

## Research Article

# Nutrients: B-Vitamin Content Methionine, Micronutrients and Oestrogen of Osun River: A River that Runs Southwestern Nigeria into the Atlantic Gulf of Guinea

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Received: March 16, 2021; Accepted: March 26, 2021; Published: October 21, 2021

## Abstract

The wide use of water from the conserved Osun-Osogbo Grove for domestic, traditional, and medical uses by indigenes necessitated the assessment of the biochemical quality of water. This study assesses the presence of water-soluble vitamin, phosphate, nitrate, amino acid, hormone, and trace metal. Water samples were taken from two different sites before, during, and post Raining sessions (April 2017-September 2019). The samples were analyzed using High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectroscopy (GC-MS), and Atomic Absorption Spectrometer (AAS). Trace metal analysis revealed an average of 0.009-0.079 mg/Kg Zinc from site one and lower in site two. The mean value of manganese at both sites was virtually the same at 0.018-0.313 mg/kg, aluminum content was 0.045-0.179 mg/Kg at site one, 0.050-0.192 mg/kg at site two, cobalt was 0.024 mg/kg at site one, 0.026 mg/kg at the site two while nickel was 0.006 mg/kg and 0.004 mg/kg for site one and two respectively. HPLC analysis shows mean Methionine content at both sites is higher than the FDA standard value of 56.6 µg/mL; site one had 74.41 µg/mL while site two had 57.11 µg/mL. The mean values of two water-soluble vitamins; Thiamine (B1) was 3.758 mg/Kg and 2.355 mg/Kg while Pyridoxine (B6) was 0.108 mg/Kg and 0.072 mg/Kg at site one and two. GCMS analysis of steroidal content revealed values below LOEL, testosterone (4.8 ng/L), and estrogen (2.4 ng/L) were still elevated while ethinylestradiol and estriol were  $\geq 1.5$  ng/L. Generally, in both sites, varying quantities of different micronutrients were detected. This study identified for the very first time the presence of water soluble vitamin, phosphate, nitrate, amino acid, hormone, and trace metal dissolved in the conserved grove water that has served as major source of water for the community from historical days especially to devotees and indigenes.

**Keywords:** Water profiling, Osun River, Micronutrient, Trace metal

## Introduction

Generally, all water bodies be it groundwater, surface water or any other forms, have other chemical components dissolved in it. Water contains small amounts of gases, minerals and organic matter of natural origin [1]. Since water acquires its constituents from contact with rocks, soil and the environment, it is natural therefore to detect other constituents in drinking water that are occurring naturally. Drinking water supplies may contain some of these essential minerals naturally or through deliberate or incidental addition. Prominent amidst these constituents are micronutrients, which are required by organisms throughout life in minute quantities to orchestrate a range of physiological functions. These may include; vitamins, amino acids, minerals as well as metals of enzymatic importance contributing significantly to the sustenance of lives. Micronutrients are vital for the proper functioning of all the body systems, enabling the body to produce enzymes, hormones, and other substances essential for proper growth and development. Although required in minute quantities, absence or decrease in quantities below body requirements may have consequences ranging from mild to severe [2]. The Osun

river-water is one of the peculiar water bodies in Southern western Nigeria. The river has a lot of myth around it, prominent of which is the therapeutic potentials of the water which has raised concern in the scientific circle and thus leading to several research documentation on the heavy metal constituent and the postulation that the water is not safe for drinking and general usage as it may constitute health consequences [3-5]. As against previous reports which concentrated on Heavy metals, our group explore the beneficial content of the widely used Osun River water, this was necessitated by the fact that despite scientific reports, indigenes and devotee kept using the water, all background checks showed there were no proclaimed scientific hazard, thus we evaluated the water from beneficial point of view with believe that our findings might support the traditional and domestic use of this water. Therefore, this study postulate, that the therapeutic constituents of the Osun river-water supersede the toxic constituents. To verify this, we evaluate the physico-chemical properties, metabolic metals, vitamins (thiamin B<sub>1</sub>, riboflavin B<sub>2</sub>, pyridoxine B<sub>6</sub>, biotin B<sub>7</sub> and cobalamin B<sub>12</sub>), methionine and oestrogen contents in the Osun River water.

## Method

### Sampling Area

This was conducted within the Osun-Osogbo Sacred grove, which is located along the bank of Osun River in Osogbo capital city of Osun State, South Western Nigeria. It is located on latitude of 7°45'05.9"N and longitude of 4°33'03.9"E, 250 km north of Lagos, land size of 75 hectares and about 350 m above sea level as indicated in Figure 1. The Groves houses hundred shrines, sculptures and it is the world heritage site [6,7].

### Collection of Water Samples

Water samples were collected from two locations namely; in the conserved region (Site X) of the Grove, with limited human activities (7°45'03.9"N and longitude of 4°33'03.9"E) and outside the Grove, where there are unlimited activities, Site Y (7°45'12.2"N 4°33'05.4"E) between April 2017-September 2019 at 7 am. Sample collection was subdivided to three, about 1000 mL each of water samples were collected in containers previously soaked in 10% HCL, washed with phosphate-free detergent, dried and pre-calibrated polythene screw capped plastic bottles. The remaining two portions were collected in clean High-Density Polyethylene (HDPE) dark

bottles for vitamins analysis, amino acid assay as well as hormone content. All collected samples were immediately transported to the Molecular Biology and Genetic Diversity Research Laboratory, Biochemistry Unit, Department of Chemical Sciences, Fountain University Osogbo. The samples were then maintained at 4°C until required for analysis.

