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Fish Kills and Physiochemical Qualities of a Crude Oil Polluted River in Nigeria

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Abstract: The impact of a spillage of Bonny light crude oil into the Miniahia eke fresh water in the Niger Delta region of Nigeria was investigated. Physicochemical parameters of the Freshwater were determined using standard analytical methods. Newly killed fish samples were collected close to the point of spill and the crude oil concentration in the water was evaluated. The physicochemical and biological parameters showed reduction of dissolved oxygen content range from 0.21 to 4.48 mg 1^{-1}. The concentration of crude oil in the freshwater ranged between 4084 and 8304 ppm in July 2001, 876 and 3015 in January 2002. The concentration of the crude oil varied in the four different species of fishes studied, and these followed the trend *lctalurus* sp. (Catfish) < *Pseudotolithus* sp. (Croakers), < *Clarias* sp.(Mudfish) < *Cichlidae* sp. (Tilapia) according to surface area covered with crude oil. The highest bacterial count values range between 4.2×10^6 - 6.9×10^6 cfu/ml. In conclusion, fishery products should factor significantly into the food security agenda because in Nigeria in particular and in the study area for example the fishery products are significant for domestic consumption because it provides approximately 22 percent of the animal protein.

Key words: Petroleum degrading bacteria, Niger Delta, Fish kills, Physicochemical parameters

INTRODUCTION

Petroleum, refinery and petrochemical industries are most desirable for national development and improved quality of life, the unwholesome and environmentally unacceptable pollution effects of the wastes from these industries which have been reported world-wide[29,8]. Petroleum exploration and exploitation have deleterious effects on the ecosystem and biodiversity in Niger Delta areas[22]. These pollution problems have been prevalent in Nigeria since the 1950s[25].

In Nigeria, oil spill did not receive attention until late 1970's, when formal documentation commenced. From available statistics, a total of 9,107 oil spill incidences occurred between 1976 and 2005 resulting in about 3,121,909.8 barrels of oil spilled into the environment[21]. In the Niger Delta Area alone, there have been over 550 reported cases of crude oil spillage since 1976, releasing about 2.8 million barrels of crude oil into the environment[22,19]. It is a recognized fact that environmental pollution problems associated with oil and gas exploration and production exist in the Niger Delta area[1,2,35].

Evidence show that both soil, marine and fresh water ecosystems are polluted by those actions taken without due consideration of the human and natural resources that are impacted upon[20,32]. Generally, crude oil is toxic to aquatic organisms, due to the presence of PAH[15]. The risk of drinking water contaminated by crude oil can be extrapolated from its effect on rats that developed hemorrhagic tendencies after exposure to water soluble components of crude oil[26,24] reported that an average of 35,000 barrels of crude oil is stolen per day in circumstances that threaten lives and the environment. Apart from the lost of lives and property through pipeline fire, the run-off from impacted sites usually degrade the quality of the fresh water sources which serves the domestic rural water supply needs of most communities in Nigeria. This has serious implications for the exploitation of fishes for food security in terms of animal protein source for the rural community.

The Environmental and Ecological Status: The surface water of the River Isiokpo was covered by a

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scum of crude oil as caption in (Plates 1 and 2). It has been reported that pipeline breakages are a source of oil pollution in Nigeria's Niger Delta region as catalogued in Table 1, and confirmed by the previous works of[27]. Since then there have been other incidences as listed in Table 1 below between July 1998 and April 2007. This is because Nigeria transports her crude oil from oil fields via pipelines to the refineries to the northern geopolitical zones. "Oil bunkering" is peculiar to Nigeria and thus made it difficult to assess whether an oil spill was through pipeline vandalization or through artificial or natural oil spills due to excessive pressure on the pipeline or otherwise.

