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RESEARCH ARTICLE

FREE FATTY LEVELS AND DISTRIBUTION IN SEDIMENTS OF NIGERIA RIVERS

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ABSTRACT

The species of free fatty acids (FFAs) in the sediments of some important rivers of South-Western Nigeria were analysed to provide baseline data / information for their levels in Nigeria river sediments. The standard methods based on lipid extraction, isolation and methylation of the free fatty acids were modified and Gas chromatograph based on flame ionization detector was used for quantitative analysis of the complex mixtures of the free fatty acids. The results reveals that palmitic acid (C16:0) is the predominant FFA species, followed by unsaturated C-18 fatty acid (C18:u). Stearic acid (C18:0), Lasuric acid (C12:0), myristic acid (C14:0) are present in medium concentration while myristoleic acid (C14:1) is present in trace quantities. The average concentrations of the FFA species in all the river sediment samples of the dry and wet seasons are 101.37±19.60 µg/kg and 98.99.46±29.65 µg/kg respectively. These represent the baseline data on which further studies on FFAs in rivers surface sediments of Nigeria could be based.

INTRODUCTION

Reports abound in literature that FFSs are present in river and ocean sediments. Nakaoka *et el.* (1994*)* reported that free unsaturated fatty acids such as oleic (C18:1) was found in toxic amount sufficient for bioluminescence test in River Ulla in East China while the FFAs and bound fatty acids distributed in the sea floor sediment showed remarkable even and odd predominance ranging from n-C14:0 to n-C32:0 (Shanchum *et al.*, 1985). Several studies have been carried out on the occurrence of trace organic compounds especially FFAs in river sediments. The fatty acid spectrum in marine sediments taken in the Sea Nicholas and Santa Barbara Basin off Southern California showed a predominance of palmitic and stearic acids and virtual absence of unsaturated acids (Williams, 1965). Fatty acid pattern of phospholipids were determined in Olomouc River and sediment and used to characterize their microbial structure (Salomonova *et. al.*, 2003). Studies have also indicated that the fatty acid lipids in superficial sediments are mainly derived from phytoplankton, bacteria and terrestrial organic matter (Laura, 2010).

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Jung-Ho *et al.*, (2006) found the FFA concentration in Ham River estuary sediment silt and coarse to be 23µg/g and 10µg/g respectively. The high level was attributed to anthropogenic impact on the coastal sediments. In the surface sediment (10 cm) of sub-tropical Pearl of Southern China. Jianfang *et, al.*, (2006), reported that the total fatty acids range from 1.28 µg/g to 42.25 µg/g dry weight. They concluded that the bacteria fatty acids in the sediment were significantly higher than water fatty acids. Several studies on isolation of fatty acids from sediment matrices revealed that the non reactive methyl ester or Trimethyl Silyl (TMS) derivatives are more easily analyzed through chromatographic method than their corresponding fatty acids (Christie, 1993). This is because the ester derivatives have a higher vapour density and lower boiling points, and hence more volatile than their corresponding fatty acids (Fatoki and Vernon, 1989). In Nigeria river sediments, there is paucity of data / information on the occurrence of levels and distribution of free fatty acids (FFAs). This study is, therefore, designed to bridge the gap and provide a baseline data / information on which FFA distribution and levels could be based.

EXPERIMENTAL

South-Western Nigeria, is approximately located on grid co-ordinates between 4^{o} 30^{1} and 9^{0} N and 2^{o} 40^{1} and 6^{o} 30^{I} E.

The study area covers a part of South-western States of Nigeria (Ondo, Osun, and Lagos States). The sampling sites are some selected major rivers in the study area (Figure 1). There are two distinct seasons in Nigeria: the wet season runs between March to November while the dry season runs from November and ends in March. The study was conducted during the wet and dry seasons between January and September, 2011. All chemicals and organic reagents used in this study were of analytical grade sodium chloride, sodium carbonate and anhydrous sodium Sulphate were heated in a muffle furnace at 450 ^{0}C for 4 hours. Fatty acid standards and their corresponding methyl esters were obtained from Sigma and Alltech and used without further purification (Purity was > 95% for all FFA and FAME standards). All sample bottles and glassware used were thoroughly washed with hot detergent solution, concentrated nitric acid, rinsed with triply distilled water and pure acetone prior to their usage.