### Water Analysis

#### Physico-chemical Analysis

##### Macroscopic Examination of Water Samples

This was performed using the protocol described by Sharif et al., [8]. It involves virtual and sensory evaluation of water samples in terms of color; odour and the presence of foreign matters were observed.

##### pH Determination

pH values of water samples were determined as described by Raphael and Emmanuel (2019) [9]. Prior to analysis, acidic and alkaline buffer solutions of pH 4 and 7 were used for calibration of the pH meter to optimize procedure. pH values of water samples were determined and pH values of less than 7 were deemed acidic, pH=7; neutral and greater than 7, alkaline.

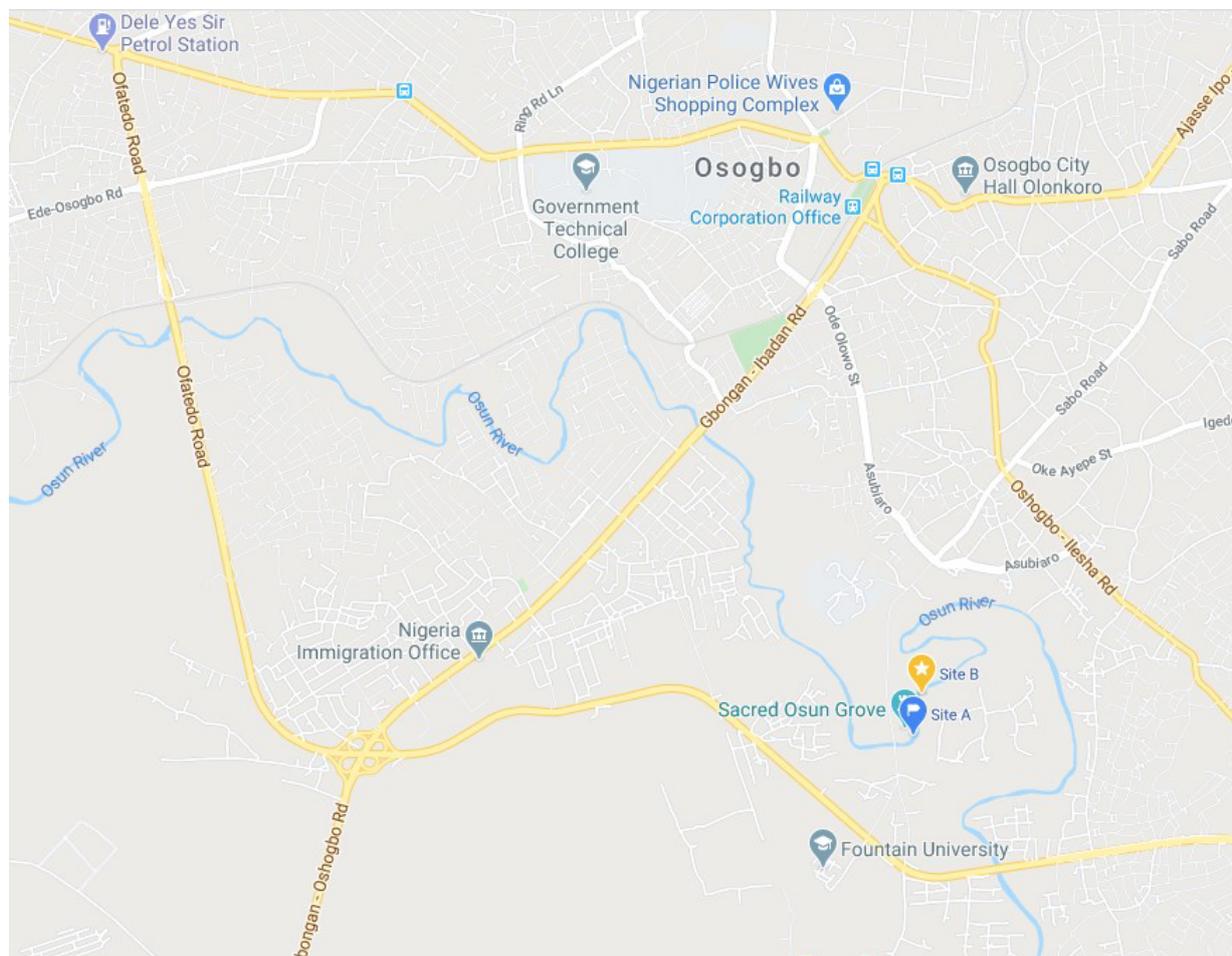


Figure 1: Graphical location of sampling location along the Osun River Path. Source: Map Data@2020 (maps.google.com).

## Metal Analysis

Water samples were filtered through a 0.22 µm polypropylene Calyx capsule filter and collected in Low-Density Polyethylene (LDPE) bottles. Samples were further acidified to pH < 2 using ultrapure grade Hydrochloric Acid (HCl), and stored at 20°C for at least one month before extraction [10]. Afterwards, samples were analyzed using Atomic Absorption Spectrophotometer (AAS) as described by Smith [11].

