Synthetic antioxidants and metallic elements as additives/contaminants in virgin palm oil

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Abstract

This study evaluated the quantitation of synthetic phenolic antioxidants and trace elements in virgin palm oil. A Reverse Phase High Performance Liquid Chromatography coupled with Ultra Violet Visible detection spectrophotometry was employed for the analysis of synthetic antioxidants. The synthetic antioxidants studied were Butylatedhydroxyanisole (BHA), Butylatedhydroxytoluene (BHT), 2-ethylhexyl 4 methoxycinnamate (EEMC) and Propyl gallate (PG). A proposed ICP-OES using oil-water micro emulsion as sample preparation method was used for metallic element analysis because it is not only reproducible, accurate and reliable but also convenient because it provided a sample extraction capable of breaking down the complex matrix in virgin palm oil. The limits of detection for BHA, BHT, EEMC and PG were found to be 0.041, 0.057, 0.06 and 0.03 mg/L respectively while the limits of quantification values were 0.56, 0.49, 0.05 and 0.04 mg/L respectively. None of the synthetic antioxidants was detected in the samples studied. This was an indication that local farmers probably preserved the oil samples prior to storage by traditional methods. The elemental analysis of virgin palm oil is necessary to decide if the oil is suitable for food, fuel or raw materials for the chemical industry. The concentrations of metallic elements found in the oil samples analysed in this study ranged between 0.006 mg/kg in Ubiaja plantation and 161.576 mg/kg in Benin City plantation respectively. Tin recorded high concentration in Benin City plantation and Pb a low concentration in Ubiaja plantation. The concentrations of metallic elements were rather high in most samples hence the oil is best suitable for use as raw material for the chemical industry. It can therefore be concluded that the level of environmental pollution on the plantation be eradicated or reduced to the barest minimum to improve the quality of the virgin palm oil for human consumption.

Keywords: Synthetic antioxidants, Virgin palm oil, Micro emulsion techniques, Metallic elements, RP-HPLC, ICP-OES

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Introduction

*Elaeis guineensis* commonly known as African virgin palm oil, having pinnate leaves with dense cluster of flowers and produces thick bright red liquid. It is a cash crop, which thrives in the tropics and native of West Africa, such as in Nigeria and others like Sri Lanka, Malaysia and Indonesia. It is important in food processing and in industry. Over fifty percent of packaged and processed foods, grocery products and cosmetics in stores contain the ingredient palm oil. Palm oil can be described as a miracle ingredient found in shampoos to biscuits and it always increase the aesthetics of products. It is versatile in the production of food because it has a unique blend of fatty acids, carotenoids and tocopherols (Adeniyi et al., 2014). It is an essential ingredient in most processed foods, (confectionaries and milk products) non-food productions (household cleaning products and cosmetics) and as a raw material for the production of biodiesel. There is a growing need for this essential vegetable oil as the world population increases extensively and there is a desire for good health and improved diets (Mancini et al., 2015). Being an essential crop for food and non-food uses, it becomes paramount to monitor the processing of the virgin oil from planting to storage of the finished products. Auto-oxidation usually sets in when edible oils are stored prior to usage. This can be seen in the deterioration of the aesthetics of the oil. Antioxidants may be added to the virgin palm oil, either natural or artificially before storage to prevent the onset of auto-oxidation and oxidative degradation of the oils during storage. Mancini et al. (2015) reported that synthetic antioxidants act as reaction inhibitors that catalyse oxygen through the transfer of hydrogen and hence inhibiting the chemical reactions which favour the production of aldehydes, ketones and peroxide radicals which are major substances of auto-oxidation. The applications of antioxidants range across a wide variety of products such as fruit concentrates and juices, snacks, confectionaries and meat. Usually manufacturers and local farmers may use antioxidants in the preservation of their products and consumer remained unaware. Local farmers may add these additives to prolong the shelf life. This could be without adequate analytical measurements or quality control. Possible health risks are associated with the intake of synthetic antioxidants.

The increased concentration of synthetic antioxidants in various analyses from various countries in foods is not strictly recognized. Synthetic antioxidants are chemically synthesized since they do not occur naturally in nature and are more harmful than beneficial to man (Kamar, 2014). At low and permitted concentrations, synthetic antioxidants could be helpful in the prevention of spoilage of food in addition to possessing anti-inflammatory and antiviral properties. Regulatory bodies responsible for the administration and control of synthetic antioxidants have not been very effective especially in developing countries. The choice of synthetic antioxidants by the manufacturer is dependent on many factors that range across value regulation and its efficacy (Akkbik et al., 2011; Pezo et al., 2008). Other synthetic antioxidants have been banned at some point in time in various countries. Generally, the guidelines for the administration and control of synthetic antioxidants vary from country to country (Pezo et al., 2008). This is a very good reason why the use of synthetic antioxidants in foods should be checked and kept under control, hence the need to assess them in this study (Hamid et al., 2010; Ayodele and Oluyomi, 2011; Itumoh et al., 2013).

Certain contamination may also arise from the oil palm plantation situated close to domestic and industrial sources of pollution (Asemave et al., 2011). The determination of elements in virgin palm oil is of economic importance because palm oil is a very important cash crop for the food and chemical industry. It is essential to determine the presence of trace and major elements in palm oil as this is one of the criteria for oil quality. Metallic elements in palm oil are deleterious because this affects the shelf life and aesthetic quality of the oil. Elements in the oil enhance the oxidation of fatty acids to esters, which affects the nutritional value and the properties of the oil. Trace elements are toxic at elevated concentrations. However, trace elements are essential to man, their presence in palm oil, which is a raw material for a variety of food and chemical industries may not be ideal for production processes (Bradhaw and Thompson, 2007). This is particularly an issue in the production of biodiesel due to the presence of such metals which consequently affects the quality of the oil.

