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Evaluation of Heavy Metals on Wetland Biodiversity of Oluwa River (Southwest Nigeria) POME Polluted Area

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ABSTRACT - The evaluation of Mg, Ca, Na, K, Cu, Mn, Fe, Hg, Zn and Pb concentrations in some component of biodiversity {Fish (*Oreochromis niloticus);*water ; soil sediment and submerge vegetation} from Oluwa River, Ondo State, Nigeria using X – ray fluorescence (XRF) technique is discussed. The river was stratified into four zones. Zone A, (ZA) was point of direct effluent discharge from the Palm Oil factory, zone B was point 50m away from ZA, towards the direction of water flow, zone C was point 100m away from ZA and zone D the control zone, was point 100m upstream from ZA without contact with the effluent. Physico-chemical parameters such as BOD, COD, DO_2, Temperature, conductivity and pH were also measured. Biodiversity samplings were carried out once weekly for 4 weeks and samples collected were prepared for analyses/measurements. The analytical samples are irradiated with high energy electrons of 109Cd to produce fluorescent X-rays was produced which passes to the Silicon-lithium detector through Mo target as a source of monochromatic X-rays. The spectrum of energy generated on the detector was processed by a Multi- Channel Analyzer to obtain analytical data. The intensity of the fluorescent X-rays on the detector is proportional to the concentration of the individual element of interest in the sample. This method can identify up to 30 or more elements at the same time. The results showed varying levels of heavy metals in the fishes, Water, Bottom sediment and submerged vegetation (biodiversity). The concentrations of Cr, Mn, Cu, and Ni in the fishes were much higher than WHO and FEPA maximum permissible limits, while the concentrations of Zn and Pb were lower than the standards. Results showed that pH; chemical oxygen demand (COD); biological oxygen demand (BOD); conductivity and temperature (T^oC) of POME were critical for the survival of aquatic organisms. Water pH; dissolved oxygen (DO); COD; BOD and T^oC were most critical ($P<0.05$) at ZA and improved along ZC, while there was no effect in ZD. There were positively high correlation between DO/pH; COD/pH; BOD/pH; conductivity/pH; T^oC/pH/COD/BOD and conductivity. Negative correlation also existed between COD/DO; BOD/DO; conductivity/DO and T^oC/DO. Regression analyses indicated high coefficient of determination R^2 between the water parameters and low R^2 between DO/T^oC which had equation as $DO = 72.0 – 2.45T^oC$. Biodiversity mineral concentration was excessively high due to POME pollution, indicating possible subtoxic effect. The results suggest that the lake is polluted with Cr, Mn, Cu and Ni and the consumption of fishes of the lake is life threatening to man. This is a confirmation that fish in river Oluwa within the polluted areas must have either migrated out of the zones or died due to POME toxicity.

Index Terms: Palm Oil Mill Effluent (POME), Acute Toxicity, River Oluwa, Biodiversity

INTRODUCTION

The existence of heavy metals in the aquatic environments has become a problem of much concern worldwide. Heavy metals can accumulate in the tissues and organs of fishes to a hazardous level without visible signs [1]. The commonest sources of heavy metals contamination may arise from industrial and municipal wastes, normal geological weathering of rocks, soil erosions, agrochemical leaching, atmospheric deposition, petroleum exploration or due to population increase, urbanization and exploitation of natural resources [1].

The heavy metals of concern include cadmium, chromium, copper, arsenic, nickel, iron, zinc, mercury and manganese. Iron, zinc, copper, nickel and magnesium are essential metals and are less toxic than non-essential metals [2]Cadmium, chromium, copper, nickel, lead and zinc exhibit aquatic toxicity when present above recommended standard. They contaminate water, plants, aquatic life and man, through bioaccumulation [3].Heavy metals form one of the major contributors to the pollution of natural aquatic ecosystems [4]

X-Ray fluorescence (XRF) is the emission of characteristic fluorescent X-rays from an analytical samples that has been excited by bombarding with high-energy elections. The technique is used for chemical and elemental analysis. It is fast, precise and non-destructive in nature. It can identify up to 30 or more elements at the same time by measuring characteristic fluorescence [5].

