



DECREASING EARTH SHIELD AN EVIDENCE OF CLIMATE CHANGE

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ABSTRACT

Climate change phenomena have being with us. Several indicators have been used to show the extent the Earth climate has change. This study aimed at calculating the total decrease in the Earth magnetic field and its Ozone layer concentration during secular variations. Two key parameters; the geomagnetic field and Ozone concentration decays were studied using Earth total field (F) and Earth Ozone concentration data in Dobson unit (DU). Geomagnetic field data were obtained from Space Physics Interactive Data Resource (SPIDR) from 1960 to 2010 for 13 observatories across the different latitudes and global Ozone concentration data from the Atmospheric Research Group of Cambridge University for the years 1955 to 2000. The data were analysed using exponential decay law. Similar decay trends were observed across the various seasons considered. Result of the analysis gave an average decay constant per year of the Earth total magnetic field (F) of 0.006 y^{-1} for an average coefficient of determination (R^2) of 0.8 across the various stations with an average decay of 185.1nT culminating to a global decrease of 615.752nT for the whole 50 years. Hence a global decay in the Ozone layer concentration of 0.02DU per year, with a coefficient of determination (R^2) of 0.7 cumulating to a reduction in the Ozone layer concentration by up to 4.624DU for the period of 45 years. This decrease in the Earth magnetic field and the Ozone layer concentration is not good for this planet. It may culminate to a decrease in the protective force of the magnetosphere, increase in lather radiations and overheating of the Earth environment. These may make life uncomfortable to living things on Earth.

KEYWORDS: Global warming, Magnetosphere, Ozone concentration, Earth shield and geomagnetic field decay.

INTRODUCTION

Climate change is a long-term shift in the statistics of the weather (including its averages). For example, it could show up as a change in climate normally (expected average values for temperature and precipitation) for a given place and time of year, from one decade to the next. (NOAA, 2007). Climate change could be natural (Orbital Variability, Solar activity Volcanic activity etc.) or anthropogenic that is human induced perturbations or changes in atmospheric green house content (Buchdahl, 2002). Over the last 100 years, average temperature near earth surface has risen little less than 1°C . This is responsible for the conspicuous increase in storms, floods and raging forest fire observed in the last ten year (Oladiran, 2010). No forcing factor, be it changes in CO_2 concentration in the atmosphere or changes in cosmic ray flux modulated by solar activity and geomagnetism, or possibly other factors, can at present be neglected or shown to be the overwhelming single driver of climate change in past centuries. (Courtillot *et al.*, 2007). Trends in the upper atmosphere, together with the global warming of the lower atmosphere are part of the global change of the Earth system. (Elias, 2011). Greyl, (2012), used the speed of the rotating magnetic fields of the sun to calculate the magnetic field activity of the sun and the number of sunspot which appears on the sun's surface, to explain the tangling of the solar magnetic field. Humphrey, (2010) estimated the total current I circulating in the core as a steady exponential decay of;

$$I = I_0 e^{-t/\tau} \dots\dots\dots (1)$$

The Magnetosphere created by the geomagnetic field protects the earth against corpuscular radiation (Belgian institute for space Aeronomy, 2013). As the Earth magnetic field reduces, it becomes

increasingly exposed to lethal charged particles, (Harold, 2010).

The earth is surrounded by a magnetic field similar to a dipole. Its magnitude at the earth surface ranges from 25nT - 65nT, (Reeve, 2010). The seven components of the Earth Magnetic field are described as

follows: Angle of Declination (D), angle of Inclination (I) Horizontal intensity (H), North component of horizontal intensity (X), East component of horizontal intensity (Y), Vertical intensity (Z) and Total intensity (F). These components are represented vectorally in Fig. 1

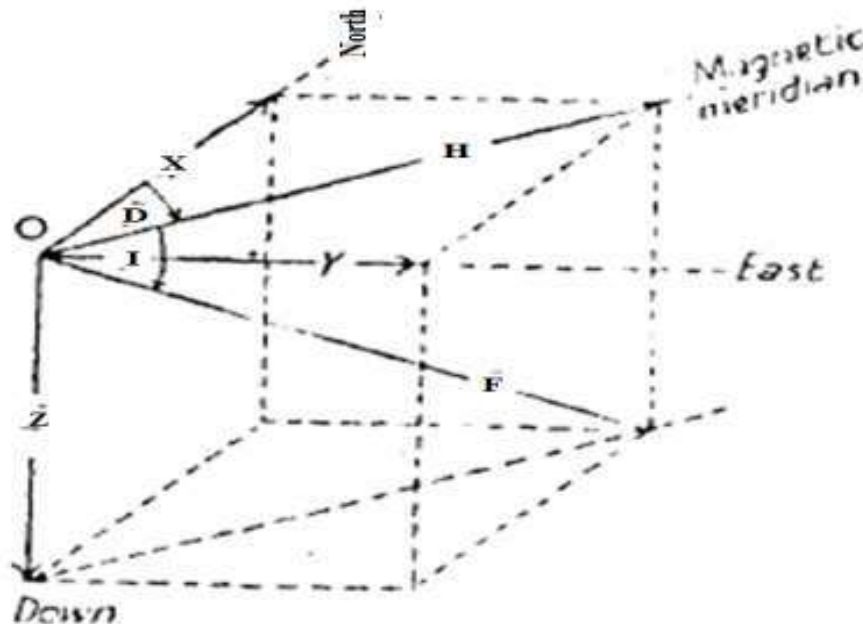


Fig 1: Vector representation of the Earth magnetic field, (Reeve, 2010).

The Earth ozone layer Concentration is fast depleting as a result of greenhouse gases emissions.

The level of ozone concentration at different altitudes and the blocking of the different bands of ultraviolet radiation is depicted in Fig 2.

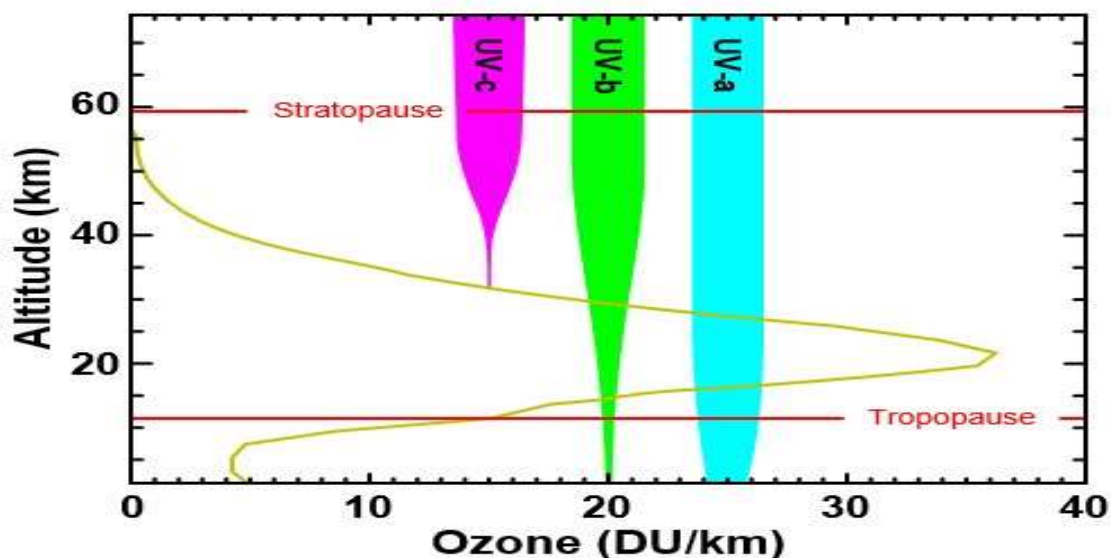


Fig 2: Ozone concentration at different altitudes, obstructing various bands of UV radiation, (Wikimedia commons, 2019).

This study will help to create awareness on the importance of the Ozone layer as a shield, the need to prevent Ozone layer depletion and the certainty of the decaying earth magnetic field culminating to global warming. When adequate cautions are taken, life will be continually enhanced on earth.

Magnetism been invoked as an evidence for connections between climate and magnetic field variations has received little attention. We therefore, analysed the geomagnetic field and ozone concentration decays using Earth total field (F) and Earth ozone concentration data in Dobson unit (DU). The study focuses at: (1) Showing that the total earth magnetic field is decaying exponentially with time over secular periods, using magnetic recorded data. (2) Understanding the implications of human

induced perturbations (e.g., increased carbon dioxide concentrations or depletion of the ozone layer) on the global climate using Ozone layer Concentration.

Data and Stations

Geomagnetic total field data **F** were obtained from Space Physics Interactive Data Resource (SPIDR) hosted by the world data centre (WDC) on <http://spider.ngdc'naoo.gv> for thirteen different stations across the globe. The locations of these stations were sampled randomly across equatorial, low latitude, mid-latitude and high latitude regions. Ozone concentration data were obtained from Atmospheric Research Group of Cambridge University, in UK. The data range for this spans from the year 1955 to 2000.

Table 1: List of Stations and their locations used in the study.

STATION	Code	LAT.	LONG.	YEAR	COUNTRY
ADISABABA	AAE	9.03	38.76	1965-1990	ETHIOPIA
ALIBAG	ABG	18.63	72.86	1935-1975	INDIA(ASIA)
APIA	API	18.63	72.86	1920-2005	WESTER N
ARGENTINE (FARADAY)	AIA	-65.25	-64.27)	1955-2005	ATATIKA
BAKER LAKE	BLC	64.32	-96.03	1975-2005	CANADA
MACQUARI E ISLAND	MCQ	-54.5	158.95	1950-2005	AUSTRALIA
M'BOUR	MBO	14.39	-16.96	1950-2005	SENEGAL
MEANOOK	MEAN	54.63	-113.33	1920-2005	CANADA
SAN JUAN	SJG	18.11	-66.16	1965-2005	USA
SITKA	SIT	57.05	-135.33	1940-2005	USA
TEOLOYUCAN	TEO	19.75	-99.19	1925-2005	MEXICO
TUCSON	TUC	37.17	-110.74	1925-2005	USA
GUAM	GUA	13.58	144.86	1960-2010	USA

The locations of these stations with respect to the globe are depicted in Fig 3



Fig 3: Map showing the locations of the stations

Method

Half-life and decay constant were calculated based on the fundamental laws of physics. Change per year in F were obtained: Minus for decrease and a plus for increase

$$t_{\frac{1}{2}} = \left\{ \frac{1}{N \ln \left(\frac{N_0}{N} \right)} \right\} T \ln 2 \dots \dots \dots (2)$$

$$\lambda = \frac{\ln 2}{t_{\frac{1}{2}}} \dots \dots \dots (3)$$

Where $t_{\frac{1}{2}}$ is the half-life and λ is the decay constant.

$$\text{Also, } F = q (E + V \times B) \dots \dots \dots (4)$$

where F is the Earth shielding force at the Magnetosphere; q is the charge on the solar wind particles streaming from the sun, E is the electric field of the Earth core, V is the velocity of the solar wind streaming out of the sun and B is the Earth core current-induced geomagnetic field. The exponential decay equation depicted by a geomagnetic field becomes;

$$F = F_0 e^{-\lambda t} \text{ or } \ln \left(\frac{F}{F_0} \right) = \lambda t \dots \dots \dots (5)$$



Results and Discussion

The results of this work are presented in Fig 4 to Fig 9 as follows;

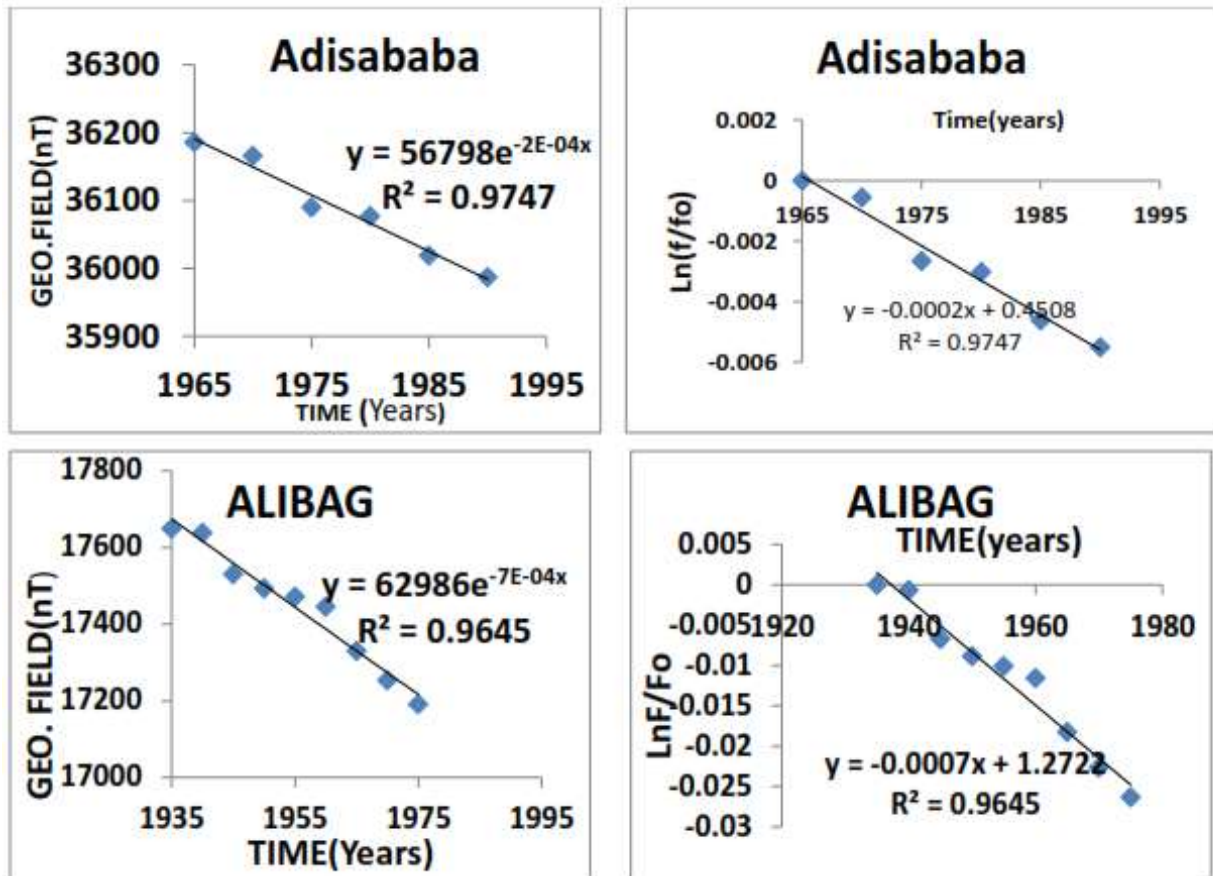


Fig 4: Total field induction intensity as a function of time for equatorial stations. Adisababa and Alibag, showed a decreasing trend in the earth magnetic field.

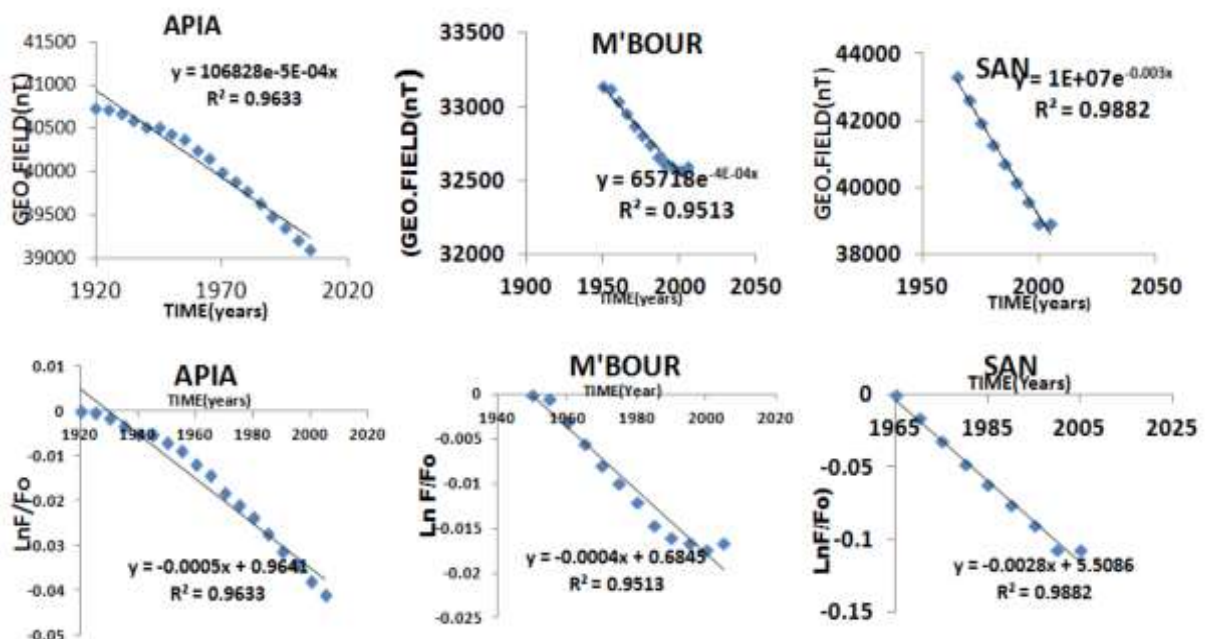


Fig 5. Total field induction intensity as a function of time for equatorial stations. Apia, M'bours and SAN showing decreasing trends in the earth magnetic field.

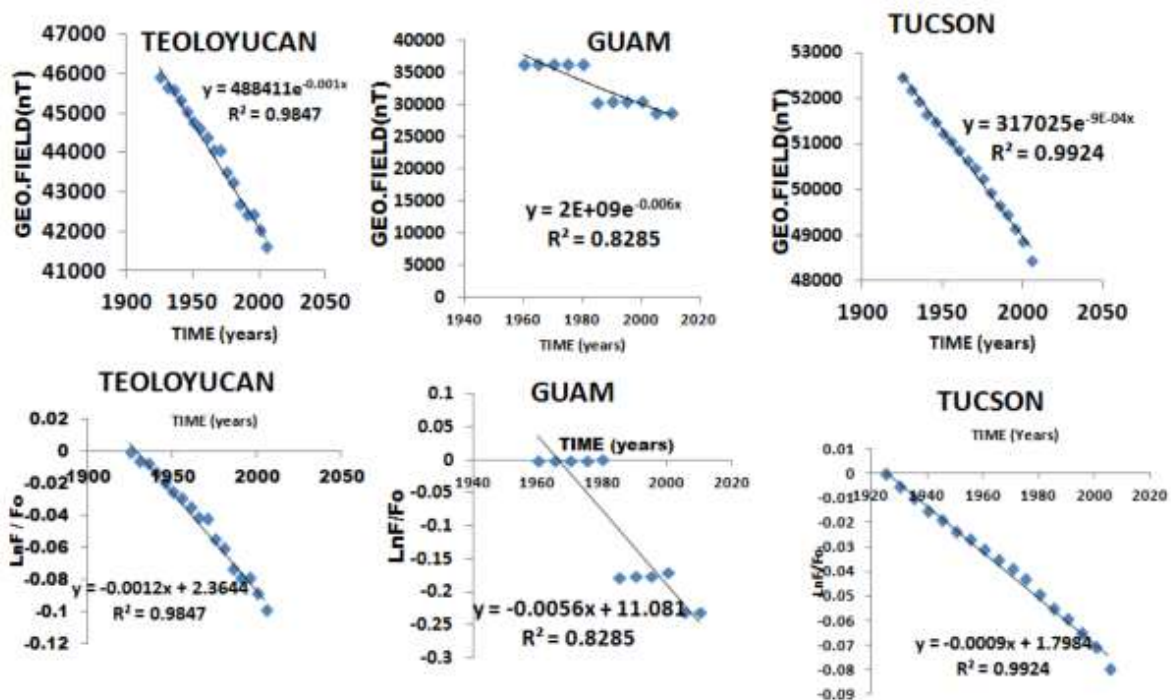


Fig 6. Total field induction intensity as a function of time for Low latitude stations Teoloyucan, Guam and Tucson all showing decreasing trend.

For the equatorial stations in Fig 4. Alibag had the highest decay constant of $7 \times 10^{-4} \text{ y}^{-1}$ corresponding to a half-life of 990.21 y^{-1} and an average decrease of geomagnetic field intensity per year by 11.45nT, this shows that there was a drastic decrease in the Earth magnetic field in Guam station whose decaying field was taken from the year 1935-1975, for a period of 40 years. M'bour in Fig 5., had the highest half-life of about 1732.87years, with a decay constant of $4 \times 10^{-4} \text{ y}^{-1}$ and the annual average

decrease of F per year obtained was 10.31nT, the geomagnetic field intensity was examine over years 1950-2005, a period of 55 years. In Fig 6, Guam had the highest decrease per year of 150.153nT compare to other equatorial station. This results are similar to the results obtain for Akoka and Ibadan which are two African equatorial stations in Nigeria using modeled data from International Geomagnetic reference field (IGRF) (Nzekwe *et al.*, 2011).

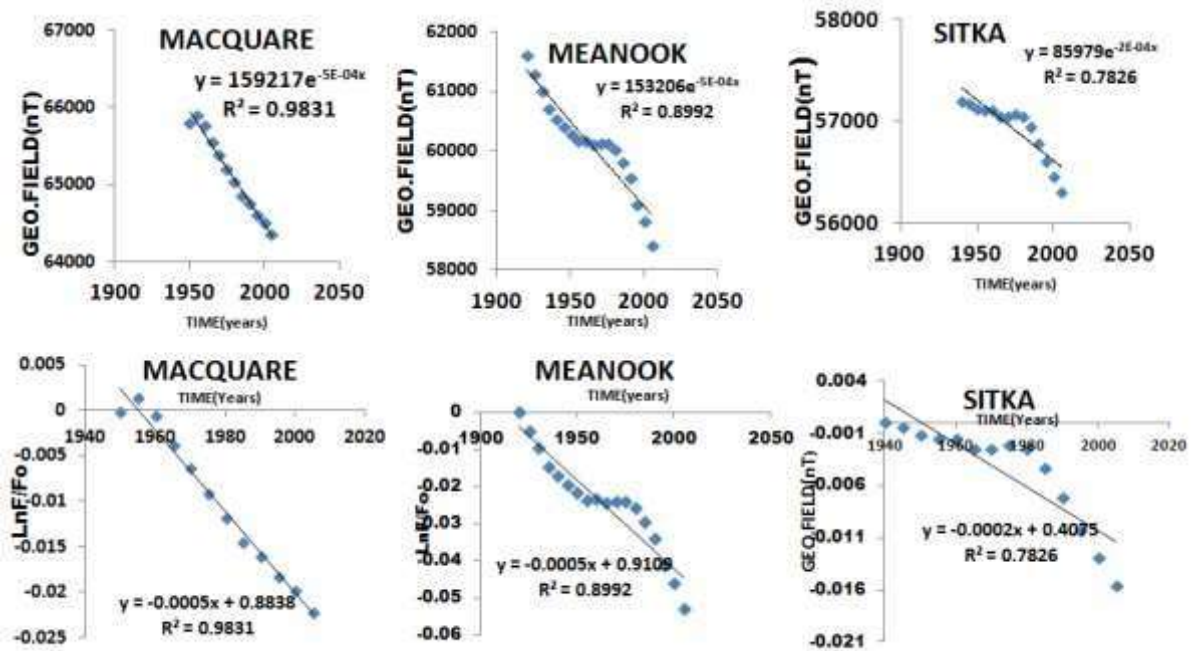


Fig 7. Decaying trend in the total Earth magnetic field induction intensity as a function of time for mid latitude stations. Macquarie, Meanook and Sitka all showing decreasing trend in earth magnetic field.