Hence, the objective of this study is the determination of four synthetic antioxidants (BHA, BHT, EEMC and PG) and metallic elements (Al, Co, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se, Sn and Zn) in virgin palm oil from fifteen independent sampling locations.

To the best of our knowledge, research has not been conducted on the simultaneous analysis of BHA,
BHT, PG and EEMC in virgin palm oil samples using HPLC and also trace elements in virgin palm oil with micro emulsion as sample preparation method by ICP-OES; both methods using UV/Vis detection techniques. The analyses provided accelerated and precise methods for the qualitative and quantitative study of synthetic phenolic antioxidants and trace elements in virgin palm oil sampled from 15 locations on oil palm plantations in south-western Nigeria.

Material and Methods

Sampling and chemicals
Virgin palm oil was sampled from fifteen locations that included the Nigerian Institute for Oil Palm Research (NIFOR) and its substations starting from 2016. More samples were collected from areas in the southern states of Nigeria where the oil palm tree was extensively cultivated. Sampling points were partitioned into four sections and a sample was collected from each section to make a grab sample. Sampling was also based on factors such as densely populated areas and areas receiving intense pollution stress. The sampling containers were sterilized and the samples preserved in a refrigerator prior to analysis in the laboratory. All chemicals were purchased from Sigma Aldrich (USA).

Preparation of micro emulsion for metal analysis
The micro emulsions were made with water, oil and alcohol and in some instances, a surfactant or a combination of co-surfactants such as medium chain alcohols propanol, butanol, pentanol, hexanol is used as a base. Additionally, the micro emulsion particles were homogenized in order to form 5 - 100 nm in size emulsion size (Xiu-Qin et al., 2009). 0.5 g of oil samples was weighed into a 100 mL polypropylene tube with lid. HCl was added (c.a 2 mL) and propan-2-ol to a final volume of 10 mL. The mixture was shaken vigorously in a shaker with specifications of an orbit and speed range of 4 mm and 4000 rpm respectively. The mixture was shaken for 30 minutes to obtain an evenly dispersed homogenous emulsion that remained stable when left for about four hours (Bradshaw and Thompson, 2007). This was kept in the refrigerator at 4°C for metallic element analysis using the ICP-OES.

Extraction procedure for virgin palm oil for synthetic antioxidant analysis
The modified extraction method was proposed by Capitan-Vallvey et al. (2002) for the determination of BHA, BHT, PG, and EEMC method was employed. Virgin palm oil samples (0.5 g) from fifteen sampling locations were weighed in individual centrifuge tubes. Acetonitrile and hexane in a ratio of 3:1 was mixed in each tube and the contents of the tubes homogenized for about 5 minutes at 1800 rpm and further homogenized at 4000 rpm for an additional 5 minutes. Centrifuged samples were allowed to separate into two layers and the non-polar phase (acetonitrile phase) was collected and extracted thrice using the same extraction technique. The re-extracted phases were again collected a 10 mL flask and volume was made to 10 mL with acetonitrile. This was kept in the refrigerator prior to HPLC analysis.

Preparation of stock solution standards for synthetic antioxidants and metal analysis
Quality type water (reverse phase osmosis) was used throughout the analysis. A concentration of 5000 mg/L of stock solution was used for the analysis and was prepared by dissolving 1250 mg/L of each of the antioxidants comprising of BHA, BHT, PG and EEMC in a 100 mL standard flask and made up to the mark with acetonitrile. 10 µg/mL of solutions were prepared to be used from the stock solution. The repeatability of the method was checked using six injections of the working standard. The linearity was determined in the range of 0.3 - 11 µg/mL. The recovery of the method was achieved by spiking 50 mg/kg of each antioxidant and analysed using similar methods (Capitan-Vallvey et al., 2002; Capitan-Vallvey et al., 2004; Reichenbach et al., 2009).

HPLC analysis and instrumentation
An ODS C18 Chromatographic column with a particle size of 5 µL was used (4.6 x 250) cm. An Isocratic system with mobile phases of 75:25 v/v methanol: water mobile phase was used. The Agilent Technologies (1200 series) RP-HPLC Instrument used for the analysis was equipped with UV/Vis detector and quaternary pumps. The maximum wavelength was 280 nm at 1 mL/min flow rate.

ICP-OES analysis and instrumentation
The Inductively Coupled Plasma Optical Emission Spectrometer (Spectro Arcos FHS 12 ICP-OES) equipped with radial viewed plasma was used for the research. The instrumentation has been extensively discussed in Olafisoye et al. (2014).
The proposed ICP-OES analysis using oil-water micro emulsion as sample preparation method was not only reproducible, accurate and reliable but also equally convenient because it provided a sample extraction capable of breaking down the complex matrix in virgin palm oil for metallic element analysis by ICP-OES (Perrin and Meyer, 2003; Maruyama et al, 2014).

**Results and Discussion**

**High performance liquid chromatography analysis for the determination of synthetic antioxidants**

**Identification assignment and integration of peaks**

![Figure 1a](image1.png)

Figure 1a: shows the chromatograph of the standard calibration curve for BHA. The retention time of BHA was within 3-4 minutes at a flow rate of 1mL/min and 50 µL injection volume.