The problem of the oil spill in the River Isiokpo spread wide, covering the entire surface of the river. Some aquatic organisms of great interest to the inhabitants were the fishes, which were conspicuously seen floating on the surface of the water days after the spill as caption in (Plate 3). The international experience of water management is a vital to fostering sustainable management and conservation of Nigeria's water and fisheries resources especially with reference to the continued oil spills from the oil and gas industry in the Niger Delta Region as shown by the impact of the oil spill in the study area.

The present study reports the impact of a spillage of Bonny light crude oil into the Miniahia eke freshwater in the Niger Delta region of Nigeria, and its resultant effect especially on fishes. Also the effect of changes in physicochemical parameters of the water on the viability of the fish habitat was examined.

2.0: Methodology:

Description of Study Sites, Oil Spill Date of Incidence: The study area is a fresh water polluted river located at Ogbodo in Isiokpo village is only 100 kilometer (km) from the oil field and pipeline carrying Bonny Shell light crude oil in Rivers state of Nigeria and within the geographical coordinates 0.05° 00' N and 0.06° 50'E. The Niger Delta is located in Southern Nigeria and is Africa's largest delta, covering about 70,000 square kilometers, and with about one-third of it made up of wetlands, and the third largest world mangrove forests. The predominant occupation in the area is fishing and farming, and location is inundated with pipelines carrying Bonny light crude oil from the oil fields to the refinery. The citizen woke up in July 2001 to find that the source of their likelihood – fresh water – has been covered with crude oil.

Sampling Techniques: Samples were collected from three strategic points at each location; zones 1 to 3. The points were located very close to the point of spill, since point of spill was inaccessible (Remediation by physical method was already going on at this zone), the source of drinking water located inside the Ogbodo Isiokpo village where the Miniahia eke river also serves as means of transportation to the farmers, and the other end of the village is surrounded by farmlands, washing of ropes, fishing nets and other materials was done at this zone including fishing activities. The fishermen nets could be seen at strategic locations around the river bank. Each zone was divided into 3 stations depending on their accessibility by foot or boat to take water samples.

Sample Collection: Water samples were collected on four different occasions during study, in the months July 2001, September 2001, January 2002, and July 2003 and transported to the laboratory for analysis. Amber bottles with tight stoppers were used for collecting water samples for chemical analyses. Temperature and pH of the water samples were measured *in situ* at different sites using a mercury bulb thermometer and a pH meter (292Mk 2PYE UNICAM), while the other physicochemical parameters were analyzed as described in the Standard Methods for the Examination of Water and Wastewater[3,10].

Sampling of Fish: Freshly dead fishes were only in evidence in the vicinity of Zone 1, station 1 during the investigation in July 2001. The affected fishes including fingerlings were collected with the assistance of a local landowner. Some of the newly dead fishes were immediately cut opened, using a sterile pair of fine scissors and forceps to check for possible tissue absorption of crude oil as photographed above (Plate 3). Samples were placed into a suitably sized cooler pack with ice and transported in cold conditions. The bacteriological specimens were stored in the deep freezer at –20°C, in the Department of Microbiology at the Obafemi Awolowo University Ile-Ife, Nigeria, until they were examined by standard methods.

Methods of Analysis:

Water Analysis and Physicochemical Parameters: All samples were analyzed as described in the Standard Methods for the Examination of Water and Wastewater[3,12] except otherwise stated. All the reagents used for the analysis were of analytical grade and obtained from BDH Chemicals Limited Poole England. Conductivity and pH were determined by the electrometric method using conductivity meter and pH meter (292Mk 2PYE UNICAM).

Analysis of the Crude Oil Concentration in the Freshwater Samples

The concentration of the crude oil in the water sample was determined using a modified method of[30,31]. 10 cm^3 sample of the water from each zone was

Plate 1: A photograph showing the oil scum on the Isiokpo River in the Study Area July 2001–Jan 2002 (Physical remediation by containment and scooping was on at the site of spill).

Plate 2: Another photograph on the Isiokpo River showing the extent of the spread of the oil scum spilled into the Study Area.