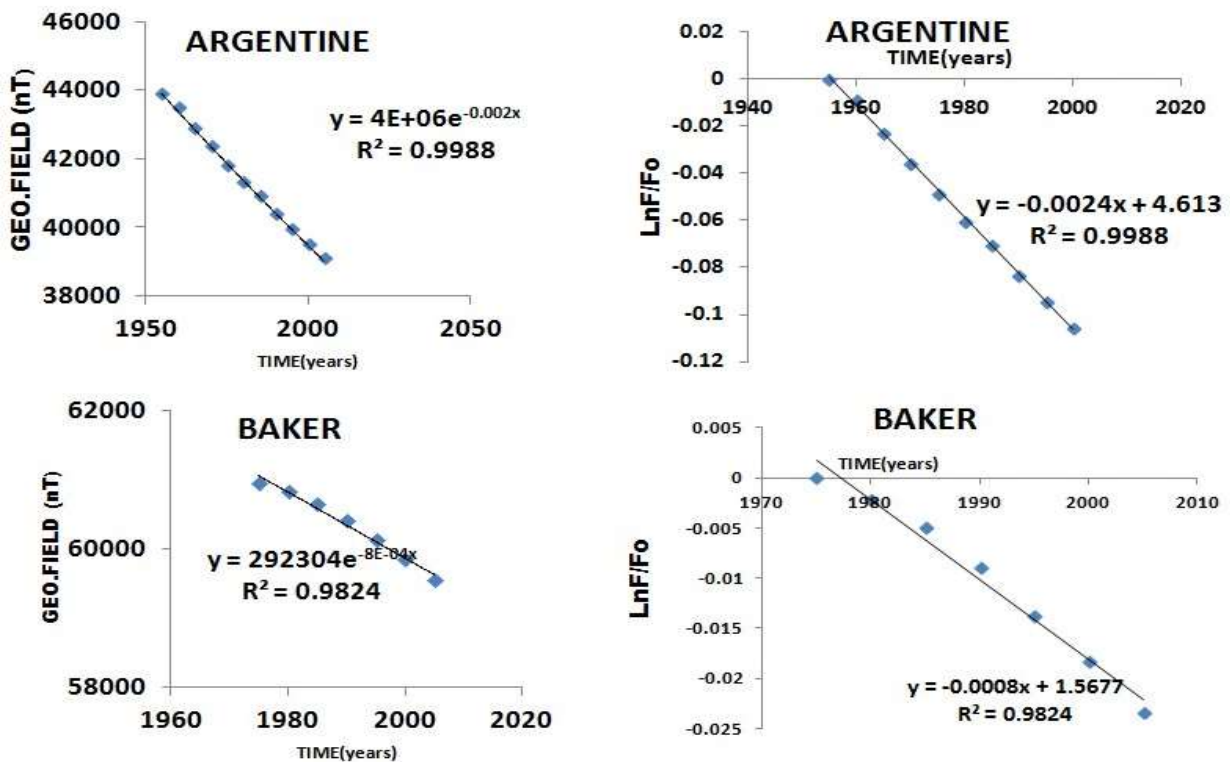


Fig 8. Decaying trend in the total Earth magnetic field induction intensity as a function of time for high latitude stations indicated on the charts.

For the mid latitude stations, Macquarie Island and Meanook in Fig 7, had the same decay constants with half lives of 6931.47 and 1386.29 years. These account for the duration taken for the Earth total geomagnetic field intensity to reduce to half its original amount (Barnes, 1971 and 1973). Fig 8, displays the decaying trend in the total Earth magnetic field induction intensity as a function of time for high latitude stations.

Similar trend were obtained for Argentine and Baker at a decay constant of 0.0024y⁻¹ and 0.0008y⁻¹ respectively. These rates at high latitude further buttressed that, the cumulative geomagnetic field intensity is reducing.

This reduction over a long period is not good for our Earth magnetosphere.

These results on geomagnetic field reduction are similar to that reported by NERC British Antarctic Survey, (2019), that the magnetic field strength has been steadily decreasing at a rate of 5-7% per century since 1840, which has led to speculations that we may be heading for a magnetic field reversal. A better solution to this is cut down anthropogenic factors. However, It won't be easy, but the technologies are already here to decarbonise electricity production, increase energy efficiency in buildings, protecting and restoring natural ecosystems, and make cropland take in more carbon than it emits. (Umair, 2019).

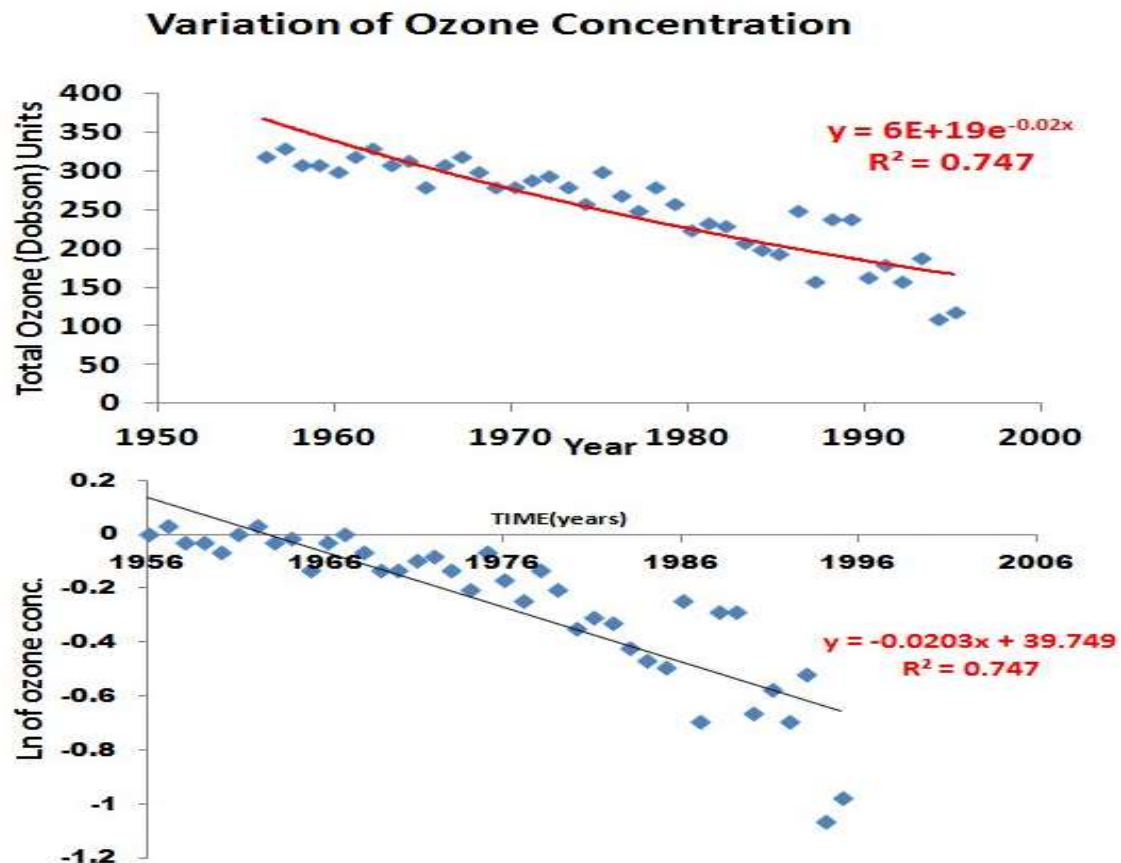


Fig 9: Global decaying trend in the ozone concentration

In Fig 9, Ozone concentration decayed at the rate of 0.02DU per year which lead to a total reduction of 4.624 DU for the periods of 45 years. This may be responsible for the antecedent ozone hole creation, because a reduction in ozone concentration by one DU means a lot to this planetary

atmosphere. (Oladiran, 2010).

Earth weakened magnetic field at the point of reversal could allow dangerous high-energy particles from space to reach the earth's surface, with potentially disastrous consequences for the atmosphere, climate and life. (Stanislav, 2007).



CONCLUSION

Total decreases of 615.752nT for the whole 13 stations were obtained from our results. While an average decay rate of 0.017nT per year was deduce from the exponential and the linear fits. Ozone Concentration decayed at the rate of 0.02DU per year which lead to a total reduction of 4.624 DU for the periods of 45 years. This may be responsible for the antecedent ozone hole creation, because a reduction in ozone concentration by one DU means a lot to our planetary atmosphere.

Geomagnetic field induction intensity decay may contribute to the earth global warming phenomenon after a long period of time. Decrease in the Earth magnetic field is not good for this planet, because it means a decrease in the protective force of the magnetosphere. Hence lather radiations may penetrate into the Earth environment. This decrease in the earth magnetic field and the Ozone layer concentration is not good for our planet. This implies a decrease in the protective force of the magnetosphere. Hence lather radiation leading to overheating of the Earth environment, low crop yield, disruption in communication and skin burn, making the atmosphere uncomfortable for man.

RECOMMENDATION

On a final note, whatever damage done to the mother earth anywhere in the world will be evenly distributed around the globe. The way forward for now is to cut down the anthropogenic factors in other to cancel the disequilibrium between the Earth core and the magnetosphere.

REFERENCES

Barnes, T.G. (1971), Decay of the earth's magnetic moment and the Geochronological implications. Creation Research Society Quarterly, 8(1),:24-29

Barnes, T.G. (1973), Electromagnetics of the earth's field and evaluation of electric conductivity, current, and joule heating in the earth's core. Creation Research Society Quarterly, 9(4): 222-230

Belgain institute for space Aeronomy (2013), Division of space physics, Article July 2013. Journal of Atmospheric and Solar-Terrestrial Physics available at <https://www.researchgate.net>departm> ent.Date accessed 25th August, 2019

Buchdahl J. (2002), Climate change Atmosphere, Climate and environmental information, Programme Aric Manchester Metropolitan University.

Courtillot V., Gallet Y., Jean M. Frédéric F. , Genevey A. (2007), Are there connections between the Earth's magnetic field and climate? Earth and Planetary Science Letters 253 (2007) 328–339

Elias G. A. (2011), possible sources of long-term variations in the mid- latitude ionosphere. The open Atmospheric Science Journal Vol. 5, 9- 15.

Geryl, (2012), The sun's Eleven year magnetic reversal theory on the sun. Eleven year oscillating theory. Obtained from ww.astrosciences.info accessed August 27,2019

Humphreys, D.R. (2010), The creation of cosmic magnetic field, Earth's magnetic field decaying steady with a little rhythm . Proceedings of the sixth ICC 213-230

Harold S. S. (2010), Thomas G. Barnes, BSMRA, and IGRF Data Leading to Half- Life calculations of the geomagnetic field decay. Geomagnetic Reference Field (IGRF) 2010 Geomagnetic Field Intensity Decay.

Oladiran E. O. (2010), Atmospheric dynamics and the regional contributions to the Earth' energy balance and global warming. An inaugural lecture, 2009/2010, Ibadan University press publishing house University of Ibadan, Oyo state.



- NERC British Antarctic Survey, (2019), The effects of long-term changes in the Earth's magnetic field on the atmosphere: understanding the past; predicting the future. Uk Research and Innovation Retrieved from, <https://gtr.ukri.org/projects?ref=NE%2FJ018058%2F1> Assesed date 26/11/2019
- NOAA (2007), Climate Change, NOAA National Weather service available at <http://www.ncdc.noaa.gov/oa/climate/research/anomalies/anomalies.html>
- Nzekwe N.M., Imafidon, L.O., Agbalagba, O.E., Oba, M.O., (2011), geomagnetic field decay contribution to global warming. International journal of science and society Yabatech. Vol 1
- Reeve W. D. (2010), Reeve Observatory Anchorage, Alaska U.S.A. (Geomagnetism). Pg. 1- 32.
- Stanislav, V., (2007) The origin of the earth's magnetic field. Available online at <https://slideplayer.com>, accessed 27, August 2019.
- Umair I., (2019), UN: The world has backed itself into a treacherous corner on climate change Countries are far off course in meeting emissions goals. More drastic actions are needed to get back on track. Retrieved from <https://www.vox.com/2019/11/26/20983626/climate-change-un-emissions-gap-report>
- Wikimedia commons, (2019) online Wikipedia, available at www.wikipedia.com, accessed 28 August, 2019.



EFFECT OF HEAT PROCESSING ON THE NUTRITIONAL VALUES OF TWO VARIETIES OF EDIBLE OIL SOLD IN LAGOS METROPOLIS

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ABSTRACT

Edible oils are used in industries and for domestic purposes ranging from preservative to processing of foods. However, methods of processing affect the nutritional quality of oils. This research examined the effect of heat processing on the nutritional qualities of two brands of edible oil sold in Lagos metropolis. The power and Turkey oil samples were purchased at Lawanson Market, Surulere, Lagos. One hundred and fifty milliliter of oil was measured into four conical flasks at room temperatures of 29°C and each of them was heated at different temperature of 40, 50 and 70°C while the control was treated at room temperature. Analyses of the oils were carried out using standard protocols. The refractive index increased with temperature same goes for saponification value, peroxide value and cholesterol levels but moisture content decreased with increase temperature. The refractive index showed a significant increase of 1.461 ± 0.7 and saponification value of 37.87 ± 3.21 . However, Iodine value, peroxide values and moisture content decreases with increased in temperature. This work showed that there was defined alteration or changes in nutritional Composition of Power and Turkey oil with variation in temperature and that frying at moderate temperature could help to achieve the best nutritional value of food during processing both in industries and domestic uses.

KEYWORDS: Refractive index, saponification value, peroxide value

INTRODUCTION

Edible oils are important part of everyday meal globally and from time immemorial; edible oil has been used for domestic and pharmaceutical purposes. Edible oil manufacturers are important employers of labour in major areas of the globe and source of income.

There are much concern expressed on the biological effect of oil containing free radicals, resulting from heating and frying. There are increasing signs that prove it can be detrimental to health. (Dobarganes et al, 2005). Frying is a profound process that causes series of changes and generates lots of radicals (Bellitz et al, 2004). In frying, whichever type, the oil is normally heated to about 50-100°C.

If the oil is fried to 500-1000°C in the presence of oxygen, the oil goes through physical and chemical reactions due to thermal reaction (Moreirra et al, 1999). There are lots of hazards generated by eating oil containing free radicals Gotch et al, (2007).

When edible oil is at high temperature for long period will produce free radical product that may be injurious to health (Lapointe et al, 2006).

However, some authors suggested that edible oils are important replacement for mineral oils in the production of quality lubricant (Farooq et al, 2011).

They can be used as another source of energy (Kalman et al, 2013), (Liland et al, 2013; Murray et al, 2014). If used properly, the oils sustain the body with natural components.

Edible oil plays prominent and sensorial roles in food product, and they are transporters of fat-soluble vitamins. They made available important fatty acid for growth. Morison and Boyd (2006). Hence, it is good to know the components of fatty acid of oil or fat to know



their behavioural pattern and to determine the likely alteration in their composition with processing as well as the stability and physical chemical properties of these products. Zambiasi and Zambiasi, (2000).

Cooking oils are used for domestic and as supplement. Some oils, edible and otherwise are used as energy for energy - based fuel.

The edible oils accounts for 80% of the world's natural oils and fat supply in edible vegetable oils include processed linseed oil and castor oil used in lubricant plants cosmetics and other industrial application.

Vegetable oil can be processed by removing the component which may have negative effect on the taste, stability appearance or nutritional value. The objective of the research is to ascertain the effect of frying on the changes in the nutritional content of two brands of edible oil sold in Lagos metropolis and to determine the appropriate temperature under which heating and frying of edible oils could be carried out in order to obtain optimal nutritive value during processing.

MATERIALS AND METHODS

The power and turkey oil samples were purchased at Lawanson market, in Surulere, Lagos, and stored at room temperature. Two hundred milliliter of the samples were measured into four graduated flask each and labeled A, B, C and D. Sample A was used as control while others were heated at different temperatures. Parameters namely acid value, cholesterol, specific gravity, moisture content, saponification value and refractive index were determined to look into the impact of varying temperature on the nutritional values of the two vegetable oils.

Analysis of Cholesterol

The cholesterol content of the samples were estimated using method described by Zlakis et al, (1953). The oils were treated with ferric chloride, acetic acid mixture and sulphuric acid and the colour developed was noted after 15

minutes and absorbance was read at 560 nm. Ten milliliter of each of cholesterol standard and samples (lipid) were measured into test tube. Three milliliter of glacial acetic acid were added to each of the test tube and also the fourth — tube to serve as blank. This was shaken to dissolve the lipids in the test tubes. Three millilitre of colour reagent was added to each of the four test tube this was vortex to mix. This was allow to stand and cool at room temperature for 20 minutes and the absorbance was read at 560nm.

Determination of Refractive Index

The refractive index was carried out as described by Hivood David (1999), the oils were measured using the Abbe, refractometer at a constant temperature of 40 °C a thermostatically controlled water bath, which uses a water circulating pump, was connected to the Abbe refractometer to maintain its temperature within ± 0.1 °C of the required temperature, the determination was then carried out.

SAPONIFICATION VALUE

Saponification value is the number of mg of KOH required to neutralize the free acid.

Two gram of the fat or oil was put into a 250ml conical flask and 25ml of alcoholic KOH solution was added to it, attach a reflex condenser and heat the flask content on a hot water bath for 1 hr with intermittent shaking, while the solution is still hot. Three drops of phenolphthalein indicator was added and titrated with KOH with the 0.5M HCl. The same procedure was repeated as above but without sample.

ACID VALUE

The acid value represents the degradation of the oil quality resulting from the hydrolysis of triacylglycerol as well as further decomposition of hydro-peroxide.

About 10 gram of oil was weighed in a specified monograph into 250 ml flask, and add 50ml of



a mixture of equal volume of ethanol and ether which has been neutralized with KOH (0.1 M) after the addition of 1ml of phenolphthalein, and then heated until the oil is completely dissolve cool and titrate with KOH (0.1 M) constantly shaking the content of the flask until a pink colour which persist for 15 seconds was obtained. The procedural blank test was also carried out in parallel with AV determination.

PEROXIDE VALUE

Two grams of solid oil was put into a 100 ml glass stopper Erlenmeyer flask, about 12ml of acetic acid chloroform solution added, stir the beaker until the sample is dissolved, in 1 ml of Mohr pipette and add 0.2ml of saturated KI solution in a stopper flask for exactly 1 min, then 12ml of deionized water was added in a stopper and shaken repeatedly to remove the iodine from the chloroform layer then triturate gently with mixing until the colour faded, using an analysing device lint of starch solution as indicator, and triturate until the grey colour evaporate in the upper layer.

TABLE 1: CHANGES IN NUTRITIONAL VALUES WITH VARIATION IN TEMPERATURE FOR POWER OIL

Sample	0°C Temp	Refractive index (0°)	Saponification value mg/KOH/g	Moisture content %	Acid value g/mg/KOH/g	Peroxide value %	Iodine value g/100g	Cholesterol % mg/dl	Specific gravity (g/cm)
A	Room testing	1.46 + 0.05	37.87+2.5	1.01+0.05	1.03 + 0.05	2.0 +0.5	114.30 + 0.12	100.57 + 4	0.92 + 0.05
B	45	1.46 ± 0.01	33.66 ± 4.5	0.02 ± 0.01	1.02 + 0.05	4.0 ± 0.5	8.8.90 + 60	93.94 ± 5	0.92 ± 0.00
C	50	1.46 ± 0.01	46.28 + 2.7	0.06 ± 0.00	1.03 + 0.05	4.0 ± 0.5	152.4 ± 90	135.89 + 9	0.92 + 0.00
D	70	1.46 ± 0.01	42.08 ± 0.00	0.34 + 0.02	1.01 + 0.4	6.0 + 0.5	82.55 + 30	212.57+12	0.92 ± 0.00

Value represent Mean SEM of triplicate determination

TABLE 2: CHANGES IN NUTRITIONAL VALUES WITH VARIATION IN TEMPERATURE FOR TURKEY OIL

Sample	0°C Temp	Refractive index (0°)	Saponification value mg/KOH/g	Moisture content %	Acid value g/mg/KOH/g	Peroxide value %	Iodine value g/100g	Cholesterol %	Specific gravity (g/cm ³)
A Standard	Room testing	1.46 ± 0.05	58.91+ 30	1.02 + 0.05	1.12 ± 0.03	6.0 + 0.50	131.0 + 40	146.97+40	0.91 + 0.03
B	45	1.59 ± 9.00	69.59 ± 0.02	1.091 ± 0.02	4.00 ± 0.5	5.6.50± 50	132.80 ± 30	132.80 ± 30	0.91 ± 0.04
C	50	1.46 ± 8.00	84.15 + 2.10	1.97+ 0.04	1.10 ± 0.00	6.0 + 0.50	248.90+60	142.40+20	0.91 +0.04
D	70	1.46 ± 0.04	105.19 + 3.20	0.34 ± 0.05	1.08 +0.00	4.0 ± 0.50	183.40±40	166.40+30	0.91 ± 0.05

Value represent Mean ± SEM of triplicate determination



Statistical Analysis

The determination was done in triplicate and data obtained were analyzed for their central tendencies and variances using statistical Package for Social Sciences (SPSS for Windows, Version 10).

RESULT

The refractive index of the Power and Turkey vegetable oils were presented in Table 1 and 2 which ranges between 1.46 ± 0.05 and 1.46 ± 0.04 . The saponification value 39.96 ± 24 ; 62 ± 21 , moisture content 0.39 ± 0.2 ; 1.03 ± 1.04 ; Acid value 1.02 ± 0.4 ; 1.81 ± 0.3 . Peroxide values 4.0 ± 0.5 35 ± 0.5 ; Iodine value $132.62 \pm 115 \pm 41$; cholesterol 13.68 ± 62 ; specific gravity 0.92 ± 0.3 ; 0.92 ± 0.4 , 14.56 ± 40 . The analysis revealed the refractive index, saponification value peroxide value and cholesterol level increased with temperature but moisture content decreased with increase in temperature. This finding revealed there is correlation between temperatures and cholesterol level of the oil, which may probably due to radical generation during frying.

DISCUSSION

The refractive index decrease with increasing temperature from $1.49 \pm 0.046^\circ\text{C}$ for both brands of oil as shown in tables 1 and 2. The oil is less dense hence the light is less refracted. The saponification value for Power was found to be 42 ± 0.00 and 105.19 ± 3.20 . For Turkey oil indicating that Power oil have higher potential for soap production after being heated Warn et al, (2011).

The moisture content also decreased with temperature from $1.02 \pm 0.056\%$ to 0.34 ± 0.05 for Turkey oil. Indicating Turkey oil is of higher quality compared with Power oil. The acid value for Turkey oil 1.88 ± 0.2 mg/KOH/g is higher compared with Power oil 1.02 mg/KOH/g. This shows that Turkey oil have higher storage quality than Power oil after been used for processing Warn et al (2011).

The low peroxide value of 4.01 ± 0.05 obtained from Power oil compared to 5.40 ± 0.05 of Turkey oil that indicated the oil can

be reused several times after being used for processing (Oghungu, 2008).

The iodine value for Power oil 89.54 ± 42 g/100mg is when compared to 198.01 ± 40 g/100g for Turkey oil indicating that the Power Oil show quite degree of unsaturated fatty acid and therefore suitable for consumption of Turkey Oil could lead to cardiovascular disease compared to Power oil. The specific gravity shows no significant difference for both oils.

CONCLUSION

The result from this study shows that the refractive index apart from the standard which had the value of 1.462, decreased as the temperature increases from 45°C to 70°C from sample B to sample D respectively.

The refractive index, showed a significant increase of 1461 ± 0.7 , saponification value of sample X are **37.87+3.21, 58.91+46.28, cholesterol level 146.97+19.43, 100.57+1112** rises with increase in temperature, while the moisture content, iodine value peroxide value decrease with temperature. Table I & II.

The relative variation in cholesterol level with increase in temperature, may probably due to increase in radicals that are generated as a result of increase in temperature.

The result of this research indicate that, there is relative alteration in the nutritional composition of edible oil with increase in temperature and that frying the oils at a moderate temperature could help to preserve the nutritional value of these oil, and the best nutritional value could be obtained from those oils when they are been used for industrial and domestic purposes.

REFERENCES

- Belitz H.D Grosch W and Shiebele P. (2004). Food chemistry. 3rd edition. Springer Verlag, pp-218-219.
- Dorbarganes C, Marquez-Ruiz G (2003), Current opinion in clinical nutrition and metabolic care 6, 157.
- Farooq M.A., Rani S Gul and Muhammed N (2011). The study of waer behaviour of 12- hydroxy stearic acid in vegetable oils. J. Appl Sci II 1381-1385.