![Figure 1b](image2.png)

Figure 1b: presents the retention time for BHT which was within 8 minutes at a flow rate of 1mL/min and 50µL injection volume.

![Figure 1c](image3.png)

Figure 1c: shows the retention time for EEMC which was within 15-16 minutes at a flow rate of 1mL/min and 50 µL injection volume.

![Figure 1d](image4.png)

Figure 1d: shows the chromatograph of the standard calibration curve for PG. The retention time of PG was within 2 minutes at a flow rate of 1mL/min and 50 µL injection volume.

The aim and objective of qualitative analysis is to identify what type of analyte is contained in the sample.

In this analysis, this was achieved by the use of reference standards. Figures 1 - 4 show the chromatograms of BHA, BHT, EEMC and PG respectively. The optimum wavelength of UV/Vis spectrometer for BHA, BHT, EEMC and PG shows maximum absorption between 275 nm and 300 nm. The UV/Vis wavelength for the RP-HPLC was fixed at 280 nm for simultaneous determination of the four antioxidants. The identified sample components were known and the peaks within the chromatogram were assigned to the known components. Hence, standards of the pure components were injected into the HPLC-UV instrument under identical conditions and peaks were assigned based on the retention times of the standards with the UV/Vis detector. The retention factor and the response of the peaks in the instrument were compared.
with the sample chromatogram. The concentrations of the standard solutions were matched to that of the real samples as peak mis-assignment due to shape defects were avoided as much as possible (Capitan-Vallvey et al., 2002; Capitan-Vallvey et al., 2004; Reichenbach et al., 2009).

The analysis was carried out in the reverse order and a more vigorous peak assignment and confidence was achieved.

### Spiking

The samples were spiked with standards for the confirmation of the identity of the sample component peaks. Table 1 presents the results of the recovery study for BHA, BHT, EEMC and PG by RP-HPLC-UV/Vis at $\lambda_{\text{max}} = 280$ nm.

Table 1: Recovery study for BHA, BHT, EEMC and PG

| Spiked (mg/L) | BHA | BHT | EEMC | PG |
|--------------|-----|-----|------|----|
| 1            | 126.2 | 103.4 | 151.7 | 101.3 |
| 2            | 108.7 | 99.4  | 109.2 | 95.3 |
| 5            | 83.4  | 77.3  | 81.7  | 89.4 |
| 10           | 62.1  | 61.3  | 66.8  | 59.4 |
| 15           | 59.9  | 44.8  | 55.2  | 50.8 |

### Quantitative identification

Matching the recorded spectra of the standard solution with that of the sample spectrum was used as a quantitative determination of the peak assignment. Quantitative peak areas were used to calculate the concentration of the compounds in the samples. Standard solutions (10) of BHA, BHT, EEMC and PG of multi linear concentrations of 1.5, 10, 15, 25, 50, 75, 100, 125 and 250 mg/L were prepared in acetonitrile. The peak areas of the four antioxidants standards (BHA, BHT, EEMC and PG) were plotted against the concentrations to obtain a wide concentration range in the samples. The samples were analysed to obtain instrument response and the response was compared with the responses obtained from the four standards. The quantitative determination of the compounds in the samples was achieved using this method under identical conditions.

### Requirements of the chromatogram

The accuracy of the quantification was affected strongly by the resolution of the peaks and the nature of the baseline around the peaks under investigation. Well separated peaks were easy to reproduce and integrate. In addition, measures were taken to ensure that other interfering peaks do not affect the integration or height of the peaks of interest.

The results show that some of the peaks were eluted on sloppy and noisy baseline that made reproducibility difficult. Other peaks were eluted with split or shoulders and tailings. The baseline and perpendicular method was used to resolve the abnormality. From the calibration curve, the concentration of the sample was calculated using the formula in equation (1).

$$\text{Concentration of sample} = \text{Response} \times RF \times M \times X$$  \hspace{1cm} (1)

Where Response is the peak area; RF is the response factor got from the calibration curve; M is a multiplier, which takes into account the purity of the standard, while D is the dilution factor (Capitan-Vallvey et al., 2002; Capitan-Vallvey et al., 2004; Reichenbach et al., 2009).

### Information from the calibration curve

#### Limit of detection (LOD)

This was calculated as the minimum concentration of the synthetic phenolic antioxidant that can normally be detected in the sample. LOD was expressed as the concentration of the lowest measure in the analysis. The samples, standards and blanks were analysed under identical conditions.

#### Limit of quantification (LOQ)

This was the lowest concentration of the synthetic phenolic antioxidant in the sample where positive identification was measured for quantification. It is thrice the LOD. Table 2 shows the validation of the analytical procedure.

| Compound | Retention time (mins) | Calibration Equation | $R^2$ | RSD (%) | LOD (mg/L) | LOQ (mg/L) |
|----------|----------------------|----------------------|-------|----------|------------|------------|
| BHA      | 3.56                 | y=1933x+12722        | 1.00  | 0.03     | 0.041      | 0.56       |
| BHT      | 15.58                | y=95893x+4268        | 0.99  | 0.09     | 0.057      | 0.49       |
| EEMC     | 8.55                 | y=20773x+2089        | 0.99  | 0.05     | 0.006      | 0.05       |
| PG       | 2.22                 | y=1941x+4903         | 0.99  | 0.07     | 0.003      | 0.04       |

### Analysis of real samples

Fifteen different virgin palm oil samples from independent palm oil plantations in the southern states...
of Nigeria were analysed. The samples were analysed for BHA, BHT, EEMC and PG contents. The real samples were analysed in triplicates.

