liver parenchyma, and depletion of red blood cells [8, 9, 10]. Ekong et al [11, 12] reported oedema and haemorrhages in the mucosa of the stomach, as well as acanthosis, hyperkeratosis, and koilocytic changes in the mucosa of the oesophagus, and the alteration of growth rate and de-mineralization of the femur bone. A recent report on the chalk showed anxiogenic potentials and cerebral cortical alteration [13].

The calabash chalk is reported to be eaten by pregnant and post-partum women, as well as children across societal class, and in different societies. This study thus investigated the elemental composition of the chalk obtained from the southern region of Nigeria and its median lethal dose using mice.

\* Corresponding Author : **M. B. EKONG**

Department of Anatomy, University of Uyo, Uyo, Nigeria

[mbe\\_flashpoint@yahoo.com](mailto:mbe_flashpoint@yahoo.com)

©Copyright 2010 BioMedSciDirect Publications. All rights reserved.

## MATERIALS AND METHODS

Two large blocks of calabash chalk (non-salted) obtained naturally from the same source and prepared by roasting in fire was obtained from a local market in Ikot Omin, Calabar. It was chopped into small pieces and grounded into fine powder with the aid of a manually operated grinder. The calabash chalk was then analysed for some major (potassium, calcium, magnesium) and trace (iron, copper, aluminium, cobalt, manganese, nickel, barium, chromine, lead, cadmium, titanium, vanadium, zinc, arsenic, silver, antimony and thallium) elements, using the flame atomic absorption spectrometer (Unicam-939).

### Analysis of the calabash chalk

Trace and major elements concentrations were measured using inductively coupled plasma-mass spectrometry (ICP-MS) on totally digested samples. One hundred milligram samples were totally dissolved by successive additions of nitric acid (HNO<sub>3</sub>) and hydrochloric acid (HCl) mixture, hydrogen fluoride (HF), and perchloric acid (HClO<sub>4</sub>) in Teflon vessels using a heating block (Digiprep, SCP Science). Ultra pure reagents were used (Normatom grade, VWR, France for HNO<sub>3</sub>, and HCl, "for trace metal analyses", Baker, SODIPRO, France). The solutions were evaporated to dryness, retaken 3 times in 2 mL of HNO<sub>3</sub> and then diluted with 50 mL of MilliQ water. The concentrations were determined by inductively coupled plasma-quadrupole mass spectrometry (ICP-QMS) (XSeries, Thermo-Electron, France).

To correct for instrumental drifts and plasma fluctuations, all solutions were spiked with rhodium (Rh) and rhenium (Re) standard solutions (SPEX, SCP Science, France) to a final concentration of 10 mg/L for Rh and 1 mg/L for Re. To minimise isobaric interferences, analysis with the Collision Cell Technology (CCT) introducing a supplementary gas mixture of H<sub>2</sub> (7 %) and He (93 %) was applied for Fe, Mn, and the 6 metals studied (Cd, Cr, Cu, Ni, Pb, Zn). The solutions were weighted at each step of the dilution and spiking operations. The data quality was controlled with lake sediment reference material SL1 (from the International Atomic Energy Agency, Vienna). The values obtained agreed within 10% of the certified values.

### Treatment for the median lethal dose (LD50)

The 'limit test' in the 'up and down' procedure was used to determine the median lethal dose (LD50). Twenty-five female albino mice were divided into 5 groups of 5 mice each. The animals were handled according to the ethical guidelines of the National Institute of Health (NIH) of the United States. A maximum of 5 animals per group were sequentially administered with calabash chalk suspension up to a test dose of 5000 mg/kg [14, 15, 16] as shown in Table 3.

Forty grams of the calabash chalk was dissolved in 1000 mL of distilled water in a glass jar. Since calabash chalk is partially miscible with water, it was administered as suspension, stirred prior to the administration. Groups 1-5 were administered respectively, 1000, 2000, 3000, 4000 and 5000 mg/kg body weight of calabash chalk suspension (Table 3). The limit test involved dosing one animal with up to 5000 mg/kg body weight. When the animal survived, two additional animals were dosed. When both animals survived, the LD50 was said to be greater than the limit dose and the test was terminated (i.e. carried to full 14-day observation without dosing of further animals).