Plate 3: A photograph showing dead and decaying fishes caught at the point of oil spilt on the Isiokpo River.

Table 1: Catalogue of Petroleum Oil Spills in Nigeria's Niger Delta 1998-2007

Oil Spills	Date
Shell Petroleum Development Company, Ibibio I, at Ikot Ada Udo in Ikot-Abasi, Akwa Ibom State	April 2007
Shell's oil spill leaks at the Nembe Creek Trunk Line	2006
Shell's oil spill, Mogho, Ogoniland of Rivers State	October, 2004
Shell's oil spill in Barrale, Ogoniland of Rivers State	October 2001-January 2002
Chevron's major oil spill in Ilaje, Ondo State	May 2002
Shell's oil spill disaster in Ogbudu, Niger Delta	June/July 2001
Gana, an Urhobo Community, impacted by Shell's oil spill	January 2000
Nigerian National Petroleum Corporation's (NNPC's) oil spill in Adeje, an Urhobo Community	January 2000
Shell's oil spills from abandoned facilities in Ogoni	1999
Shell's oil spills in Bille in Niger Delta's Rivers State	November 1999
Chevron's oil spill in Ilaje, Ondo State	July 1998

Source: (Udo, 2008; WWW. Waado.org/Environmental 3/3/2007)

extracted with dichloromethane. The residual oil retained on the filter paper was extracted with 10.0 ml of dichloromethane (CH_2Cl_2). The remaining content of the flask was thoroughly shaken with another 10.0 ml of dichloromethane (CH_2Cl_2) for about two minutes and transferred onto the filter paper. The filtrate of both extractions was collected in a clean, dry and pre-weighed conical flask. The oil-solvent mixture was treated with anhydrous sodium sulphate, in order to remove the residual water from the extract. 50 g of sodium sulphate was added and shaken vigorously to remove all traces of water that may have been present in the aqueous medium. The extract was reduced to a volume of 10 ml by evaporative concentration. Hydrocarbon oil concentrations in the water sample were extrapolated from a standard curve obtained by preparing various concentrations of the crude oil (0.10, 1.0 and 10.0 mg/ml) with absorbance's (0.01, 0.1and 0.3) at a wavelength of 430 nm. The concentration of the crude oil in water was calculated according to the following equation:

Equation:

$$C = \frac{R \times D}{V}$$

C = % crude oil (ppm)
R = Concentration from graph
V = Volume of sample (ml)

Where,

$$D = \frac{Va}{Vb}$$

Va = Total volume of solvent
Vb = Volume of Extract (10ml)

Bacterial Isolation and Cultivation: Freshly dead fish were examined for the presence of bacteria by cultivation. Four Fish species were studied; *Ictalurus sp.* (Catfish), *Clarias sp.* (Mudfish), *Pseudotolithus sp.* (Croakers), *Tilapia sp.* (Cichlidae). Fishes were externally washed with sterilized water to reduce potential contamination with skin bacteria. Sterile cotton swabs were used to sample the fish mouths, gills and the body. Each swab was spread on sterile nutrient agar plates and on tryptic soy agar (Difco) with 1% NaCl (TSA-1) as well as on selective media for *coliforms* such as MacConkey Agar[10]. Test samples were incubated under the same conditions and were carried out in triplicates, along with appropriate positive and negative controls at different temperatures. Readings were taken every 24 h for up to 5 days for bacterial growth and up to 7 days for pigment production. The viable count was assessed by the agar-surface viable count method and brown diffusible pigment productions were evaluated on TSA[4]. The most abundant colonies were selected and obtained in pure culture for characterization and identification.