The retention factors and response of the peaks presented in all the samples were compared against that of the standards for BHA, BHT, EEMC and PG. The peaks of the real samples could not be assigned tentatively. There was no agreement with the peak of the samples and standards since all the antioxidants under studies were not detected in the analysis of the real samples. Hence, there was no close match between the chromatograms of the real samples and standards. Hitherto quantitative analysis of the real sample peaks was also integrated and identified for the determination of the concentration of the samples. The response of the samples containing the unknown concentration was also matched against that of the response of standards to determine the amount of antioxidants present. This was closely observed to avoid peak mis-assignment due to peak size and shape effects. The analysis was performed under a reverse phase condition to increase the confidence of the results. None of the four antioxidants was detected in any of the real samples studied.

**Inductively coupled plasma optical emission spectroscopy analysis for the determination of metals**

Table 3a and 3b showed the results. Detectable levels of the metallic elements were observed as most of them exceeded the WHO permissible limits for metallic elements in vegetable oils (WHO, 2011). Lead is not essential to man neither is it beneficial in any form. Environmental and health problems are caused by lead metals. Adverse effect of lead is seen in mental retardation and biological toxicity in children. The concentration of lead recorded was highest in Onishere plantation (0.847 mg/L). The World Health Organisation, WHO (2011) permissible limit for lead in vegetable oils is 0.01 mg/L and most of the palm oil samples analysed recorded elevated levels above this limit. An exception was Ubiaja plantation that recorded 0.006 mg/L. Sources of lead in the environment are from the burning of fossil fuels, mining and manufacturing industries whose main raw material is lead. Such industries include the paint industries, leaded petrol or gasolines servicing and drilling industries, coal burning and metal smelting industry, lead cell battery industry, lead mines, lead paints and plumbing industries. Concentration of lead in the environment could also be from anthropogenic sources such as the exhaust pipes of vehicles or machines being ran on leaded petrol and electronic waste such as leaded batteries (Chibuike and Obiora, 2014; Olafisoye et al., 2014; Anju and Banarjee, 2011). Bitumen deposits, lead acid battery factories and glass-making industries in and around the vicinity of the plantation may contribute to particulate lead concentrations which may get into the oil during processing. Generally, all the machineries used for processing were run on leaded petrol/diesel and could be a contributory factor of high lead concentrations on the oil palm plantations. Similar studies on soils sampled on the oil plantations have shown that lead is a potentially mobile element and soluble in soil water and under acidic conditions of the soil, it becomes mobile and can bio-accumulate in the palm fruit (Yasmin et al., 2000; Olafisoye et al., 2013; Olafisoye et al., 2014).

A recent study on the bioavailability and accessibility of metallic elements in the soil on the oil palm plantations revealed the environmental quality of manganese in soil solution (Olafisoye et al., 2016). This shows that Manganese is bound in loose forms of precipitates to organic matter and clays in the speciation studies in the soil of the oil plantation under investigation. Manganese is known to be an essential element for the proper functioning of plants. It is also a potentially mobile metallic element in soil and is usually bound to the organic phases in speciation studies. Potentially mobile elements also exist in the mobile phase as carbonate, sulphides or organic forms. This metallic element can be released into soil solution under conditions which are acidic and made accessible to be utilized by plants. Manganese, a beneficial trace metal contains antioxidant properties and is a very active component of the mitochondria preventing plants and animals from cellular damage and oxidative stress. This makes it an essential metallic element that may be toxic at elevated concentrations. It is also accessible to plants since it is soluble in soil solution and not bound to the crystalline lattice of the soil. Hence, the elevated concentration of the manganese in soil could be transferred to the oil palm fruit from which the palm oil is pressed. Elevated concentrations of manganese in palm oil is not beneficial as accumulation is likely under acidic conditions.
Studies on plant and human health has also revealed the devastating effects of Manganese in humans (Levy and Nassetta, 2003; Lawrence et al., 2009). Also previous studies on the oil palm plantation have shown that the soil has a pH of between 4 and 5 which is acidic. The palm oil samples analysed did not reveal elevated levels of Manganese element. Since the concentrations of Manganese in all the oil samples under investigation were below the WHO (2011) permissible limits of Manganese also for vegetable oils, the bioaccumulation of Manganese in vegetable oils in this present study may not pose a danger or health risks to plants and humans when consumed and will be an excellent raw material and lubricating oil for the food and chemical industry.

Cadmium is a metallic element that is bound in the exchangeable fractions in the soil. It is useful in various application due to its malleable and ductile properties. Elevated concentrations of Cadmium are likely to be found in electronic dump sites due to the dumping of Nickel-Cadmium batteries, pigments from paints and the coating and use of stabilizers in plastics and electroplating of steel material parts. Cadmium may be found in the earth crust in minute concentrations but anthropogenic activities mostly from electronic waste and the application of fertilizers.
have greatly increased the concentration of Cadmium in the environment thus far. Naturally cadmium can also be leached into water bodies and soil through the weathering of rocks and in air through volcanoes and the burning of bush fires. Anthropogenic contribution of Cadmium in the environment is mostly from human activities especially effluents from industries, electronic wastes burning of fossil fuel and more. Major pathways in which Cadmium is transferred to humans is via the food chain and the metal has the tendency to concentrate in the viscera organs of aquatic animals. People living in areas where hazardous waste are dumped and industrial areas are also at high risk of Cadmium exposure. Symptoms of Cadmium toxicity are manifested in stomach ulcers, mutations, bone maladies, infertility, nervous, immune and psychological disorders. Cadmium is the most assessable metallic element to pollutants because it is the most mobilisable (Aziz et al, 2014). Cadmium is found in the soluble fraction or the non-residual fraction in speciation studies, it is not found in the insoluble or the residual fraction of the soil. The results presented in Table 3a revealed that the concentration of Cd in oil on the plantations ranged from 0.127 (Badagry/Abak plantation) to 0.762 (Igede-Ekiti plantation). All the plantations under study had elevated concentrations of Cadmium, which exceeded the WHO (2011) permissible limits for Cadmium (0.005 mg/L) in vegetable oils. Cadmium is not an essential element and has no known nutritional benefits to man. Although Cadmium may naturally be found in deposits in the earth crust as explained earlier, possible elevated sources of anthropogenic Cadmium poisoning in the vegetable oil samples may be from the use of fertilizers and insecticides in unwholesome farming practices (Bernhoft 2013; Tran and Popova, 2013).