## RESULTS

The presence and quantities of the different metallic elements in the calabash chalk is presented in Table 2. Analysis revealed the following; magnesium (1100±100 mg/kg±SD), aluminium (16000±15000 mg/kg±SD), potassium (5500±500 mg/kg±SD), calcium (160±14 mg/kg±SD), titanium (11000±1000 mg/kg±SD), vanadium (125±10 mg/kg±SD), chromine (130±10 mg/kg±SD), manganese (40±5 mg/kg±SD), iron (15000±1500 mg/kg±SD), cobalt (4.1±0.2 mg/kg±SD), nickel (25.5±1.3 mg/kg±SD), copper (15.5±0.7 mg/kg±SD), arsenic (11.5±0.8 mg/kg±SD), silver (0.50±0.03 mg/kg±SD), cadmium (0.76±0.04 mg/kg±SD), antimony (0.42±0.02 mg/kg±SD), barium (200±10 mg/kg±SD), thallium (0.33±0.02 mg/kg±SD), lead (57±3 mg/kg±SD), zinc (<100mg/kg).

The mice were administered a calabash chalk suspension of 40 grams dissolved in 1000 mL distilled water, and the groups were administered a maximum of 5000 mg/kg body weight calabash chalk suspension. At 5000 mg/kg body weight of the single dose treatment of calabash chalk, no mortality and/or toxicity was recorded. Therefore, calabash chalk may have a median lethal dose (LD50) of over 5000 mg/kg body weight (Table 3).

**Table 1: Energy dispersive X-ray fluorescence spectroscopy (EDXRF) analysis of calabash chalk samples as reported by Dean et al (2004)**

Element	Within sample variation Mean±sd (mg/kg) (n = 5)	Between sample Variation Mean±sd (mg/kg) (n = 5)	Comparison of two experimental means t-Test significant
Aluminum (Al)	8856±176	8630±152	No
Potassium (K)	1618±25	1372±63	Yes
Titanium (Ti)	8052±134	7230±98	Yes
Chromine (Cr)	52.5±1.6	49.6±5.9	No
Manganese (Mn)	24.1±2.2	24.0±2.1	No
Iron (Fe)	14 770±86	14 402±155	No
Nickel (Ni)	15.5±0.8	15.0±1.3	No
Copper (Cu)	1.8 ±0.5	3.1 ±0.5	No
Zinc (Zn)	26.9±0.9	25.6±1.8	No
Arsenic (As)	Nd	nd	
Rubidium (Rb)	13.4±0.2	12.0±0.6	Yes
Strontium (Sr)	85.9±1.1	78.2±3.8	No
Yttrium (Y)	17.4±0.3	19.8±1.6	No
Zirconium (Zr)	355.3±1.4	337.7±24.2	No
Niobium (Nb)	72.9±0.62	67.1±2.6	Yes
Cadmium (Cd)	nd	nd	
Tin (Sn)	6.2 ±0.7	6.2 ±0.7	No
Antimony (Sb)	nd	nd	
Barium (Ba)	226.5±6.0	230.9±5.9	No
Cerium (Ce)	266.2±7.4	243.6±9.6	No
Mercury (Hg)	nd	nd	
Lead (Pb)	42.5±1.2	36.4±2.8	No

nd- Arsenic, cadmium, antimony and mercury were not detected in the samples analyzed

Dean et al (2004)

