Confluence Journal of Pure and Applied Sciences (CJPAS)
Faculty of Science, Federal University Lokoja, Kogi State, Nigeria

Vol. 2, No. 1, June 2018
ISSN: 2616-1303 | **Web:***www.cjpas.fulokoja.edu.ng*

SAFETY ASSESSMENT OF SELECTED BRANDS OF TOOTHPASTE IN ZARIA, KADUNA STATE, NIGERIA

Stephen Eyije Abechi*, Elizabert Ogbe, Casimir Emmanuel Gimba

Department of Chemistry,
Ahmadu Bello University, Zaria, Nigeria.
E-mail: ***abeshus@yahoo.com***

ABSTRACT

Toothpaste is a personal hygiene product used on daily basis by many. The chemical composition of toothpaste varies from one brand to another and even among several presentation of the same brand. The manufactures do not inform the consumers of the composition of toothpastes in detail. This work sought to evaluate the safety level of some chemical constituents and the microbial load of selected toothpastes.Ten different brands of toothpastes were analyzedfor physicochemical properties, bacterial load, fluoride, heavy metal levels including titanium concentrations using Standard methods. The pH of these toothpastes was found to range from 5.20 to 8.42. Only 60% of the sampled toothpastes fell within the National Agency for Food and Drug Administration (NAFDAC) recommended pH range of 6.5 to 7.5. The moisture and volatile constituents of the toothpastes were between 28.03% and 46.19%, which are within the range set by NAFDAC. The fluoride concentration of the sampled toothpastes ranged from 770± 13.46ppm to 1220 ±5.00ppm. None of the toothpastes fluoride level was above the maximum limit of 1500 ppm set by NAFDAC. However, three were found to be below the minimum set value of 850 ppm. The results from the microbial load of the toothpastes showed that they were all sterile as no bacterial or fungal isolates were identified in any of the sampled toothpastes. Traces of titanium metals were found in all the sampled toothpastes ranging from 1.44×10^3 mg/kg to 6.25×10^3 mg/g. Traces of Cd, Mn, Ni, Cu and Zn were detected in the toothpaste samples. Lead was below detection limit in all the sampled toothpastes. The presence of Ni, Mn, Cd, Cu and Zn calls for caution in the usage of these products as they accumulated in the body and could build up to the risk level.

Keywords:fluoride, heavy metals, health hazards, toothpaste, physicochemical properties

1.0 INTRODUCTION

A mark of quality of toothpaste will depends largely on the level of its safety for usage. The safety to a large extent is determined by the level of constituent's chemicals and the physicochemical characteristics. Toothpaste are composed of substances which act as abrasive, pigment, foam inducer, humectants, thickener, stabilizer, solvent, sweetener, therapeutic agent and enamel hardener (Souza-Rodrigues *et al*, 2015).

***Corresponding Author**

How to cite this paper: Abechi, S. E., Ogbe, E., &Gimba, C. E. (2018). Safety assessment of selected brands of toothpaste in Zaria, Kaduna State, Nigeria. Confluence Journal of Pure and Applied Sciences (CJPAS), 2(1), 48-55.

The primary purpose of brushing the teeth with a dentifrice is to clean the accessible tooth surface ofdental plaque, stainsand food debris. Modern developments in toothpaste formulation have led to the addition of agents to provide therapeutic, as well as cosmetic benefits, thereby exposing consumers to possible health problems. Toothpaste contains largely of fluoride, titanium, heavy metals as impurity, and microbes as a result of deterioration. Physicochemical parameters such as pH and moisture content form part of quality assessment of toothpaste (***Mangilal and Ravikumar, 2016)***.The pH value of toothpaste gives indication of the inorganic ingredients present,such as calcium carbonate and phosphate (**Bhat, and Teki, 2013).** Physicochemical

Vol. 2, No. 1, June 2018
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properties have been used to evaluate safety of toothpastes in literatures (***Mangilal and Ravikumar, 2016;*** Sekar and Ariffin, 2016; **Mohammad *et al*, 2017**).Iqbal*et al*. (2011) formulated toothpaste and evaluated the standard physiochemical parameters along with the antimicrobial activity.