## Vitamins and Methionine Analysis

This was performed using the liquid chromatographic method as described by Cortés-Herrera et al., [12]. Water samples for vitamin and methionine analysis were filtered through 0.22 µm polypropylene Calyx capsule filters and collected in High-Density Polyethylene (HDPE) dark bottles and stored frozen until analysis. Dissolved B-vitamins and methionine were extracted and pre-concentrated in solid-phase extraction onto a C18 resin before analysis.

## Nutrients Analysis

Phosphate and nitrate analysis were performed according to the protocol described by Environmental Protection Agency [13].

## Phosphate Analysis

Standard solutions were prepared by accurately measuring 10 mL of the stock solution into a 250 mL volumetric flask and made up to volume with distilled H<sub>2</sub>O. Varying volumes of the standard were then measured (5 mL, 10 mL, 15 mL, 20 mL and 25 mL) into separate labeled 100 mL volumetric flasks. The test water sample was diluted by a factor 10, before 25 mL of diluted sample was transferred to a 100 mL volumetric flask, then made to mark using dilute distilled water. All solutions were kept for 30 minutes to allow colour development before reading absorbance at 880 nm. Concentrations of the test samples were calculated from the standard curve.

## Nitrate Analysis

Standard solutions were prepared by measuring 2 mL of the stock solution and made up to a 100 mL with distilled water. Varying volumes of the standard were measured into as separate beaker then interfering organic and metallic substances were removed by treating with 20 mL mercury (II) chloride solution. Two different volumes of each test sample were also subjected to similar treatment. The pH of all samples was adjusted to 11 with 50% sodium hydroxide (NaOH) and then filtered to remove insoluble pellet. The initial flow through was discarded before allowing complete filtration. Then 2 mL of each filtrate was transferred into a beaker, and 1 mL of 1% sodium salicylate solution was added, mixed well, and left to evaporate to dryness. It was later dried in the oven for 20 minutes at 105°C.

Oven incubated samples were allowed to cool to room temperature, and then dissolved with 2 mL concentrated tetraoxosulphate (VI) acid (H<sub>2</sub>SO<sub>4</sub>), 15 mL distilled water was added after the solution had cooled to room temperature followed by addition of 15 mL of the sodium hydroxide-potassium sodium

tartrate. The mixture was allowed to stand at room temperature for one hour and absorbance read at 420 nm.

## Oestrogen Analysis

River-water samples were prepared and analyzed as described by Xiao et al 2001 using 8 ng/L estradiol II as internal control in each calibrated sample. The samples were then subjected to 131 GCMS using the spitless technique, using 0.75 min period on an HP-5MS capillary column (15 m 132 x 0.25 mm I.D., 0.25 mm film thickness) and 5% diphenyl - 95% dimethyl siloxane liquid phase. The oven temperature was maintained at 65°C for 1 min and then programmed to 220°C at 40°C per min, then to 255°C at 5°C per min and finally to 330°C at 20°C per min and maintained at 330°C. The injector and transfer lines were 330°C. Methane (99.99%) was used as the reagent gas in the negative ion mode with source pressure of 160 Pa.

## Data Analysis

All data were presented in tables, figures and charts were used to express the different concentrations of micronutrients, vitamins and heavy metals.

## Results

### Physico-chemical Analysis

Table 1 shows the macroscopic (colour, odour and foreign matter) characteristics and pH values for water samples collected from both sites. Water samples from both sites had similar characteristics with Site X having a slightly high alkaline pH value.

### Metal Analysis

Plate I show a screenshot of the result of metal analysis for Al, Zn, Cd, Cu, Ni, Co, Pb, Mn and Cr for samples collected from site 1 and site 2. The average zinc content in Site 1 was 0.079 mg/Kg, while that of site 2 was below detection. The manganese (Mn) content was practically the same for both sites, while the Nickel (Ni), Cobalt (Co) and Aluminum (Al) levels were almost the same throughout the study period in both study sites.

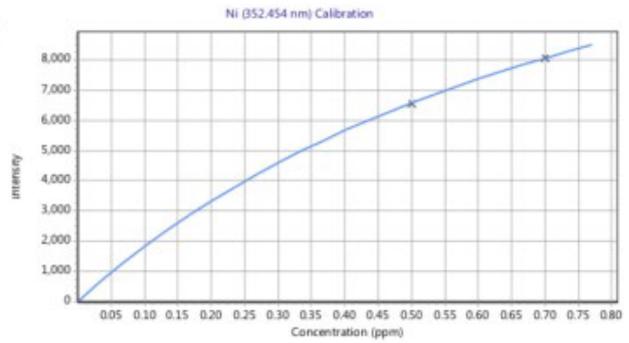
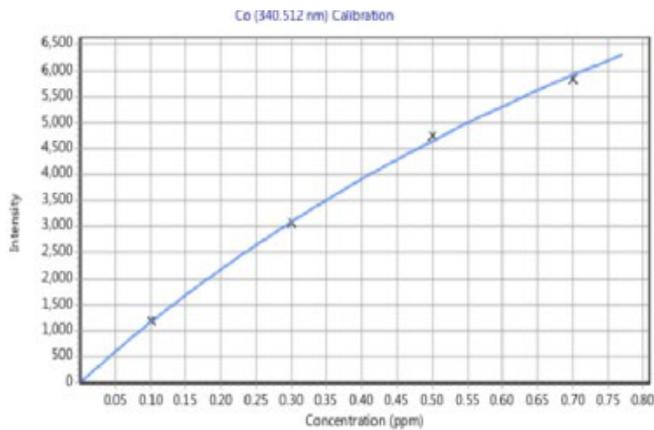
Furthermore, phosphate and nitrate analysis performed on the water samples yielded relatively lower concentrations. Average phosphate content of site 1 was observed to be 0.027 mg/Kg while Nitrate content was 0.082 mg/Kg.