Palm oil is obtained from the fleshy mesocarp of oil palm fruits (Elaeis guineensis) which contain 45-55% oil [6]. In 1978, Okitipupa Oil Palm Mill factory was established, and since then the palm oil mill effluent is being discharged into River Oluwa [3] & [6]. The effluent from the discharge is a mixture of sterilizer condensate from the hydrocyclone unit, sludge from bottom sludge tanks and others from factory drains. Presence of the effluent in the river constitutes environmental harzard as it causes oxygen sag apart from unpleasant odour and other

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bioactivities with detrimental effect on aquatic life ([8]and [9]). The major characteristics of industrial waste waters that affect receiving water quality includes, organic matter, suspended solids, dissolved solids, nutrients, toxic matter and pathogens ([10]). Parameters such as pH, dissolved oxygen (DO), BOD, COD, ammonia and suspended solids have been used in monitoring water quality and extent of pollution of Nigerian rivers ([11], [12]; [13]), and other rivers ([14]; [15]).

Therefore, the present study evaluated the effect of POME on biodiversity of river Oluwa using such indices as water pH, BOD, COD, conductivity, suspended and dissolved solids, and heavy metal concentrations on the fish, water, submerged vegetations and bottom sediments.

MATERIALS AND METHODS

River Oluwa is enclosed between latitude 6°30'N and longitude 4°48'to 4°55E rising from Ayepe-Olode in Osun State. It transverses Ondo town, crosses the Lagos/Benin Expressway at Ore and eventually discharges into the Atlantic Ocean at Ayetoro. The main tributaries of River Oluwa are rivers Ofara (6°29, 04N 4°44,44E) and Erinodo (6°29,64N4°44,02E) (Akinsorotan 2005). The river bank is characterized by shore line vegetation interspaced with dove-tailing branches. River Oluwa receives palm oil mill effluent (POME) from Okitipupa Palm Oil mill Plc., at Okitipupa. The study site was established along the river behind the factory site with sampling zones/points at 50m intervals and made up as follows: Zone A (ZA) - the point of discharge of effluent into the river, Zone B (ZB) - 50m away from ZA, towards the direction of water flow (down stream), Zone C (ZC) - 100m away from ZA, Zone D (ZD)- control area, 100m upstream from ZA. Samples were collected from the four zones for analyses. POME effluent, water, fish, submerged vegetation and bottom sediments were collected from each sampling zones once a week for four weeks. Fishermen/divers were employed to collect samples at the bottom of the river. Cast nets were used by the fishermen to catch the tilapia fingerlings used in the study. The samples were collected and stored at -20°C prior to analyses. The chemical analyses of the samples were conducted according to the methods of [16].

The modified version of Emission– Transmission (E-T) method ([17], [18] and [19]) was used. The fish sample, *O.niloticus,* was de-scaled and oven dried to constant weight at 105°C. Submerge vegetation samples were sundried; while bottom sediment samples were also sundried. The dried samples were ground to powder, sieved to grain size of less than 125µm and quartered to give representative sample weight. 0.5g of the powdered fish samples was weighed and three drops of organic binder were added to each and were pressed with 10 tons hydraulic press to form pellet of each fish samples. Three replicate of pellets of each fish sample were prepared.

Determination of Heavy metals in fish by Energy Dispersive X- ray Fluorescence (EDXRF). The modified version of Emission Transmission (E-T) ([17], [18] and [19]) method was used. The pellet of each fish sample was put into X –ray fluorescence spectrophotometer sample holder and it was bombarded with high energy electrons of 109Cd (22.1kv). Fluorescent X –rays was produced which passes to the silicon lithium detector, through Mo target as a source of monochromatic X –rays. The spectrum of energy generated from the detector was processed by a Multi – Channel Analyzer (MCA) to obtain analytical data. The intensity of the fluorescent xrays on the detector is proportional to the concentration of the individual element of interest in the sample. This investigations were conducted in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, published by the Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, First Edition, 1988; the National Research Council (NRC) publication Guide for Care and Use of Laboratory Animals (copyright 2010, National Academy of Science).

RESULTS

The physico-chemical properties of POME are presented in Table 1, which showed that the pH was acidic and critical to survival of aquatic organisms. The COD, BOD and T°C values were far above the values for other rivers in Nigeria. While conductivity was far less than values reported for other rivers. This indicates danger to aquatic lives where POME was discharged. Also the high levels of minerals concentration in POME is an indication of possible minerals toxicity to the biodiversity in the discharged areas.

Table 2 presents physico-chemical properties of water samples from different zones/strata in river Oluwa, the study site. The pH of water in ZA was acidic ($P<0.05$) and improved towards water in ZD which had almost neutral pH. DO concentration in ZA and ZB was critical ($P<0.05$) and could possibly not support any lives. However, DO, improved in ZC and ZD to acceptable levels for tropical warm water fishes. BOD of the water was dangerously high ($P<0.05$) in ZA and ZB, but improved to desirable levels in ZC and ZD.

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Characteristic of the COD was similar to that of BOD. Water T°C and conductivity were high ($P<0.05$) in ZA. While T°C was still within tolerable ranges, conductivity was very low in comparison with values from other Nigerian rivers.