Copper is a trace element which possesses antioxidant properties with properties such as malleability, ductility, tensile strength and a good conductor of heat and electricity. Copper and its alloy find good use in the electrical industry, agriculture and in construction. Copper is a noble metal and widespread use has increased recently causing an elevated increase in its concentration in the environment. Effluent water polluted from industries which use a form of copper as raw material are discharged into streams and water bodies and more sources of Copper pollution is the burning of fossil fuels, mining, use of Copper plumbing materials in houses and use of Copper containing pesticides and fertilizers. Particulate Copper in the air are also washed into soil via deposition, landfills, waste disposals and runoffs from rain. These are a few anthropogenic contributions of Copper in the environment. Copper compounds settle and are bound to either water sediments soil particles or organic matter and hence travel in surface water as free ions. Humans living in industrial areas where Copper is processed may experience occupational and long term exposure to Copper. Manifestation of symptoms of Copper toxicity is seen as irritation, stomach ache, vomiting and diarrhoea. Chronic toxicity includes liver and kidney damage, brain and nasal tumours, hepatic cirrhosis, renal and eye defects, genetic disorders, Wilson’s disease and death. Copper persists in the soil and is non-biodegradable especially in acidic soils and in the presence of soil organic matter. Hence copper exists as free ions, soluble in solution rather than bound to water sediments or soil particles especially when copper containing fertilizers/pesticides are used. This is detrimental and a large threat to human existence as Copper is easily absorbed by the food chain. Copper is a trace element and essential for the proper functioning of vital organs in plants and animals. It is a component of antioxidant enzyme ferroxidase. This assists in the regulation of iron and the metabolism of carbohydrate from storage. Notwithstanding, elevated concentrations of Copper become lethal to plants and animals. Previous speciation studies on soil on the oil palm plantations revealed Copper exists in the immobile fraction of the soil that is insoluble in soil solution and poorly oxidised or reduced. Table 3a showed that the concentrations of Cu in the palm oil samples ranged from 0.022 mg/L to 0.446 mg/L. This was above the WHO (2011) permissible levels for Copper in vegetable/palm oil. Studies on the toxicity of Copper had revealed that elevated levels of Copper element are toxic and this causes bioaccumulation and biomagnifications in the oil palm fruits. Being a semi mobile metallic element, bio-accessibility and bioaccumulation is enhanced since its mobility is increased at lower soil pH. Adverse effects of this are seen as manifested (Ek et. al, 2001). Anthropogenic sources of Copper on the oil palm plantation could be from effluents of coin and electrical wiring industries, burning of fossil fuels, mining, use of Copper plumbing materials in houses and use of Copper containing pesticides and fertilizers. Cobalt being a mobile element is fairly mobilised in the soil. It is a trace element which is essential for the treatment of blood disorders, foetal development and
exists in the diet as vitamin B\textsubscript{12} (Permenter et al., 2013; Zhang et al., 2010). The concentrations of Cobalt in the oil samples studied ranged between 0.098 mg/L in Abak and 0.665 mg/L in Nsukka plantation respectively. Although Cobalt occurs naturally in the environment, anthropogenic sources may arise from the burning of fossil fuels and the smelting of metals. Cobalt may also be found in effluents of porcelain and paint industries. Cobalt being a fairly mobile element, hence the concentration of Cobalt in the soil will be assessable to plants and may bio-accumulate in the fruits causing accumulation and bioaccumulation in the fruit. In addition, production processes can also accumulate Cobalt in the palm oil fruit. Elevated concentration of Cobalt is toxic and manifested in humans as symptoms such as blood and lung disorders (Bhattacharya et al., 1995). WHO (2011) permissible limit for Cobalt in vegetable oils is 0.060 mg/L. All the oil plantations investigated revealed elevated concentrations of Cobalt in the palm oil. This buttresses the results from previous speciation study on the oil palm plantations which show that the concentration of Cobalt was above the permissible limits for Cobalt in soil and this is in agreement with the bioaccumulation of the Cobalt in the fruits of the oil palm tree. Chromium is easily bound in soil organic matter when studied in speciation studies. Chromium finds wide applications as raw materials in the electroplating, leather, tobacco, steel and textile industries, alloy and metal/ceramic industries. Chromium exists as Chromium (III), (IV) and (VI). Chromium (VI) being more the most lethal. Chromium is a trace metal necessary for proper functioning of tissues and organs and is found naturally in foods like meat, fruits, whole grains and yeast and Chromium (III) being the essential trace element for the body system functions. Deficiency of Chromium (III) is manifested in heart maladies, nose irritations, stomach ulcers decreased metabolism and diabetes and elevated concentration in humans is detrimental and manifested in skin lesions, respiratory disorders and immune system deficiency, kidney and liver disorders, mutations, infertility, skin cancer and death. Hexavalent Chromium being the most toxic. High concentration of acids in soils can also influence Chromium uptake by crops. Plants usually absorb only chromium (III). This may be the essential kind of chromium, but when concentrations exceed the permissible levels, negative effects can be seen. Chromium is not known to accumulate in the bodies of fish though elevated concentrations of chromium, due to the disposal of metal products in surface waters, can destroy the gills of fish that swim near the point of disposal. In animals Chromium can cause respiratory problems, a lower ability to fight disease, birth defects, infertility and tumour formation. Chromium normally exists as Cr\textsuperscript{6+} in soil solution. Cr\textsuperscript{3+} is used extensively in industry hence it is readily available in the environment (Baralkiewicz and Siepak, 1998). In the presence of elevated soil pH and organic matter, Cr\textsuperscript{3+} is metabolised to Cr\textsuperscript{6+}, which is the toxic form of Chromium. Although Chromium may be an essential metallic element required in trace amounts for metabolism in plants and animals and enzyme activity, elevated concentrations especially Cr\textsuperscript{6+} is toxic and lethal to life. WHO (2011) permissible limit for Chromium in vegetable oil is 0.1 mg/L. The plantations under study revealed lowest and highest concentrations of Chromium as 0.011 mg/L (Onishere plantation) and 0.998 mg/L (Ubiaja plantation) respectively. Invariably the results in Table 3b revealed that most of the oil samples investigated showed high concentrations of Chromium metallic element.