**Table 2: Analysis of calabash chalk showing the concentration of different elements**

Elements analyzed	Concentration of elements (mg/kg±SD)
Magnesium (Mg)	1100±100
Aluminium (Al)	160000±15000
Potassium (K)	5500±500
Calcium (Ca)	160±14
Titanium (Ti)	11000±1000
Vanadium (V)	125±10
Chromine (Cr)	130±10
Manganese (Mn)	40±5
Iron (Fe)	15000±1500
Cobalt (Co)	4.1±0.2
Nickel (Ni)	25.5±1.3
Copper (Cu)	15.5±0.7
Arsenic (As)	11.5±0.8
Silver (Ag)	0.50±0.03
Cadmium (Cd)	0.76±0.04
Antimony (Sb)	0.42±0.02
Barium (Ba)	200±10
Thallium (Tl)	0.33±0.02
Lead (Pb)	57±3
*Zinc (Zn)	<100mg/kg

Results are presented as Mean ± Standard deviation

\*A precise figure for zinc (Zn) concentration could not be arrived at because of some analytical problems. So Zn concentration can be said to be lower than 100 mg/kg.

**Table 3: Calabash chalk treatment for the determination of median lethal dose (LD50) in mice**

Groups n=5	Dose of calabash chalk suspension (mg/kg)	Mice mortality
1	1000	Non
2	2000	Non
3	3000	Non
4	4000	Non
5	*5000	Non

\*Ld<sub>50</sub> is over 5000 mg/kg, and the administration was discontinued

## DISCUSSION

The calabash chalk is reported to be eaten by pregnant and post-partum women, as well as children across societal class, and in different societies [17]. Thus, it poses a risk to the consumers due to the various inherent toxic constituents [4, 5, 7, 18]. This study, thus investigated the elemental composition of the chalk and its median lethal dose using mice.

Analysis of the chalk revealed the following minerals; iron, magnesium, potassium, manganese, calcium, copper, zinc among others. These minerals are known to be beneficial in the biological systems of both plants and animals [19, 20, 21, 22, 23].

The presence of these minerals portrays the chalk as useful for nutritional purposes, which may justify its consumption.

Other elements present, and whose effects on biological system is uncertain includes; barium and titanium [24, 25]. However, the presence of metals such as aluminium, lead, arsenic, chromine, vanadium and cadmium in the chalk depending on its composition and bioavailability, may however not be beneficial. The harmful/toxic nature of these metals have also been established [26, 27, 28]. Thus, the usefulness of the chalk may not supercede the potential toxic effects due to the presence of these adverse elements. However, it is reported that the bioavailability of these elements in similar chalk samples may not result in any serious consequences [29], a situation that may also apply in this study. Hence, calabash chalk may play a dual role in the biological system due to its mixed beneficial and adverse chemical composition.

The calabash chalk sample in this study exhibited concentration values for trace elements in soil sample generally [30, 31], which indicates its soil-like nature. However, this is at variance with a previous report where the concentration values for trace elements was below the average [29]. The clay-rich calabash chalk sample in this study also showed probably significant absorption potential for other pollutants not reported. Previous reports has shown the likely presence of microbes, as well as persistent organic pollutants [4, 29].

The composition of the chalk in this study is to an extent, at variance with that of previous studies [4, 29]. Their report on calabash chalk's composition did not include cobalt, vanadium, arsenic, antimony and silver. Also, the concentration of the elements in the present study was higher compared with these other studies [4, 29]. Another report by Campbell [5], showed that the calabash chalk contained both arsenic and lead. The actual concentration of these elements were not provided.

The difference between this study and the previously reported ones could be due to differences in the environment where both chalks were obtained. In the study by [32], the samples were obtained in Jos and Zaria in northern Nigeria, while Dean et al [4] obtained theirs in a retail store in Newcastle upon Tyne, with its origin not known. Campbell [5] also did not revealed the origin of the calabash chalk analyzed. In the present study, the chalk was obtained in southern Nigeria which is replete with crude oil and other natural mineral reserves [32, 33, 34]. These mineral deposits could have influenced the concentration of the reported elements found in the calabash chalk of this study.

Though the actual quantities of arsenic in calabash chalk has not been reported previously, the quantity of lead as reportedly contained in the chalk is approximately 40 mg/kg [4], an amount that high when food safety levels is not supposed to exceed 1 mg/kg [35, 36]. Consumption of the chalk sample would result in an undesirable increase in lead intake because as high as 60g of calabash chalk, with an average of 20g can be consumed a day [29]. The lead level involved may amount to about 4.5 fold the safety guideline, before even taking into account any additional exposure from other sources [37]. A report also has it that young adults with higher blood lead levels were more likely to have major depressive disorder (MDD) or panic disorder [38].