Sample Extraction and Analysis

Accurately weighed 10g of each sediment sample was put into a pre-extracted Whatman extraction thimble and fitted to the Buchi extraction system (Alternative to Soxhlet extractor). 100 mL portion of the pentane-acetone (1:1 v/v) (judging by the results obtained in previous experiments) was put into the beaker and fitted to the system. The heating time for the extraction was set at 30 minutes. At the end of the triplicate extractions, the extracts were allowed to cool, pooled together and then concentrated to 20 mL. The clean- up technique used involves washing each of the sediment extract with 10 mL of 0.1M Na_2CO_3 solution (3 portions) and shaking for 10 minutes each time using a mechanical shaker set at 250 rpm. The Na_2CO_3 extracts were combined and acidified to pH≤2 with 2M HCl and the FFAs were further extracted with 10 mL x 3 portions of diethyl ether each time.

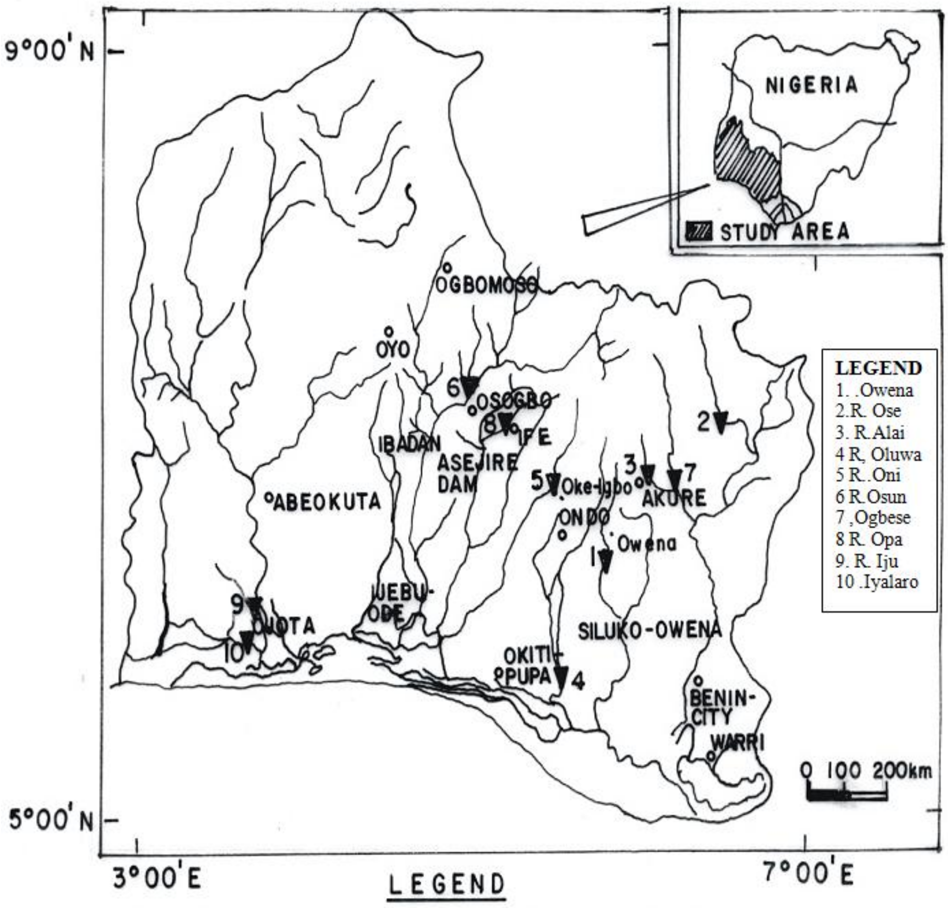

Figure 1. Map of South-Western Nigeria showing major rivers and sampling sites

Sample Collection

Sixty (60) sediment samples were collected during dry and wet seasons from ten randomly selected rivers. (Ala, Oluwa, Ogbese, Ose, Owena, Oni, Opa, Osun, Iju, and Iyaalaro) in South-Western Nigeria (Figure 1). The river sediments were collected at 30 minutes intervals from the bottom surface of the river with a metal grab and transferred into a 500 mL amber glass bottle with a Teflon-lined cap. About 10 mL of pentane (HPLC grade) was added prior to the extraction of the FFAs to prevent biological degradation and improve the extractability of free fatty acids in undissociated form (Fatoki and Vernon, 1989; Matsumoto, 1981). Samples were later stored at 4 ^{o}C.