- Gotoh N, Ai-Iwasawa H, Watanabe R, Osato and S wada (2007). Oxidation of fats and oils in instant noodles stored under various condition. *Journal of Food Lipids*, 14; 350-365.
- David (1999). X-ray and extreme ultraviolet radiation. Principles and applications. P60. ISBN 0.521-0.2997.
- Holden, P. and N. Purcell (2000): *The corrupting sea*, London Blackwell.
- Kamalan B.S., Medale L, Laroque G Corraze and Panserat S (2013). Metabolism and fatty acid profile in fat and lean with vegetable oil.
- Lapointe A. Couillard C., Lermieux S (2006). Effect of dietary factors on oxidation of low density lipoprotein particles.
- Lilard N.S., Rosenlund M I, Brattewild L madsen and Torstensen (2013). Net-production of Atlantic Salmon (FIFO). Fish in fish out with dietary plant proteins and vegetable oils. *Aquacult Nutri* 19; 289-300.
- Moreira R.G., Castel-Perez M.E. and Barrufetia (1999). Deep fat frying fundamental and application. Aspen Publishers. Gaithersburg. MD.
- Morrison, R.T., Boyd, R.N. (2006): "Organic chemistry" 61st edition Prentice Hall India Private Ltd, New Delhi pg. 1120 — 1125.
- Murray D.S., Hager D.R., Topcher and Kainz M.J. (2014). Effect of partial replacement of dietary fish meal and oil by pumpkin kernel cake and repressed oil on fatty acid composition and metabolism in Arctic Charr (*Salvelinus alpinus*) *Aquacult*: 431-8591.
- Gunstone, F.D. (1989): "Oils and fats, past, present and future. Pp. 1 — 16 in fats for the future edited by R.C. Cambie Chichester Ellis Horwood Ltd.
- Ogungu M.M. (2008). Inhibitory effect of onion and garlic extract on the rancidity of palm and palm kernel oils. *Journal Chemistry Society of Nigeria* Vol 33 No. 1, pp. 43-44.
- Senanayake, S.P.I.H and Shahidi, F. (2002): structured lipid acylolysis of gamma — linolenic acid rich oils with n — 3 — polyunsaturated fatty acid. *Journal of Food Lipid* 4 pp. 309-323.
- Toussant — Samsat, M. (1992): *A history of food* Oxford Blackwell.
- Warn A.A., Gunu S.Y., Jega A. and Aisha J.A. (2011). Soap production from shea and butter. *International Journal of Applied Science*. Vol 5, No 4: 410-412.
- Warn A.A., Wawata I.G., Hassan I.G., Gunu S.Y. and Aujan K.M. (2011). Extraction and phytochemical analysis of some selected Northern Nigerian industrial oil. *Scholars research library. Archives of Applied Science Research*. Vol. 3 No.4, pp 536-541.
- Zambiasi, R.Z. and Zambiasi, M.W. (2000): Vegetable oil oxidation effect of endogenous component. *Revista da Sociedade Brasileira de Engenharia de Alimentos* 34(1) pp. 22-32



PRODUCTION OF PRINTED CIRCUIT BOARD FOR ELECTRONIC APPLIANCES

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ABSTRACT

Technology advancement promotes all round development for a nation. In Nigeria today, technology is encouraged through electronics among upcoming scientists and technologists for sustainable human capital and science development. Electronics is very fundamental and important to man, and electronic appliances rely on Printed Circuit Board (PCB) for component interconnection and firmness. It provides basic connection for any electronic gadget. PCB production is one area that offers opportunity to undergraduates of electronics and science to improve on circuit design and production which in turn promotes economic and technological advancement. This paper describes PCB production using the etching method and presents buffer circuit, online data logger circuit and a general purpose microcontroller boards through this method. The production process began with circuit design using Computer Aided Device (CAD) tools known as Proteus, iron (III) Chloride was the etchant for the copper boards during production. Afterwards, the drilling machine was used to bore holes on the boards according to the circuit design where needed components should be placed. Besides the three circuits earlier mentioned, this method has also been used to produce circuit boards for the following systems: bistable generator, audio amplifier, power supply unit and others. The outcome was cost efficient and encouraged homemade electronic appliance.

Keywords: Printed circuit board, Proteus, Etchant, Technology advancement.

INTRODUCTION

Electrical and electronic devices are made up of circuits which are used to monitor their operations and to trace faults when they malfunction. They are used in a huge variety of manufactured products (Charles & Matthew, 2004), ranging from everyday popular items such as lamps to robotic machines used in industries.

According to Elaine and Paul (2005), the brain inside every electronic device is the printed circuits assemblies mounted on printed circuit boards after design for interconnection of components and mechanical supports. Circuits were always constructed on bread boards, perf boards and strip boards through laborious processes of point-to-point and wrap wiring before the advent of the Printed Circuit Boards (PCB) (www.visually.com). This latter method makes manufacturing circuits cheaper, faster (en.wikipedia.org) and operator wiring errors are eliminated (www.worldlibrary.org).

PCBs provide mechanical support for the electronic components mounted on its nonconductive (Ebnesajjad, 2013) substrate (glass reinforced plastic), and interconnect the components to each other through conductive copper tracks (Ryan, 2009; Kraig, 2011); thereby create input/output connections so that the electronics can interact with the users.

Kristin (2015), stated among other advantages of PCB production as having minimum chance of wrong wiring and short circuit.

Production of PCBs is cheaper than sending your boards out to Fabrication Company. The knowledge and practice of producing PCBs will also help before a mass production using automation, to save one of a lot of money if few prototypes are made and tested in case something goes wrong with the design.



Nevertheless, the focus of Polytechnic Education in science and technology is to train and produce technologists who would be able to design, produce and troubleshoot electrical circuits- fixing appliances when they break down. When an electronic gadget develops faults, these faults are often traced to its circuit board. The entire circuit board production process begins with circuit designs, which implies working on a circuit board. Being familiar with this production process will help to improve electronic skills. Introducing the production of PCBs into Nigerian education curriculum especially at the polytechnic level will be of great significance which in turn will result into advancement in technology.

Ericsson reveals that the ongoing growth of the smartphone market is not likely to slow down anytime soon, and predicts 90% of world's population aged over six having a mobile phone by the year 2020 (Ericsson, 2015). This infers that the demand for electronic gadget will continue to increase.

Scottish printed circuit board make 25% of UK sales market creating 1800 job opportunity (Turok, 1993). The approach of indigenous production will help raise researchers in electronic field of optimising its performance over time, and create job opportunity. Countries that have stake in electronic industry will be able to increase their internal revenue with increase in demand, because for every need of electronic gadget comes the need for PCB. In spite of the fast developing electronic industry, Nigeria that housed part percentage of world consumer does not pose the technology of the printed circuit board. However, this research paper aimed at providing method of indigenous production of PCBs that will encourage designers of electronics system.

Following the patent of Charles Ducas in 1925, he described his very first design as "printed wires" which is close to PCB; he specially designed it for a radio receiver (US patent, 1925).

Schatzel (2009) pointed out a trend continuing in the electronics industry where standard epoxy glass and polyimide substrates are substituted for ceramic based substrates which effects high reliability packages; achieved as a

result of consistent raw material properties and improved fabrication accuracy. Of course, increased processing power results in increased heat generation, and at such, improved fabrication processes that allow smaller via, smaller trace line width/spaces and pad size combined with designs that have multiple internal conductive layers routinely reach 20 to 25 layers; make printed circuit boards becoming very dense. This increased processing power is affecting printed circuit board designs for space electronic applications, and additional heat must be conducted away from a microprocessor or power device through the printed circuit board to the board frame or chassis. This could be achieved for processing devices by utilizing thick copper clad layers and thermal via to transfer the resultant heat, but increasing the copper thickness is an increase in mass which defeat the goal of miniaturization of electronics package.

According to Mao et al (2011), China being the largest PCB manufacturing country in the world is greatly enhanced, due to rapid development of printed circuit board (PCB) industry during the 1990s and 2000s. This made Chinese universities, institutes and PCB manufacturers, in recent years, to conduct much basic researches on high-end PCB technology; results of which unfold improved technological methods of analysis, solutions to signal/power integrity (SI/PI) and electromagnetic compatibility (EMC) problems, approaches of electro-thermal modelling, analysis and co-design, all for PCB performance enhancement or improvement.

Chunfei et al (2013) pointed out that FR4 is commonly used in industry to build printed circuit boards, but signals propagating in such media have significant attenuation as data rate increases, gating the solution space. In their paper, hybrid PCB stackup was used to investigate improvement on full channel signal integrity performance; in which simulations for SATA3 and PCIE3 showed noticeable improvement. Though low loss materials are very costly for most of commercial platforms but must be considered so as to enable longer board routing; however, using hybrid of this sort is normally less costly than all low loss



PCB stackup, thus achieving a good compromising between cost and performance for PCB design and manufacturing.

MATERIALS AND METHODS

The following is the table of list of materials used for the research work

Table 1: Materials Used

S/N	NAME	DESCRIPTION
1	Proteus	CAD - Electronic Design Automation (EDA)
2	Copper Board	A material of non-conductive substrate coated with conductive substrate (copper) on either side
3	A4 paper	Glossy A4 paper
4	Drilling machine	Machine with ½ inches drilling bits for boring holes
5	Dry Iron	Resistive joule heating material
6	Liquid Iron (III) Chloride	Etchant
7	Petrol/kerosene	Organic solvent

Sources: www.newenergytimes.com; www.totalpfs.co.uk; mospi.nic.in; www.gustaf-galke.de; newton.pbk-solutions.de; epdf.tips; download.quickheal.com; and www.digitek.ru

The method of producing printed circuit board is the same pattern but resource apply may vary. The method described in this paper is the application of locally sourced material for the production of PCB. There are basically two stages involve in the production of PCB, namely – virtual development stage and manufacturing method.

Virtual development stage

Computer-aided design (CAD) employs the use of computer systems to aid the production, modification, analysis, or optimization (www.renovision.in) of any design; applicable almost in all fields. There are basically two types of CAD tools, namely – Electronic design automation, EDA used in electronic systems and – Mechanical design automation, MDA used in mechanical design. The CAD tool used in this research work is the EDA, and it is used in the two ways of PCB development design, namely – PCB schematic design and PCB layout design.

PCB Schematic Design

PCB design is the selection of circuit component from the application (CAD) library and the 2D drawing of circuit tracks to link the terminal of different circuit component. In this stage, the EDA (Proteus) was used to select component and to create interconnect between the selected circuit components.

PCB Layout Design

PCB layout design is the 3D graphical development of the PCB schematic drawn. It is the next stage of design after PCB schematic design, selected circuit component are placed on the board virtually using the EDA computer based application. This application also allows post-production simulation, which showed the expected circuit design.

Manufacturing Method Toner Transfer

After the design of the circuit tracks, the first process in the manufacturing stage is the toner transfer. Toner is a mixture of carbon and iron oxide powder used for laser printer. The laser printing method was adapted to print out the circuit tracks designed on a glossy A4 paper. Glossy paper was specially chosen because whatever is printed on it can be transferred onto another surface by using heat.

The circuit footprint (tracks) was printed on a glossy A4 paper as shown in the figure above, the printed tracks was then placed on a cleaned copper board surface pressed by a dry pre-heated iron. The circuit footprint was printed on the copper board using this method.

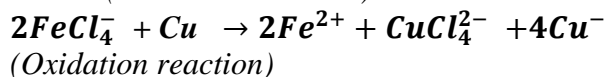
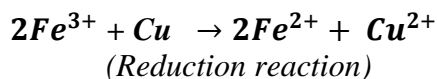
Chemical Etching

Chemical etching is the process of eliminating unwanted part of copper clad from its composition using baths of temperature-regulated (Zhang, 2014) etchants.

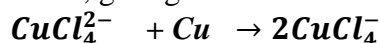
According to Clyde and Happy (2007), the etched out part is the unwanted portion of the copper foil in the base substrate.

Etchant however, is the chemical (always in solution) used for etching and there are different types of etchant but Iron (III) Chloride was used in this process, because of its availability, health

consideration and cost efficiency. The chemical reaction involves Redox reaction written below:



When all iron (III) (Fe^{3+}) is converted/reduced to iron (II) (Fe^{2+}), then the $CuCl_4^{2-}$ ion in turn also oxidizes remaining copper on the circuit board, giving:



The circuit footprint (i.e. region coated with toner ink as in figure 4 below) will not be etched because they are insulated from the chemical

reaction by polymerized iron and carbon powdered composed in the toner ink.

Drilling

This is the process of creating holes on the PCB for component's terminal. This ensures provision for component's interconnection through soldering. However, this process only applies to "through hole" devices; other components like surface mount devices (SMDs).

RESULTS

Using the methods outlined above, several useful circuit boards were designed and produced. Figure 1-4 show the final virtual development of a buffer board while figure 5 shows the outcome of the design.

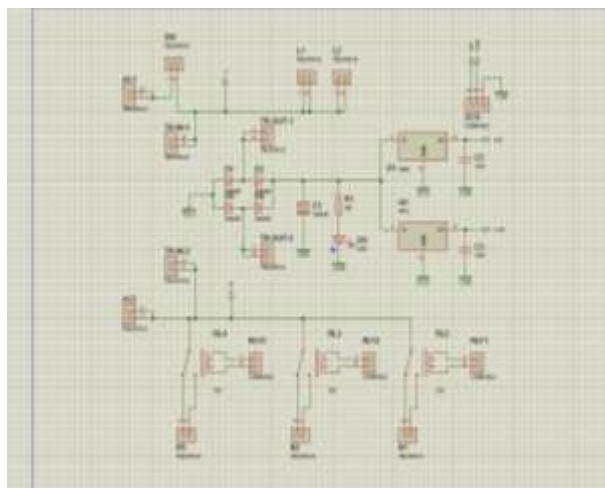


Figure 1: Schematic of a Buffer board

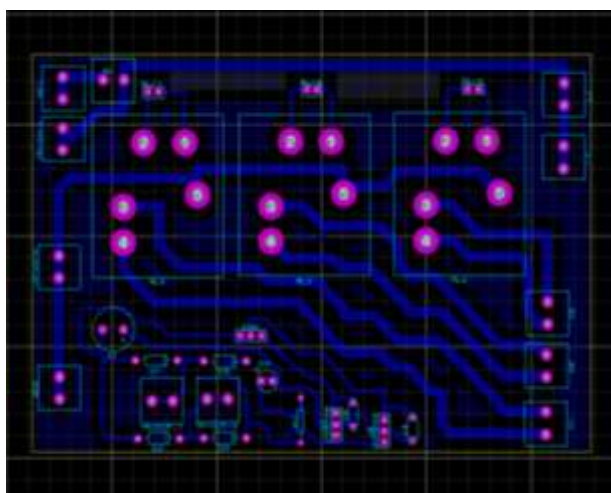


Figure 2: Layout design

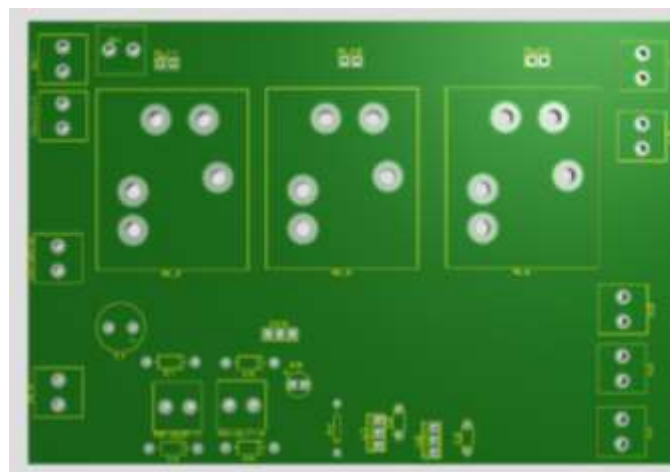


Figure 3: 3D simulation (Top view)

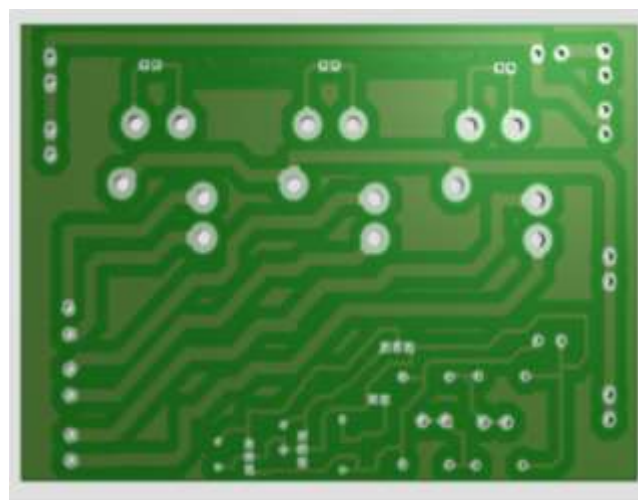


Figure 4: 3D simulation (Bottom view)

Pradal, 2008 had similar outlook of Figure 3 and Figure 4 in his description of printed circuit boards design flow. The buffer boards can be used in home and industrial automation, when there is need to switch heavy current load using a microcontroller

that uses sensor input like light sensors, proximity sensors, temperature sensor and others. It simply serves as an interface unit between an embedded system and load output.

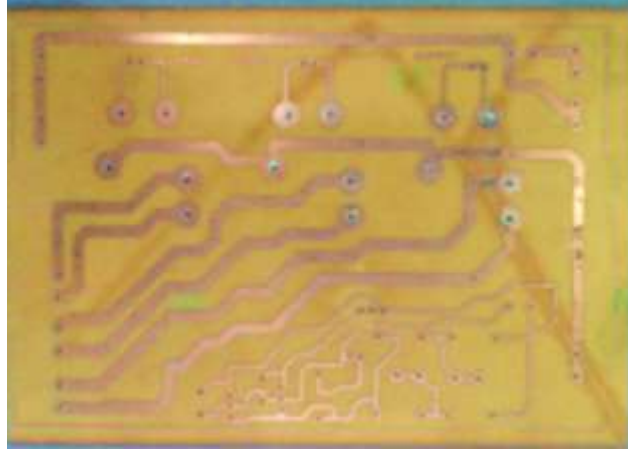


Figure 5: Final outcome of design

Figure 6-10 show the virtual development stages of a general purpose microcontroller board. a general microcontroller board was designed for a CY8C29466-24PXI cypress microcontroller IC to server multiple

function of taking several inputs from multiple sensors; and displaying the output on LCD display or using these input signals to drive relays on a buffer board (whose circuit is shown in Figure 1-5).

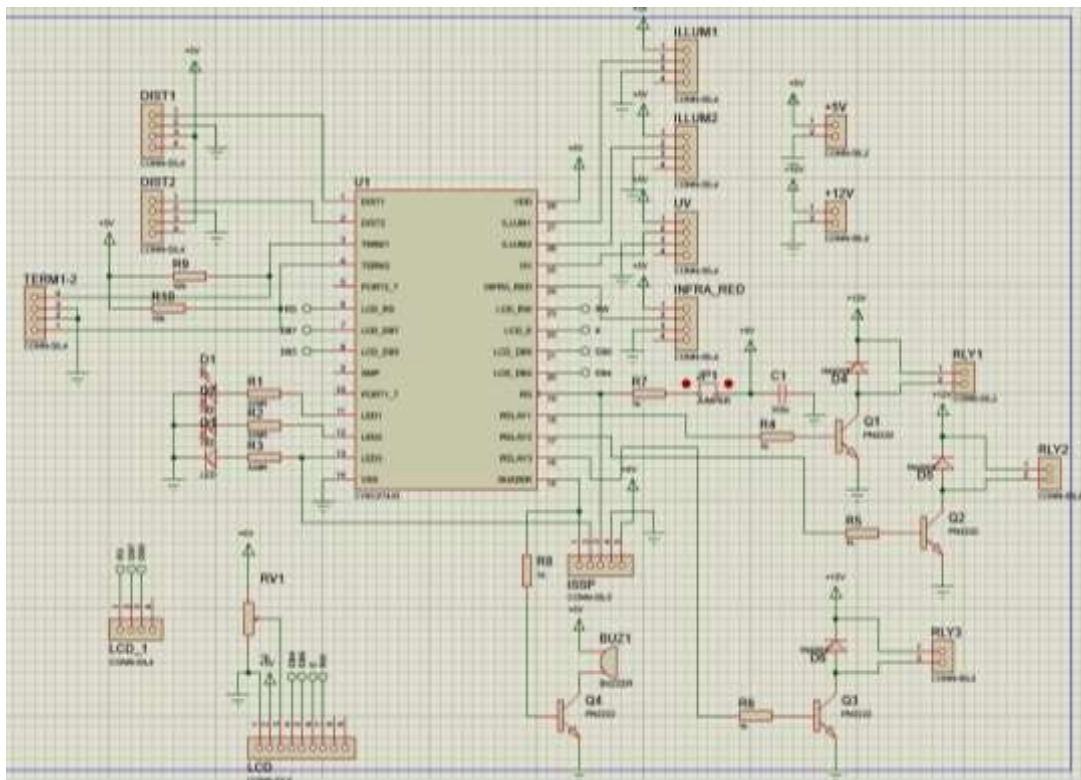


Figure 6: Layout Design

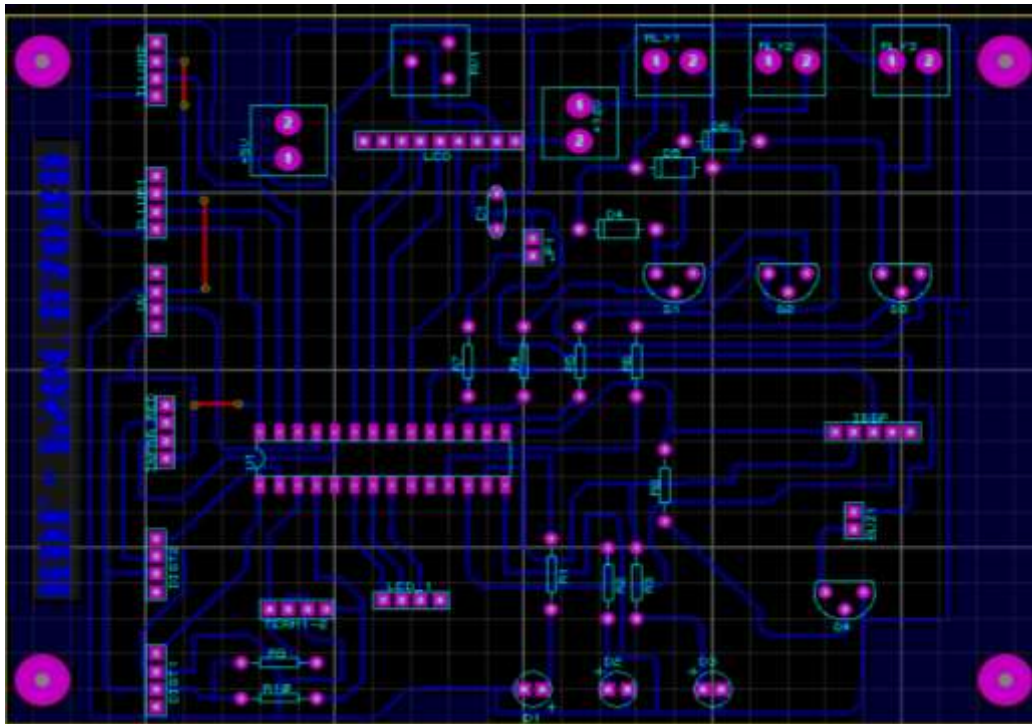


Figure 7: system schematics

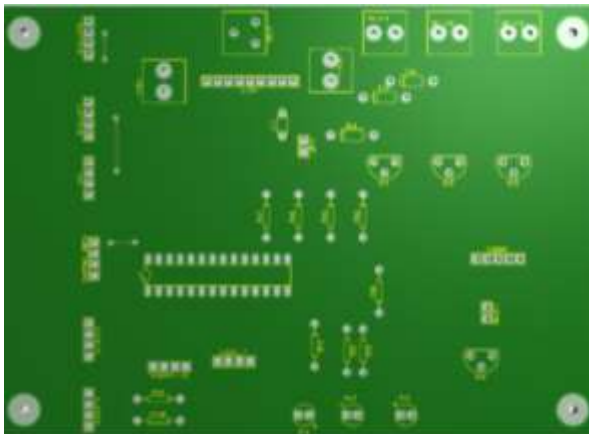


Figure 6: 3D simulation (Top view)

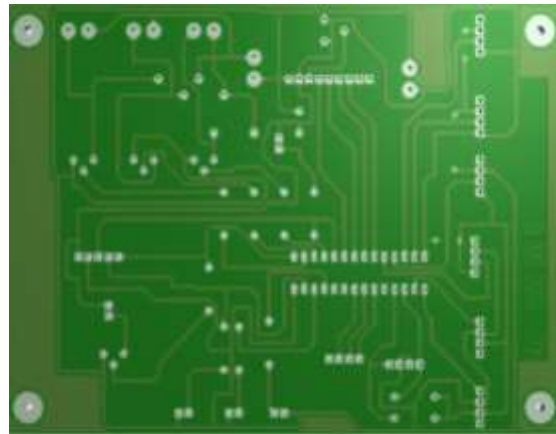


Figure 7: 3D simulation (Bottom View)

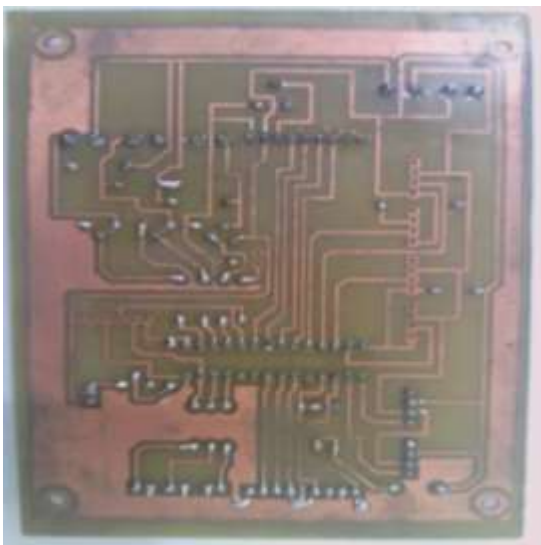


Figure 8: Copper side of final product



Figure 9: Component side of the final product

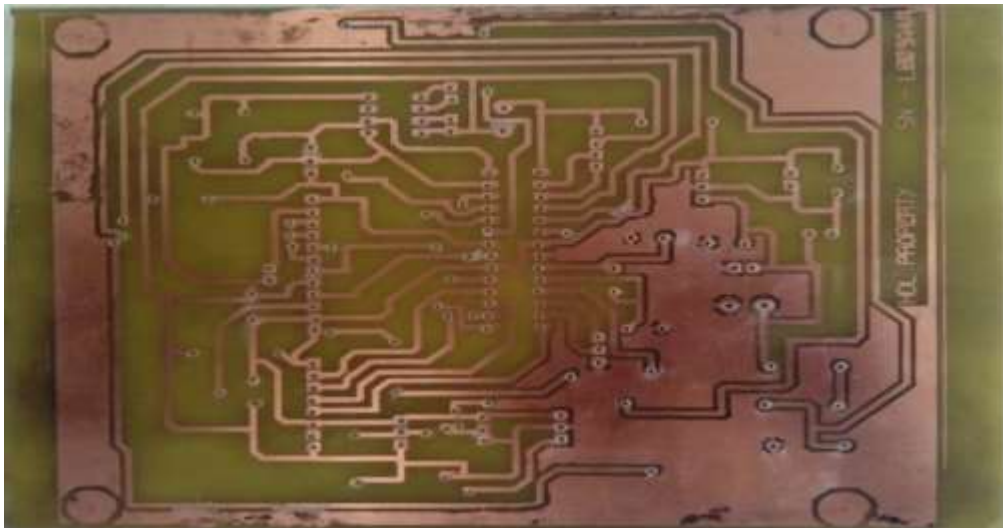


Figure 12: PCB of an online data logger circuit

Other circuit designed using this method are bistable generator, LM386 audio amplifier, Fluid gauge, touch alarm, online data logger circuit, change of period alarm circuit among others. Figure 12 is the picture of another PCB produced using this method.