Iron is released in more ways into the environment through anthropogenic pollution. Iron metal has several useful purposes and a versatile raw material in most industrial works and packaging industry. Anthropogenic sources of Iron pollution may be from casings of wells, pumps, iron pipes and crude oil drilling. Effluents from landfills may also leach the metallic element into soil water. Iron is an essential trace element required by both plants and animals for the effective functioning of systems and organs. Deficiency of iron in both plants and animals are manifested as chlorosis and anaemia. Some food such as potatoes, vegetables, meat and dairy products contain traces of Iron. The human body requires haemoglobin for the transportation of oxygen to all parts of the body. Occupational hazard chances are increased in workers living and working in areas where Iron dust is prevalent and signs and symptoms of Iron toxicity are eye and lung disorders, mutations, infertility and some forms of cancer. Lack of iron in the blood causes anaemia in patients. Elevated levels of iron are detrimental as iron persists and is highly biodegradable and combines with lethal elements such as arsenic to produce toxic, hazardous compounds which affects the ecosystem and alters the food chain. Toxicity of Fe is manifested in oxidative stress, anaemia, reduced growth and death (Xing and Liu, 2011; Ajala and Onwukeme, 2012). Iron is also a semi...
mobile element. It is an essential element and a component of the enzyme haemoglobin. WHO (2011) permissible limit for Fe in vegetable oil is 0.2 mg/L. Onishere and Abak plantations recorded 0.211 mg/L and 0.673 mg/L elevated levels of Fe in the study. Crude oil drilling and processing near Abak plantation may be responsible for the elevated concentration of Fe in the palm oil sampled from the plantation.

Zinc is a trace element which is very essential to plants and animals for the development of strong bones and teeth, metabolism, wound healing and growth. It is a very important commercial metal/element that is used extensively by miners and smelters. Zinc may also be released into the environment through industrial activities such as the burning of coal and in the production of steel. Plants and animals living in areas where Zinc is used extensively in industries are susceptible to accumulation and bio magnification of high levels of Zinc through pathways such as air water and food crops planted on contaminated soils from hazardous waste sites and in fishes. Deficiency of Zinc in animals and plants are manifested by Chororosis which is the yellowing of leaves. The offset of necrotic spots occurs at the death of tissues and also the bronzing of the leaves. Deficiency of Zinc in humans/animals is more pronounced physically in facial disorders such as blemishes, skin cancer, loss of appetite, decrease sense of taste and smell, inability to fight infections, retarded growth, cognitive and hedonic tone, immune system and psychological disorders, stomach cramps, nausea, vomiting and cancer. Exposure is seen in ingestion of canned foods coated with Zinc and water flowing through Zinc pipes. Metal fume fever which is usually manifested in occupational hazard is an illness which causes chills, fever and excessive sweating in workers exposed to high levels of Zinc dust or fumes in industries. Nickel is a noble metal that has found increased use in the production of alloys, turbines, engines, propeller shafts in boats which are characterized by high tensile strength, ductility and the ability to resist corrosion and heat in the form of stainless steel. Nickel is also used in the production of rechargeable batteries, jewelleries and coins and in oil refineries. Nickel is an essential element found naturally in the earth crust in minute traces and as enzymes in some leguminous crops, chocolates, fats and the dried leaves of some teas. Elevated uptake of Nickel metal through soil, water, leaves of oil fruits is detrimental and lethal. Toxicity is manifested in dizziness, mutations, infertility, heart, lungs, skin and prostate cancer. Nickel persists in the environment, is toxic, non-biodegradable and a carcinogen. Pathways of Nickel is from dissolution of Nickel dust in air from precipitation and runoffs especially in areas where incinerators and electronic dumpsites are prevalent. It can also be parts of effluents which are carried into water bodies and soil. Nickel is an immobile element but become more mobile in acidic soils and is soluble in groundwater which is toxic when absorbed by plants. Nickel is known to cause some forms of cancer in humans. Zinc, nickel and selenium are immobile elements and their concentrations in the oil samples under investigation were relatively high. Under favourable conditions, these metals may be released from their residual crystalline and immobile phases into soil solutions for absorption by plants (Hajo and Lothar, 2009; Laura et al., 2010). Zn, Ni and Se have been considered by researchers as essential elements with great antioxidant properties protecting the body against the actions of free radicals and usually toxic when concentrations are elevated (Sieprawska et al., 2015). For the proper functioning of the respiratory and cardiovascular organs in human, it requires selenium. It actively participates in the activity of the thyroid hormone. Its strength as an antioxidant enzyme is manifested in the inhibition of the HIV/Aids (Oguntibeju et al., 2010). Ni and Zinc assist vitamin C and E by enhancing fertility and the prevention of oxidative stress by free radicals. Plants take in metallic elements via the soil solution and ideally, this reflects the level of environmental pollution of the area. It is erroneous to base environmental quality of the area on the total metal concentrations rather than the availability of the metallic elements in soil solution (Chibuike and Obiora, 2014).