The combined diethyl ether extracts were dried under anhydrous Na_2SO_4 filtered and evaporated to dryness with gentle stream of nitrogen gas and methylated with BF_3-methanol. A computerized Gas chromatography based on Flame Ionization Detector (GC-FID) (Varian Chrompack VA 3800 model), equipped with split injector was used for the analysis and characterization of the free fatty acids in the extracts. The parameters used for optimization of separation and GC conditions were determined. The capillary column used was ZB–5, 30 m x 0.25 mm i.d. 0.25 µL film thickness. The stationary phase was 5%-phenyl-95%-dimethylpolysiloxane. The GC/FID injector temperature condition was set at 260 ^{o}C; Split ratio 1:50; FID temperature was 300 ^{0}C; Temperature Programming was 100 ^{o}C (initial) and final temperature at 280 ^{0}C for 7min, held for 1min; flow rate 0.8ml/min. Precisely, 0.1 µL of the dissolved residue of the methylated fatty acids and n-butyl benzoate internal standard were injected into a GC for the analysis of the FFA methyl esters. Identification of the free fatty acids was performed by comparison of the relative retention times of the methyl esters of fatty acids standard with those of FFAs in the samples.

RESULTS AND DISCUSSION

Figure 2 indicates the representative chromatographic peaks of FFAs in the sediment samples in both seasons while the free fatty acid levels of the river sediments sampled in dry season are shown in Table 1.

In the dry season, the average concentration of lauric acid (C12:0) was 60.58±14.8 µgkg^{-1} across all the rivers with lowest and highest concentrations at river Osun and Oluwa respectively. Myristoleic acid (C14:1) was non detectable in these rivers (Ala, Ogbese, and Ose) and at concentration below 10 µg/kg from other rivers (Iju, Oni, Osun and Owena). The average concentration in all the rivers was 16.40 ± 6.17 µg/kg. Myristic acid (C14:0) was detected in all the samples at a much higher concentration. The least concentration at river Owena (40.95±34.95 µg/kg) was higher than the highest of C14:1 (38.17±5.7 µg/Kg). The highest concentration (149.54±6.17µg/kg) was found in river Iju, Palmitic acid (C16:0) recorded the highest concentration ranged (70.78±59.17 µg/kg to 509.45±37.83 µg/kg) of all the FFAs determined.

Table 1. Mean* levels (±sd) of FFAs (µg /kg) in some major river sediments of South-Western Nigeria in dry season

River	C12.0	C14.0	C14.1	C16.0	C18.0	C18.u	Overall mean
Ala_S	23.83	ND	104.84	138.17	93.46	89.38	89.94
	±1.69		±11.67	±19.09	±7.45	±4.92	±7.47
Iju_S	55.83	8.33	149.54	509.45	411.88	160.21	215.88
	±12.54	±13.23	±8.38	±37.83	±64.20	±33.27	±28.24
Ijalaro_S	68.39	38.17	93.78	254.75	131.67	194.83	130.27
	±26.27	±5.71	±12.25	±107.06	±36.55	±37.45	±37.55
Ogbese_S	36.61	ND	140.83	144.5	169.38	94.54	117.17
	±8.49		±11.02	±12.21	±25.32	±10.93	±11.33
Oluwa_S	198.44	28	68.28	157.5	123.25	110.72	114.37
	±56.52	±2.61	±10.83	±47.75	±59.30	±26.44	±33.91
Oni_S	53.83	7.15	57	219.78	132.63	78.21	91.43
	±2.72	±9.07	±8.29	±31.02	±18.60	±13.06	±68.37
Opa_S	20.95	27.67	56.61	112.95	105.25	69.29	65.45
	±3.83	±6.22	±4.76	±9.42	±8.81	±3.05	±6.01
Ose_S	79.00	ND	69.39	285.67	116.17	114.83	99.18
	±1.14		±48.81	±18.00	±15.38	±19.96	±17.21
Osun_S	20.89	4.8	67.67	70.78	74	44.34	47.08
	±1.92	±5.59	±12.10	±59.17	±16.56	±9.52	±17.47
Owena_S	48.06	0.67	40.95	76.45	13.3	78.08	42.92
	±28.69	±.73	±34.95	±42.28	±2.41	±10.49	±21.03
Overall	60.58	16.40	84.89	197.00	137.10	103.44	101.37
	± 14.38	±6.17	±16.31	±38.38	±25.47	±16.91	±19.60