Use of fluorides has been the foundation of caries counteractive action and the use of fluoridated toothpaste is the most widely recognized types of caries control being used today (***Mangilal and Ravikumar, 2016)***. Fluoride and its derivatives are used to strengthen the enamel and prevent cavities (Sekar and Ariffin, 2016). However, Studies have shown that overconsumption offluoridated toothpastes among children is associated with health risks such as fluorosis, which causes changes in tooth enamel ranging from spots to stains to deep pitting depending on the severity (Souza-Rodrigues *et al*, 2015; Corey and William, 2017). Toothpaste used multiple times a day, and often swallowed by young children, poses risks for bioaccumulation(Corey and William, 2017).Chronic fluoride intake is capable of decreasing insulin signal and causing insulin resistance. The use of denfrices with lower fluoride content is therefore recommended, especially for diabetic children for whom excessive fluoride consumption may lead to health implications (Chiba, 2012). Acute fluoride poisoning which occurs at doses as low as 0.1 to 0.3 mg per kg of body weight generally presents in the form of gastric pain, nausea, vomiting, headache, dizziness and flu-like symptoms (Akiniwa 1997, Gessner, 1994). A child weighing 10kg would only need to ingest 1 to 3grams of paste to experience one or more of this symptoms.

Heavy metals are not acceptable as ingredient in personal hygiene products. Compounds of these metals however, are sometimes added as ingredient in toothpastes. Zinc and copper compounds are added to prevent dental plaque formation. In large amounts, ingestion of heavy metals can be dangerous. Generally, humans are exposed to these metals by ingestion (drinking or eating) or inhalation (breathing). A more common source of human exposure is in the use of oral health products like toothpastes, which is used on daily basis.In all cases, however, the prevailing concentration of heavy metals in toothpaste should not be above 20mg/kg (Popova and Marinova, 2007). Titanium oxide is added as whitening and thickening agent in toothpaste. There is no known biological role for titanium. There is a detectable amount of titanium in the human body and it has been estimated that we take in about 0.8 mg/day. However, it passes through the human system without being adsorbed. It is not a poisonous metal and the human body can tolerate titanium in large doses.

Toothpastes are not all identical in composition and should be indicated according to each patient's needs. Differences in the presence and/or concentration of all these components in toothpastes may cause undesirables side effects (Souza-Rodrigues *et al*, 2015). The addition of unnecessary and potentially toxic ingredients such as sweeteners, artificial colorings, flavorings and other additives that facilitate the composition of toothpaste in appearing more like a food product do not improved oral health (Corey and William, 2017). The majority of all the unnecessary ingredients were found to have known contamination risks (Corey and William, 2017). Information on oral health implications of the toothpastes in terms of their claims and the claim ingredients in their respective formulations is scanty. There is also the need for continuous monitoring of the level of these chemicals ingredients in both the old and new products in the market to keep the manufacturers in check and to ensure protection of consumers. This work sought to evaluate the level of some physicochemical properties and the microbial load of selected toothpastes in the open market in Zaria-Nigeria.

2.0 MATERIALS AND METHODS

All chemicals used were BDH products of analytical grade purity.All solutions used for these analyses were prepared usingdeionizeddistilled water.

2.1 Sample Collections and Preparation

Ten (10) brand of widely distributed toothpaste samples were purchased from open/local markets in

Zaria-Kaduna State, Nigeria and label A to J. The ten toothpaste samples consisted of six (6) locally made and four (4) imported toothpastes. All the samples were in molten form (gel-like).

2.2 Moisture and Volatile Content

Two grams of toothpaste was weighed in a suitable tarred dish. It was then dried at 105±2°C in oven to constant weight. The moisture and volatile matter percent by weight was then calculated thus;

$$\frac{(M - M_1)\ x\ 100}{M}$$

Where, M_1 = weight in grams of the material after drying, M = weight in grams of the paste taken for the test.

2.3 Determination of pH Value

Ten grams of the toothpaste was weighed in 100cm^3 beaker. 45cm^3 of freshly distilled and cooled water was then added and mixed well. The pH of the solution was determined using a pH meter (JENWAY 3505).