Environmental pollution arising from aluminium and tin are usually from utensils on dumpsites and in landfills. The leachate from such sites seeps into soil water causing environmental pollution (Roseland et al., 1990). Al and Sn are not essential elements and have no biological importance to life. Nervous system disorder and Alzheimer diseases are signs of Al toxicity (Sparling and Lowe, 1996; Vardar and Unal, 2007). Tin element toxicity is seen in symptoms such as intestinal and urinary tract infections. WHO (2011) permissible limits of Al and Sn in vegetable oils are 0.05 and 0.01 mg/L respectively. All the plantations under study showed elevated levels of Al and Sn. Organotin compounds have been studied to be carcinogenic. However, recently, some of the compounds are useful in agriculture as insecticides,
fungicides and in the treatment of some forms of cancer (Wesdock and Arnold, 2014).

Total concentration of metallic elements (mg/L) analysed in the water and soil from previous findings and virgin palm oil samples presently on the oil palm plantations were presented in Tables 4 (a-c). This assessed the possible human health threats linked with the intake of palm oil contaminated with toxic metals. Contaminated palm oil with essential metallic elements above the recommended values also become toxic. A possible route of human contact to slow poisoning is from the ingestion of contaminated food. Several sources of contamination of the oil palm plantations were identified in this study. Soil acidity and/or alkalinity are a major contributing factor to the bioavailability and accessibility of metallic elements in the soil.

Smith and Giller (1992) confirmed a decrease in the bioavailability and accessibility of metallic elements with increasing pH. This fact was attributed to the formation of insoluble complexes coupled with the removal of hydroxides and carbonates from soil solution. Low pH values have also been associated with a reduction on particle soil size and organic matter. Other factors, which varied significantly in the bioavailability and mobility of metallic elements in the soil were redox reactions, cation exchange capacity, soil chemistry amongst other soil properties (Mukherjee, 1998)

| Table-4a: Total concentration of Cd, Co and Cr (mg/L) |
|-----------------------------------------------|
| Plantation      | Soil  | Water | Oil |
| Cd              |       |       |     |
| Abak            | 1.09 ± 0.2 | 0.26 ± 0.01 | 0.127 ± 0.001 |
| Acharu          | 1.09 ± 0.2 | <0.26  | 0.139 ± 0.001 |
| Agbarho         | 1.09 ± 0.2 | 0.26 ± 0.01 | 0.683 ± 0.001  |
| Ago-Emokpae     | 1.09 ± 0.2 | 0.26 ± 0.01 | 0.140 ± 0.001  |
| Apoje           | 1.28 ± 0.1 | 0.33 ± 0.03 | 0.266 ± 0.002  |
| Badagry         | 4.26 ± 0.2 | 0.39 ± 0.02 | 0.127 ± 0.001  |
| Benin city      | 1.33 ± 0.1 | 0.26 ± 0.02 | 0.557 ± 0.002  |
| Igede-Ekiti     | 0.39 ± 0.2 | 0.26 ± 0.01 | 0.762 ± 0.002  |
| Apomu-Ikire     | 0.27 ± 0.1 | 0.26 ± 0.03 | 0.138 ± 0.001  |
| Iresa-apa       | 1.32 ± 0.1 | 0.27 ± 0.01 | 0.139 ± 0.001 |
| Nsukka          | 1.12 ± 0.1 | 0.27 ± 0.01 | 0.611 ± 0.001  |
| Okitipupa       | 0.12 ± 0.1 | 0.27 ± 0.01 | 0.389 ± 0.002  |
| Onishe          | 0.37 ± 0.1 | 0.32 ± 0.03 | 0.445 ± 0.0050 |
| Ubiaja          | 0.32 ± 0.1 | <0.32    | 0.137 ± 0.001  |
| Umuabi          | 0.32 ± 0.1 | 0.32 ± 0.03 | 0.131 ± 0.001  |