### Methionine and Vitamins Analysis

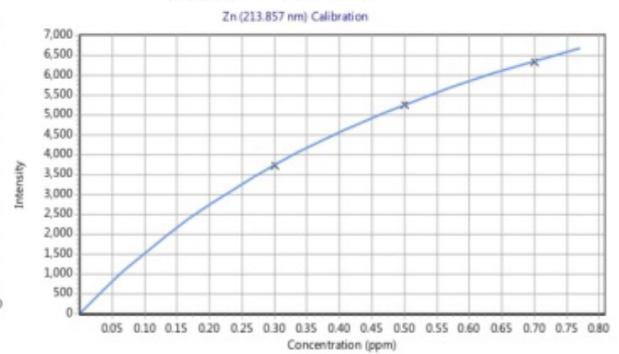
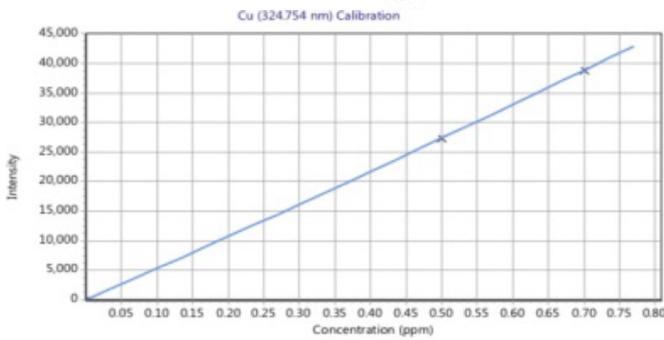
Figure 2 shows the Methionine content at site 1 was 74.41 µg/mL while site 2 was 57.11 µg/mL. The mean values of two water-soluble vitamins; Thiamine (vitamin B1) content of site 1 was 3.758 mg/Kg and 2.355 mg/Kg at site 2 and B6 (Pyridoxine) was 0.108 mg/Kg in site 1 and 0.072 mg/Kg at site 2 as indicated Figures 3.

Table 1: Macroscopic characteristics and pH of water samples from both sites.

Samples	Colour	Smell	pH	Foreign matters
Site 1	Light Brown	None	10.1	Slight debris
Site 2	Light Brown	None	8.2	Same as above

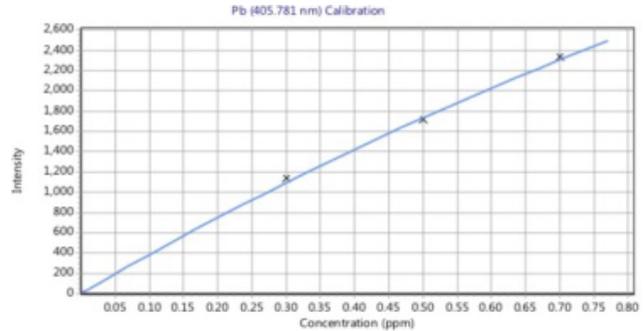
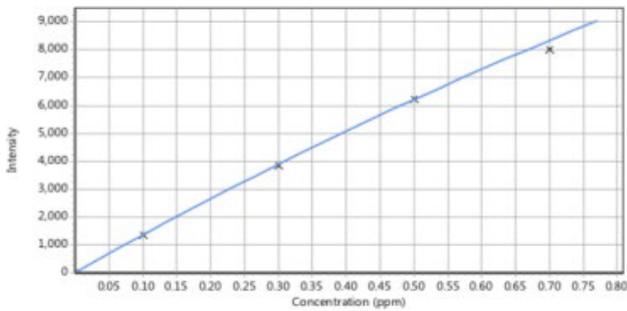


**Ni (352.454 nm)**  
 Intensity = (20360.278 \* Concentration - 0.000) / (1 + 1.099 \* Concentration)  
 Correlation coefficient: 1.00000



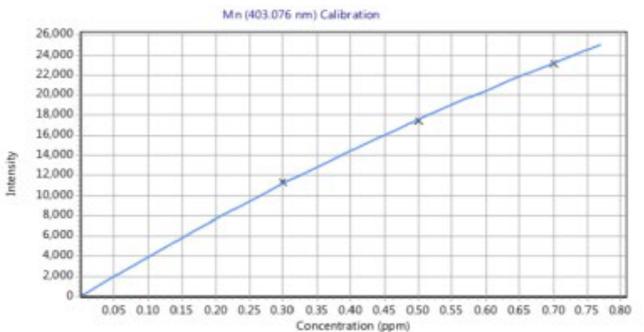
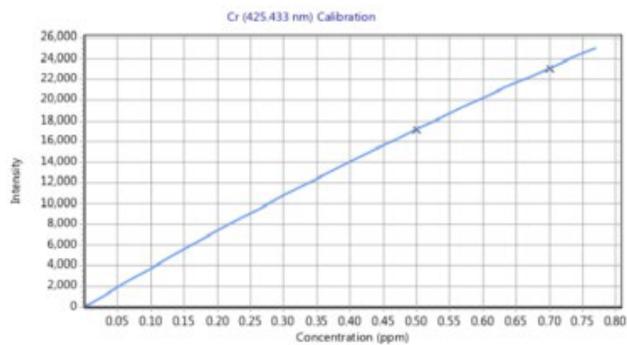
**Cu (324.754 nm)**  
 Intensity = (52804.943 \* Concentration + 0.000) / (1 - 0.069 \* Concentration)  
 Correlation coefficient: 1.00000