Exposure to the higher levels of lead and arsenic in the present study by pregnant and breast feeding women, poses a risk to the mental development of their developing unborn babies and breast feeding infants, respectively [5, 7, 18]. The high lead level may also

result cancers of the urinary bladder, lungs and skin [7], because lead, a toxic metal is known to induce a broad range of physiological, biochemical, and behavioural dysfunctions in laboratory animals and humans [39], including central and peripheral nervous systems [40], haematopoietic system [41], cardiovascular system [42], kidneys [43], liver [44], and reproductive systems [45, 46]. However, the nervous system damage is considered the most serious. Neurological and visual alterations (including retina) have been reported with low lead concentration particularly in the developing nervous system [40].

In this study, calabash chalk showed the LD50 of over 5000 mg/kg body weight. This indicates that calabash chalk has low acute toxicity hazard because of the high LD50 of over 5000 mg/kg body weight. Thus, this chalk may be classified under 'Hazard Category 5' in the Globally Harmonised Classification System for Chemical Substances and Mixtures (GHS) [14, 15, 16].

## CONCLUSIONS

Analysis of calabash chalk has shown several metals and metalloid with many being biologically beneficial, and some either of unknown function or adverse. Though it may be relatively non-toxic in acute usage, the chronic usage may be toxic; hence, its consumption is discouraged.

## ACKNOWLEDGEMENT

I appreciate the International Brain Research Organization (IBRO) whose support to France gave me the opportunity to associate with Dr. Philip Vermeer and Dr. Laure Bally-Cuif. Their contact allowed for the analysis of the chalk sample by the Climate and Environment Sciences Laboratory, CNRS-CEA-UVSQ, at Gif-sur-Yvette, France. Indeed Louise Bordier performed the sample digestion and the trace element determination, while Sophie Ayrault performed the data treatment.