Table 3 presents the correlation matrix between the water parameters from the water strata. There were positively high correlation between DO/pH; BOD/pH; COD/pH/BOD; conductivity/pH/BOD/COD and T°C/pH/BOD/COD and conductivity. This explains direct relationships between the variables. Also high negative correlation existed between BOD/DO; COD/DO; conductivity/DO and T°C/DO. This means inverse relationships between the parameters that an increase in one parameter led to a corresponding decrease in others.

The regression equations of the water parameters are presented in Table 4, which showed high values of coefficient of determination R^2 between the parameters, and low R^2 between DO/T°C, which also had regression equation as DO =72.0 -2.45T°C. The regression equations also showed the dependency of each variable on others, and they might be useful in the prediction of water parameters around the studied water strata.

Table 5 presents the values of suspended and dissolved solids. Both parameters were highest ($P<0.05$) in ZA and ZB and reduced along ZC and ZD. Generally, the parameters were at very low concentration that might not support good aquatic production. This also led to low levels of conductivity in the water.

Biodiversity minerals concentration (Table 6) indicated that both the major and minor elements were excessively high in the samples. In most cases, highest concentration was observed in samples from ZA. This high volume of minerals concentration could be toxic to aquatic lives. The high mineral concentration in the samples is due to high levels also contained in POME (Table 6). The table also showed that POME dissolved in the water to the extent that highest concentration of minerals was observed in the bottom sediments. POME also adsorbed to submerged vegetations which led to very high mineral concentrations in the samples. There were high concentrations of Cu and Pb in the bottom sediments. However, no traces of Hg were found in the samples. Also the high concentration of minerals in the fish samples is an indication that the fish might have fed on POME directly, eaten the submerged vegetations or even taken it as solutes in water.

DISCUSSION

The influence of discharged palm oil mill effluent (POME) on biodiversity resources of River Oluwa, Ondo State was investigated with a view to suggesting safe levels for management policy decisions. The physico-chemical properties of POME clearly indicated that it is a pollutant, as its presence in water changed the physical and chemical qualities of water to critical levels that could hardly support aquatic productivity. In 1997, Aiyesanmi & Ipinmoroti studied the impact of POME on some water quality variables in the same river and concluded that POME constituted environmental hazards as it changed the colour of the water and with high values of pollution characteristics.

The minerals concentration in the effluent is at toxic levels. This also resulted in concentrations of very high (subtoxic) levels of minerals in the fish, water, vegetation and bottom sediments in the areas investigated. This level of minerals could lead to minerals toxicity in aquatic ecosystem, as the minerals balance ratios have been altered. Minerals concentrations were least in the water samples, while concentrations were higher in the vegetation and bottom sediments than in the fish samples. This finding is in line with that of [20] and [21] which showed that metals concentrations in water samples from three rivers in Ondo State were very much lower than the corresponding concentrations in the sediments. However, the observation that metals concentrations in fish was higher than those in sediments [20]; [21] contrast the observation from the present study which showed that higher metals were concentrated in vegetations and sediments than in fish. This discrepancy could be ascribed to absorption/adsorption of high concentrations of POME by/on the vegetations and sediments. From a similar study, [22] also reported that metals concentrations showed large variation among samples from different water bodies.

As expected, high concentration of POME in the areas resulted in increased water temperature with corresponding increase in BOD, COD, conductivity and reduction in dissolved oxygen concentrations. Conductivity is the expression of the levels of ions in the water that maintains ionic balance and offer buffer in the ecosystem. But the levels observed in the studied areas were far below values reported for good productivity in tropical rivers [13] and pond waters [21]. This could be attributed to very high concentration of POME which interfered with ionic dissociations.

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Naturally, in self purifying tropical rivers, COD values are higher than BOD because the former is a more complete process [13], but the very large gap between the values obtained from the studied areas is evidence of very low biological activities in the areas which could be attributed to POME toxicity. This also showed high oxidation and concentrations of organic materials in the areas. The extremely, high levels of BOD and COD in POME resulted in a rapid consumption of the DO in the receiving water leading to a phenomenon referred to as oxygen sag. This process has also been described by [8], [23] & [21]. [24] similarly reported oxygen sag in a river in South Western Nigeria due textile mill effluent pollution.

The acidic pH condition of POME led to acidic water quality. This has implication of killing the organisms or reducing aquatic productivity. [21] suggested that the acidity of the water could be ascribed to the presence of free fatty acids (FFA) in the effluent.