**Zn (213.857 nm)**  
 Intensity = (17333.031 \* Concentration - 0.000) / (1 + 1.296 \* Concentration)  
 Correlation coefficient: 1.00000



**Cd (228.802 nm)**  
 Intensity = (13911.464 \* Concentration - 0.000) / (1 + 0.239 \* Concentration)  
 Correlation coefficient: 0.99943

**Pb (405.781 nm)**  
 Intensity = (3971.933 \* Concentration - 0.000) / (1 + 0.295 \* Concentration)  
 Correlation coefficient: 0.99966



**Cr (425.433 nm)**  
 Intensity = (38451.406 \* Concentration - 0.000) / (1 + 0.236 \* Concentration)  
 Correlation coefficient: 1.00000

**Mn (403.076 nm)**  
 Intensity = (41095.628 \* Concentration + 0.000) / (1 + 0.343 \* Concentration)  
 Correlation coefficient: 0.99994

Plate 1: A screenshot of trace metal analysis for both sites.

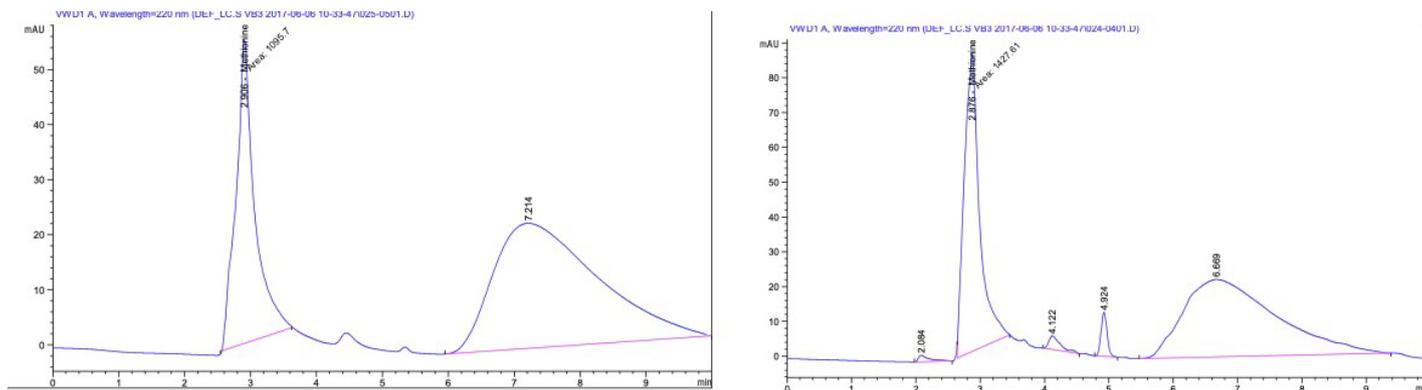


Figure 2: HPLC spectra of Methionine content of Site 1 (a) and 2 (b).

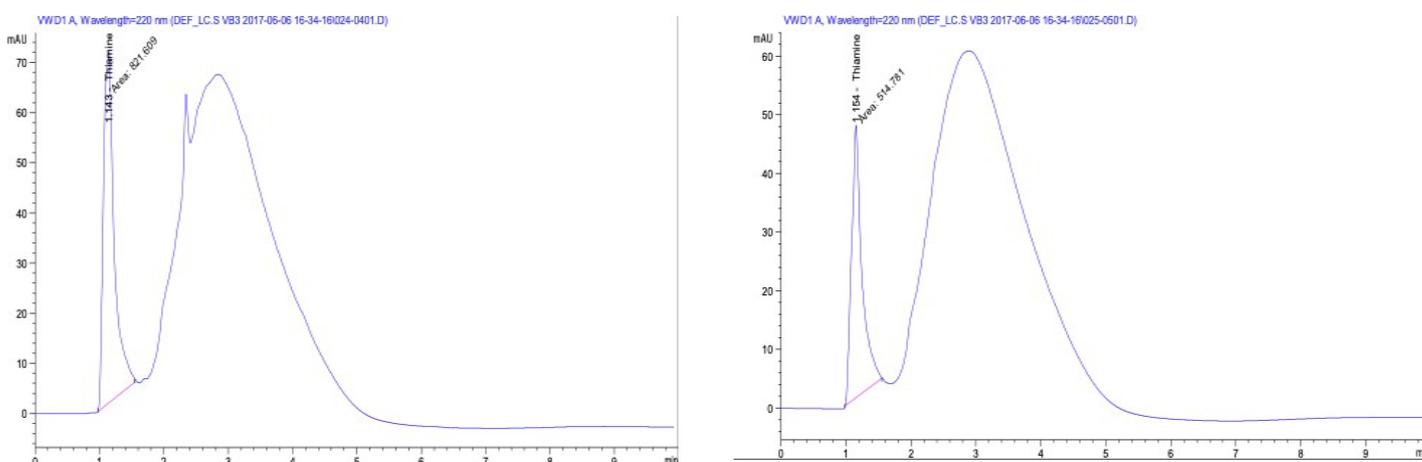


Figure 3: HPLC spectra of Thiamine content of Site 1 (a) and 2 (b).