## REFERENCES

- Food Standards Agency. Lead contamination of calabash chalk category b: for action. Retrieved December 2, 2008 from [www.food.gov.uk](http://www.food.gov.uk), 2002.
- Guilford County Department of Public Health. Calabash chalk: lead and arsenic in remedy for morning sickness 2010. Retrieved January 3, 2013 from <http://www.co.guilford.nc.us/blogs/dph/wp-content/uploads/2012/01/calabash-chalk-032510-logo.pdf>.
- Health Protection Agency. Lead in calabash chalk. Development of a UK children's environment and health strategy. Chemical hazards, 2008. Retrieved January 4, 2013 from [http://www.hpa.org.uk/webc/HPAwebFile/HPAweb\\_C/1204186222593](http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1204186222593).
- Dean JR, Deary ME, Gbafa BK, Scott WC. Characterization and analysis of persistent organic pollutants and major, minor and trace elements in Calabash chalk. *Chemosphere*. 2004; 57:21-25.
- Campbell H. Calabash chalk (calabar stone, la craie, argile, nzu, mabele), 2002. Retrieved May 1, 2011 from [http://www.docstoc.com/docs/54253409/calabashchalk-\(calabar-stone-La-Argile-Nzu-Mabele\)](http://www.docstoc.com/docs/54253409/calabashchalk-(calabar-stone-La-Argile-Nzu-Mabele)).
- Dooley EE. The beat. *Environ Health Perspect*. 2010; 118:A200-A201.
- Health Canada. Calabash chalk may pose health risk for pregnant and breastfeeding women. Ontario, 2007. Retrieved January 16, 2012 from [http://www.hc-sc.gc.ca/ahc-asc/media/advisories-avis/\\_2007/2007\\_136-eng.php](http://www.hc-sc.gc.ca/ahc-asc/media/advisories-avis/_2007/2007_136-eng.php).
- Akpantah AO, Ibok OS, Ekong MB, Eluwa MA, Ekanem TB. The effect of calabash chalk on some hematological parameters in female adult Wistar rats. *Turk J Hematol*. 2010; 27:177-181.
- Ekong MB, Akpantah AO, Ibok OS, Eluwa MA, Ekanem TB. Differentia effects of calabash chalk on the histology of liver of adult Wistar rats. *Internet J Health*. 2009; 8.
- Ekong MB, Ekanem TB, Abraham KE, Akpanabiati MI, Peter AI, Edagha IA. Effects of calabash chalk on hematology indices and histomorphology of the spleen of growing Wistar rats. *Instasci J Med Sci Clin Res*. 2012; 2:1-7.
- Ekong MB, Ekanem TB, Sunday AO, Aquaisua AN, Akpanabiati MI. Evaluation of calabash chalk effect on femur bone morphometry and mineralization in young Wistar rats: a pilot study. *Int Appl Basic Med Res*. 2012; 2:107-110.
- Ekong MB, John EE, Mbadugha CC, Bassey EI, Ekanem TB. Effect of calabash chalk on the histomorphology of the gastro-oesophageal tract of growing Wistar rats. *Malays J Med Sci*. 2012; 19:30-35.
- Ekong MB, Peter AI, Ekanem TB, Eluwa MA, Mbadugha CC, Osim EE. Calabash chalk's geophagy affects gestating rats' behavior and the histomorphology of the cerebral cortex. *International Journal for Brain Science*. 2014; 2014. <http://dx.doi.org/10.1155/2014/394847>.
- Dixon WJ. Staircase bioassay: the up-and-down method. *Neurosci Biobehav Rev*. 1991; 15:47-50.
- Organization for Economic Co-operation and Development. Acute oral toxicity-up-and-down procedure. OECD Guidelines for the testing of chemicals, Guideline 425. Paris, OECD, 2001, pp 1-26.
- Rispin A, Farrar D, Margosches E, Gupta K, Stitzel K, Carr G, Greene M, Meyer W, McCall D. Alternative methods for the median lethal dose (LD50) test: the up-and-down procedure for acute oral toxicity. *ILAR J*. 2002; 43:233-243.
- Abrahams PW, Follansbee MH, Hunt A, Smith B, Wragg J. Iron nutrition and possible lead toxicity: an appraisal of geophagy undertaken by pregnant women of UK Asian communities. *Appl Geochem*. 2006; 21:98-108.
- Shannon M. Severe lead poisoning in pregnancy. *Ambulat Pediatr*. 2003; 3:37-39.
- Bitanihirwe BK, Cunningham MG. Zinc: the brain's dark horse. *Synapse*. 2009; 63:1029-1049.
- D'Elia L, Barba G, Cappuccio F, Strazzullo P. Potassium intake, stroke, and cardiovascular disease: a meta-analysis of prospective studies. *J Am Coll Cardiol*. 2011; 57:1210-1219.
- Mehtar S, Wiid I, Todorov SD. The antimicrobial activity of copper and copper alloys against nosocomial pathogens and Mycobacterium tuberculosis isolated from healthcare facilities in the Western Cape: an in-vitro study. *J Hospital Infect*. 2008; 68: 45.
- Stepura OB, Martynow AI. Magnesium orotate in severe congestive heart failure (MACH). *Int J Cardiol*. 2008; 131:293-295.
- Rouault TA. How mammals acquire and distribute iron needed for oxygen-based metabolism. *PLoS Biol*. 2003; 1: e9.
- Centers for Disease Control and Prevention. Toxicological profile for barium and barium compounds. United States department of health and human services, 2007. Retrieved August 21, 2007 from <http://www.atsdr.cdc.gov/toxprofiles/tp24.pdf>.
- Emsley J. Titanium. Nature's building blocks: an a-z guide to the elements. New ed. Oxford, England, Oxford University Press, 2001, pp 506-510.
- Ferreira PC, Piai Kde A, Takayanagui AM, Segura-Muñoz SI. Aluminum as a risk factor for Alzheimer's disease. *Revista Latino-Americana de Enfermagem*. 2008; 16:151-157.
- Reimer KJ, Koch I, Cullen WR. Organoarsenicals. distribution and transformation in the environment. Metal ions in life sciences. Cambridge: RSC Publishing, 2010, pp 165-229.
- Sanders T, Liu Y, Buchner V, Tchounwou PB. Neurotoxic effects and biomarkers of lead exposure: a review. *Rev Environ Health*. 2009; 24:15-45.