DISCUSSION

In electronic industry today, countries like United State of America, Japan, Scotland, and China are dominating the consumer electronic market, this serves as economy boast for these countries as they get to sell to other countries. Nigeria on the other hand imports almost all the electronics equipment used.

The approach described in this paper can be adapted in designing PCBs at cheap production cost which is necessary for virtually all circuits found in any electronic gadget no matter how small such a gadget may seem. The following are the likely results to expect:

Encouraging upcoming scientists and engineers in their designs and packaging. Leading to making their products attractive and more durable to compete with the ones being imported and branding it to give it an outlook fit for the market within and outside Nigeria.

Likewise, if the circuit production process described above is included in the science and technical curriculum as a compulsory skill to be learnt, it will help in bridging the gap between theory of circuit design as a course taken in the institutions and hands-on experience (practicals) on circuit design and

production of circuit boards (Khandpur, 2005). This in turn will result to producing technology relevant science and technology graduates useful to the nation.

Notwithstanding, sustainable human capital is a great asset to a nation. Graduates that have developed and acquired this skill will no doubt contribute to boosting the nation's economy and promote all round development. Therefore, solving part of the problem of unemployment through job creation training programs they can provide. Finally, it is very important for scientist and technologist to acquire this skill to give circuit design a facelift hence improving our electronics.

REFERENCES

- Charles Alexander and Matthew Sadiku (2004): 'Fundamentals of Electric Circuits' 5th edition. McGraw-Hill publishers, U.S.A
- Chunfei Ye ; Xiaoning Ye ; Lopez, E. ; Maoxin Yin ; Hsu, J. ; Su, T. (2013): 'Improve signal integrity performance by using hybrid PCB stackup'. IEEE Journal: 122-125.
- Clyde Coombs and Happy Holden (2007): 'Printed Circuits Handbook' 7th edition, McGraw-Hill Education, U.S.A. download.quickheal.com (12/09/2015; 1:22pm) Internet source
- Ebnesajjad, Sina (2013): 'Release Film Application, Polyvinyl Fluoride'
- Elaine, R and Paul, S, (2005): "Developing Printed Assemblies: From specifications to mass Production" first Lulu edition.



- en.wikipedia.org (12/09/2015; 1:02pm)
Internet source epdf.tips (04/09/2015; 2:43pm) Internet source Ericsson (2015): Ericsson Mobility Report: <http://www.ericsson.com/mobility-report>
- Junfa Mao, Jianjie Li, Min Tang, Linsheng Wu (2011): 'Performance enhancement research for printed circuit board manufacture in China'. IEEE Journal, 12 –19.
- Khandpur, R.S, (2005): "Printed Circuit Board: Design, fabrication and Testing" Tata McGraw Hill Education private limited.
- Kraig Mitzner (2011): 'Complete PCB Design Using OrCad Capture and Layout' pages 443-446. Newness www.broom02revovlry.com
- Kristin Chung (2015): 'The Advantages and Disadvantages of Printed Circuit Boards' www.dredging-m3.com mospin.nic.in (07/09/2015; 2pm) Internet source
- newton.pbk-solutions.de(07/09/2015; 12:43pm) Internet source Printed Circuit Boards: http://www.worldlibrary.org/articles/printed_circuit_board
- Ryan V. (2009) <http://www.technologystudent.com/pcb/pcb2a.htm>
- Schatzel D.V. (2009). Improving heat transfer performance of printed circuit boards. IEEE Journal: 31 - 37
- Turok L. N. (1993). The Growth of an Indigenous Electronic Industry: Scottish Printed Circuit Boards. JEER Journal: 67 – 70.
- U. S. Patent (1925): <http://www.google.com/patents/US1563731>
- www.wikivisually.com (07/09/2015; 3:01am) Internet source
- www.digitek.ru (07/09/2015; 2:24am) Internet source
- www.gustaf-galke.de; (08/09/2015; 2pm) Internet source
- www.newenergytimes.com; (07/09/2015; 2:45am) Internet source
- www.totalpfs.co.uk; (08/09/2015; 2:13pm) Internet source
- www.renovision.in; (07/09/2015; 2am) Internet source
- Zhang, Yun Dian, and Jiang Peng Wang (2014): 'Processing Technology of Quartz Pendulous Reed by Chemical Etching' Applied Mechanics and Materials



PARASITIC INFECTION AMONG WASTE MANAGEMENT WORKERS IN LAGOS.

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ABSTRACT.

Waste collection is an important environmentally beneficial job but it is associated with several health hazards. It is a possible source of infection to waste workers, especially those of parasitic organisms. A parasitological study was conducted to determine the prevalence of parasites among Lagos Waste Management Authority (LAWMA) staff. A total of 100 staff consisting of 30 males and 70 females aged between 25- 55 years working as sweepers, drivers, refuse collector, and those in the maintenance department were included in the study. Their stool samples were collected and analyzed microscopically to determine parasitic infection. Well-structured questionnaire was also administered to the respondents with questions such as: washing of hands after work, eating while working. The result showed a prevalence of 11% with 3 (10%) males and 8(11%) females being positive. Four parasites were identified namely *Ascaris lumbricoides*, (72.7%), *Trichuris trichiura* (9%), *Schistosoma mansoni* (9%) and Hookworm (9%). 14% of the sampled individuals had primary school education, 23% had secondary education and 63% had no formal education while those that had tertiary education were not found infected at all. Maintenance of good personal hygiene and wearing of protective gadgets while at work should guide the operation of waste workers to reduce the level of parasitic infection.

Keywords: Waste, Parasites, Hygiene, Prevalence, Contamination.

INTRODUCTION

Parasitic infections have a global distribution and constitute substantially to public health problems in developing countries. Intestinal parasitic infections have been documented by World Health Organization (WHO) to be widespread where poverty prevails, where sanitation is deficient and where more health awareness and care are needed (Hesham *et al.*, 2004). Municipal waste comprises of all types of solid waste which are generated both by households and commercial establishments are usually collected by waste management workers, which exposes them to an array of infection, particularly parasitic infection (Eassa *et al.*, 2016). Waste are unhygienic and foul smelling thereby attracting disease transmitting vectors such as flies, mosquitoes, fleas, and rats and ruin the aesthetic appeal of the environment. Domestic refuse in Lagos alone has been estimated to be 4.5 million tonnes yearly (Ogbonna *et al.*, 2002). Several occupational levels are involved in solid waste management industry including garbage collectors, street sweepers, waste sorters, truck drivers, machine operators, supervisory and administrative staff, with varying degree of exposure and job hazards (Tooher *et al.*, 2005). Little is known about the types and magnitude of the health risks, particularly those of parasitic infections, associated with waste management workers with different exposures (Fuhrimann *et al.*, 2016).

Waste management authorities will be prompted to devise more secured management practices if information on basic epidemiological data of the health impact of waste management implementation are known (Eassa *et al.*, 2016). Thus, the aim of this study is to



determine the prevalence of parasites that can be contacted from collection and constant exposure to waste and refuse and predisposing factors among waste handlers.

MATERIALS AND METHODS

Study Area

The study was carried out among Lagos State Waste Management Authority (LAWMA) staff located in Mushin Local Government Area (LGA) of Lagos State, Nigeria. It lies in latitude 6° 33' 28" North and longitude 3° 19' 33" East. A total of 100 staff consisting of male and female aged between 25- 55 years which comprised of sweepers, drivers, refuse collector, and those in the maintenance department participated in the exercise.

Sample Collection.

Wide mouthed screwed cap sterile bottles were given to the staff for the collection of their stool samples. Structured questionnaires were distributed among the participating staff for the collection of their demographic information such as age, sex, wearing of protective clothing and personal hygiene habits. The stool samples were collected between 7:30a.m and 8:30a.m the following morning properly labelled and were transported to the laboratory in a cold box filled with ice packs.

Laboratory Examination of Stool Samples.

The specimens were stored in the refrigerator and were worked upon in sequence as collected from the respondents.

Macroscopic Examination.

The macroscopic analyses of stool samples were carried out by scrutiny of the samples for the appearance and consistency. They were subsequently classified as brownish, blackish, greenish and yellowish; or as formed, semi – formed and loose stool.

Microscopic Examination.

Parasitological *diagnosis* of the parasites was carried out by analysing stool samples for presence of eggs or cysts using the concentration technique according to the method used by Okoronkwo (2004) and Fuhrmann *et al.*, (2016). The slide was then mounted and examined for parasite eggs under x10 and x40 objective of the microscope.

Ethical Considerations.

Consent to conduct the study was gotten from the manager in charge of the company located in Mushin. Consent was also obtained from the waste handlers after the purpose of the study was explained to them and assurance of confidentiality. Voluntary participants were asked to give a verbal informed consent before starting the interview and sample collection.

Statistical Analysis.

The analysis of data collected was done using Analysis of Variance (ANOVA) and categorized based on gender, age group, job specification and use of anthelmintic drugs by respondents. Results were subjected to statistical analysis with significance level at $P < 0.05$. SPSS version 20 was the statistical software employed.

RESULT.

The parasitological findings conducted on the waste management workers revealed a prevalence of 11%. The parasites isolated in the stool sample include *Ascaris lumbricoides*, *Trichuris trichiura*, *Schistosoma mansoni* and Hookworm, out of which *Ascaris* had the highest prevalence rate of 72.7% while the others had equal prevalence rate of 9% each.

10%(3) of a total number of 30 males examined were infected and 11.4% (8) of the 70 females examined were infected (Table 1). There was no statistically significant difference between the infected male and female workers since the p value (0.764) is greater than 0.05.



Table 1: Parasitic Infection based on sex

Sex	No Examined	No Infected (%)	<i>Ascaris lumbricoides</i>	<i>Trichuris trichiura</i>	Hookworm	<i>Schistosoma mansoni</i>
Male	30	3(10)	1	1	1	0
Female	70	8(11.4)	7	0	0	1
Total	100	11(11)	8(72.7)	1(9)	1(9)	1(9)

The infection according to age is shown in Table 2. The age group 41-45 had the highest infection rate with a prevalence of 14.3%, *Ascaris lumbricoides* had the highest occurrence in this age group (50%). Individuals

in age group 20-25 had no infection. The difference among the age groups and parasitic infections was found not to be statistically significant (p value (0.981) > 0.05).

Table 2: Parasitic Infection based on Age

Age	No Examined	No Infected (%)	<i>Ascaris lumbricoides</i> (%)	<i>Trichuris trichiura</i> (%)	Hookworm	<i>Schistosoma mansoni</i>
20-25	6	0(0)	0(0)	0(0)	0	0
26-30	15	1(6.7)	1(6.7)	0(0)	0	0
31-35	16	1(6.3)	0(0)	1(6.3)	0	0
36-40	22	3(13.6)	3(13.6)	0(0)	0	0
41-45	35	5(14.3)	4(11.4)	0(0)	0	1
46 and above	6	1(16.7)	0(0)	0(0)	1	0
Total	100	11(11)	8(8)	1	1	1

Comparison of infection rate with the use of anthelmintic drugs by respondent is shown in Table 3. Those that used anthelmintic drugs more than 6 months before sample collection

had the highest prevalence of infection 15.4 %. Also, there was no statistical significance found between parasitic infection and the use of Anthelmintics since the p value (0.179) is greater than 0.05.

Table 3: Comparison of infection rate with the use of anthelmintic drugs by respondents

Use Of Antelmintic Drugs	No Examined	No Infected (%)	<i>Ascaris lumbricoides</i>	<i>Trichuris trichiura</i>	<i>Schistosoma mansoni</i>	Hookworm
0-3months	39	3(7.7)	1	1	1	0
4-6months	9	0(0)	0	0	0	0
More than 6months	52	8(15.4)	7	0	0	1
Total	100	11(11)	8	1	1	1

Infectivity according to job specification is shown in figure 1 with sweepers having the highest prevalence rate of 12.3% followed by

refuse collectors (9.5%) while the drivers were found to have no infections.

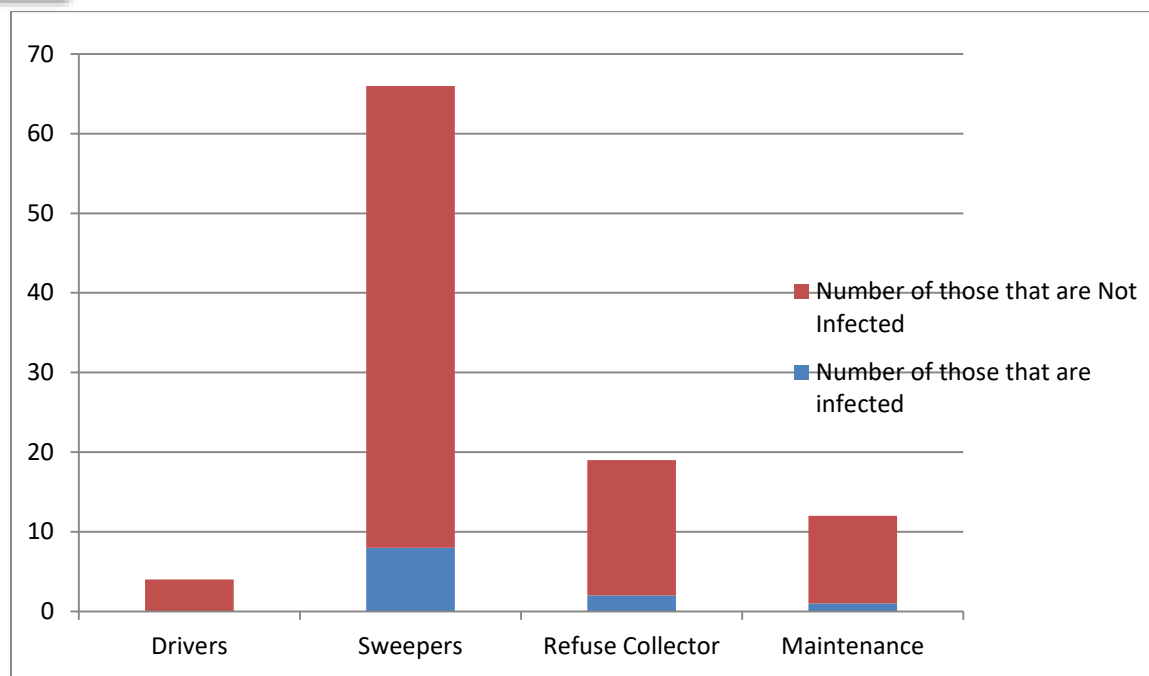


Figure 1: Bar chart showing parasitic infection based on job specification

Based on the educational level of sampled individuals, 14% of those that went to only primary school were infected, 23% of those that had up to secondary education were

infected, and 63% of those that had no formal education were infected, while those that had tertiary education were not infected at all.

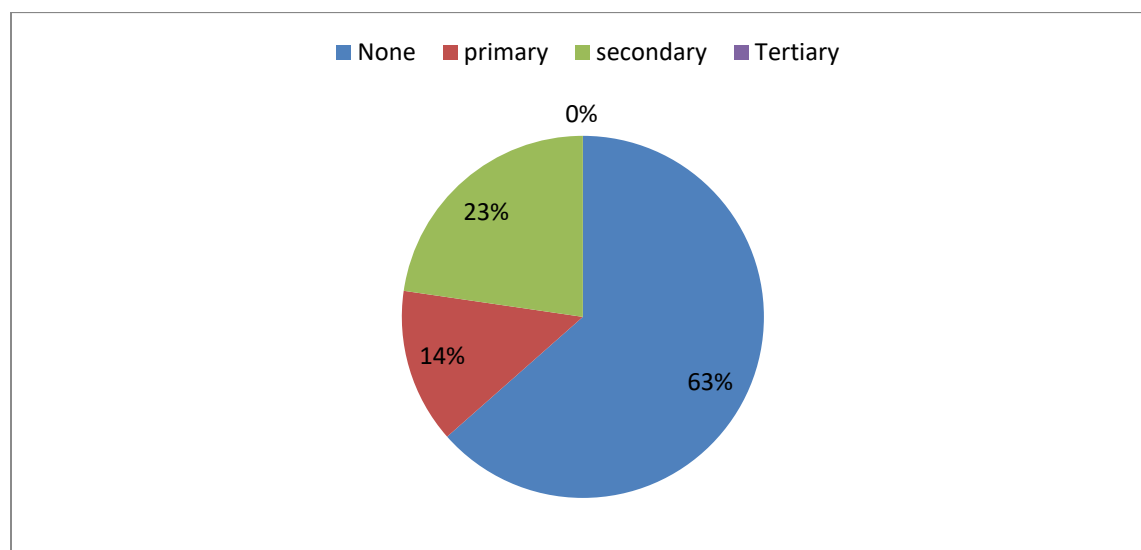


Figure 2: Pie chart showing parasitic infection based on level of education



DISCUSSION

The overall prevalence of intestinal parasites in this study was found to be 11% with *Ascaris lumbricoides* being the most frequently encountered parasite. Many other researchers such as Eassa *et al.* (2016), Iboh *et al.* (2015), Ewis *et al.* (2013), Adeyeba and Akinbo (2002), Onyido *et al.* (2009), have all shown that refuse dumps are significant source of transmission of important parasites. The infection rate in this study is low and contradicts the findings of Okoronko (2004) when he carried out a similar study on waste management workers in Jos, Plateau state and he discovered a higher percentage of parasites distributed among the surveyed workers. The low prevalence observed in this study appears to be due to the hygiene practises such as washing of hands and taking of bath after work observed among the workers during the period of sample collection and from the responses given in the questionnaires. On the contrary, Ewis *et al.* (2013) found higher rates of parasitic infestations (21.7%), which included parasites such as *Entamoeba histolytica*, *Giardia lamblia* and *H. nana*, among the sweepers and waste collectors examined. They put it down to lack of knowledge and poor hygienic practises of the sweepers who could have neglected safety preventive measurements provided for them such as wearing protective gloves, face masks and washing hands after work and before eating. Four parasites were isolated in this study; these include *Trichuris trichiura*, *Ascaris lumbricoides*, *Schistosoma mansoni* and hookworm. This result agrees with the deductions of Hunt (2001) that the levels of helminth infection in solid waste is high. Ascariasis has been reported by several workers, and Adeyeba and Akinbo (2002) reported that *Ascaris* was the most frequently encountered parasite in their findings which is in line with the findings of this present study. *A. lumbricoides* eggs are

very resistant to harsh environmental conditions. This may account for the ubiquitous nature of egg distributions and hence high prevalence.

The prevalence of intestinal parasitic infection by the sex of people sampled revealed that 3(10%) males of 30 sampled were infected and out of 70 females sampled, 8(11.4%) were infected as seen in **Table 1**. This showed that the prevalence of intestinal parasitic infection was higher in females than their male counterpart. This could be as a result of women being more involved in this sector. There was however no statistically significant difference found between the infected male and female workers.

The study showed that people in the age range of 41-45 had the highest infection rate (Table 2). This could be as a result of decrease in the level of their immune system but there was no statistically significant difference between prevalence of infection and age.

The nature of the occupation of individuals examined was found to influence the infection rate. Sweepers recorded higher positive rate (12.3%) (Figure 1) because they are always on the street and some of these parasites are air borne and could also be transmitted through oral faecal route. This result is in contrast with the study of Okoronkwo (2004) who reported that drivers had the highest rate of infection 81.1%, then refuse vehicle loaders 69.3%, sweepers 63.5% and technical staff 53.3%.

Sampled individuals with tertiary education were not found infected from the results of this study while some of those that had up to primary or secondary education were found infected. The infection rates among this group could be due to the educational level of the individuals involved. Many of the individuals working as waste collectors have low levels of education and low socio-economic status, and as a way of surviving become workers for waste management



authorities. Moreso, Abd El-Wahab *et al.* (2014) reported in their findings that about 75 of the workers do not wear the recommended personal protective clothing. However, in this study there is no significant association between occupation and parasitic infection. From the questionnaire, parasitic infection was also found to be higher among those that had taken anthelmintic drugs more than six months before the study was conducted. There was significant difference between the use of anthelmintic drugs and parasitic infection. This implied that the use anthelmintic drug reduces the cases of parasitic infections.

In conclusion, it can be deduced that poverty, lack of education, low level of environmental sanitation, and ignorance of simple health precautions makes this workers to become

infected not only with parasitic infections but a wider variety of pathogenic organisms. Transmission of potentially pathogenic organisms can be minimized if better waste management can be implemented by making waste technologies more contained, modifying working methods, regular education of workers on the use of protective clothing and other personal protective devices, and constant enlightenment of the general public on the need for regular and proper disposal of wastes.

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REFERENCES

- Abd El-Wahab E. W., Eassa S. M., Lotfi S. E., El Masry S. A., Shatat H. Z., Kotkat A. M. (2014). Adverse Health Problems Among Municipality Workers in Alexandria (Egypt). *International Journal of Preventive Medicine*, 5(5): 545–556.
- Adeyeba O.A., Akinbo, J.A.(2002) Pathogenic intestinal parasites and bacterial agents in solid wastes. *East African Medical Journal*, 79: 604–10.
- Eassa S. M., El-Wahab E. W. A., Lotfi S. E., El Masry S. A., Shatat H. Z., Kotkat A. M. (2016). Risk Factors Associated with Parasitic Infection Among Municipality Solid-Waste Workers in an Egyptian Community. *The Journal of Parasitology*, 102(2): 214-221.
- Ewis A.A., Rahma M.A., Mohamed E.S., Hifnawy T.M., Arafa A.E. (2013). Occupational Health-Related Morbidities Among Street Sweepers And Waste Collectors At Beni-Suef, Egypt. *Egyptian Journal of Occupational Medicine*, 37 (1): 79-94
- Fuhrmann S., Winkler M. S., Kabatereine N. B., Tukahebwa E. M., Halage A. A., Rutebemberwa E., Medlicott K., Schinder C., Utzinger J., Cissé G. (2016). Risk of intestinal parasitic infections in people with different exposures to wastewater and fecal sludge in Kampala, Uganda: a cross-sectional study. *PLoS Negl Trop Dis*, 10(3).
- Hesham M. S., Edariah A. B., Norhayati, M. (2004). Intestinal parasitic infections and micronutrient deficiency: a review. *The Medical Journal of Malaysia*, 59(2), 284-293.
- Hunt C. (1996). Child waste pickers in India: the occupation and its health risks. *Environment and Urbanization*, 8(2): 111-118.
- Hunt C. (2001). A review of the health hazards associated with the occupation of waste picking for children. *International Journal of Adolescent Medicine and Health*, 13(3): 177-190.