Histopathological changes in Nile tilapia caught in the polluted areas are indications that though the fish were still alive at the time of sampling, that they already had chronic disease conditions which would result in death with time. This observation is in consonance with the report of [25] who described that apart from massive killing of African catfish and Nile tilapia fingerlings by an ichthyotoxic plant (*Parkia clappertoniana*), that sublethal concentration of the toxin has chronic histopathological conditions on the survived fishes which could constitute serious unconscious threat to fish conservation.

The 96h LC_{50} of Nile tilapia which is 9.19 ml L^{-1} [7] is a pointer to what happens to the organisms in the river when hundreds ml L^{-1} of POME is discharged. Therefore, safe level of POME to be released in the water must be lower than 9.19 ml L^{-1} and the value could be even lower when micro-organisms are considered. The revelation from the bioassay test on how the fish were attempting to jump out of the experimental tanks when in contact with POME toxicant [7], suggests that most of the fish in polluted areas might have migrated out of the zones. This finding was corroborated by fishermen in the area (names not disclosed) who confirmed that many species and adult fish have migrated out of the zones leaving few hard survived tilapia and tilapia fingerlings. They also confirmed that at the point of discharge (ZA) that one can hardly see any fishes that occasionally fingerlings of tilapia dive into the areas to feed on POME, and later move back to less polluted areas. The low fish catch in the studied areas also supports the work of [21] who described that the levels of BOD and COD resulted in environmental pollution which manifested in the decline output of fish production in the down stream part of the river as reported by fishermen. In the course of data collection for this study, the fishermen also reported about fish kills at the point of effluent discharge due to high POME concentration and toxicity during the peak of dry seasons. [7] also reported that discharge of POME in the river has caused a reduction in the number of fishes due to migration and absence of some fish species at the point of discharge which has affected the economic benefits of the fishermen.

Table 1: Physico-chemical properties of the raw Palm Oil Mill Effluent (POME)

Appearance	Yellowship and emulsified
pH	4.83± 0.04
COD (mg kg^{-1})	1547.4 ±21.1
BOD (mg kg^{-1})	1048.9 ±16.5
Conductivity (µs/m)	9.85± 0.00
Temperature (°C)	38.5± 0.00

Table 2. Physico-chemical properties of the various studied water strata in Oluwa River

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Table 4: Linear regression analysis of the physic-chemical properties of water samples from the different strata along Oluwa river

STRATAS → PARAMETERS ↓	A	B	C	D
pH	5.43 ± 0.20^{a}	6.38± 0.07^{b}	6.97± 0.03^{c}	7.20± 0.01^{d}
Dissolved oxygen (DO) mg kg^{-1}	1.25± 0.05^{a}	2.80± 0.15^{b}	5.88± 0.15^{c}	9.28 ±0.08^{d}
Biochemical oxygen demand (BOD) (mg kg^{-1})	91.1 ± 0.64^{a}	39.0 0.34^{b}	10.2±0.04^{c}	6.21±0.04^{d}
Chemical oxygen demand (COD) (mg/kg)	261.6± 2.18^{a}	127.0± 0.62^{b}	57.6± 0.45^{c}	9.54± 0.18^{d}
Conductivity (µs/m)	1.73 ±0.03^{a}	0.57± 0.12^{b}	0.53± 0.01^{b}	0.06± 0.00^{c}
Temperature (°C)	28.9 ± 0.13^{a}	27.0 ± 0.00^{b}	27.0± 0.00^{b}	26.8 ± 0.25^{b}

Relationship	R^2 value (%)	Regression equation
Conductivity/DO	72.1	Conductivity = 1.54-0.17DO
DO/Temp	49.9	DO = 72.0-2.45Temp
COD/BOD	97.9	COD = 12.5+2.77BOD
Conductivity/BOD	91.2	Conductivity =0.087+0.017BOD
BOD/Temp	82.9	BOD = -917+34.8Temp
Conductivity/COD	94.1	Conductivity = 0.005+0.006COD
COD/Temp	80.9	COD =-2522+96.2Temp
Conductivity/Temp	88.2	Conductivity = -17.1+0.65Temp
DO/pH	78.8	DO = -20.2+3.85pH

Table 3. Correlation between the physico-chemical properties of water samples from the various strata in River Oluwa.