| Table-4b: Total concentration of Cu, Fe and Pb (mg/L) |
|-----------------------------------------------|
| Plantation      | Soil  | Water | Oil |
| Cu              |       |       |     |
| Abak            | 39.11 ± 0.2 | 1.01 ± 0.01 | 0.070±0.002 |
| Acharu          | 12.92 ± 0.1 | 0.99 ± 0.02 | 0.117±0.001 |
| Agbarho         | 45.06 ± 0.4 | 1.31±0.09  | 0.075±0.001  |
| Ago-Emokpae     | 35.08 ± 0.1 | 0.10±0.01  | 0.070±0.003  |
| Apoje           | 33.22 ± 0.1 | 0.12±0.01  | 0.083±0.002  |
| Badagry         | 69.11 ± 0.9 | 0.12±0.01  | 0.184±0.003  |
| Benin city      | 23.02 ± 0.2 | 0.01±0.01  | 0.091±0.002  |
| Igede-Ekiti     | 33.41 ± 0.1 | <0.13     | 0.090±0.001  |
| Apomu-Ikire     | 22.63 ± 0.1 | 0.10±0.01  | 0.109±0.001  |
| Iresa-apa       | 30.82 ± 0.2 | 1.24±0.01  | 0.082±0.002  |
| Nsukka          | 28.56 ± 0.1 | 1.37±0.03  | 0.022±0.001  |
| Okitipupa       | 33.78 ± 0.2 | 1.24±0.02  | 0.332±0.002  |
| Onishe          | 37.99 ± 0.1 | 0.88±0.02  | 0.446±0.006  |
| Ubiaja          | 30.22 ± 0.1 | 1.01±0.01  | 0.076±0.005  |
| Umuabi          | 25.52 ± 0.1 | 0.56±0.01  | 0.101±0.007  |
The pH of the soil studied on the oil palm plantation ranged between 4 and 7. Under these conditions, metallic elements in the soil solution are bioavailable and accessible to the plants grown on these soils due to increased mobility. This is hazardous to humans and the food chain due to the fact that the crops bioaccumulate toxic metals in their edible parts especially their fruits (Wei et al., 2005).

Bioaccumulation and biomagnifications of metallic elements in plant tissues is influenced by factors such as pH, moisture content of the soil, soil organic matter and nutrient load. Rupa et al. (2003) studied an elevation in the uptake of metallic elements such as Zn, Cr, Fe and Fe due to the occurrence of soil organic matter. In this study, the relationship between soil factors and metallic elements determined the bioaccumulation and accessibility of the toxic elements in the palm oil.

The soils analysed on the plantations were acidic, an indication of elevated concentrations of the metallic element in the oil samples. This prompted an increase in mobilization and uptake of the metallic elements in the palm oil. Itanna (2002) emphasized the influence of morphology and physiology of plants as an important factor in mobility, bioaccumulation and the elimination of metallic elements in soils. Toxic metallic elements en-route to humans and the food chain via consumption of food crops. This is especially hazardous and a critical health risk issue when the metallic elements exceed the permissible levels in palm oil.

The food chain as a pathway of toxic metallic elements is vital in risk assessment in developing countries like Nigeria since ingestion of palm oil remains deregulated. Palm oil is a stable ingredient for the preparation of most dishes in Nigeria and consumed regularly by the population at large. Scientists such as Horiguchi et al. (2004) implied that the intake of metallic elements does not equate the concentrations absorbed by humans. He further explained that part of the absorbed dose is eliminated through various biochemical processes. The remainder portion becomes bioaccumulated and biomagnified in humans. This study can be used to assess crop pollution and prospective risk assessment.

Comparison of the concentration of metallic elements in the water and soil of the oil palm plantations from previous findings to that found in the palm oil in this study show that the concentration of the metallic elements varied considerably. Metallic elements have the ability to be translocated from soil and water to various parts of the plants. Invariably researchers have studied the oil palm tree extensively as an effective crop in remediation technology. It is a cheap choice to phytoremediation because it is low in maintenance and eco-friendly. Soils are cleaned up as the oil palm tree translocate metallic elements from soils/water to various parts of the plant such as the fruits or stems. This provides interesting tools to sites contaminated by toxic metals. Cunningham and Ow (1996) have studied the accumulation of metallic elements in parts of the oil palm tree such as the stem and the root. Research is limited in the area of the virgin palm oil which is the oil palm fruit to decide if accumulation may be higher in such parts.

**Conclusion**

The research determined the simultaneous analysis of synthetic antioxidants in virgin palm oil samples using HPLC and trace elements in virgin palm oil, with micro emulsion as sample preparation method by ICP-OES. Both methods used UV/Vis detection techniques. Synthetic antioxidants were absent in the virgin palm oil in all the plantations under study. Most of the plantations recorded significant levels of the metallic elements. The virgin palm oil is not suitable for food at its present state due to the presence of elevated concentrations of the metallic elements; but can be used in the raw material/chemical industry and in the production of biodiesel. The maximum concentration of metallic elements allowed in virgin palm oil used as food is much lower than that used as
biodiesel or raw material for the chemical industry. It is recommended that the level of environmental pollution on the plantation be eradicated or reduced to the barest minimum to improve the quality of the virgin palm oil for human consumption. In addition, agronomical practices, pre-planting and post planting activities should be done under sanitary conditions. This would potentially reduce metallic elements improving the quality of virgin palm oil. Individuals and governmental bodies should address point and non-point sources of the environmental pollution on the oil palm plantation. This is of paramount importance considering the fact that the oil palm tree is an essential crop. The analytical methods may be employed for routine laboratory analysis.

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Contribution of Authors

Olafisoye OB: Conceptualized part of the research, performed the experiment, data collection and collation, carried out data analysis and wrote the manuscript.
Oguntibeju OO: Conceptualized the research, supervised the work and edited the manuscript.
Osibote OA: Conceptualized the research, supervised the work and edited the manuscript.