Characterization of the Bacteria Isolates: Bacteria strains identification was conducted based on colony morphology, cell morphology, and biochemical tests. The morphological, cultural and physiological characteristics of the isolated bacteria were compared with data from the taxonomic scheme of Bergey's Manual of Determinative Bacteriology[9,16]. Gram staining, oxidase test, motility, susceptibility to the vibriostatic compound O/129 (2,4-diamino-6, 7-

diisopropylpteridine), and growth on thiosulfate citrate bile sucrose agar (Difco) were the main assays employed to identify the organisms[14]. All colonies suspected to be *E. coli* were biochemical confirmed by their ability to produce indole from tryptophan using Kovac's reagent[17].

RESULT AND DISCUSSION

Results: Results on physico-chemical parameters (Table 2) revealed that the lowest recorded value of dissolved oxygen obtained in this study was 0.21 mgl^{-1} (Table 2) before the physical remediation started, while the highest of 4.48 mgl^{-1} was recorded in July 2003 after scooping and containment of the crude oil. The pH ranged of 4.3-7.2 was recorded during the period of investigation. The highest temperature recorded in the study area ranged between 23.5 ^{o}C -32.4 ^{o}C and turbidity values ranged from 37 to 237 NTU, while total alkalinity ranged from 18.4 to 119.5 throughout the sampling period as presented in table 2.

Profile of Crude Oil Concentration in Water and Fish Samples: The highest levels of the polluting oil measured in the freshwater was 8304 ± 376.24 ppm in zone 1, station 1, recorded in July, 2001 as presented in Fig. 1., while the lowest recorded value of 61 ± 3.09 ppm was obtain in July 2003 in zone 3 station 3, throughout the sampling period as shown in Fig.4.

The profile of the polluting oil varied in the four different species of fishes as summarized in (Table 3). On the whole, the individual fish species of the catfish (*Ictalurus sp*.) had the largest surface area of the polluting crude oil concentration followed by the species of croaker (*Pseudotolithus sp*.), Mudfish (*Clarias sp*.) and *Cichlidae* (*Tilapia sp*.) which had the smallest surface area covered with crude oil sample.

Bacterial Population on the Various Parts Examined: Using cultural characteristics, cell morphology and biochemical characteristics, four bacteria species detected in these fishes, as well as *Aeromonas* strains isolated are indicated in (Table 4) *Escherichia coli, Bacillus substilis, Proteus vulgaris, Pseudomaonas aeruginosa* and *Aeromonas spp* were identified. From this table, it is seen that the highest bacterial counts was 6.9×10^{6} cfu/ml, and this was encountered on the body of the fish, while the bacterial counts were 4.2×10^{6} cfu/ml in the gills.

Discussion: Detailed results of the water quality samples and measurements taken from the study area are presented in table 2. Physical measurements were taken on site, as many variables as possible with the available field instrumentation, especially of those variables such as temperature, dissolved oxygen, and pH that are prone to change prior to analysis in the laboratory.

Results recorded for dissolved oxygen (DO) in freshwater samples range from 0.21 to 4.48 mg/l. The low level of DO in our samples may be attributed to the presence of the crude oil spread on the river, and this must have resulted in the depletion of DO levels in the study area. This is in agreement with the work of[6] who reported that the presence of crude oil spilt could inhibit O_2 penetration into the water where fishes live thus bringing about O_2 tension. Moreover, the type of life in a natural water body will depend upon the amount of DO Present. In accordance with the set guidelines of[37], DO of 6-9 mg/l is required for the survival of aquatic life, thus the low dissolved oxygen levels that were recorded at all three Zones before scooping and containment of the polluting oil in the water, between July 2001-July 2003 presented in (table 2) are indicative of water quality problems, and the oxygen levels are low enough to be problematical to the survival of fish.

The pH range of 4.3-7.2 was recorded between July 2001 and July 2003. Previous studies had shown that low pH is toxic to fish and other aquatic lives[5]. The generalized short-term effects of acidity upon fish shows that pH of 4 – 4.4 is harmful to adult fish of many types which have not been progressively acclimated to low pH, and most fish are killed within hours at pH levels of 3.0-3.4[20,11,28]. The pH values obtained in this study are highly acidic and below the standard of 6.5 – 8.5 recommended by World Health Organisation and Department of Petroleum Resources for potable water[37,13].