- Iboh C. I., Effiom O. E., Ekor S. C., Inyang A. (2015). Parasitological Evaluation of Un-Disposed Refuse Dumps in Calabar South, Cross River State, Nigeria. *International Journal of Pure and Applied Zoology*, 3(3): 232-239.
- Ogbonna D. N., Ekweozor I. K. E., Igwe F. U. (2002). Waste management: A tool for environmental protection in Nigeria. *AMBIO: A Journal of the Human Environment*, 31(1): 55-57.
- Okoronkwo M. O. (2004). Intestinal parasites among waste-handlers in Jos metropolitan area of plateau state, Nigeria. *Sahel Medical Journal*, 7(1): 13.
- Okoronkwo M. O., Onwuliri C. O. E. (1998). Intestinal parasites from refuse dumps and abattoir wastes in plateau State. *Nigeria Journal of Medical Laboratory Science*, 7: 25-33.
- Onyido A.E., Okolo P.O., Obiukwu M.O., Amadi E.S. (2009). A survey of vectors of public health diseases in un-disposed refuse dumps in Awka Town, Anambra State, Southeastern Nigeria. *Res. Journal Parasitology*, 4: 22-27
- Toohar R., Griffin T., Shute E. (2005). Vaccinations for waste-handling workers. A review of the literature. *Waste Management Resources*, 23, 79-86.



POLYCYCLIC AROMATIC HYDROCARBON IN SOME SPECIES OF DIFFERENTLY PROCESSED SMOKED FISH

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are generated during incomplete combustion of certain materials such as coal, oil, wood, fossil fuels, garbage, tobacco and charbroiled meat; especially under high temperature pyrolysis. They are known to have genotoxic, carcinogenic, mutagenic and cytotoxic properties. Different smoking methods have been used over time to preserve venison and varieties of fish, among other food items. The level of PAHs in fishes smoked using charcoal, firewood and sawdust and laboratory oven drying were studied. Samples of three fish species namely Atlantic mackerel, Atlantic herring and Atlantic hake purchased from a cold room depot in Lagos were smoked and PAH formed were extracted using 3:1 v/v mixture of n-hexane and dichloromethane in a soxhlet extractor. Clean up of the extract was carried out with activated silica gel and sodium sulphate powder in a column. The extracts were then analysed for the 16 named US_EPA PAH pollutants using gas chromatograph with flame ionization detector (GC-FID). The results obtained showed that fish samples processed by charcoal smoking gave the highest PAHs content with a range of 17.42 ppb to 162.54 ppb; while sawdust and firewood processed samples gave range of 1.22 ppb to 104.17 ppb and 2.84 ppb to 105.63 ppb respectively.

The level of PAHs in Atlantic mackerel and Atlantic hake gave benzo(a)pyrene contents of 18.77 ppb and 17.42 ppb respectively which all exceeds the 10 ppb threshold limit of carcinogenicity set by European Commission (EC). Thus, smoking as a means of preservation through any of these method and regular consumption of these fish species may increase the risk of cancer in human health.

KEYWORDS: Smoking, fish, polycyclic aromatic hydrocarbon (PAH), pyrolysis, carcinogenic.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are made up of two or more fused aromatic rings that may be formed naturally (volcanoes, forest fire) or from anthropogenic sources (wood burning, incinerators, incomplete combustion or high temperature pyrolysis of coal, oil, wood, fossil fuels, garbage or other substances such as tobacco and charbroiled meat) (Mottier, Parisod, & Turesky, 2000)

Although several PAHs exist naturally, only 16 have been classified as “priority pollutants” based on their source, toxicity and carcinogenicity (USEPA, 1993; Alexander, et al., 2008). Smoking is a major source of PAHs domestic contamination and it has been one of the commonly used methods of



fish preservation by the Nigerian populace. The occurrence of PAHs in food over time has drawn so much interest not only because of their carcinogenic, mutagenic and cytotoxic properties but also due to the likelihood of affecting human health (Zelinkova & Wenzl, 2015; Josephson, 1984; Haugen, et al., 1986 and Davis, Fellin, & Otson, 1987). Since smoked fish is a common food item and ingredient in Nigeria cuisine, regular monitoring is required to be able to establish when level of PAHs exceed regulatory threshold and hence adequately advise consumers on the potential health hazards. This study was conducted using three traditional smoking methods (sawdust smoking, charcoal smoking, and firewood smoking) and laboratory oven drying method for three fish species purchased from a cold room depot located at Makoko area of Yaba, Lagos State, Nigeria. The objectives of the study are to determine the level of PAHs in smoked fish and comparatively assess the traditional methods used in smoking so as to determine which method(s) produces the least PAHs.

MATERIALS AND METHODS

Sampling

The fish samples were purchased at a cold room depot at Makoko area of Yaba Local Government area/Mainland of Lagos State Nigeria. Three species of fish namely; Atlantic herring (sawa), Atlantic mackerel (titus) and Atlantic hake (panla) were chosen because of their perceived high demand. For each fish species, twelve pieces were collected randomly from the same fish pack.

Sample pre-treatment

The fishes were washed thoroughly under tap water to remove all internal and external dirt and then rinsed with distilled water. Three

samples of each fish species were smoked for 5 hours on three different traditional ovens using firewood, saw dust and charcoal. Thereafter the smoked fishes were put separately inside air tight ice bags and transported to the laboratory in a cooler packed with ice. The samples for laboratory oven drying were dried for 48 hours at 105°C in the laboratory. All parts of the samples (smoked and oven dried) were grounded using kitchen blender, packaged and labelled in air tight ice bag and kept in the freezer prior to extraction and clean-up process.

10g of pulverized sample was extracted for 6 hours using 3:1 v/v hexane: dichloromethane mixture in a soxhlet extractor. The resulting extract was concentrated to 5 mL using a rotary evaporator. This procedure was carried out in duplicate for each sample and was repeated for all the samples (Olabemiwo, Alade, Tella, & Adediran, 2011)

For the clean-up, a chromatographic column packed with activated silica and 1cm top layer of sodium sulphate was used.

1mL of the extract was immediately added to the prepared column and eluted incrementally with dichloromethane solution. 10mL of the eluent was accurately measured into a labelled sample bottle. The resulting extracts were then concentrated to 2 mL on a rotary evaporator and the residues dissolved in 2 mL of dichloromethane and kept in air tight glass vial with rubber cap, labelled according to the smoking method used and refrigerated prior to gas chromatographic analysis (Nkpaa, Wegwu, & Essien, 2013). This procedure was repeated for other samples

Instrumental Analysis

The procedure was adopted from that used by Olabemiwo (2013).



The concentrated samples were analyzed using a gas chromatograph with a flame ionization detector. The gas chromatograph was calibrated with five serially diluted standard solutions (20 – 100 µg/mL) from a 1000 µg/mL stock solution of all 16 hazardous USEPA PAHs.

The peaks obtained were identified by comparing their retention times with the retention times for standard PAHs under the same conditions for analysis of samples (Olabemiwo, 2013).

Results and Discussion

The results obtained for the analysis of the PAH contents are as presented in Table 4.1. It shows that the most abundant PAHs was naphthalene with mean concentration of 94.53 ppb while Indeno (123_cd) pyrene (3.40 ppb) was the least abundant.

Distribution of PAHs content in fish samples according to smoking methods:

1. Saw dust smoked samples

For fish samples processed with saw dust smoking method, 7 PAHs were detected in Atlantic herring- sawa (SS) namely; naphthalene, acenaphthalene, benzo(v)fluoranthene, benzo(a)pyrene, dibenzo(a)anthracene, benzo(ghi)perylene and indeno (123_cd) pyrene, while for Atlantic mackerel -titus (ST) and Atlantic hake - panla (SP) 6 PAHs were detected as named for Atlantic herring except Indeno(123_cd)pyrene that was not detected. This shows that saw dust smoking is a source of PAHs due to the high level of smoke produced by it; this is in agreement with the findings from Risk Assessment Studies (RAS), (2004) and Ubwa *et al.* (2015).

2. Fire wood smoked samples

Six (6) PAHs were detected in Atlantic herring (FS) namely; naphthalene, acenaphthalene, benzo(v)fluoranthene, benzo(a)pyrene, dibenzo(a)anthracene, benzo(ghi)perylene but no PAHs were detected in Atlantic mackerel (FT) and Atlantic hake (FP). This might be due to the soft wood used to smoke Atlantic herring while hard wood was used to smoke Atlantic mackerel and Atlantic hake. Smoke produced from hard wood is better due to its less chemical components, it also provides moderate temperature and less smoke (Akpan, Lodovici, & Dolara, 1994). Silva *et al.* (2011) inferred similarly that level of smoke produced increases with temperature of smoking.

3. Charcoal smoked samples

For these samples 16 PAHs were detected in Atlantic herring (CS) and Atlantic mackerel (CT) while none was detected in Atlantic hake (CP). It has been established that during charcoal grilling, fat drippings on the hot charcoal is able to generate more PAHs on the food which increases with increase in the fat content, temperature and grilling time (Lijinsky & Shubik, 1964). The values for CS ranged from 17.42 ppb for benzo(a)pyrene to 162.54 ppb for naphthalene. Ubwa *et al.* (2015) and Silva *et al.* (2011) reported in their study that charcoal gave lower level of PAHs. This may be due to the different samples used (cat-fish and sole) and different sources of charcoal because charcoals have their different source such as: lump charcoal which is made directly from hardwood, pillow shaped briquettes are made from saw dust and other wood by-product, with a binder such as starch and extruded charcoal made from raw ground wood or carbonized wood (Ogunsanwo, Aiyelaja, & Uzo, 2007).

4. Oven dried samples

For these samples 16 PAHs were detected in Atlantic mackerel (OT) while 15 PAHs were detected in Atlantic herring (OS) and Atlantic hake (OP). Oven drying method recorded the highest number of PAHs. This might be due to fact that the time taken (48 hours) to dry the samples could have contributed to the level of PAHs. The result did not agree with Olabemiwo *et al.* (2011) who reported that oven drying method recorded the least PAHs in their analysis after drying the fish samples for longer time (144 hours).

Concentration of individual PAHs in different fish species:

1. Atlantic mackerel (titus)

Figure 4.1 shows that the concentrations of individual PAHs in titus occurred in the oven dry method (OT) with 19.64 ppb benzo(a)pyrene. Amos-Tautua *et al.* (2013) reported a concentration of 2.41 µg/g for benzo(a)pyrene in roasted Atlantic mackerel. The values for OT and CT show that some PAHs were high in charcoal method, while some were high in oven dry method but indeno(123_{cd})pyrene was detected in charcoal and not in oven dry method. However, sawdust smoking method produced lower PAHs contents while firewood smoking method recorded no PAHs.

2. Atlantic herring (sawa)

The results of PAHs in Atlantic herring in Figure 4.2 shows that charcoal smoked samples gave the highest values for naphthalene, benzo(v)fluoranthene chrysene, benzo(ghi)perylene, fluorene, phenanthrene, while sawdust, firewood and oven drying methods recorded lower values. This may be because charcoal produced more smoke and hence more PAHs than the other smoking sources (Bouloubassi, Fillaux, & Saliot, 2001).

3. Atlantic hake (panla)

The distribution of PAHs in Atlantic hake is presented in Figure 4.3. It shows that oven drying method recorded the highest individual PAHs and also recorded the highest values for naphthalene, fluorine and chrysene; while the least of individual PAHs were recorded in sawdust smoking method. However, no PAHs

was recorded with firewood and charcoal smoking methods.

Concentrations of total PAHs in the smoked fishes:

The concentrations of total PAHs in the smoked fishes is presented in Figure 4.4. The figure shows that the charcoal smoked samples have the highest values of 902.87 ppb for Atlantic herring (sawa) and 906.26 ppb for Atlantic mackerel (titus), followed by the samples smoked by oven drying method with total PAHs content of 181.874 ppb in Atlantic herring (sawa) and 903.89 ppb in Atlantic hake (panla). The samples smoked using firewood recorded the fewest PAHs content with a total concentration of 162.432 ppb in Atlantic herring (sawa). Samples smoked using sawdust recorded the least total of PAHs content ranging from 147.779 ppb in Atlantic hake (panla) to 162.068 ppb in Atlantic herring (sawa). Ubwa *et al.* (2015) studied the effect of conventional smoking methods on levels of polynuclear hydrocarbons in selected smoked fish species. Their results revealed that saw dust smoking method had the highest concentration of total PAHs followed by smoking method and least for charcoal smoking method. The observed differences between the result of this present study and that of Ubwa *et al.* (2015) might be due to the type of sawdust, charcoal and firewood used for the smoking process and the different types of fish species used in this study.

The benzo(a)pyrene levels of 19.64 ppb obtained for oven dried sample of Atlantic mackerel (titus), followed by 17.42 ppb and 18.77 ppb in charcoal smoked samples of Atlantic mackerel (titus) and Atlantic herring (sawa) respectively exceeded the recommended maximum limit of 10 ppb threshold of carcinogenicity set by European Commission (EC) (FSAI, 2006). Generally the values in Table 4.1 shows that other samples were at the threshold limit of carcinogenicity.

CONCLUSION

The level of PAHs in the fish samples analysed in this study was determined by the three traditional methods of smoking as well as the oven drying method. The results show that oven drying method is not a totally safe method of preservation due to the intense heat

produced by it. It caused increased level of PAHs in fish species processed by it, thereby putting human health at high risk. However, firewood smoking produced the lowest level of PAHs. This implies that the use of hardwood instead of softwood is safe for smoking because of the less chemical component produced by the charcoal smoking. It is important to note the source of the charcoal before it is used to smoke fish; this will greatly reduce the level of PAHs in smoked food items.

REFERENCES

- Akpan, V., Lodovici, M., & Dolara, P. (1994). Polycyclic aromatic hydrocarbons in fresh and smoked fish samples from the three Nigerian cities. , 53: 246-253. *Bulletin of Environmental Contamination and Toxicology*, 53, 246-253.
- Alexander, J., Benford, D., Cockburn, A., Cravedi, J.-P., Dogliotti, E., Di Domenico, A., . . . Verger, P. (2008). Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on Polycyclic Aromatic Hydrocarbons in Food. *The European Food Safety Authority Journal*, 724, 1-114.
- Amos-Tautua, B. M., Inengite, A. K., Abasi, C. Y., & Amirize, G. C. (2013). Evaluation of polycyclic aromatic hydrocarbons and some heavy metals in roasted food snacks in Amassoma, Niger Delta, Nigeria. *Africa Journal of Environmental Science and Technology Africa Journal of Environmental Science and Technology*, 7(10), 961-966.
- Bouloubassi, I. J., Fillaux, J., & Saliot, A. (2001). Polycyclic hydrocarbon in Surface Sediments from Changjian (Yangtze river) Estuary. East China Sea. *Marine Pollution Bulletin*, 42, 1335-13462.
- Davis, C. S., Fellin, P., & Otson, R. (1987). A Review of Sampling Method for Polycyclic Aromatic Hydrocarbon in Air. *JAPCA*, 37, 1397-1408.
- FSAI. (2006). *Investigation into Levels of Polycyclic Aromatic Hydrocarbons (PAHs) in Food on the Irish Market*. Dublin: Food Safety Authority of Ireland. Retrieved from https://www.fsai.ie/uploadedFiles/PAH_levels.pdf
- Haugen, A., Becher, G., Benestad, C., Vahakangas, K., Trivers, G. E., Newman, M. J., & Harris, C. C. (1986). Determination of Polycyclic Aromatic Hydrocarbons in the Urine, Benzo(a)pyrene Diol Epoxide-DNA Adducts in Lymphocyte DNA, and Antibodies to the Adducts in Sera from Coke Oven Workers Exposed to Measured Amounts of Polycyclic Aromatic Hydrocarbons in the. *Cancer Research*, 46(8), 4178-4183.
- Josephson, J. (1984). Polynuclear Aromatic Hydrocarbons. *Environmental Science and Technology*, 18, 93-95.
- Lijinsky, W., & Shubik, P. (1964). Benzo(A)Pyrene and other Polynuclear Hydrocarbons in Charcoal-Broiled Meat. *Science*, 145(3627), 53-55.
- Mottier, P., Parisod, V., & Turesky, R. J. (2000). Quantitative Determination of Polycyclic Aromatic Hydrocarbons in Barbecued Meat Sausages by Gas Chromatography Coupled to Mass Spectrometry. *Journal of Agricultural Food Chemistry*, 48, 1160-1166.
- Nkpaa, K. W., Wegwu, M. O., & Essien, E. B. (2013). Assessment of Polycyclic Aromatic Hydrocarbons Level in Two Commercially Important Fish Species from Crude Oil Polluted Waters of Ogoni Land and their Carcinogenic Health Risks. *Journal of Environment and Earth Science*, 3(8), 128-137.
- Ogunsanwo, O. Y., Aiyeloja, A. A., & Uzo, C. (2007). Production Technique and the Influence of Wood Species on the Properties of Charcoal in Nigeria, A Case Study of Oyo State. . *Agricultural Journal*, 2(1), 131-133.



- Olabemiwo, O. M. (2013). Levels of Polycyclic Aromatic Hydrocarbons in Grilled/Roasted Maize and Plantain Sold in Ogbomoso, Nigeria. *International Journal of Basic & Applied Science*, 13(3), 87-93.
- Olabemiwo, O. M., Alade, A. O., Tella, A. C., & Adediran, G. O. (2011). Assessment of Polycyclic Aromatic Hydrocarbons Content in Smoked *C. gariepinus* and *T. guineensis* Fish Species Available in Western Nigeria. *International Journal of Basic & Applied Science*, 11(2), 135-150.
- Risk Assessment Studies (RAS). (2004). *Polycyclic Aromatic Hydrocarbons in Barbecued Meat*. Food and Environmental Hygiene Department. Hong Kong: The Government of the Hong Kong Special Administrative Region.
- Silva, B. O., Adetunde, O. T., Oluseyi, T. O., Olayinka, K. O., & Alo, B. I. (2011). Effects of the methods of smoking on the levels of polycyclic aromatic hydrocarbons (PAHs) in some locally consumed fishes in Nigeria. *African Journal of Food Science*, 5(7), 384-391.
- Ubwa, S. T., Abah, J., Tarzaa, L., Tyohemba, R. L., & Ahile, U. J. (2015). Effects of Traditional Smoking Methods on the Concentrations of Polynuclear Aromatic Hydrocarbons (PAHs) in Some Species of Smoked Fish Traded in Benue State, Nigeria. *Journal of Food Research*, 4(2), 119-127.
- USEPA. (1993). *Summary of the Emissions Characterization and Non-cancer Respiratory effects of Wood Smoke*. (T. V. Larson, & J. Q. Koenig, Eds.) Seattle, WA: EPA 453-R-93-036.
- Zelinkova, Z., & Wenzl, T. (2015). The Occurrence of 16 EPA PAHs in Food – A Review. *Polycyclic Aromatic Compounds Taylor & Frances open select.*, 15(2-4), 248-284.



PERINATAL VITAMIN B12 SUPPLEMENTATION IMPROVES GLUCOSE TOLERANCE, INSULIN RESPONSE AND EXPRESSION OF ADIPONECTIN LEVEL IN OFFSPRING OF RATS

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ABSTRACT

Perinatal nutrition, besides providing nutrients required for an effective foetal growth and development is now known to participate in the programming of metabolic disorders such as glucose intolerance, dyslipidemia and obesity. The present study investigated whether dietary enrichment with Vitamin B12 could improve glucose metabolism in the offspring of rats. Female Sprague-Dawley rats (n=18) were fed *ad libitum* for 4 weeks, a control diet (n=6) or the same supplemented with vitamin B12 (25 mg/kg, n=12) before mating with males. Four dams from the VitB12 diet fed and their offspring were shifted at birth to control diet. At postnatal day 22, six offspring of VitB12 dams were weaned to control diet while offspring of the remaining VitB12 dams continued on VitB12 diet. Glucose tolerance, insulin resistance, insulin level and expression of adiponectin were assessed in the male offspring at week 10. Food intake and body weight were comparable among control and VitB12 diet fed female dams. At birth, VitB12 diet fed offspring weighed significantly higher than controls ($p<0.05$). Interestingly lactational VitB12 supplementation produced a higher body weight gain on day 70; however post-weaning supplementation yielded a lower weight gain compared with control. Despite the significant ($p<0.05$) difference in insulin tolerance, VitB12 offspring had comparable fasting plasma glucose and glucose tolerance with control. With the exception of TG that was significantly ($p<0.05$) lower, there was no difference between the groups for serum CHOL, HDL and LDL. Serum insulin and adiponectin of the VitB12 offspring also remained with control values.

These results suggest that VitB12 supplementation may influence long term glucose homeostasis without affecting food intake. Although the data here are insufficient to conclude that VitB12 supplementation is beneficial or deleterious to the offspring metabolism, the observed improvement in insulin sensitivity is very fascinating and warrant further mechanistic investigation.

KEYWORDS: Cholesterol, maternal, offspring, glucose, adiponectin

INTRODUCTION

An adverse gestational environment is associated with chronic metabolic disorders, in particular obesity, insulin resistance and type 2 diabetes mellitus (Barker, 2004). Results from human and animal studies have given rise to the concept of developmental programming, which proposes that challenges during an organism's intrauterine development evoke a persistent physiological response in adult life. Although the effect of maternal micronutrients on adult chronic diseases in the offspring has been the topic of many studies, the effect of micronutrient (especially trace element) is poorly understood. It has been reported that 2 billion people worldwide suffer from at least 1 form deficiency of micronutrients. Early life nutritional exposures, combined with changes in lifestyle in adult life, can result in increased risk of chronic diseases (Petry et al., 2000). The notion that the intrauterine environment may influence the development of the foetus is not novel. However, the concept that foetal development impacts on adult diseases has arisen relatively recently and led to a revival



of interest in the influence of the in-utero environment on the foetus and neonate (Armitage et al., 2005).

Many nutritional factors play a role in foetal programming including individual nutrients, energy restriction, inadequate diets and timing of insult during pregnancy or post-natal (Waterland and Garza, 2002). Foetal intrauterine growth restriction (IUGR) occurs in humans as a consequence of poor maternal nutrition. Developmental plasticity allows the foetus to adapt tissue structure in response to environmental changes. The long-term post-natal consequences of developmental plasticity have been described by a number of terms including programming and metabolic imprinting (Waterland and Garza, 2002). Programming is described as any situation where a stimulus or an insult during development induces a permanent physiological response.

Although micronutrient deficiencies during gestation have been associated with adverse pregnancy consequences (Allen, 2005), their effects on the long-term health of the offspring are not still novel. For a number of reasons, it is essential to hypothesize that deficiency of minerals and vitamins during critical stages of development will have long lasting health consequences. The field of developmental origins of health and disease has now established the link between small size at birth and chronic disease risk in adulthood and old age (Allen, 1998). In addition, magnesium and zinc are important for insulin sensitivity, storage, and secretion and altered zinc metabolism has been implicated in the development of type 2 diabetes and its complications (Chausmer, 1998).

Glucose is the primary source of energy for the body cells. Glucose is absorbed from the small intestine and circulated via the bloodstream for body cells to assimilate. Cellular absorption is aided by the hormone insulin produced by the pancreas (Naslund *et al.*, 1999). Typical fasting levels of blood glucose lie between 3.3 mmol/L and 6.1 mmol/L (Cryer, 1992).

Vitamin B12 is a water soluble vitamin that is naturally present in some foods, added to others available as dietary supplement and

prescription medication. It exists in several forms and contains the mineral cobalt (Herbert et al., 1996). It is required for proper red blood cell formation, neurological function, and DNA synthesis (Herbert et al., 1996). It is an essential component of one carbon metabolism which modulates methylation of DNA and protein. Its deficiency increases plasma homocysteine, an independent risk factor for recurrent, spontaneous, early pregnancy losses (Nelen, 2000).

During gestation, the developing fetus is completely dependent on his mother and maternal environment for nourishment. Human and experimental studies demonstrated that the outcomes of inadequate nutrition during pregnancy may extend to adulthood and could lead to glucose intolerance or insulin resistance. Faced with ample available calories, such individuals are at risk of developing dyslipidemia, glucose intolerance and other manifestations. However, the effect of early life nutritional Vitamin B12 supplementation on glucose metabolism has not been fully elucidated. In view of the forgoing literature, this study sought to unravel designed the effect of perinatal Vitamin B12 supplementation on the risk of glucose metabolism in male offspring.

MATERIALS AND METHOD

Diet

Vitamin B12 with NAFDAC number: 04-7011 supplement was obtained from Bernados pharmaceutical company, Idi-Araba, Lagos. The dosage of Vitamin B12 was 25mg/kg body weight.