2.4 Microbial Purity Test

The Nigerian Industrial Standard (NIS) method for microbiological examination for toothpaste was used to determine the approximate bacterial load of each sample. The first 5cm^3 of toothpaste was squeezed out and discarded, so as to ensure that the paste used for this analysis is not contaminated, since it is possible that the paste might have been exposed to bacteria from the environment. 1g of the paste was then weighed into 9cm^{3}of 0.1% peptone water (diluents). It was then shake until the paste was evenly suspended. Petri dishes containing the sterile plate count agar were heated in boiling water until the agar has completely melted. The containers were then allowed to cool to 45°C and kept in a water bath at 45°C until required. 0.1cm^3 aliquots of the sample after shaking was transferred to a petri dish each and incubated for the specified periods to permit the development of visual colonies for counting

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2.5 Determination of Fluoride Content

Fluoride concentration of the sampled toothpaste was determined using the HI 83200 multi-parameter bench photometer. One gram of dried toothpaste was dissolved in 100cm^3 distilled water in a flask. The (1cm^3)solution was carefully transferred into a sample cuvette, 4 drops of alizarin and zirconylacid were then added and the solution made up to the mark of the cuvette. The blank solution was prepared in the same way but without the sample. 4 drops of alizarin and 4 drops of zirconyl acid were added into another cuvette and made up to the mark with distilled water. The cuvette caps are then replaced and inverted several times to mix. The fluoride content was then determined using the HI 83200 multi-parameter bench photometer

2.6 Determination of Titanium

Five gram of the toothpaste was weighed and oven dried. It was then crushed and one gram was weighed and pelletized. The concentration of titanium was determined using XRF.

2.7 Determination of Heavy metals

Sample preparation for heavy metals in toothpastes was done according to the method by Popova and Marinova (2007). 1g of toothpaste weighed in a crucible was heated cautiously on a sand bath, until the evolution of vapour seizes, after which, 1cm^3 of concentrated HNO_3 was added until the absence of nitrogen oxide was detected. The residue was introduced into a cold furnace and the temperature was increased at 100°C / hour up to 800°C. The ash was treated with 1cm^3 HNO_3 (1:1) and let to fume carefully on a sand bath to dry. The residue obtained is then dissolved in hot distilled water, acidified by the addition of several drops of HNO_3, and filtered through a filter into a 50ml volumetric flask and made up to the mark. The concentrations of Pb, Cd, Mn, Zn, Ni and Cu in the toothpaste were then determined using AAS.

3.0 RESULTS AND DISCUSSION

3.1 Bacteriological Examination

The analysis of the microbial load of the toothpastes (Table 1) shows that they were all sterile as no

bacterial or fungal isolates were identified in any of the sampled toothpaste. This result is as expected as the presence of bacteria in toothpaste poses high health risk to human. This effect includes nausea, vomiting, stomach pains, diarrhea, weakness, and fever or chills/sweating, headache. According to the Standard Organization of Nigeria (SON) standard for toothpaste, the tolerable limit for the total viable count (TVC) of microorganisms per gram of toothpaste is 300cfμ/g. E. coli, Salmonella and *pseudomonas arugenisa* must be absent. Hence, the result obtained from the present study shows that the microbial load of the toothpastes meets the criteria set by Standard Organization of Nigeria.

Table 1: Results obtained from bacteriological examination of toothpaste.

Sample label	TVC	E.Coli	Salmonelia	*Peudomonas arugenisa*
A	0.00	Nil	Nil	Nil
B	0.00	Nil	Nil	Nil
C	0.00	Nil	Nil	Nil
D	0.00	Nil	Nil	Nil
E	0.00	Nil	Nil	Nil
F	0.00	Nil	Nil	Nil
G	0.00	Nil	Nil	Nil
H	0.00	Nil	Nil	Nil
I	0.00	Nil	Nil	Nil
J	0.00	Nil	Nil	Nil
WHO/SON	300	Nil	Nil	Nil

NIL: Not detected.

3.2 Physicochemical Parameters of the Samples

The pH of the toothpastes was found to be between 5.20 and 8.42 (Figure 1). Sample H, a herbal toothpaste was found to be alkaline; it had the highest pH value of 8.42. About 40% of the sampled toothpaste are acidic (with pH values are less than 7), 40% are neutral and about 20% are basic (with pH values greater than 7). The pH value gives an indication of the inorganic constituents in toothpaste. Acidic pH encourages the growth of mouth bacterial that causes dental carries (Oyewale, 2005). According to the Nigerian Industrial Standard, recommended pH for toothpaste is between 6.5 and 7.5. Only 60% of the sampled toothpaste fell within this range.