Results in Table 2 show that the water temperature varied in the different zones and did not follow a particular trend. The turbidity value obtained for this study between July 2001 and January 2002 were extremely high compared to the[37] turbidity limit set for freshwaters (5 NTU). The relatively high levels of turbidity could be attributed to the presence of the polluting crude. Also the increased turbidity of the water must have reduced the light availability to photosynthetic organism which in turn would have caused $C0_2$ accumulate beneath the water as a dissolution of oxygen is prevented and this result to unavailability of oxygen in the water.

Department of Petroleum Resource has no Limits for SO_4^{2-}, NO_3^{-}, and NH_4^{+} ions. However, the results obtained for SO_4^{2-} is lower than[37] standard as could be observed from the results (Table 2). Although, gastrointestinal irritation, dehydration, and catharsis have been linked to high sulphate concentrations in drinking water, W.H.O does not have any recommended value for sulphate in drinking water,

Table 2: Physicochemical parameters of the water samples from the polluted river

						JULY 2001							
Source	Temp (°C)	DO (mg l^{-1})	Conductivity (µS cm^{-1})	pH	Turbidity NTU	NO_3^- (mgl^{-1})	SO_4^{2-} (mgl^{-1})	PO_4^{3-} (mgl^{-1})	Ca^{2+} (mgl^{-1})	Mg^{2+} (mgl^{-1})	TotalHardness $MgCaCO_3l^{-1}$	Total Alkalinity $MgCaCO_3l^{-1}$	Cl^- (mgl^{-1})
Zone 1	25.1	0.7	635	4.4	193	0.05	18.9	0.03	29.6	10.5	117.3	18.6	0.39
Zone 2	23.5	1.5	404	4.3	104	0.08	21.4	0.04	23.0	7.2	86.7	36.2	0.21
Zone 3	25.5	2.5	364	5.4	66	0.15	23.5	0.09	11.5	5.2	50.1	44.3	0.27
						SEPTEMBER 2001							
Source	Temp (°C)	DO (mg l^{-1})	Conductivity (µS cm^{-1})	pH	Turbidity NTU	NO_3^- (mgl^{-1})	SO_4^{2-} (mgl^{-1})	PO_4^{3-} (mgl^{-1})	Ca^{2+} (mgl^{-1})	Mg^{2+} (mgl^{-1})	TotalHardness $MgCaCO_3l^{-1}$	Total Alkalinity $MgCaCO_3l^{-1}$	Cl^- (mgl^{-1})
Zone 1	25.0	0.44	643	4.4	200	0.05	18.0	0.008	23.2	9.2	95.6	20.0	0.36
Zone 2	24.0	0.77	424	5.4	131	0.04	20.3	0.018	22.8	5.8	80.9	71.6	0.19
Zone 3	25.2	1.6	373	5.4	90	0.14	23.1	0.06	7.42	4.8	48.3	28.2	0.29
						JANUARY 2002							
Source	Temp (°C)	DO (mg l^{-1})	Conductivity (µS cm^{-1})	pH	Turbidity NTU	NO_3^- (mgl^{-1})	SO_4^{2-} (mgl^{-1})	PO_4^{3-} (mgl^{-1})	Ca^{2+} (mgl^{-1})	Mg^{2+} (mgl^{-1})	TotalHardness $MgCaCO_3l^{-1}$	Total Alkalinity $MgCaCO_3l^{-1}$	Cl^- (mgl^{-1})
Zone 1	26.2	0.21	646	4.4	237	0.03	17.8	0.007	22.0	5.41	89.6	18.4	0.35
Zone 2	28.0	0.37	409	5.4	162	0.06	19.1	0.01	20.4	5.39	73.1	42.0	0.14
Zone 3	29.6	1.1	395	5.5	104	0.13	nd	0.044	10.7	4.69	45.6	48.4	0.27
						JULY 2003							
Source	Temp (°C)	DO (mg l^{-1})	Conductivity (µS cm^{-1})	pH	Turbidity NTU	NO_3^- (mgl^{-1})	SO_4^{2-} (mgl^{-1})	PO_4^{3-} (mgl^{-1})	Ca^{2+} (mgl^{-1})	Mg^{2+} (mgl^{-1})	TotalHardness $MgCaCO_3l^{-1}$	Total Alkalinity $MgCaCO_3l^{-1}$	Cl^- (mgl^{-1})
Zone 1	28.7	3.70	978	5.6	79	0.40	213.41	0.04	41.2	38.2	157.2	106.4	105.5
Zone 2	31.5	4.48	1112	7.2	54	0.01	209.05	nd	52.0	37.8	128.0	98.2	122.8
Zone 3	32.4	4.46	1023	7.0	37	0.03	234.09	0.07	48.1	35.2	136.5	119.5	110.3