Laboratory Animals

Virgin female Sprague-Dawley rats weighing between 120-150g (n=18) were obtained from the Physiology department of the College of Medicine of the University of Lagos and acclimatized for two weeks before the experiment. They were each fed with standard rodent chow and water *ad libitum*. The experimental procedures adopted were in accordance with the provisions of the Experimentation Ethics Committee on Animals Use of the College of Medicine of the University of Lagos, Lagos State and the United States National



Academy of Sciences Guide for the Care and Use of Laboratory Animals. A control diet (n=6) or the same supplemented with vitamin B12 (25 mg/kg, n=12)

Mating

Female rats were mated overnight with proven male breeders and a vaginal smear which was washed with normal saline solution (NaCl, 0.9% w/v) was collected to evaluate the presence of spermatozoa (de Oliveira *et al.*, 2016). Pregnant rats were transferred to individual cages and allocated at random to one of four groups to be fed either a control diet or VB12 supplemented diet. Food and water were available for all animals. At day 21 after birth, the pups were weaned and fed with control diet until the end of the experiment and grouped (six animals per group). Four dams from the VitB12 diet fed and their offspring were shifted at parturition to control diet. At weaning (day 22), six offspring of VitB12 dams were weaned to control diet while offspring of the remaining VitB12 dams continued on VitB12 diet.

Determination of body weight

The animals were weighed at birth, and recorded at weekly interval using a Duet top loading weighing scale (Salter, England).

Determination of fasting blood glucose

The fasting blood glucose level after overnight fasting was determined with Acu – chek glucose meter (Rani *et al.*, 2016). Briefly, blood samples were drawn from tail vein carefully from each rat to measure fasting blood glucose level.

Determination of oral glucose tolerance

The animals were fasted overnight prior to the test. A zero time (baseline) blood sample was drawn, which was designated zero (0) minute's glucose each rat was given an oral glucose load of 2g/kg body weight of glucose solution (D-(+) Glucose 99.5%; Sigma product). Blood samples were drawn from tail vein after the glucose load at intervals of 30, 60, 120 and 180 min for measurement of glucose level. The glucose level was measured with Acu – chek glucometer and glucose strips (de Oliveira *et al.*, 2016)

Determination of insulin tolerance

Insulin tolerance test (ITT) measures glucose levels subsequent to a standardized insulin load. It gives an estimate of the insulin sensitivity of the animals. Animals were fasted overnight as previously referenced in glucose tolerance test; a bolus of insulin 0.75IU/kg body weight was administered intraperitoneally, and blood glucose was measured at different time points during 120 min

Blood collection

Five (5ml) of blood sample was taken by retro-orbital puncture. Blood was allowed to clot for 1 hour at 4°C, then centrifuged at 3,000 rpm for 10 minutes and the serum samples were kept at -20°C until assayed (Alessandra *et al.*, 2008).

Lipid profile analysis

Serum levels of CHOL, TG, HDL and LDL were assayed using an automated Analyzer (Roclie, HITACHI)

Determination of serum insulin

The Enzyme-linked immunoabsorbent assay (ELISA) test was used in the determination of fasting serum insulin level with the aid of rat insulin ELISA kit (Elabscience, Wuhan, China). Briefly; 50µl of the samples were pipetted into an eppendorf tube. 100µl of the conjugate was added to the samples labeled accordingly. 50µl of the standard were also pipetted into another eppendorf tube, followed by the addition of 100µl of the conjugate. Another was created as blank and was incubated alongside with the standard and sample at room temperature (22 – 28°C) for 2 hours and the absorbance was read at 450nm against blank (Morakinyo *et al.*, 2018).

Determination of serum Adiponectin level

The parameter was determined using enzyme-linked immunoabsorbent assay (ELISA) rat adiponectin kit (Elabscience Biotechnology Co., China). The procedure specified in the manufacturer's manual for the kits was followed. A 96-well microtiter plate was used to conduct the analysis.

A volume of 50µl of the sample was pipetted into an eppendorf tube. 100µl of the conjugate was added to the sample labeled accordingly. 50µl of the standard was also pipetted into another eppendorf tube,

followed by the addition of 100µl of the conjugate. After an appropriate time has elapsed for maximum color development, the enzyme reaction was stopped by the addition of 100µl stop solution to the standard, sample and blank and the absorbance was read at 450nm against blank

Statistical Analysis

Data were recorded as Mean \pm standard error of the Mean. Statistical difference between

the means was determined by one way ANOVA. The Tukeys' post-hoc test as used to identify differences between individual means. The confidence interval was set at 95%, so that in all cases results with a value of $P \leq 0.05$ were accepted as being significant (GraphPad Prism 5, GraphPad Software, Inc., La Jolla, Calif., USA).

RESULTS

Table 1: Reproductive performance in female rats fed with control and Vitamin B12 supplemented diet.

PARAMETERS	CONT	PG	PGL	PGL+
Conception (%)	100	66.7	85.8	90.0
Still birth (%)	0	0	0	0
Average Litter size (n)	8	9	10	11
Weight gain during pregnancy(g)	169.75	172.38	175.78	177.34
Mean Weaning weight	36.00 \pm 1.63	39.66 \pm 2.31	40.1 \pm 0.31	40.8 \pm 0.21
Death of offspring before weaning (n)	4	3	2	3

KEY: CONT=Control
PG=Pregnancy

PGL=Lactation
PGL+=Pregnancy/lactation

At birth (week 0), VB12 supplemented offspring had higher weight gain compared to the control, $p < 0.05$ with significant increase in body weight at week 10 in PGL and PGL+ offspring ($p < 0.05$). Interestingly,

all the VB12 supplemented offspring had lower percentage weight gain compared to the control, ($p < 0.05$). The results are illustrated in table 2.

Table 2: Effect of Vitamin B12 supplementation on weight change in Control and VB12 supplemented offspring of Sprague-Dawley rats

GROUPS	IBW (initial body weight)	FBW (Final body weight)	WC (Weight change)	% WC
CONTROL	2.25±0.19	65.50±3.30	63.25±2.56	2811.11±14.52
PG	3.95±0.26*	63.33±2.33	59.38±3.01*	1503.29±23.90*
PGL	4.00±0.35*	88.33±3.91*	84.33±2.77*	2108.25±18.93*
PGL+	4.25±0.16*	54.66±3.48*	50.41±2.03*	1186.11±30.01*

*= $P < 0.05$ when compared with the control group

Key: IBW, FBW WC % WC

KEY: CONT=Control
PG=Pregnancy

PGL=Lactation
PGL+=Pregnancy/lactation

The fasting blood glucose was significantly elevated in PG group when compared with control ($P < 0.05$).

At 30 minutes, the glucose level was significantly lower in PG offspring ($p < 0.05$), compared with control and significantly higher in PGL offspring when compared with the control. At 60 min, the blood

glucose level was significantly lower in PG offspring and significantly higher in both the PGL and PGL+ offspring ($p < 0.05$), compared with control. At 120 min the blood glucose level was significantly higher ($p < 0.05$), in PGL and PGL+ offspring compared with control. The results are illustrated in figure 1.

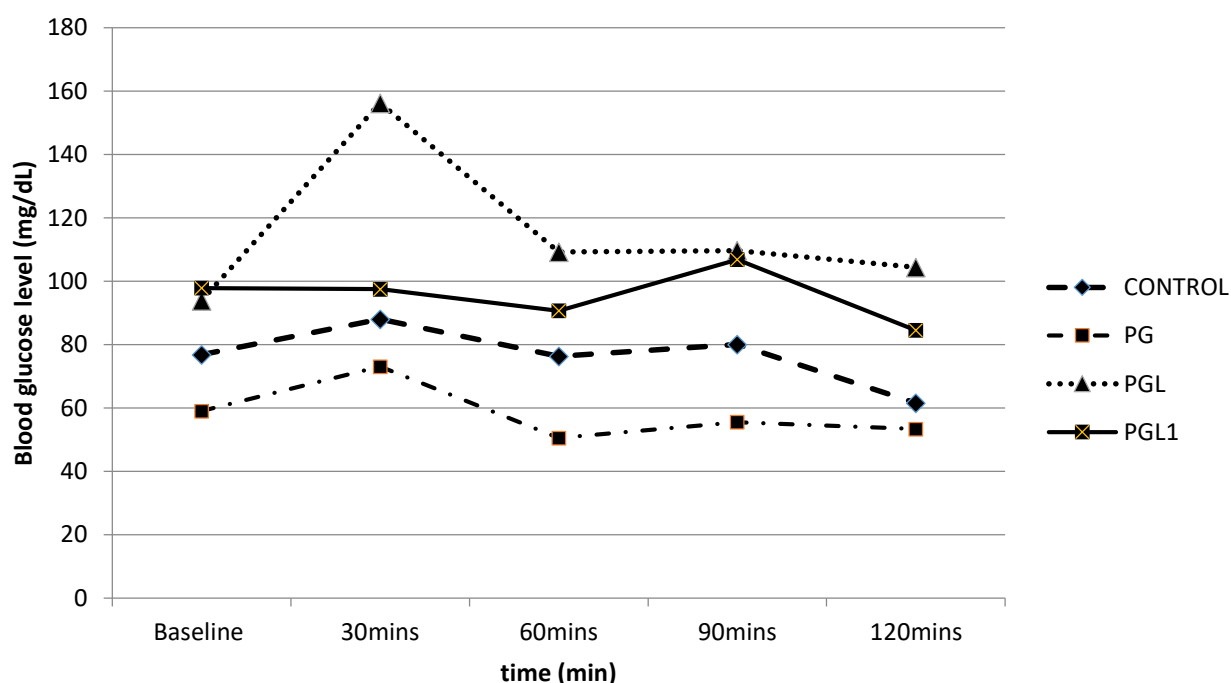


Figure 1: Effect of Vitamin B12 supplementation on blood glucose levels during OGTT in control and Vitamin B12 supplemented offspring of Sprague-Dawley rats

KEY: CONT=Control
PG=Pregnancy

PGL=Lactation
PGL+=Pregnancy/lactation

The fasting blood glucose during intraperitoneal insulin sensitivity test in VB12 supplemented offspring was significantly elevated compared with control, ($p<0.05$).

The blood glucose during 120 minutes intraperitoneal insulin sensitivity test was significantly decreased compared with control, ($p<0.05$). The results are illustrated in figure 2.

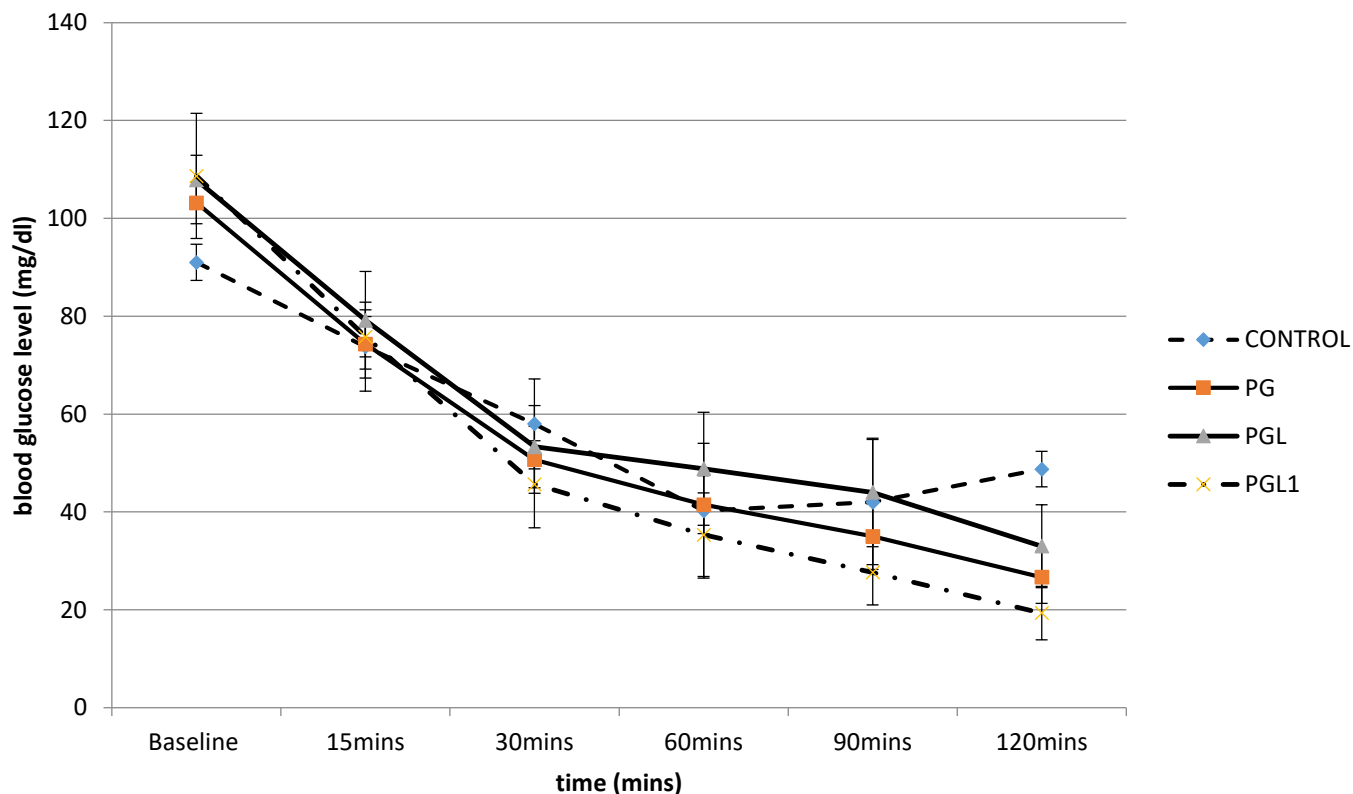


Figure 2: Effect of Vitamin B12 supplementation on blood glucose levels during ITT in control and Vitamin B12 supplemented offspring of Sprague-Dawley rats

KEY: PG=Pregnancy
PGL=Lactation

PGL+=Pregnancy/lactation

With the exception of triglyceride that was significantly ($p<0.05$) lower, there was no difference between the groups for serum

cholesterol, CHOL high density lipoprotein, HDL and low density lipoprotein, LDL. The results are illustrated in table 3.

Table 3: Effect of Vitamin B12 supplementation on serum levels of CHOL, TG, HDL and LDL in control and Vitamin B12 supplemented offspring of Sprague-Dawley rats

PARAMETERS(mmol/dl)	CONTROL	PG	PGL	PGL+
CHOL	2.95±0.13	3.26±0.31	3.55±0.43	2.92±0.08
TG	2.36±0.18	1.48±0.15 *	1.30±0.28 *	1.70±0.07 *
HDL	1.58±0.09	1.41±0.11	2.08±0.26	1.44±0.10
LDL	0.64±0.16	1.16±0.34*	0.91±0.12	0.70±0.16

*= $P<0.05$ when compared with the control group

KEY: CONT=Control
PG=Pregnancy

PGL=Lactation
PGL+=Pregnancy/lactation



The serum insulin level was significantly reduced in all the VB12 supplemented offspring ($p < 0.05$) compared with the control, while the serum adiponectin level

was significantly higher in PG and significantly lower in PGL and PGL+ offspring ($p < 0.05$) compared with the control. The results are illustrated in table 4.

Table 4: Effect of Vitamin B12 supplementation on serum level of adiponectin control and Vitamin B12 supplemented offspring of Sprague-Dawley rats

PARAMETERS	CONTROL	PG	PGL	PGL+
INSULIN(pg/ml)	77.33±21.45	19.50±2.98 *	23.83±2.63 *	21.33±5.73 *
ADIPONECTIN (ng/ml)	1.40±0.10	1.92±0.22 *	0.58±0.13 *	0.76±0.12 *

*= $P < 0.05$ when compared with the control group

KEY: CONT=Control
PG=Pregnancy

PGL=Lactation
PGL+=Pregnancy/lactation

DISCUSSION

Vitamin B12 is important for neural development, myelination of nervous system and its deficiency results in neurological disorders (Bourre et al., 2006). Birth weight was determined as a measure of growth curve. Result from the current study showed a significant increase in birth weight and weaning weight of the offspring born to Vitamin B12 supplemented diet dams compared with the control. Emerson et al., (1947) reported a decrease in mean birth weight due to maternal Vitamin B12 restriction. In addition, Venu et al., (2004) reported that Vitamin B12 administration to young Vitamin B12 deficient young rats increased food intake and enhanced their growth. Lawlor et al., (2002) reported that birth weight of the offspring is inversely related to maternal insulin resistance and the present study shows a progressive increase in weekly body weight. The current study suggests improved growth in offspring of VB12 supplemented rats.

OGTT, ITT, and fasting serum insulin were measured as indices of glucose homeostasis. The current study showed a significant increase in blood glucose level during oral glucose tolerance test in VB12 only in offspring exposed to VB12 supplemented offspring during lactation while the gestational exposure showed decreased blood glucose level. This showed post natal

Vitamin B12 supplementation may stimulate glucose tolerance in rat offspring. Kalle et al., (2013) had earlier reported that Vitamin B 12 rehabilitation was responsible for improved glucose tolerance initially observed in Vitamin B12 deficient rat offspring.

The result showed significant decrease in blood glucose level during ITT which suggest an improved insulin sensitivity in VB12 supplemented offspring. This strongly agrees with Kalle et al., (2013), who reported that maternal Vitamin B12 supplementation was responsible for modulating insulin sensitivity in rat offspring. In the current study, adiponectin was determined as index of food intake regulation. Also, the current study showed significant decrease in all VB12 supplemented offspring compared with control suggesting hypoinsulinemia in the offspring. The results showed significant increase in serum adiponectin level in PG and significant decrease in PGL and PGL+ offspring.

Adiponectin is an adipocyte-derived hormone that has been proposed to play an important role in energy homeostasis (Trujillo and Scherer, 2005). In sharp contrast to leptin, plasma adiponectin levels are negatively correlated with body fat, decreasing with obesity and increasing in response to weight loss. Hence, the current

study suggests increase food intake in PG offspring and decrease food intake in PGL and PGL+ offspring.

In conclusion, these results suggest that VitB12 supplementation may influence long term glucose homeostasis without affecting food intake. Although the data here are insufficient to conclude that VitB12 supplementation is beneficial or deleterious to the offspring metabolism, the observed improvement in insulin sensitivity is very fascinating and warrant further mechanistic investigation.

REFERENCES

- Allen, L.H (1998). Biological mechanisms that might underlie iron's effects on fetal growth and preterm birth. *J Nutr*; **131** (2, Suppl 2):581–9.
- Barker, D.J., Bull, A.R, Osmond C, Simmonds SJ (2004). Fetal and placental size and risk of hypertension in adult life. *Bmj*; **301**(6746):259–62.
- Bourre, J.M (2006). Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirements for brain Part 1: Micronutrients. *J Nutr Health Aging* 10: 377–385.
- Chausmer, A.B (1998). Insulin and diabetes. *J Am Coll Nutr*; **17**:109–15.
- Cryer, P.E. (1992). Glucose homeostasis and hypoglycaemia. In William's Textbook of Endocrinology. Wilson JD, Foster DW, Eds. Philadelphia, Pa., W.B. Saunders Company, p.1223–125
- De Oliveira, D., Bisht, V., Rodrigues, G., Reis, M. G. (2015). Patterns in leptospira shedding in Norway rats (*Rattus norvegicus*) from Brazilian slum communities at high risk of disease transmission
- Emerson, G., Wurtz, E., Zanetti, M.E (1949). Vitamin B12-a growth factor for young rats. *Fed Proc* **8**: 381–382.
- Garland, H.O. (1992). New experimental data on the relationship between diabetes mellitus and magnesium. *Magn Res*; **5**:193–202.
- Herbert, V. (1996). Vitamin B12. Present Knowledge in Nutrition, 7th ed. Washington, DC: International Life Sciences Institute Press, pp191–205.
- Kalle, A.K., Lalitha, A., Pavithra, D., Padmavathi I.J.N., Manisha, G. (2011). Maternal dietary folate and/or vitamin B12 restrictions alter body composition (adiposity) and lipid metabolism in Wistar rat offspring. *J Nutr Biochem*; **24**: 25–35.
- Lawlor, DA., George, D.S., Shah, E. (2002). Birth weight of offspring and insulin resistance in late adulthood: cross sectional survey. *BMJ* 325: 359.
- Nelen, W., Blom, H., Steegers, E., Den, H.M., Thomas, C. (2000). Homocysteine and folate levels as risk factors for recurrent early pregnancy loss. *Obstet Gynecol* **95**: 519–524.
- Morakinyo, A.O., Iranloye B.O., Ogunisola, O.A. (2018). Glucometabolic effects of single and repeated exposure to forced-swimming stressor in Sprague-Dawley rats. *Endocr Regul.*; **52**(2):85–92. doi: 10.2478/enr-2018-0010.
- Petry, C.J and Hales, C.N. (2000). Long-term effects on offspring of intrauterine exposure to deficits in nutrition. *Hum Reprod Update*; **6**: 578–586.
- Taylor, C.G. (2005). The pancreas, and diabetes: insights from rodent studies and future directions. *Biometals*; **18**:305–12.
- Taylor, P.D., Poston, L. (2005). Developmental programming of obesity in mammals. *Exp Physiol.* Mar; **92**(2):287–98.
- Venu, L., Harishankar, N., PrasannaKrishna, T., Raghunath, M. (2004). Maternal dietary vitamin restriction increases body fat content but not insulin resistance in WNIN rat offspring up to 6 months of age. *Diabetologia*; **47**(9):1493–501.
- Waterland, R.A. and Jirtle, R.L. (2002). Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol.*; **23**(15):5293–300.



HEAVY METALS IN SOME COMMON VEGETABLES SOLD IN SOME MARKETS IN LAGOS STATE, NIGERIA.

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ABSTRACT

Heavy metals disrupt the biochemical and biological activities in human body. Throughout the globe, significant interest are given to heavy metals due to their mutagenic, teratogenic and toxic effects even at very low concentrations. The risk to human health by heavy metals: Copper (Cu), Zinc (Zn), (Lead) Pb, Cadmium (Cd) and Nickel (Ni), through the intake of common vegetables: *Solanum lycopersicum*, *Cochorus olitorious*, *Telfaira occidentalis*, *Solanum macrocarpon* and *Capsicum frutescens*, were assessed using Atomic absorption spectroscopy (AAS). Vegetables samples were collected from markets in three cities, (Mushin, Yaba and Surulere) in Lagos State, Nigeria. Of all the three locations, the results showed the presence of the heavy metals in all the samples collected from Mushin, and this is an indication of the presence of high level of pollution from vehicular exhausts and industrial activities in the area. Also, the results revealed that, heavy metals mean concentrations and standard deviations were in the range of Cu (1.15 ± 0.66 to 2.57 ± 0.44)mg/kg, Zn (1.05 ± 0.92 to 2.16 ± 1.28)mg/kg, Pb (0.02 ± 0.01 to 0.04 ± 0.03)mg/kg, Cd (0.01 ± 0.00 to 0.02 ± 0.01)mg/kg and Ni (0.25 ± 0.16 to 0.40 ± 0.24)mg/kg respectively. When compared with standards: World Health Organisation (WHO), and Food and Agriculture Organisation (FAO), the concentrations fell within the range of safe values, indicating the vegetables were safe for consumption without the risk of the toxicants in the environment. However, in the biological systems of people, there could be bio-accumulation of these heavy metals after long period of consumption, thus posing potential health challenges to consumers.

KEYWORDS: Vegetables, Heavy metals, Atomic absorption Spectroscopy, Pollution.