The moisture and volatile matter constituents of the toothpastes analyzed were between 28.03 and 46.19. The tolerable limit for the moisture and volatile matter in toothpaste as specified by SON is 50%. All the sampled toothpastes were found to conform to this specification. The summary of these results are as shown in Figure 1.

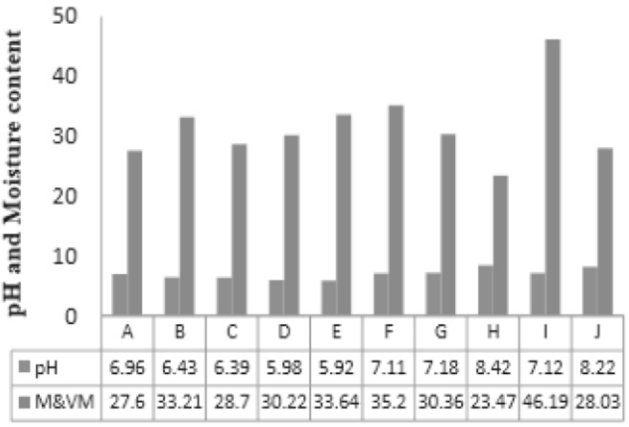

Figure 1 pH and moisture content of toothpastes.

Figure 1 shows that the pH obtained for the imported toothpastes sampled, conform to the pH specification for toothpastes, except for E (5.92±0.001) and J (8.22±0.002) which are slightly below and slightly above the minimum and maximum specified values respectively.

3.3 Fluoride Concentration in Toothpaste Samples

The average fluoride content obtained from three determinations for each of the toothpaste is as shown in Figure 2. The fluoride concentration of the toothpastes was found to be between 770±13.46 ppm and 1220±5.00 ppm. The Claimed Active Fluoride Ingredient (CAFI) and their fluoride content in percentages (%) as well as the Claimed Equivalent Fluoride Concentration (CEFC) in ppm, Calculated Fluoride Concentration (CFC) for each of the ten toothpaste sampled and the allowed permissible levels of fluoride in toothpaste as set by NAFDAC is as shown in Table 2. According to the National Agency for Food and Drugs Administration and Control (NAFDAC) and Standard Organization of Nigeria (SON), the recommended minimal and maximal permissible levels of fluoride in toothpaste are 850 ppm and 1500ppm, respectively. As can be seen from Figure 2, the highest fluoride concentration was observed

Vol. 2, No. 1, June 2018
ISSN: 2616-1303 | **Web:***www.cjpas.fulokoja.edu.ng*

in the sample labeled C (1220±5.00) with D having the least fluoride concentration (770±13.46). Although, toothpaste samples labeled A, E and F were claimed to contain fluoride as high as 1100ppm, 1450ppm and 1450ppm respectively.

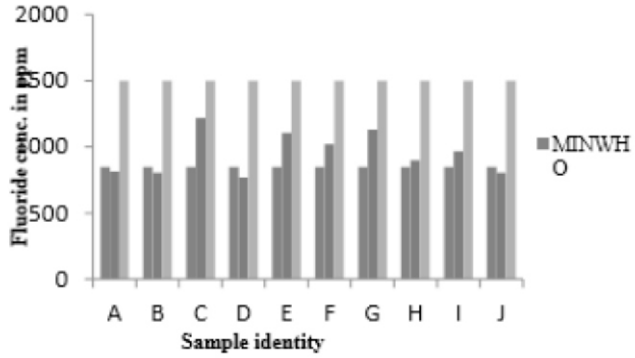

Figure 2: Comparison of fluoride concentration of the sampled toothpaste with standards

The result of the analysis showed that the fluoride concentration in these toothpastes were not actually as claimed. The fluoride levels in the toothpastes were 816.67±20.82, 1106±33.00 ppm and 1021 ±33.00ppm respectively.

Table 2: The claimed fluoride content and fluoride concentrations of the toothpastes.