### Oestrogen Content

Over the stretch of the study period, the hormone values declined during the raining session by half from their maximum values for testosterone (4.8 ng/L), estrone (8.8 ng/L), ethinylestradiol (6.1 ng/L), and estrogen (4.9 ng/L) in site 1 estrogen (4.8 ng/L) and ethinylestradiol (2.4 ng/L) while estrogen was about  $\geq 1.5$  ng/L in site 2 as indicated in Figures 4 and 5.

Also, the hormone values declined by half from their maximum mean values for testosterone (3.3 ng/L), estriol (8.8 ng/L), ethinylestradiol (6.1 ng/L), and estrogen (4.9 ng/L). From 67 to 100 km mark, testosterone (4.8 ng/L) and estrogen (2.4 ng/L) were still elevated while ethinylestradiol and estriol were  $\geq 1.5$  ng/L.

### Discussion

Assessment of the biochemical quality of Osun river water becomes highly necessary due to the high traditional, domestic, human activities and the discharge of industrial wastes into the water body. The exposure of humans, animals and plants to such contaminated water may lead water borne diseases which in severe cases cause damage to the body resulting to high level mortality [14]. Owing to the high local mythology ascribed to the Osun River, this research was carried out to give a background scientific knowledge on the constituents of the river, which are likely to aid understanding the role of some of these constituents in the acclaimed properties of

the river water.

The results from this study revealed that the Osun river water is slightly brownish and highly alkaline pH. This is in concordance to the findings of Shomar [15], who reported alkaline pH for zamzam water and disagrees with the reports of Yusuf et al., [5] which reported a weak alkaline pH in Saba River. The slightly brownish colouration might be attributed to the dissolved organic materials, environmental pressure due to human activities from settlements along the river, flood inflow from rainfall and rituals performed during festival that attract thousands of people (NCMM, 2005) [6], other anthropogenic factors which affect the properties of the water [16] and inorganic contaminants, such as metals, are also common causes of color. In general, the point of consumer complaint is variable, ranging from 5 to 30 color units, although most people find color objectionable in excess of 10 color units. Other contaminants that may be related to change in watercolor include aluminum, copper, foaming agents, iron, manganese and 214 total dissolved solids (Scherer, 2019).

The alkaline pH=10.1 of site 1 is higher when compared with Zamzam water with pH 8 [15] and Mediterranean Sea water pH 8 [17]. Alkaline water are rich in minerals and attributed with health benefits such as ability to balance body pH, antioxidant, detoxification properties and generally optimized body immunity [18]. This could be attributed to the local use of the water from Osun River for therapeutic purposes.

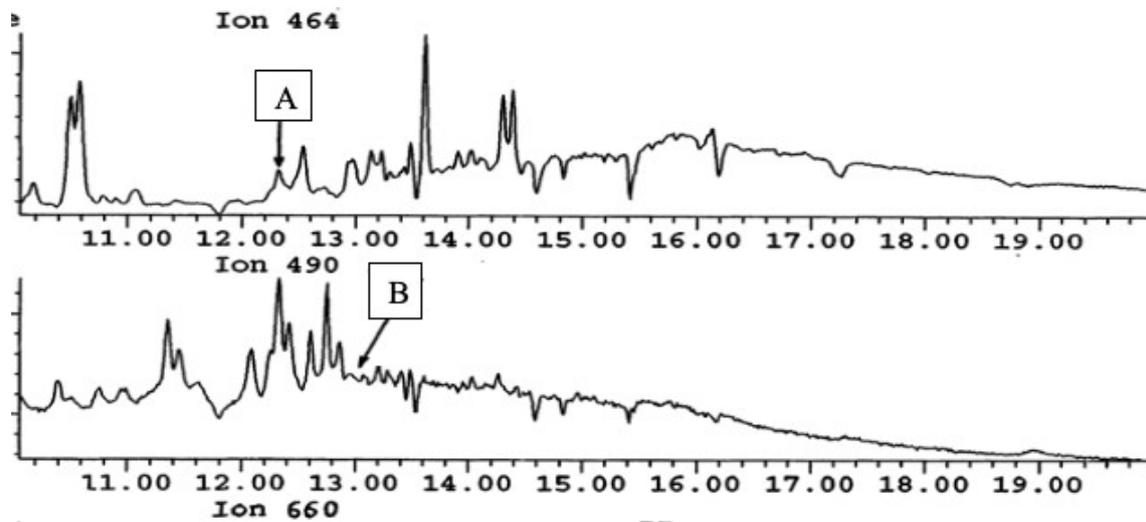


Figure 4: Chromatographic spectra of Estrone (A) and Ethynylestradiol (B).