INTRODUCTION

Heavy metals have been known to be very harmful because of their long half-lives biologically, and they are non- biodegradable in nature. They can also accumulate in different parts of the body of organisms (Suruchi and Khanna, 2011). Heavy metals are made of metalloids and metals, having the atomic density that is higher than 4g/cm^3 . Although, the term “heavy metals” is defined loosely, it is universally known, and greatly applicable to the ecosystems of both the terrestrial and freshwater widespread of contaminants (Duffus, 2002). They are among the lists of significant forms of contaminants that are usually present, both in tissues and on surfaces of dry vegetables. Toxic and essential elements are also included, with concentrations of over a wide range. In earth's crust, heavy metals naturally occur at low concentrations, but due to various anthropogenic activities, their concentrations have been drastically increased (Badawy *et al.*, 2013). The various sources of introduction into the environment includes effluents, chemicals, large amount of waste substances and energy from homes and industries (Mohod, 2015). Moreover, pollutions into the environments include emissions from the vehicular exhausts, industrial activities, thus, increasing their levels of contamination in plants, soils and residents along roads with heavy traffic loads (Ghrefat and Yusuf, 2006). Because of yet no effective method for total removal of heavy metals from the body, their chronic low level intakes in human beings and other animals result in adverse effects (Babemuka and Mubofu, 1999). Prolonged consumption of foodstuffs with dangerous concentrations of heavy metals leads to the chronic accumulation of the heavy metals in human



liver and kidney, causing the disruption of several biochemical processes, and bringing about bone, kidney, cardiovascular, and nervous diseases (WHO, 1992; Jarup, 2003). One of the great issues that have been a major worldwide public concern is food safety. In the last decades, increasingly food safety demands, encouraged the conduct of research, regarding the danger accompanied with consumption of foodstuffs contaminated by pesticides, toxins and heavy metals (D'Mello, 2003). Vegetables contain proteins, carbohydrates, minerals, vitamins, with some trace elements, and they constitute a significant part diet for humans (Nazemi, 2012). Consumption of vegetable edible parts is one of the major ways through which soil heavy metals cause havoc to human health. Since vegetables are rich with all these nutrients, they constitute a major part of daily diets in many households and are widely used as food (Oluwole *et al.*, 2013). Human health is highly dependent on vitamins and minerals from vegetables and greatly contain pectin substances, hemicellulose and cellulose, from which they receive their firmness and texture. (Sobukola and Sairo, 2007). Vegetables contains very important fibres that keep human health in good form. During digestion, acidic substances formed are neutralized by agents such as vegetables (Thompson and Kelly, 1990). In urban communities, their consumption is gradually increasing because of education and exposure of many people to other cultures. This is as a result of increase in awareness of vegetable food values (Fisseha, 2002). Atmospheric and soil contamination as results of the presence of heavy metals is a serious threat to vegetables safety and qualities. Factors like, atmospheric depositions, climate, plant maturity degree, during the period of harvest and heavy metals concentrations in soils, along with the nature of soil usually influence the heavy metals uptake and bioaccumulation in vegetables (Scott *et al.*, 1996). Animals and human health are greatly affected by heavy metals dietary intake. The high occurrence of cancer of the upper gastrointestinal, has been observed to be caused by heavy metals toxicity, (Cu, Cd and Pb) in high concentrations in vegetables and

fruits (Turkdogan *et al.*, 2003). Due to the potential toxicity of heavy metals, their phytotoxicity in plants leads to plant metabolism disorders, reduction in nutrient uptake, and reduction in molecular nitrogen fixation ability for leguminous plants, thus resulting to weak plant growth, Chlorosis and yield depression (Guala *et al.*, 2010). These heavy metals usually accumulate in the soil through sewage water disposal, they are not naturally abundant in the soil (Kalaskar, 2012).

Five types of metal were focused in this research. They are copper (Cu), cadmium (Cd), zinc (Zn), lead (Pb) and nickel (Ni). Copper is an important trace element that is very useful to the health of all organisms because it is needed in metabolic processes and organs proper functioning. Low concentration of copper in plants helps in chlorophyll production, protein synthesis and utilization. In fact, copper is a component of metalloenzymes, acting as an electron acceptor or assisting in mammalian nutrition. Hence, copper is an important element in mammals. However, certain levels of copper in vegetables has been associated with cancer. Moreover, there could be many adverse health effects as results of high level intake of copper. For example, excessive intake of copper for humans brings about the following symptoms; severe headaches, arthritis, depression, renal and hepatic damage, widespread capillary damage, irritation of the central nervous system, dry skin, severe irritation of gastrointestinal pathways, possible necrotic changes in the kidney and liver and many others (Badawy *et al.*, 2013). Researches have shown that most organisms take in copper primarily from food consumption and drinking of water. Accidental ingestion generally results into acute copper toxicity; however genetic disease or predisposition may be caused by adverse effects of high intake of copper by members of the population (Stern *et al.*, 2007). Copper is one of the key constituents of complex cytochrome c. oxidase, a respiratory enzyme. Therefore, copper is a trace dietary mineral. Hence, it is very essential in living organisms. Mainly, copper is found in the bone, muscle and liver



in humans (Johnson and Larry, 2008). Nickel occurs in the environment at low level. Most compounds of nickel are blue or green. Dissolving nickel is very slow in dilute acids, but when nitric acid is added, it becomes inactive like iron. It is one of the main materials used in steel production and other metal products. High concentrations of nickel can exist in polluted soils with growing food. Smoking, drinking water, inhalation, and consumption of contaminated food are some of the means through which humans are exposed to nickel. It causes cancer, respiration failure, birth defects and hearth failure (Abdu, 2013). Nickel in high concentrations causes severe necrosis and chlorosis in some plants and a group of other anatomical changes and abnormalities of growth. Enzymes activity, mineral metabolism and other numerous metabolic activities in plants are regulated by nickel (Adu *et al.*, 2014). Oral consumption forms the major source of exposure to nickel, because nickel is essential to plants (Haber *et al.*, 2017). Nickel is naturally found in both food and water and may be very toxic and carcinogenic. Lead is a contaminant that is widely spread in the soil. In most part of the world, one of the most prevalent public health problem is lead poisoning. Reports have been given regarding the accumulation of lead in edible in animals and aquatic plants (He *et al.*, 2003). When fetuses, infants and children are exposed to lead, it results to some neurobehavioral effects and developmental and on them. It elevates adults' blood pressure. Lead has long been known to be neurologically and physiologically toxic to humans. Dysfunction in the liver, brain, kidney, and reproduction system, resulting in sickness and death may be due to acute lead poisoning (Odum, 2000). It has been observed that even at extremely low concentrations, lead poses a threat (Kazemipour *et al.*, 2008). Haemoglobin synthesis inhibition resulting to chronic and acute damage to the peripheral nervous system (PNS) and central nervous system (CNS), cardiovascular system is caused by lead poisoning.

Lead also causes retardation in children. Exposure to Cadmium are associated with bone and kidney damage. It causes cardiovascular diseases. Cadmium has also been observed as a potential carcinogenic substance and can cause human lung cancer. Its toxicity also affects the sperm and thus, reduces birth rate. Various man made activities, involving fertilizer applications, burning of fossil fuels, plastic weeds and sewage sludge are responsible for large concentration of cadmium in the soil (Suruchi and Khanna, 2011). It has also been reported that both cadmium and lead accumulate in the non-edible and edible structures of vegetables. For zinc, its' knowledge of toxicity is minimal, but it has been reported to be associated with copper metabolism (Barone *et al.*, 1998). In fact, an essential trace element for animals including humans is zinc (Maret, 2013). According to Ladipo and Doherty (2011), zinc is described as the least toxic of all the heavy metals found among human diet, and it is useful in maintenance and proper immune system functioning. When zinc is too much in diet, it is not as detrimental to human health as at when there is deficiency of it (Ladipo and Doherty, 2011). An acute oral zinc dose may provoke the symptoms such as dyspeptic nausea, hepatic parenchyma damaging, pancreatitis, vascular shock, vomiting, tachycardia, and diarrhoea (Salgueiro *et al.*, 2000). Most concentrations of zinc are found in the bones, brain, liver, kidney and muscles. Some parts of the eye and the prostate have been observed to contain the highest concentrations (Wapnir, 1990).

MATERIALS AND METHOD

Study area

This research project was carried out in Lagos state, with the location of latitude; 6°27' 14.65" North and Longitude; 3°23' 40.81" East, which is 35 meters above the sea level in elevation. In Nigeria, Lagos is the main mega city, with the population of about 9,000,000 inhabitants (Koleayo *et al.*, 2017). It is a suitable area for the research study, considering the proximity of the sampling area (markets) and laboratory area.

**Collection of samples**

Five samples: *Solanum macrocarpon* (Garden egg), *Solanum lycopersicum* (Tomato), *Capsicum annuum* (Bell pepper), *Telfairia occidentalis* (Ugu) and *Corchorus olitorius* (Ewedu) were each collected (purchased) from three different markets in Lagos, Nigeria on the 5th of October, 2017.

The markets visited include Yaba, Surulere and Mushin markets. Labelled polythene bags were used in packing the vegetable samples. These were taken to the laboratory where acid digestion and heavy metals analyses were done using atomic absorption spectroscopy (AAS).

Table 1: Vegetables samples collected from the three markets.

BOTANICAL NAME	FAMILY	ENGLISH NAME	LOCAL NAME
<i>Telfairia occidentalis</i>	Cucurbitaceae	Fluted pumpkin leaves	Ugu (Igbo)
<i>Corchorus olitorius</i>	Malvaceae	Jute leaves	Ewedu (Yoruba)
<i>Solanum macrocarpon</i>	Solanaceae	Garden egg	Igba (Yoruba)
<i>Capsicum annuum</i>	Solanaceae	Pepper	Bell pepper
<i>Solanum lycopersicum</i>	Solanaceae	Tomato	Tomato

Quality assurance/ reagents

Analytical grade was the standard of all the reagents that were used. Dilution, preparation of standards and reagents, were done by double distilled water. Liquid soap was used in washing all glass wares and plastic containers and they were well rinsed using water, soaked in 10% nitric acid for 24hours. They were thoroughly cleaned using double distilled water after which they were dried to avoid any contamination (Wodaje, 2015).

Sample digestion

The samples were digested by taking 2g of each sample. Each sample was weighed and put into digestive tubes. Ten millilitres (10 mL) of Trioxonitrate (v) acid (HNO_3) was poured and mixed. Then, they were arranged on the Q wireless (Questron Technology) digester equipment placed in the fume cupboard. The equipment was then powered on and the temperature was allowed to rise to 120 degree Celsius (120°C). The digester was stopped and allowed to cool for three hours. It was then treated with deionized water. The volume was then made up to twenty five millilitres (25ml) and then filtered into the samples bottles. It was then

taken for Atomic absorption spectrophotometer (AAS) analysis.

Heavy metals analysis

The samples were analysed for copper (Cu), lead (Pb), nickel (Ni), cadmium (Cd), and zinc (Zn). Each sample was placed in 250 mL conical flask and digested with HNO_3 . The digests were filtered and levelled up to the mark in volumetric flask of 50 mL with deionised water. Digested samples were placed into the element lamp, Hollow Cathode Lamps (HCL). Each lamp corresponds to the heavy metals to be tested (element of interest). The lamp was then placed back in the atomic absorption spectrophotometer (Type- Buck Scientific; Model- 210 VGP). A nebulizer was also attached. The analysis began and the result was displayed on the atomic absorption spectrophotometer (AAS) digital screen.

RESULTS AND DISCUSSION

The summary of the results received from this study is seen in Table 2 and Figure 1 respectively. All the metals studied were observed to be present in the vegetable samples at some certain concentrations, except few cases; Nickel was not detected in

C. olerious, collected from Mushin market. Lead was not detected in *S. macrocarpon*, *T. occidentalis* and *C. olerius*, from Yaba market. Cadmium was only detected in *T. occidentalis* and *S. lycopersicum* in Yaba market also. In Surulere market, Lead was only detected in *C. annuum* and partially and in *C. olerious*. Cadmium was not detected in *S. lycopersicum* and *T. occidentalis*. For *C. olerious*, the concentrations of copper and lead were higher in samples obtained from Mushin. Also, high concentrations of zinc, cadmium and nickel were found in samples from Surulere market. High concentrations of cadmium and nickel were found in samples of *T. occidentalis* from Yaba market, also with high concentrations of copper in Surulere market. Moreover, concentrations of copper, zinc and nickel were found in higher concentrations in samples of *C. annuum* from Surulere market. Higher concentrations of zinc and nickel were also recorded for samples of *S. lycopersicum* in Surulere market, with higher concentration of copper in Mushin market. Surulere also has the higher

concentrations of copper and nickel for samples of *S. macrocarpon*, with higher concentrations of zinc, lead and cadmium in Mushin market. However, in all, copper reflects the highest concentrations of metals as reported in the samples of *C. olerious*, collected from Mushin area. The mean concentration and standard deviation of copper ranged from 1.53 ± 0.44 mg/kg to 2.57 ± 0.44 mg/kg. The values are less than food and agriculture organization (FAO)/World health organization (WHO) permissible levels with standard of 40 mg/kg. Moreover, in comparison with the research work of Adu *et al.* (2014) from Agboju and Iba markets, the mean concentration of copper ranged from 0.004 mg/kg to 0.013 mg/kg respectively. Also in comparison with Babatunde *et al.* (2014) in which the mean concentration of copper ranged from 0.028 mg/kg to 1.342 mg/kg, the values are also less than the FAO/WHO standard. Hence, these show that the levels of copper in the vegetables are within the range of safe values in accordance to FAO/WHO standard.

Table 2: Statistical analysis of the levels of Heavy Metals in the selected Vegetables from the three markets (n=6).

Selected Vegetables	COPPER (mg/kg)	ZINC (mg/kg)	CADMIUM (mg/kg)	NICKEL (mg/kg)	LEAD (mg/kg)
<i>Solanum macrocarpon</i>	1.53 ± 0.44	2.16 ± 1.28	0.02 ± 0.01	0.35 ± 0.17	Nil
<i>Solanum lycopersicum</i>	$1.15^* \pm 0.66$	1.05 ± 0.92	0.01 ± 0.00	0.30 ± 0.37	0.03 ± 0.02
<i>Capsicum annuum</i>	1.43 ± 0.91	1.81 ± 1.21	0.02 ± 0.01	0.40 ± 0.24	0.04 ± 0.03
<i>Telfairia occidentalis</i>	$2.57^* \pm 0.44$	2.09 ± 0.61	0.02 ± 0.01	0.25 ± 0.16	Nil
<i>Corchorus olerius</i>	2.24 ± 84	1.47 ± 0.50	0.01 ± 0.01	0.30 ± 0.09	0.02 ± 0.01
Standard	40mg/kg	60mg/kg	0.2mg/kg	1.5mg/kg	0.3mg/kg

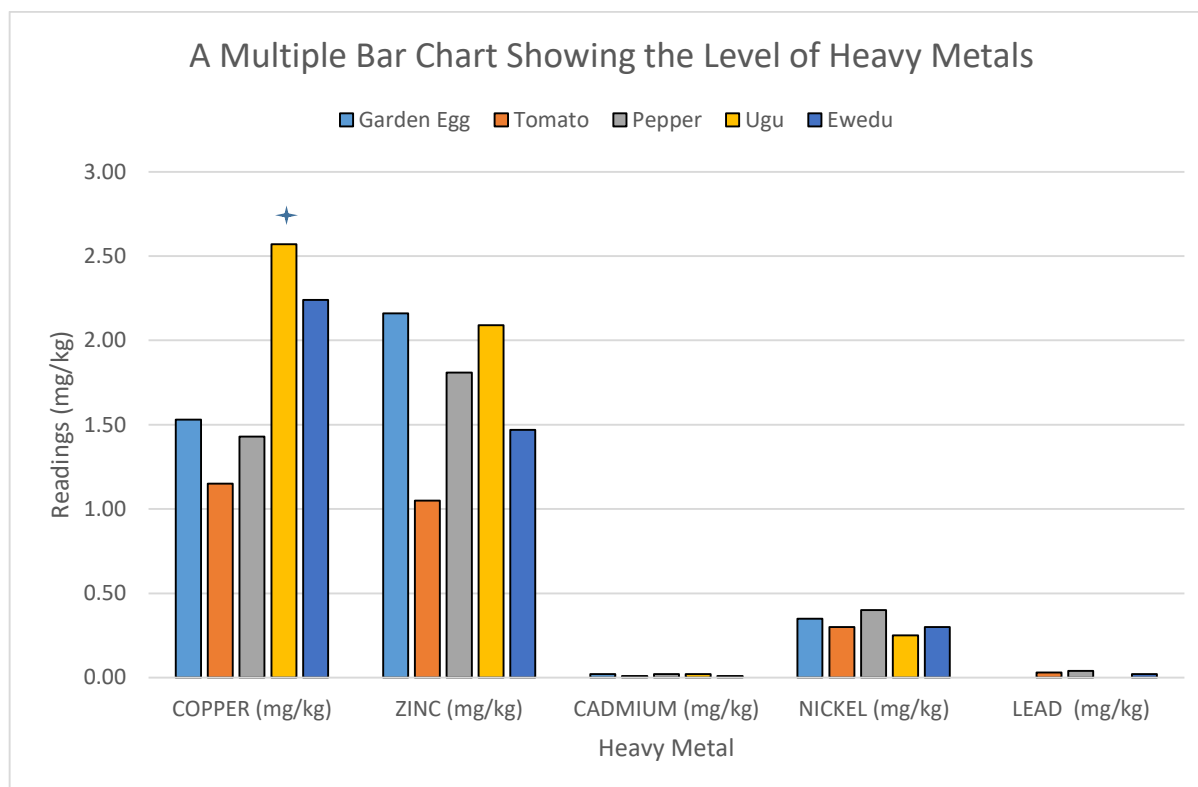


Figure 1: Graphical representation of the levels of heavy metals in the selected vegetables.

The mean concentration and standard deviation of zinc ranged from $1.05 \pm 0.92 \text{ mg/kg}$ to $2.16 \pm 1.28 \text{ mg/kg}$. Comparing it with the work of Doherty *et al.* (2012), in which the zinc concentration in vegetables also collected from the markets ranged from 0.078 to 0.091 mg/kg. Although, these values are less than the FAO/WHO permissible levels with standard of 60 mg/kg, it is significantly less than the result from this study. Since Mushin market was also among the locations where samples were collected in the study of Doherty *et al.* (2012), it shows that the significant increase in zinc concentration could be due to persist industrial exhausts in the area. The results of the research carried out by Babatunde *et al.* (2014), shows the mean concentration of zinc which ranged from 18.243 mg/kg to 45.223 mg/kg. The concentration of cadmium in the samples ranged from 0.01 ± 0.00 to 0.02 ± 0.01 . Again, comparing this with the results of the work done by Akan *et al.* (2013) in Borno State, with mean concentration of Cadmium; 0.02 mg/kg, it could be seen that the concentrations are almost the same. Exposure to cadmium have been observed to generally exist in industrialized areas and Cadmium chronic

exposure can cause renal disease, fragile bones and chronic obstructive lung disease (Adepoju- Bello *et al.*, 2013). The mean concentrations and standard deviations of Nickel ranged from $0.25 \pm 0.16 \text{ mg/kg}$ to $0.40 \pm 0.24 \text{ mg/kg}$. These values are however less than the FAO/WHO permissible levels with standard of 1.5 mg/kg. This result is also in consistency with the result of Adu *et al.* (2014), which mean concentrations, which ranged from 0.005 mg/kg to 0.023 mg/kg. Thus, the values are within the safe limit of FAO/WHO standard. As stated earlier, lead was not detected in some of the vegetable samples particularly in Yaba and Surulere markets. However, the mean concentration and standard deviation of lead content levels detected in other vegetable samples ranged from $0.02 \pm 0.01 \text{ mg/kg}$ to $0.03 \pm 0.02 \text{ mg/kg}$. The values are also less than the FAO/WHO permissible levels with standard of 0.3 mg/kg. The result of this study is in line with the result of Adu *et al.* (2014), which ranged from 0.008 mg/kg to 0.123 mg/kg. From the work of Ladipo and Doherty (2011), there are no traces of lead content from their results, hence the presence of lead in some of the vegetable samples might be indicators of the exhaust released from

vehicles or industries. However, mean concentration of lead content from the result of Babatunde *et al.* (2014) shows to range from 0.167mg/kg to 0.826mg/kg which is higher than the safe limit of FAO/WHO standard. Pollutants in farm soil, irrigation water or pollution from traffic in highways might have resulted in high levels of Lead in some plants (Qui *et al.*, 2000).

Moreover, Table 2 shows the mean and the standard deviation ($\bar{x} \pm SD$) of the vegetables in the markets combined. Analysis of variance shows that the level of copper is significantly different only in *Solanum lycopersicum* (Tomato) and *Telfairia occidentalis* (Ugu) ($p < 0.05$) while its level is not significantly different in *Solanum macrocarpon* (garden egg), *Capsicum annum* (pepper) and *Corchorus olitorius* (ewedu) ($p > 0.05$). There is no significant difference in the level of Zinc, Cadmium, Nickel and Lead among the selected vegetables ($p > 0.05$).

CONCLUSION

Since the concentrations of the selected heavy metals (copper, zinc, cadmium, nickel and lead) in the selected vegetables *Solanum lycopersicum*, *Cochorus olitorious*, *Telfaira occidentalis*, *Solanum macrocarpon* and *Capsicum frutescens* are all less than the World health organization (WHO) and Food and Agriculture Organisation (FAO) standards, it implies that the concentrations of the selected heavy metals present in the selected vegetables are within the acceptable range of consumption for humans. However, bioaccumulation of these metals could occur in peoples' biological systems after long period of consumption, thus posing potential health challenges to consumers.

REFERENCES

Adepoju-Bello, A.A., Okeke, C.P., Bamgbade, I. and Oguntibeju, O.O. (2013). Determination of the concentration of selected heavy metals in indigenous plant: *Telfairia occidentalis*. *Alternative and Integrative Medicine*. 2:112.

Akan, J. C., Kolo, B. G., Yikala, B. S. and Ogugbuaja, V. O. (2013). Determination of Some Heavy Metals in Vegetable Samples from Biu Local Government Area, Borno State, North Eastern Nigeria. *International Journal of Environmental Monitoring and Analysis*, 1(2): 40-46.

Adu, A.A., Aderinola, O.J. and Kusemiju, V.V. (2014). Assessment of trace metals in commonly edible vegetables from selected markets in Lagos state, Nigeria. *Current World Environment: An International Research Journal of Environmental Science*. 9(3):22- 34.

Badawy, R. K., El-Gawad, A. M. A., and Osman, H.E. (2013). Health risks assessment of heavy metals and microbial contamination in water, soil and agricultural foodstuff from wastewater irrigation at Sahl El-Hessania area, Egypt. *Journal of Applied Sciences Research*, 9(4): 3091-3107.

Babatunde, A.I., Oyelola, O.T. and Bamidele, I. (2014). Assessment of zinc copper and lead contamination in soils and vegetables from some farmlands in Lagos metropolis, Lagos, Nigeria. *Communications in applied sciences*. 2(1): 129-140.

Bahemuka, T.E. and E.B. Mubofu, (1999). Heavy metals in edible green vegetables grown along the sites of the Sinza and Msimbazi rivers in Dares Salaam, Tanzania. *Food Chem.*, 66: 63-66.

Barone, A., Ebesh, O. and Harper, R.G. (1998). Placental copper transport in rats: Effects of elevated dietary zinc on fetal copper, iron and metallothionien. *Journal of Nutrition*, 128: 1037-1041.

Doherty, V. F., Sogbanmu, T.O., Kanife, U.C. and Wright, O. (2012). Heavy metals in vegetables collected from selected farm and market sites in Lagos, Nigeria. *Global Advanced Research Journal of Environmental Science and Toxicology*, 1(6):137-142.