Note:-S= Sediment sample; sd = Standard Deviation ND = Not Detected. Note : C12:0 = Lauric acid; C14:1 = Myristoleic acid; C14:0 = Myristic acid; C16:0 = palmitic acid; C18:0 = stearic acid, C18:u = unsaturated FFAs. * means value for three replicate analyses

Table 2: Mean* levels (±sd) of FFAs (µg /kg) in some major river sediments of South-Western Nigeria in wet season

RIver	C12:0	C14:1	C14:0	C16:0	C18:u	C18:0	Overall Mean
Ala_S	16.28	14.50	38.02	81.57	67.46	40.54	43.06
	±4.53	±1.87	±4.82	±6.27	±11.61	±5.20	±7.47
Iju_S	221.78	ND	36.33	205.28	99.92	64.62	125.59
	±17.94		±47.88	±36.20	±10.41	±8.21	±28.24
Ijalaro_S	40.73	6.87	75.11	295.67	138.42	172.13	121.49
	±6.25	±10.78	±40.85	±140.21	±31.96	±23.38	±37.55
Ogbese_S	2.95	12.33	59.17	130.72	141.63	75.00	70.30
	±0.83	±1.21	±6.42	±32.46	±42.53	±25.59	±11.33
Oluwa_S	180.83	17.04	29.62	144.26	138.29	80.50	98.42
	±38.46	±29.79	±25.13	±27.87	±13.58	±32.38	±33.91
Oni_S	25.33	5.60	242.84	298.12	194.63	134.25	150.13
	±2.92	±1.57	±122.33	±333.63	±128.29	±73.38	±68.37
Opa_S	7.15	13.83	57.00	219.78	132.63	78.21	84.77
	±9.07	±2.72	±8.29	±31.02	±18.60	±13.06	±6.01
Ose_S	22.94	1.50	23.22	249.75	121.84	99.08	86.39
	±1.97	±1.76	±50.67	±39.59	±21.17	±6.79	±17.21
Osun_S	82.20	22.89	79.00	152.17	164.17	124.29	104.12
	±1.05	±3.30	±4.55	±25.96	±7.14	±21.35	±17.47
Owena_S	51.61	0.50	106.28	183.17	4.74	287.28	105.60
	±5.76	±36.19	±23.89	±43.54	±0.56	±22.87	±21.03
Overall	65.18	10.56	74.66	196.05	120.34	115.59	98,99
	± 8.88	±9.91	±33.48	±71.68	±28.59	±23,22	±29.29

Note:-S= Sediment sample; sd = Standard Deviation ND = Not Detected. Note : C12:0 = Lauric acid; C14:1 = Myristoleic acid; C14:0 = Myristic acid; C16:0 = palmitic acid; C18:0 = stearic acid, C18:u = unsaturated FFAs. * means value for three replicate analyses

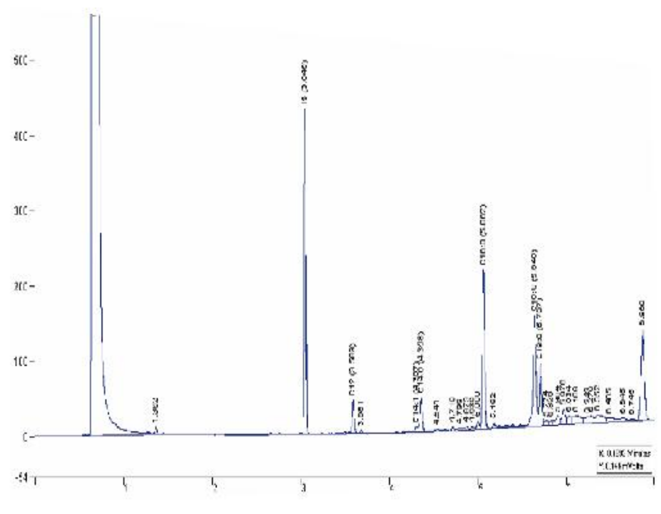

Figure 2. Representative peaks of FFAs in the sediment samples

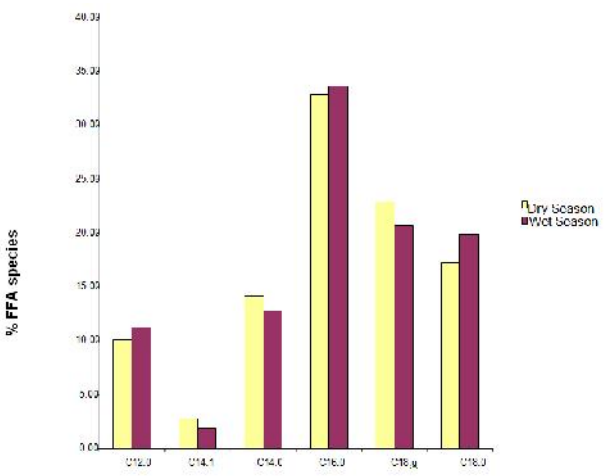

Figure 3. Percentage composition of FFA species in some surface sediments of South-Western Nigeria