29. Abrahams PW, Davies TC, Solomon AO, Trow AJ, Wragg J. Human geophagia, calabash chalk and undongo: mineral element nutritional implications. *Plos One*. 2013; 8(1): e53304.
30. Pais I, Jones JB. *The handbook of trace elements*. St. Lucie Press, Boca Raton, FL. 1997
31. Slagle A, Skousen J, Bhumbra D, Sencindiver J, McDonald L. Trace element concentrations of three soils in central Appalachia. *Soil Survey Horizons*. 2004; 45(3):73-85.
32. Abraham NM. Functional education, militancy and youth restiveness in Nigeria's Niger Delta: the place of multi-national oil corporations (MNOs). *Afr J Politic Sci Inter Relations*. 2011; 5:442-447.
33. Orime OCN, Igwe CF. Environmental sociology and sociology of health: a step in accomplishing the principle of sustainable environmental safety in the Niger Delta region of Nigeria. *Journal of Environmental Management and Safety*. 2011; 2:108-122.
34. Udoudoh FP. Oil spillage and management problems in the Niger Delta, Nigeria. *Journal of Environmental Management and Safety*. 2011; 2:137-155.
35. European Food Safety Authority. EFSA panel on contaminants in the food chain (CONTAM). *EFSA Journal*. 2010; 8(4):1570.
36. European Union Commission Regulation (2001). Setting maximum levels for certain contaminants in foodstuffs. No. 466/2001.
37. European Community Comments for the Codex Committee on Food Additives and Contaminants, 35th Session, Arusha, Tanzania, 17-21 March 2003. Agenda Item 16(c)--CX/FAC 03/28. Proposed draft code of practice for the prevention and reduction of lead in food.
38. Bouchard MF, Bellinger DC, Weuve J, Matthews-Bellinger J, Gilman SE, Wright RO, Schwartz J, Weisskopf MG. Blood lead levels and major depressive disorder, panic disorder, and generalized anxiety disorder in U.S. young adults. *Arch Gen Psych*. 2009; 66:1313-1319.
39. Goyer RA. Results of lead research: prenatal exposure and neurological consequences. *Environ Health Perspect*. 1996; 104:1050-1105.
40. Bressler J, Kim KA, Chakraborti T, Goldstein G. Molecular mechanisms of lead neurotoxicity. *Neurochem Res*. 1999; 24:595-600.
41. Desilva PE. Determination of lead in plasma and studies on its relationship to lead in erythrocytes. *Brit J Industrial Med*. 1981; 38:209-217.
42. Khalil-Manesh F, Gonick HC, Weiler EW, Prins B, Weber MA, Purdy RE. Lead-induced hypertension: possible role of endothelial factors. *Am J Hyperten*. 1993; 6:723-729.
43. Humphreys DJ. Effects of exposure to excessive quantities of lead on animals. *Brit Vet J*. 1991; 147:18-30.
44. Sharma RP, Street JC. Public health aspects of toxic heavy metals in animal feeds. *J Am Vet Med Assoc*, 1980; 177:149-153.
45. Lancranjan JI, Popescu O, Gavenescu I, Serbanescu KM. Reproductive ability of workmen occupationally exposed to lead. *Arch Environ Health*. 1975; 30:396-401.
46. Rom WN. Effects of lead on reproduction. In: Infante PF, Legator MS (eds). *Proceedings of a workshop on methodology for assessing reproductive hazards in the workplace*, National Institute for Occupational Safety and Health, Washington DC, 1980, pp 33-42.