Sample ID	Sample Type	CAFI	CAFC (%)	CEFC (ppm)	CFC(ppm) ±SD
A	Local	NaF	NS	1100	816.67±20.82
B	Foreign	$NaF.PO_4$	0.76	NS	805.00±8.66
C	Local	NaF	0.32	NS	1220.00±10.00
D	Local	NaF	0.306	NS	770.00±26.46
E	Foreign	NaF	NS	1450	1106.67±11.54
F	Foreign	NS	NS	1450	1021.67±17.55
G	Local	$NaF.PO_4$	NS	NS	1130.00±26.45
H	Local	NS	NS	NS	900.00±20.00
I	Local	$NaF.PO_4$	0.85	NS	966.67±30.55
J	Foreign	NS	NS	NS	805.00±33.00
NAFDAC	NS	NS	NS	850-1500	-

The concentration of fluoride in A, B, D and J were found to be below the minimum permissible limit set by NAFDAC. The concentration of fluoride in toothpastes depends on such factors as presence of ingredients which reacts with the fluoride to form insoluble compounds and therefore makes fluoride unavailable to fulfill its therapeutic function[6]. All the toothpastes sampled were purchased from the open market and these samples could have been exposed to temperatures as high as 32°C which could lead to the degradation of these samples. This may as well contribute to the non-conformance of A, B, D, and J to the acceptable standard. The fluoride levels of toothpastes C, E, F, G, H and I (60% of the sampled toothpastes) as analyzed, conforms to the limit set by the regulatory bodies.

The fluoride levels in these toothpastes are within the specified limits. However, it is risky to consume even as low as a pea size of the toothpaste since mild exposure to fluoride (6-8ppm in water) is known to have adverse effect on human, especially children, pregnant women and old people (Adejumo*et al*., 2010). It is now estimated that blood fluoride level of just 95ppb produce an increase in glucose level and a decrease in insulin (Menoyo*et al*., 2005). Strikingly, this level is routinely exceeded by about 5 to 10% of children using fluoride toothpaste (Daniel and Agho, 2010, Akpata*et al*., 2006). Figure 2 shows the fluoride levels of the samples as compared with other standard.

3.4 Titanium and Other Heavy Metals.

Titanium metal and other selected heavy metals were detected in all the sampled toothpaste, with the exception of lead, which was below the detection limit. X-Ray spectrophotometer was used to analyze titanium, while Atomic Absorption Spectrophotometer was used to determine Pd, Cd, Ni, Cu, Zn and Mn in the samples.The result obtained from these analyses showed that all the toothpastes sampled contained some levels of titanium in the range of 1.44×10^{3}mg/kg to 6.25×10^{3}mg/kg, with toothpaste labeled D having the highest titanium concentration of 6.25×10^{3}mg/kg.The concentrations of titanium in the sampled toothpastes were found to be very

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ISSN: 2616-1303 | **Web:***www.cjpas.fulokoja.edu.ng*

similar, except for D, a local toothpaste, which had a very high titanium concentration of 6.25×10^{3}mg/kg (Figure 3). C and I had the lowest concentration of 1.44×10^{3}mg/kg.

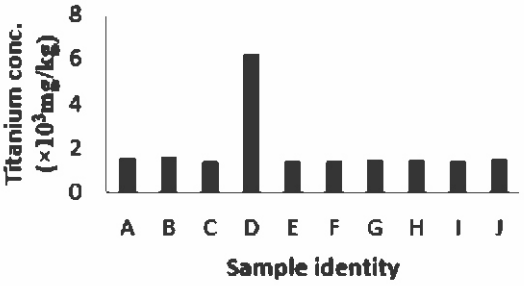
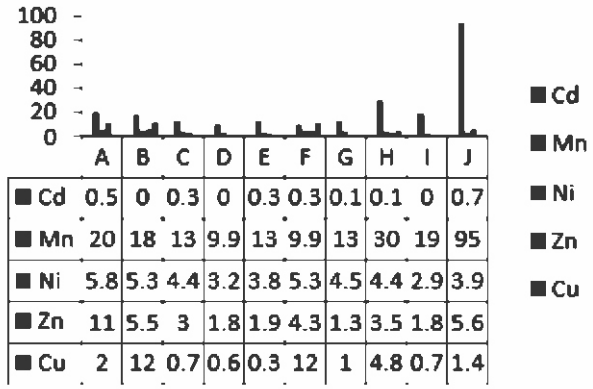


Figure 3: Concentration of titanium in the sampled toothpastes.