- D'Mello, J.P.F. (2003). Food safety: Contaminants and toxins. Cambridge, CABI Publishing, pp32-65.
- Duffus, J.H. (2002): "Heavy Metals" meaningless term. *Pure and Applied Chemistry* 74:793-807.
- FAO/WHO Codex alimentarius international food standards (1993, 1995, 2001, 2005). General standard for contaminants and toxins in food and feed. Codex standard.
- Fisseha, I. (2002). Metals in Leafy vegetables grown in Addis Ababa and toxicological Implication, *Ethiopian Journal of Health Development*, 16(3): 295-302.
- Ghrefat, H. and Yusuf, N. (2006). Assessing Mn, Fe, Cu, Zn and Cd pollution in bottom sediments of Wadi Al-Arab Dam, Jordan. *Chemosphere*, 65: 2114-2121.
- Guala, S.D., Vega, F. A. and Covelo, E.F. (2010) The dynamics of heavy metals in plant-soil interactions. *Ecological Modelling*, 221, 1148-1152.
- He, H.P., Corke, H. and Cai, J.G. (2003). Supercritical Carbon dioxide Extraction of oil and squalene from Amaranthus Grain. *Journal of Agricultural and Food Chemistry*, 51: 7921-7925.
- Jarup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin* 68: 167-182.
- Johnson, M.D. PhD and Larry E. Ed. (2008). Copper. *Merck Manual home health handbook*. Merck Sharp and Dohme corporation, a subsidiary of Merck and Corporation Incorporation.
- Kalaskar, M.M. (2012). Quantitative analysis of heavy metals from vegetable of Amba Nalain Amravati District. *Der Pharma Chemica*, 4(6):2373-2377
- Kazemipour M., Ansari M., Tajrobehkar S., Majdzadeh M., and Kermani H.R. (2008). Removal of lead, cadmium, zinc, and copper from industrial wastewater by carbon developed from walnut, hazelnut, almond, pistachio shell, and apricot stone. *Journal of Hazardous Materials*, 150, 322-327.
- Koleayo O.O., Kelechi L.N., Olutunde O.B. and Olapeju A. A. (2017). Nutritional composition and heavy metal content of selected fruits in Nigeria. *Journal of Agriculture and Environment for International Development*, 111 (1): 123-139.
- Ladipo M.K. and Doherty V.F. (2011). Heavy metal levels in vegetables from selected markets in Lagos, Nigeria. *African journal of food science and technology*. 2(1):018-021. ISSN: 2141-5455.
- Maret W. (2013). Zinc and Human disease. *Metal ions in life Sciences*, 12:389-414.
- Mohod, C.V. (2015). A Review on the concentration of the Heavy Metals in vegetable samples like Spinach and Tomato grown Near the Area of Amba Nalla of Amravati City. *International Journal of Innovative Research in Science, Engineering and Technology*. 4(5):2788-2792.
- Nazemi, S. (2012). Concentration of Heavy Metal in Edible Vegetables Widely Consumed in Shahroud, the North East of Iran. *Journal of Applied Environmental and Biological Sciences*, 2(8): 386-391.
- Odum H.T. (2000). Back Ground of Published Studies on Lead and Wetland. In: Howard T.
- Odum (Ed), Heavy Metals in the environment Using Wetlands for Their Removal, Lewis Publishers, New York USA, pp. 32.
- Oluwole, S.O., Makinde S.C., Yusuf, K.A., Fajana, O.O. and Odumosu, A.O. (2013). Determination of Heavy Metal Contaminants in Leafy Vegetables Cultivated By the Road Side. *International Journal of Engineering Research and Development*, 7: 01-05.
- Qui, X.X., Huang, D.F., Cai, S.X., Chen, F., Ren, Z.G. and Cai, Y.C. (2000). Investigations on vegetables pollution and pollution sources and its control in Fuzhou, Fujian



- Province. *Fuijian journal of agricultural science*, 15: 16-21.
- Scott, D., Keoghan, J.M. and Allan, B.E. (1996). Native and low-input grasses-a New Zealand high country perspective. *New Zealand Journal of Agricultural Research*, 39: 499-512.
- Sobukola, O.P, Dairo O.U. (2007). Modeling drying kinetics of fever leaves (*Ocimum viride*) in a convective hot air dryer. *Nigerian Food Journal*, 25(1):145-153.
- Stern B.R., Solioz M., Krewski D., Aggett P., Aw T. C., Baker S., Crump K., Dourson M., Haber L., Hertzberg R., Keen C., Meek B., Rudenko L., Schoeny R., Slob and Starr T. (2007). Copper and human health: biochemistry, genetics, and strategies for modeling doseresponse relationships. *Journal of Toxicology and Environmental Health, Part B*, 10, 157–222.
- Salgueiro, M.J., M. Zubillaga, A. Lysionek, M. Sarabia and Caro, R. (2000). Zinc as an essential micronutrient: A review; *Nutrition Research Journal*, 20: 737-755.
- Suruchi and Pankaj Khanna, (2011). Assessment of Heavy Metal Contamination in Different Vegetables Grown in and Around Urban Areas Research Journal of Environmental Toxicology, pp. 1-18.
- Turkdogan, M.K., Kilicel F., Kara, K. and Tuncer, I. (2003). Heavy metals in soil, vegetables and fruits in the endemic upper gastrointestinal cancer region of Turkey. *Environmental Toxicology and Pharmacology*, 13: 175-179.
- Wodaje A.T. (2015). Assessment of some heavy metals in selected cereals collected from local markets of Ambo city, Ethiopia. *Journals of cereals and oil seeds*. 6(2):8-13.
- Wapnir, R.A. (1990). Protein nutrition and mineral absorption. Boca Raton, Florida: CRC press. ISBN 0-8493-5227-4.
- WHO, (1992). Cadmium, environmental, health, criteria. Geneva: World Health organization, 134.



EVALUATION OF PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITIES OF *ADANSONIA DIGITATA* EXTRACT AGAINST SELECTED CLINICAL ISOLATES

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ABSTRACT

Adansonia digitata is a deciduous tree spread throughout Africa. Its root bark has been used in alternative medicine. The aim of study is to analyse the secondary metabolites and antimicrobial activities of the root bark extracts of *Adansonia digitata* against some fungal and bacteria isolates. Powdered *Adansonia digitata* root bark was extracted with hexane, ethanol and water by maceration. The extracts were tested for antimicrobial activities against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Candida albicans* and *Cladosporium oxysporum* using agar well diffusion and micro-broth dilution techniques. The extracts were applied at concentration of 12.5 – 100 µg/ml against fungi and bacteria isolates. But n-hexane extract inhibit growth of all isolates at all concentration except for *C. oxysporum*, which has inhibition at 100 µg/ml and no inhibition on *E. coli* at all concentration. While ethanolic extract has no inhibition of *E. coli* and *C. Albicans* at all concentration but has inhibition on other isolates at 25 µg/ml. Moreover, aqueous extract has no inhibition on the fungal isolates but has inhibition on the bacteria from *E. coli* to *S. typhi* at 25, 50, 100 µg/ml respectively. The presences of secondary metabolites were examined from the extracts using standard techniques. Results of antimicrobial activity showed that n-hexane extracts of this plant were more effective than other extracts on the isolates. The results of phytochemical screening indicated the

presence of alkaloids, saponins, flavonoid, phlobatanins and terpenoids in the extracts. This reveals that the root of *Adansonia digitata* has the inherent capacity for the manufacturing medicine for the cure infection caused by these clinical isolates

KEYWORDS: *Adansonia digitata*, isolates, extracts, antimicrobial activities, secondary metabolites.

INTRODUCTION

Infectious diseases caused by microorganisms are great challenges to general populace in spite of the immeasurable development in human drug. Their effect is largely enormous in developing countries due to drug resistant to these microorganisms as well as relatively insufficient development of medicine (Okeke *et al.*, 2005). Therefore there must be continuous search for new antimicrobial substance and exploration of all necessary measures must be employed. Medicinal plants serves as means of developing new drugs conformed to the financial status and the social and way of life of the general populace since, they have little adverse impacts (Datsugwai & Yusuf, 2017). Plant derived bioactive compounds such as alkaloids, flavonoids, phenolics, saponins, and tannins protect plants from parasites such as fungi, bacteria, viruses, and nematodes. (Marimuthu *et al.*, 2011). Improvement on antibiotic treatment



appeared to have rectified almost all prominent challenges in infectious diseases. Subsequently, the drastic revolution of immunity in bacteria have been found inhibited by multi-drugs before now develop a great challenges (Ndiaye, 2005). *A. digitata* which is known as Baobab is from the Malvaceae family, which enjoy longevity up to one hundred years and above (De Caluwé *et al.*, 2010). It has been confirmed that the leaves are also used as alternative medicine in combating wide range of infection and diseases in some parts of Africa (Abiona *et al.*, 2015). The strong fibres of the bark are used in making leather wares. The smooth fibers produced by the interior part of the bark are used more specially for weaving than the external bark (Yusha'uet *al.*, 2010). The extracts all parts of this useful tree *A. digitata* have been characterised to possess phytochemicals (Samatha and RamaSwamy, 2015) and rich in some vitamins derivatives (Samatha and RamaSwamy, 2016) Literature reviews on baobab has provided informations on its phyto-chemistry, pharmacology, the species taxonomy and other ideas needed (De Caluwé *et al.*, 2010) Many compounds have been extracted from fruit pulp, seed oil, root polyphenols, and other source of food supplement. From pericarp employing column chromatography epicatechin was isolated which have strong anti- oxidant property and may also enhance survival in diabetic mice. From roots 3 commercial drugs used in modern medicine were derived from plants following the ethnobotanical and ethno-medical knowledge (Arokiyaraj *et al.*, 2012). Anti-bacterial activity of *Adansonia digitata* stem bark extracted was carried out on clinical bacterial isolates of *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Staphylococcus species* using disc diffusion and micro broth dilution techniques. In phytochemical screening it was confirmed to possess alkaloids, flavonoids, reducing sugars, steroids. Presence of flavonoids may be the reason

for their anti-bacterial activity. Root bark is also employed in traditional medicine. It was found to contains steroid and glycosides (Yusha'uet *al.*, 2010). This research is designed at determining the secondary metabolites and antimicrobial activities of the root bark extract against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Candida albicans* and *Cladosporium oxysporum*

MATERIALS AND METHODS

(a) Sample collection

The root bark of *Adansonia digitata* was peeled with the aid of sterile knife at Ijebu-North Local Government Area of Ogun state. The plant root was authenticated and identified by a botanist at Science laboratory technology department. The scrapped samples were air-dried and blend into powdery form by employing mortar and kept subsequent use.,

(b) Preparation of Extracts Powder obtained was subjected to maceration extraction using different solvents according to their increasing polarity i.e. Hexane, Ethanol (60⁰c), and Water. Fifteen grams of the sample powder was weighed into 150ml each of n-hexane, ethanol and water in various conical flasks, kept it was shake at regular intervals for two weeks, later filtered and then evaporated the filtrate at 30°C (Daniel, 1991).

(c) Phytochemical screening:

The secondary metabolite screening was carried out on the plant samples to test for alkaloids, anthraquinone glycoside, saponins, cardiac glycoside, tannins, flavonoids, phenol, steroid and terpenoids according to the standard analysed by Abiona *et al.*, (2015) , Lawal *et al.*, (2014) and Oloyede *et al.*, (2010).

Extract yield: The yield was estimated using the formula:

$$R (\%) = \frac{M_1 \times 100}{M_0}$$



where R(%): Extract yield; M_1 : Weight of extract in gram(g); M_0 : Weight of powder sample in gram of root barks of *A. digitata* employed by extraction (g).

(d) Antimicrobial assay on extracts

i. Test organisms: The bacterial and fungal microbes used were; *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Candida albican* and *Cladosporium oxysporum*. All species were obtained from the human clinical cases isolated from Olabisi Onabanjo University Teaching Hospital Sagamu, which were cultured in nutrient agar and sabouraud dextrose agar (SDA) then stored at 4 °C.

ii Standardization of Inoculum: A sterile wire loop was used to pick a loopful of the test isolate and emulsified in 3 – 4mls of sterile physiological saline. The turbidity of the suspension was compared with that of 0.5 McFarland Standard (Yushau, 2010).

iii Preparation of concentrations of root bark extract of baobab tree Employing sterile dilution technique, 2 g of the aqueous, ethanolic and n-hexane extracts were dissolved separately in 2 ml of water to give a concentration of 1000 mg/ml (highest stock solution) after which it is serially diluted with distilled water and were made to produce various

concentrations of 100 µg/ml, 50 µg/ml, 25 µg /ml and 12.5 µg/ml. The tubes were labelled for various concentrations they contained and consumed instantly. (Ekwe & Elenglam, 2005).

iv Antimicrobial activity was determined by agar well diffusion method using Nutrient agar for bacteria and Potato dextrose for fungi.

Each plate was inoculated with 20 mg/ml microbial suspension having a concentration of 10^8 cells/ml 0.1ml extract was added to each well. The bacterial plates were incubated at 37°C for 24 hours and those containing fungi were incubated at 25°C for seven days. The antimicrobial activity was observed as inhibition zone which was compared with standard. MIC was also determined by broth dilution method. The cultures were diluted in nutrient agar broth at a density adjusted to turbidity of 0.5 Mac Farland standards. Equal volume of each extracts and nutrient broth was mixed in test tubes; 0.1ml standard inoculums were added to each tube. The lowest concentration of the extract that effects visible bacterial growth and compared with standard regarded as MIC (Rodge and Biradar, 2013).

(e) Statistical analysis: All experiments were performed in triplicates (unless indicated otherwise).

RESULTS

Table 1: Phytochemical Screening of *Adansonia digitata*

Phytochemical screening was performed to determine and to identified the components available in a particular plant sample. The secondary metabolite

screening of *A. digitata* root bark indicate the availability of alkaloids, saponins, flavonoid, phlobatanins and terpenoids as shown in table below:



1. Alkaloids	
a. Mayers	++
b. Dragendorff	+++
2. Anthraquinone Glycoside	-
3. Cardiac glycoside	-
4. Saponins	
a. Frothing	+++
b. Emulsion	+++ (oil droplet)
5. Tannins	-
6. Phloba-Tanins	++
7. Flavonoid	+
8. Phenol	-
9. Steroids	-
Terpenoids	++

Key: + Trace, ++ Moderate, +++ Abundant, - Absent

Extract efficiency:

The percentage of total mass of sample extracted from 50g of the sample by various solvents (water, ethanol and hexane) is presented in the Figure 1. Yields for the

various extracts revealed the values between 4.36 %,8.80% and 8.80%. The highest yield was produced by ethanol above all other samples than all other solvents on the plant used.

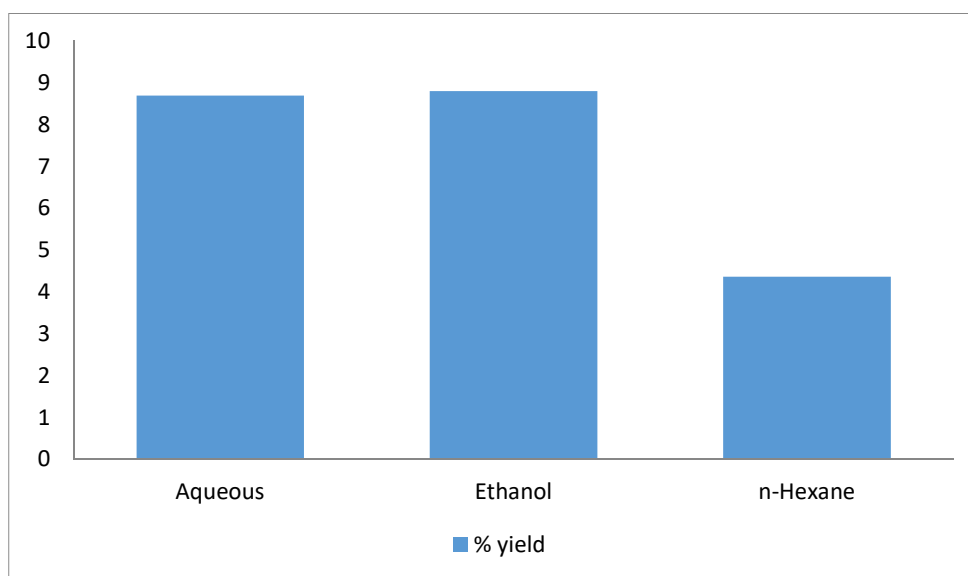


Fig 1: % yield of Solvents

Table 2; Zone of inhibition and minimum inhibitory concentration Antimicrobial assay was carried out using agar well diffusion method against three bacterial isolates and two fungal isolates. Table 2 show the summary of the microbial growth zone of inhibition for aqueous, hexane and ethanolic extracts of *A. digitata*. Hexane extract exhibit significant antimicrobial activity against *S. aureus*, *Salmonella typhi* and *C. albican*, It exhibit less antimicrobial activity against *C. oxyporum* and *E. coli*

and Ethanolic extract exhibit antimicrobial activity against *S. aureus*, *S. typhi* and *C. oxyporum*. and it does not exhibit antimicrobial activity against *E. coli* and *C. albicans* While aqueous extract exhibit antimicrobial activity against *S. aureus*, *S. typhi* and *E. coli* and no activity against *C. oxyporum* and *C. albicans*. The peak antimicrobial activity was revealed by n-hexane extract against *S. aureus*, *S. typhi* and *C. albican*. Whereas extract is not effective against *E. coli* and *C. albicans*.

Solvents	Organisms	12.5	25	50	100	MIC
Aqueous	<i>E. coli</i>	6.0±0.0mm	7.33±0.58mm	10.0±0.0mm	11.0±1.0mm	25.0
	<i>S. aureus</i>	6.0 ± 0.0	6.6 ± 1.15	9.66 ± 0.58	13.0 ± 1.0	0.78
	<i>S. typhi</i>	6.0 ± 0.0	6.0 ± 0.0	7.66 ± 1.53	16.66 ± 0.58	12.5
	<i>C. albican</i>	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	50.0
	<i>C. oxyporum</i>	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.25
Ethanol	<i>E. coli</i>	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	50.0
	<i>S. aureus</i>	6.0 ± 0.0	6.66 ± 1.15	9.04 ± 1.73	11.66 ± 4.93	6.25
	<i>S. typhi</i>	6.0 ± 0.0	6.66 ± 1.15	9.33 ± 2.31	12.33 ± 1.52	12.5
	<i>C. albican</i>	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	1.56
	<i>C. oxyporum</i>	6.0 ± 0.0	8.33 ± 1.15	10.33 ± 1.53	13.00 ± 2.00	12.5
Hexane	<i>E. coli</i>	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.00.0	25
	<i>S. aureus</i>	7.0 ± 1.73	9.33 ± 1.53	12.0 ± 1.53	15.66 ± 0.58	0.79
	<i>S. typhi</i>	6.33 ± 0.58	6.66± 1.78	10.00 ± 3.58	13.66 ± 1.15	6.25
	<i>C. albican</i>	6.33 ± 0.58	8.66 ± 1.15	8.0 ± 2.65	9.33 ± 3.51	3.125
	<i>C. oxyporum</i>	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	9.66 ± 3.51	3.125

DISCUSSION

The need for the investigation of modern antimicrobial compounds due to production of drug resistance in human pathogens against usually administered antimicrobial drugs is crucial. Higher plants produce different bioactive compounds having various physiological functions. The bioactive substances may be a origin of compounds that contain antimicrobial impacts and therefore certain specimen for the production of modern antimicrobial drugs (Latifou *et al.*, 2012). High yield of extract was obtained after mercuration extraction employing ethanol, with extract possessing gummy texture with yellow colour. The high yield of this extract could be due to the high level of solubility of these compounds present in low polar solvent (Yusha'u *et al.*, 2010). There are disparities

in the colour of the extract due to the extraction solvent. The colour of ethanol is yellow, aqueous is greenish brown while n-hexane is lemon has powdery texture and all the extracts had gummy texture which correspond with the work of Yusha'u *et al.* (2010). The results of bioactive compound screening of ethanol, aqueous and n hexane extracts of *A. digitata* using mercuration extraction method indicate the availability of alkaloids, saponins, flavonoid, phlobatanins and terpenoids. Table 2 shows that as the concentration increases the zone of inhibition increases and vice versa. There is significant inhibitory effect on the isolate by n-hexane of which the inhibitory effect was more pronounced on *S. aureus*, *S. typhi*, *C. albican* at all concentrations and less in *C. oxyporum* while no effect was observed on *E. coli*. In addition, ethanolic extract has inhibitory effect on *C. oxyporum*, *S. typhi* and *Staphylococcus aureus* at concentration of

25µg/ml, whereas but not effective on *C. albican* and *E. coli*. While aqueous extract has inhibitory effect at on *E. coli* at concentration of 25µg/ml, *S.aureus* concentration of 50µg/ml and *S.typhi* at concentration of 25µg/ml and no effect on *C.albicans* and *C. oxyforom*. This research work shows that n- hexane has the maximum antimicrobial activity which is due to its polarity and the presence of flavonoids which also show the ability to inhibit microbes which are working against antibiotics by Linuma et al. (1994). Saponins are important type of glycosides which posses soapy characteristics. It has also been made known that saponins are active antifungal compounds (Najafi *et al.*, 2010). Alkaloids are commonly found to have antimicrobial properties (Nahal *et al.*, 2015). The study also revealed that sample is broad spectrum because it inhibits gram negative bacteria, gram positive bacteria and fungi.

CONCLUSION

The findings back up the maximum degree of antimicrobial activity of *Adansonia digitata*. Broad spectrum of antimicrobial activities of this species may assist in the evolution of new sets of antibiotic chemicals that could serve as selective agents against infectious diseases, chemotherapy and control. This study has indicated the certainty of employing this plant developing drug for the treatment of wide range of microbial infection.

REFERENCES

- Abiona D.L., Adedapo Z. Suleiman M.K.(2015) Proximate Analysis, Phytochemical Screening and Antimicrobial Activity of Baobab (*Adansonia digitata*) Leaves. *IOSR Journal of Applied Chemistry*. **8**, (5): 60-65
- Arokiyaraj, S., Sripriya, N., Bhagya, R., Radhika, B., Prameela, L., Udayaprakash, N.K. (2012). High rates of multidrug resistance in *Enterococcus faecalis* and *E. Faecium* isolated from inpatients and outpatients in Taiwan. *Journal of Diagnostic Microbiology and Infectious Disease* **75**, 406–411.
- Craig, G. M. (1991): The Agriculture of the Sudan. Oxford University Press, USA. Pp. 32-58.
- Daniel M (1991). Methods of Phytochemistry and plant economic Botany, Kalyani Publishers, New Delhi.
- De Caluwé E, Halamová K, Van Damme P (2010). *Adansoniadigitata* L. – A review of traditional uses, phytochemistry and pharmacology. *Afrika focus*, **23**: 11-51.
- Harborne J.B.(1973). Phytochemical methods, A guide to modern techniques of plant analysis. London, New York: Chapman and Hall Ltd; p 49-188.
- Igboeldi, L. C., Addy, E.O.H. and Salami, L. I. (1997): Effects of some processing techniques on the antinutrient contents of baobab seeds (*Adansoniadigitata*). *Bioresource Technology* **59** (1): 29 – 31.
- Lardy J and Walsh C (2004). Lessons from natural molecules. *Nature* **432** 829-837.
- Latifou Lagnika, Madjid Amoussa, Yann Adjovi and Ambaliou Sanni (2012). Antifungal, antibacterial and antioxidant properties of *Adansoniadigitata* and *Vitexdoniana* from Bénin pharmacopeia, *Journal of Pharmacognosy and Phytotherapy*. **4**(4), pp. 44-52,
- Linuma M, Tsuchiya H, Sato M, Yokoyama J, Ohyama M, Ohkawa Y, Tanaka T, Fujiwara S, Fujii T (1994). Flavanones with potent antibacterial activity against methicillin resistant *Staphylococcus aureus* qwe. *J. Pharmacol.*, **46** (11): 892-895.
- Maghrani M, Zeggwagh N, Michel J, Eddoules M (2005). Antihypertensive effect of *Lepidium sativum* L. in spontaneously hypertension rats, *Journal of Ethnopharmacology*, **100**(102):193 – 197.

- Marimuthu, J., Mithraja, M.J., Mony Mahesh, M., Paul, Z. M., Jeeva, S., (2011). Phytochemical studies on *Azollapinnata* R. Br., *Marsileaminuta* L. And *Salviniamolesta* Mitch. *Asian Pacific Journal of Tropical Biomedicine* **S26-S29**
- Mohammed S. S. Datsugwai Ashiru S. Yusuf (2017) Phytochemical analysis and antimicrobial activity of baobab (*Adansoniadigitata*) leaves and steam bark extracts on *Staphylococcus aureus* and *Escherichia coli*, *J. BioSci. Biotechnol.*, **6(1)**: 9-16
- Nahal Boudierba Nora, Kadi Hamid, Moghtet Snouci, Meddah Boumediene and Moussaoui Abdellah (2015). Phytochemical and antibacterial screening of *Citrullus colocynthis* of South-west Algeria, *Journal of Chemical and Pharmaceutical Research*, **7(5)**: 1344-1348
- Najafi Shahla, Nima Sanadgol Nima, Batool Sadeghi Njad, Maryam Ashofteh Beiragi and Ehsan Sanadgol (2010). Phytochemical screening and antibacterial activity of *Citrullus colocynthis* (Linn) Schrad against *Staphylococcus aureus*, *Medicinal Plants Research* **4(22)** 2321-2325.
- Ndiaye AOK (2005). Les Enterobacteries secretrices de beta-lactamases à spectre élargi. Thèse de Docteur en Pharmacie. Univ. Cheikh Anta Diop de Dakar. 69p
- Okeke IN, Laximaninarayan R, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF, Pablos Mendez A, Klugman KP (2005). Antimicrobial resistance in developing countries. Part-1: recent trends and current status. *Lancet Infectious diseases* **5** 481-493.
- Samatha T, Ramaswamy N (2015). In vitro preliminary screening of bioactive compounds of different parts of *Adansoniadigitata* L. a globally endangered tree, *International journal of Phytomedicine*; **7(4)**: 402-410. 16.
- Samatha T and RamaSwamy N (2016). Determination of Total Phenolics and Flavonoids in Different Parts of Globally Endangered Tree Species *Adansoniadigitata* L. *Int. J. Ayu. Alt. Med.*; **4(1)**: 24-29.
- Sandhya V. Rodge and *S.D. Biradar (2013). preliminary phytochemical screening and antimicrobial activity of *Citrullus colocynthis*. (linn.) *Indian Journal of Plant Sciences.*, **2 (1)**. 19-23
- Sofowora A, E (1993). Recent trends in research into African medicinal plants. *J Ethnopharmacol*, **38**: 209-14.
- M. Yusha'u, M. M. Hamza, and N. Abdullahi (2010). Antibacterial activity of *Adansoniadigitata* stem bark extracts on some clinical bacterial isolates, *International Journal of Biomedical and Health Science*. **6(3)**: 129 – 135