Parameters	pH	DO_2	BOD	COD	Cond	Temp
pH	1.00					
DO_2 (mg kg^{-1})	0.89	1.00				
BOD (mg kg^{-1})	0.96	-0.87	1.00			
COD (mg kg^{-1})	0.96	-0.92	0.99	1.00		
Conductivity (µs/m)	0.92	-0.85	0.96	0.97	1.00	
Temperature (°C)	0.84	-0.71	0.91	0.90	0.94	1.00

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Table 5: Suspended and Dissolved solid of the different strata (mg l $^{-1}$) along Oluwa river

Strata	Suspended solid	Dissolved solid
A	0.34± 0.02[a]	9.40± 0.21[a]
B	0.25± 0.01[b]	3.05± 0.05[b]
C	0.09± 0.58[c]	0.48± 0.18[c]
D	0.04± 0.01[c]	0.11± 0.00[d]

Table 6a. Mineral composition (major elements) of Nile tilapia, water, soil and vegetation samples from the different water strata along Oluwa river.

	STRATA	Mg	Ca	Na	K
Nile tilapia	A	337.8 ±0.25	243.5 ±0.50	321.8 ±3.70	265.0 ±10.5
(mg kg $^{-1}$)	B	299.0± 0.50	313.6 ±0.60	368.0 ±9.55	387.4 ±3.15
	C	255.8± 0.30	317.5 ±0.00	387.9 ±3.65	396.8 ±2.70
	D	206.2± .30	172.8 ±2.25	196.9 ±0.65	170.6 ±0.40
Water	A	68.0 ±0.45	82.8 ±0.53	70.3 ±0.12	68.9 ±0.33
(mg l $^{-1}$)	B	55.6 ±0.71	64.7 ±0.53	66.2 ±1.05	54.1 ±0.21
	C	88.6 ±0.11	60.8 ±0.08	57.9 ±0.31	70.3 ±0.18
	D	70.3 ±0.27	48.1 ±0.00	45.7 ±0.24	48.7 ±0.24
Bottom	A	593.4 ±0.95	560.6 ±0.60	354.2 ±2.25	341.5 ±1.05
sediment	B	479.4 ±0.95	480.7 ±0.15	342.3 ±0.55	269.1 ±1.60
(mg kg $^{-1}$)	C	320.8 ±0.40	471.0 ±0.65	325.2 ±0.40	258.4 ±1.25
	D	219.4 ±0.20	322.5 ±0.35	212.7 ±0.15	158.6 ±0.60
Submerged	A	489.3 ±0.20	481.9 ±0.40	416.6 ±8.60	514.0 ±2.00
vegetation	B	347.8 ±0.20	346.2 ±0.35	332.2 ±1.35	492.2 ±1.85
(mg kg $^{-1}$)	C	313.6 ±0.10	325.6 ±0.45	323.5 ±2.00	454.5 ±2.50
	D	290.9 ±0.15	251.9 ±0.10	320.7 ±1.30	451.9 ±1.10
Raw palm oil mill (mg/1)		298.2±0.80	282.7 ±1.35	260.6 ±0.60	291.5 ±0.95

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Table 6b. Mineral composition (minor elements) of Nile tilapia, water, soil and vegetation samples from the different water strata

STRATA		Zn	Hg	Cu	Mn	Pb	Fe
Nile tilapia	A	141.9 ±1.60			63.8 ±1.25		2.21 ±0.50
(mg kg^{-1})	B	173.5± 3.05	ND	ND	82.3 ±0.75	ND	1.69 ±1.00
	C	192.3± 3.25			101.7 ±0.85		1.05 ±190
	D	39.5± 1.05			127.2 ±1.80		0.45 ±2.10
Water	A	3.48 ±0.03			0.034 ±0.04		0.07 ±0.03
(mg l^{-1})	B	2.08 ±0.03	ND	ND	0.01 ±0.00	ND	0.05 ±0.04
	C	1.49 ±0.39			0.02 ±0.00		0.03 ±0.00
	D	4.47 ±0.07			0.05 ±0.00		0.01 ±0.00
Bottom	A	154.6 ±0.60		70.6 ±0.20	81.3 ±0.35	14.7 ±0.25	451.6 ±0.40
sediment	B	122.1 ±1.30	ND	26.2 ±0.25	72.2 ±0.20	4.15 ±0.15	419.7 ±0.45
(mg kg^{-1})	C	117.9 ±0.10		22.6 ±0.20	66.5 ±0.35	40.6 ±0.60	388.7 ±0.50
	D	58.6 ±0.20		3.70 ±0.10	38.5 ±0.30	ND	241.3 ±0.35
Submerged	A	7.30 ±0.20					10.2 ±0.33
vegetation	B	5.35 ±0.15	ND	ND	ND	ND	7.32 ±0.19
(mg kg^{-1})	C	3.90 ±0.10					7.34 ±1.16
	D	2.85 ±0.15					4.99 ±0.01
Raw palm oil effluent (mg/ l)		137.1±0.10	ND	ND	ND	ND	192.7 ±0.85

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