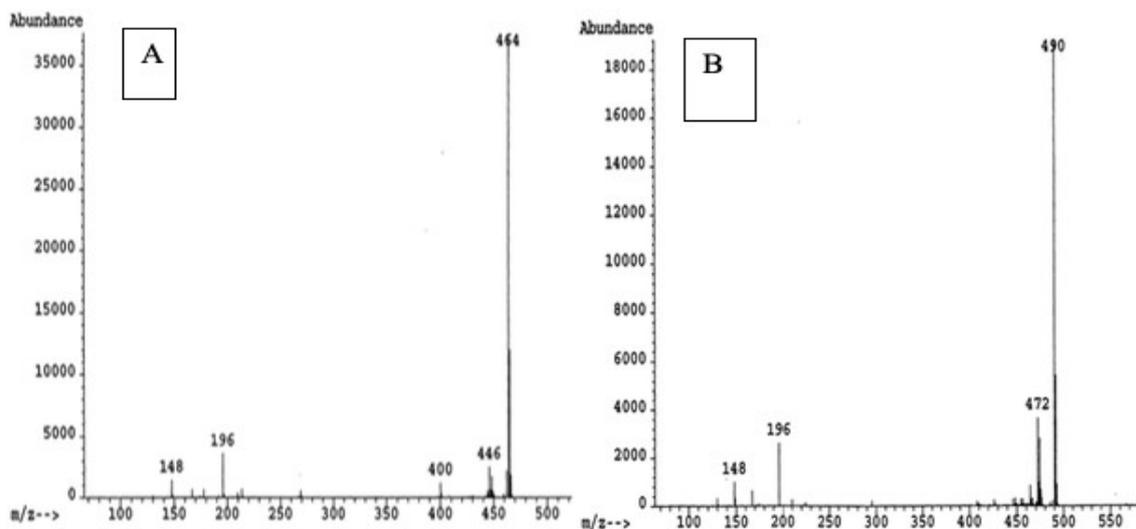


Figure 5: Mass spectra derivative Estrone (A) and Ethynylestradiol (B).

The presence of vitamins in drinking water has been of particular interest due to the role vitamins play in metabolism, especially the vitamin B complex family known to play significant role as cofactor in enzyme catalyzed reaction such as dehydrogenase complexes [19]. Prominent among these vitamins are thiamine used in the synthesis of the cofactor Thiamine 224 pyrophosphostate, pyridoxine and its role in the glycogen synthesis pathway as well as amino acid metabolism. In this study, the vitamins and methionine concentrations along the Osun river follow different trend, for instance, site one was observed to be richer in methionine (74.410 g/Kg), thiamine (3.75823 g/Kg) and pyridoxine (0.108020 g/Kg; 0.622776 g/Kg) when compared with site two where methionine (54.11 g/Kg), thiamine (2.35473 g/Kg), pyridoxine (0.0715691 g/Kg) values were detected respectively. Conversely, an increase of vitamin B1 and B6 is observed in site one, when compared with site two, however, the values were lower than those reported for Moulouya river by Tovar-Sanchez et al., [20], other vitamins such as B12 were not detected in the water samples. Opposite responses in the various B-vitamins is not rare since their availability in water is governed by the specificity of the predominant

phytoplankton species for those vitamins [21]. In this study, different values of vitamins (i.e., B1 and B6) were observed in the main worship area where the phytoplankton assemblages changed from dominance of diatoms to dinoflagellates mainly due to the fact that devotees tend to continuously drop sacrifices at this portion of the river. These might also give basis for the consistence slight brown colouration of the Osun water, going by the ability of dinoflagellates to generate “red tides”. In their report, Radi, et al., [22] established the relationship between dinoflagellate cyst assemblages and hydrographic conditions, productivity and nutrient concentrations; they suggested that dinoflagellate cyst assemblages can be used to reconstruct primary productivity, temperature and salinity. Sa~nudo-Wilhelmy et al., [21] emphasized the regulatory role of Vitamins in metabolic activities of marine plankton. Because of their high bacterial activities, freshwater sources (such as rivers and groundwater) are considered important sources of vitamin B1 and B6 [22-24].

The National Agricultural Library reported the role of trace metals such as: zinc, copper, manganese, etc. in the influence on

reproduction and development. In a similar report by Rasheed et al., [25] reported  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  play an important role in biochemical processes. Looking at the trace metal zinc, the value 0.079 g/Kg was obtained for site one, and -0.015 g/Kg for site two. Zinc, an essential metal which is needed for hormone regulation, immune builder and fertility in women was detected in the river sample at 7 mg in each liter of water taken from the river, compared with standard FDA value of (3-5) mg/L. Aluminum content was observed to be 0.179 g/Kg, site one and 0.192 g/Kg for site two, compared with standard FDA value of 0.05-0.2 mg/L; this implies that for every liter of Osun water taken, 0.2 mg of aluminum is contained in it. The concentration of Cr in surface water represents the industrial activity [26]. Surface water contains chromium in the range of 0.004 to 0.007 mg/L [27]. Chromium, cadmium, copper and lead levels in the Osun River water were below detection 16 indicating. However, Manganese (Mn) which is an essential component of biochemical reactions that affects bone, cartilage, brain and energy supply but toxic in higher concentration was detected. In this present study, the concentration of Mn was 0.313 g/Kg for both sites and do not exceed the permissible limit for drinking water set by various organizations. The concentration obtained was comparable with the European Commission, World Health Organization (WHO) and United States Environmental Protection Agency (USEPA) prescribed guideline (Table 2 in [28]). Arsenic was 0.842 g/Kg for site one, and 0.569 g/Kg for site two, compared with 7.29 g/L reported by Fahad et al. (2016) for Zamzam. Although arsenic may cause low birth weight and spontaneous abortion, long-term chronic health effects, such as skin disease, skin cancer, it was and is still applied for pharmaceutical and medical purposes in curing asthma and hematological illnesses. In their report, Stein and Tallman described the use of Arsenic Trioxide (ATO) as a new era in chemotherapeutic of Acute Promyelocytic Leukemia (APL) [29]. A growing body of literature demonstrates the feasibility and efficacy of ATO, usually given with ATRA, in the treatment of patients with newly diagnosed APL. However, he mentioned reports of potential unintended toxicities, which included impaired fertility in both men and women. Second edition textbook of Biopharmaceutical Biochemistry and Biotechnology also describe biologic agent as any other trivalent organic arsenic compound applicable to the prevention, cure or treatment of disease or conditions of human beings [30].