The result obtained from the analysis of other heavy metals is as shown in Figure 4. Lead was absent in all the toothpaste samples, while traces of Cd, Ni, Zn, Cu and Mn were detected in all the sampled toothpastes. This can be due to the persistent nature of these elements as contaminants, while some of them were actually present in some materials used in the manufacture of the toothpaste. Pepper mint herb, used in toothpaste is an excellent source for manganese and copper.



	A	B	C	D	E	F	G	H	I	J
Cd	0.5	0	0.3	0	0.3	0.3	0.1	0.1	0	0.7
Mn	20	18	13	9.9	13	9.9	13	30	19	95
Ni	5.8	5.3	4.4	3.2	3.8	5.3	4.5	4.4	2.9	3.9
Zn	11	5.5	3	1.8	1.9	4.3	1.3	3.5	1.8	5.6
Cu	2	12	0.7	0.6	0.3	12	1	4.8	0.7	1.4

Figure 4: Concentration of the selected heavy metals in the sampled toothpastes (mg/kg)

Some compounds of these elements are sometimes deliberately added as ingredients in the toothpaste e.g. zinc and copper compounds are added to prevent dental plaque formation.

The presence of cadmium in the samples were in trace amount (0.05-0.7mg/kg) in the sampled toothpastes and was absent in others (B, D and I). Even though cadmium level in the toothpaste is very low, it is good to note that, the slow release of cadmium may also cause harm to the human body. It targets blood vessels and heart tissue, as well as the kidneys, lungs and brain, and results in heart disease, hypertension, liver damage, suppressed immune system and other nasty symptoms (Ashworth and Allowway, 2004).

Among the different samples analyzed, manganese was found at higher concentration and ranged from 9.9±0.0005 to 94.5±0.0005mg/kg. Manganese is required in small amounts in the human body, it has been recommended that an estimated level of 2 to 5 mg of manganese per day is safe and adequate intake for adults, meaning that, the consumption of as low as one gram of toothpaste is enough to pose danger to one's health.

The level of nickel in the sampled toothpaste fall within the range of 2.85 ± 0.0004 and 5.8 ± 0.0004 with the selected foreign toothpastes having a higher average concentration of 4.538 ±0.827. These concentrations are not very alarming, but it is necessary to avoid swallowing toothpastes while brushing, as this metal have the ability to accumulate in the body and this can be harmful to the human body system. Ingesting too much nickel can cause gastrointestinal distress, increased red blood cells, kidney stress, chronic bronchitis, reduced lung function and in some cases lung cancer. There is currently no recommended daily intake for nickel, however, according to the national agricultural library; the tolerable upper in take level of nickel is one milligram in adults.

The level of zinc in the toothpaste analyzed ranged from 1.3 ± 0.0002 mg/kg to 11.05 ± 0.0004 mg/kg. Zinc compounds such as zinc citrate is often used in toothpaste and mouth wash to prevent dental plaque formation and gingivitis. However, only a small amount of zinc is required by the human body. Comparison of the average concentration of zinc in both the foreign and local toothpastes showed that the foreign toothpaste has higher concentration of zinc (4.413 ±1.814) than the local toothpastes (3.725 ± 3.357).

The concentration of copper in the sampled toothpastes ranged from 0.3±0.0002 mg/kg to 12.05±0.0001mg/kg. The average daily requirement

for copper in the adult human has been established at 2mg and for infant and children at 0.05mg/kg bw.

4.0CONCLUSIONS

The pH of the toothpastes was found to be between 5.20 and 8.42. Only 60% of the sampled toothpastes have their pH within the set standard. The moisture and volatile matter constituents of the toothpastes analyzed fell within 28.03 and 46.19 %, and below the upper limit of 50%. The fluoride level in these toothpastes are within the specified limits and there was no case of microbial contamination. The result obtained from the analysis of heavy metals indicated thatPb was absent in all the toothpaste samples, while traces of Cd, Ni, Zn, Cu and Mnwere detected in all the samples. Toothpaste therefore, serves as a source of human exposure to heavy metals. These metals could accumulate in the body of users of toothpaste to a dangerous level, hence caution must be taken in the use of these products.

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Vol. 2, No. 1, June 2018
ISSN: 2616-1303 | **Web:***www.cjpas.fulokoja.edu.ng*

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