Apart from C12:0 (Oluwa), C18:O) Ogbese and C18:u (Osun) the levels of palmitic acid was higher than all the FFAs across the entire river sediments. The levels of unsaturated carbon [carbon-18 FFA, (C18:u)] was equally high. Apart from significantly low level (13.30±2.41µg/Kg) at Owena, all other concentrations were above 70.0 µg/kg and the highest being 411.88±64.2 µg/Kg at River Iju. The level of stearic acid (C18:0) was slightly lower than that of C18:u across the all locations except at river Iyalaro (194.8 µg/kg) and river Owena (78.1µg/kg). The average mean across all locations was 103.44±16.91µg/kg. The overall mean concentration of the FFA species in all the river sediments from site to site ranged from 42.9±21.0 µg/kg to 215.9±28.2 µg/kg. The highest and least overall FFAs' sediments burden were found in Iju and Owena, rivers contributing 215.9±28.24 µg/kg and 42.9 ± 21.0 µg/kg FFAs respectively. Data of FFAs contribution during the wet season (Table 2) shows that in the wet season, the overall FFAs' sediment burden was highest in Oni river, contributing 150.1±68.4 µg/kg FFAs overall level and least in Ala river contributing an overall mean level of 43.1±7.5µg/kg FFAs. The overall mean concentration of the FFA species in all the river sediments from site to site ranged from 10.6±9.9µg/kg to 196.14±71.7µg/kg. The trend in FFA contribution follow the same pattern as in dry season with palmitic acid (C16:0) and myristoleic acid (C14:1) generally recording the highest and least concentrations. However, unlike in the dry season, C14:1 was detected in all the samples, though at low concentration except at river Iju. The distribution pattern and trend of all the FFAs are displayed in Figure 3. By interpretation, palmitic acid contributed 32.9 and 33.7 % of the FAs in the river sediments sampled in both dry and wet season respectively. This implied the predominance of C16:0 in the river sediments. From Tables 1 and 2, it is obvious that the unsaturated C14:1 levels are generally lower in both dry and wet seasons than all other FFAs in the river sediments investigated in this study. The reason for this may also be attributed to evaporation or oxidation during drying, storage, extraction, and methylation procedures for this volatile FFA. Other FFA species (C12: 0, C14: 1, C14:0, C18:u and C18:0) ranged between 1.73% and 21.98 % for dry season and 2.21% and 23.23% for wet season samples. Paired samples t- test analysis of surface sediment samples reveals that there were no significant differences between the levels of some FFAs species (C14:1 and C16:0) of the dry and wet season sediment samples at 95 % confidence level.

However, significant differences were observed between the levels of some FFAs (C14:0, C18:u and C18:0) in the sediment samples of both seasons. The observed variation in the levels of these FFAs between the two seasons cannot be explained in terms of biological interaction with the dissolved and particulate FFAs because these interactions are not properly understood (Stauffer and Macintyre, 1970) and also the organisms present were not sampled. Perhaps some of these FFAs were lost to evaporation and autoxidation more in the dry season than wet season. The presence of free fatty acids in river sediments of the study area is expected because many of these rivers received effluents from agricultural waste products, and municipal wastes and sewage discharge without pre-treatment. All these wastes find their ways into the sediments through surface run-off. Statistical analysis of data indicates that the FFA levels in the sediments of dry and wet seasons are not significantly different, even though they varied with locations. Therefore, seasonal variation is not a major determining factor for the relative amount of FFAs in the river sediments of the study area.

Conclusion

It is established in this study that free fatty acids are present in river sediments of South-Western Nigeria in varying degrees, the predominant FFA species being palmitic acid (C16:0) followed by unsaturated C-18 fatty acid (C18:u) and stearic acid (C18:0). Lauric acid (C12:0) and myristic acid (C14:0) are present in medium concentration while myristoleic acid (C14:1) is present in trace quantities. It has been possible to obtain, as a baseline data, information and a picture of the approximate distribution of FFAs in river sediments of Nigeria.

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