Copper, cadmium, and lead had relatively no value (-0.006 g/Kg) when tested for in the Osun water; knowing that lead is harmful to the body, it was satisfactory to know the lead content of the Osun water was below detectable level at the period. After obtaining the values 74.410  $\mu\text{g}/\text{mL}$  for the first site, and 57.110  $\mu\text{g}/\text{mL}$  for the second site, and knowing that methionine is an essential amino acid required for initiation of protein synthesis. It was satisfactory to know the methionine content is high when compared with standard FDA value 56.6  $\mu\text{g}/\text{mL}$ . This might imply that an individual taking Osun water takes in over 55  $\mu\text{g}$  of water dissolved methionine per every mL of the water. Vitamin B1 (Thiamine) content gotten in site one was 3.758  $\mu\text{g}/\text{mL}$  and site two was 2.355  $\mu\text{g}/\text{mL}$  compared with standard of 1.5 mg/l. Hence, it shows that if one takes a mL of Osun water, the thiamine

content obtained from it is over 3  $\mu\text{g}$  compared with the RDA value of 1.1 mg. Vitamin B6 (Pyridoxine) value obtained was 0.108  $\mu\text{g}/\text{mL}$  for site one, while 0.072  $\mu\text{g}/\text{mL}$  was observed for site two and this shows that for every mL of the Osun water taken in, 0.1  $\mu\text{g}$  of 17 pyridoxine is contained in it.  $\text{PO}_4^{3-}$  value observed from the Osun water did not exceed the stipulated standard of 0.02 g/Kg, as the value obtained was 0.027 g/Kg. The  $\text{NO}_3^-$  value obtained was 0.082 g/Kg. In summary, it was observed that higher nutrients levels was obtained from the first site, which is within the grove and the believed center of most of the spiritual activities of the devotees, and this is due to the natural conservation present over the river.

Occurrence of metals such as Cu, Zn and Fe in water is also of importance considering the role of metals as cofactors of enzymatic activities and protein structure. In natural surface waters, the concentration of zinc is usually below 0.010 mg/L, while in groundwater 0.010-0.040 mg/L [31,32]. Essential amino acids such as methionine found in some water bodies have be attributed to environment or climatic conditions of the water. Micronutrients indirectly serve as the catalyst to release the energy from the macronutrients.

Due to the high bacterial activities, freshwater sources (such as rivers and groundwater) are considered important sources of vitamin B<sub>1</sub> and B<sub>6</sub> and Baren-cohen et al., reported that hormones in readily measured quantities can be transported along a considerable distance from the source of pollution [33]. Several literatures have shown that steroid hormones produced by humans and animals constantly excreted into the environment found their ways into underground water and rivers [34-36]. This work concentrated on naturally occurring hormones such as estrone (E<sub>1</sub>) and estradiol-17b (E<sub>2</sub>) which were reported to exert physiological effect at concentrations above LOEL (Lowest observable effect level). E<sub>2</sub> is abiotically converted to E<sub>1</sub> thus, they are generally considered as oestrogen. The LOEL for E<sub>2</sub> and E<sub>1</sub> were report as 14 and 3.3 ng/L, respectively [37-50] while ethinylestradiol is 1 ng/L [33]. the mean values of steroid detected in the Osun River water over the study period shows the hormone content were lower doing pre-raining season but the content were both above the LOEL. Ethinyl estradiol binds to the estrogen receptor complex and enters the nucleus, activating DNA transcription of genes involved in estrogenic cellular responses. This agent also inhibits 5-alpha reductase in epididymal tissue, which lowers testosterone levels and may delay progression of prostatic cancer. In addition to its antineoplastic effects, ethinyl estradiol protects against osteoporosis. In animal models, short-term therapy with this agent has been shown to provide long-term protection against breast cancer, mimicking the antitumor effects of pregnancy.

In conclusion, this study established the presence of micronutrient, trace metals, water soluble vitamin, methionine and hormone content of the Osun River water that maybe associated with metabolic and physiological processes. Thus, this study report for the very first time the presence of water-soluble vitamin, phosphate, nitrate, amino acid, hormone, and trace metal dissolved in the conserved grove water that has served as major source of water for the community from historical days especially to devotees and indigenes.

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**Citation:**

Sneineh MA (2021) Nutrients: B-Vitamin Content Methionine, Micronutrients and Oestrogen of Osun River: A River that Runs Southwestern Nigeria into the Atlantic Gulf of Guinea. *Nutr Res Food Sci J* Volume 4(1): 1-9.