values reported are means of three replicates
DO = dissolved oxygen
nd = Not detected

Table 3: Profile of Crude Oil Concentration in Fish Mouth

Species of fishes Caught	Surface area (cm^2)	Concentration of oil (ppm)
1(I)	4.07	0.192
(ii) *lctalurus sp.*	4.80	0.187
(Catfish) (iii)	8.05	0.165
2(I)	4.00	0.136
(ii) *Clarias sp.*	6.50	0.170
(Mudfish) (iii)	3.20	0.132
3(I)	6.50	0.180
(ii) *Pseudotolithus sp.*	5.00	0.172
(Croakers) (iii)	3.90	0.112
4(I)	0.91	0.086
(ii) *Tilapia sp.*	0.84	0.52
(Cichlidae) (iii)	0.78	0.41

Source: Field Study, July 2001

Table 4: Bacterial population on the various parts of the fishes examined.

	Source		
Bacterial isolates	Mouth	Gills (cfu/ml)	Trunk
Escherichia coli	-	2.3×10^6	1.6×10^6
Bacillus substilis	-	1.1×10^6	2.6×10^6
Proteus vulgaris	-	0.3×10^6	1.2×10^6
Pseudomaonas aeruginosa	2.8×10^6	4.2×10^6	6.9×10^6
Aeromonas spp.	1.0×10^6	3.3×10^6	1.7×10^6

+ Present; - Absent,
Source: Field Study, 2001

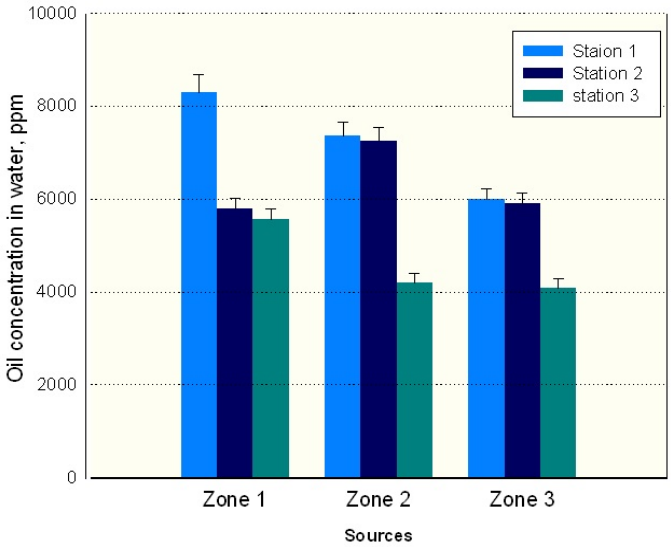

Fig. 1: Oil concentration in water before containment and scooping (July, 2001)

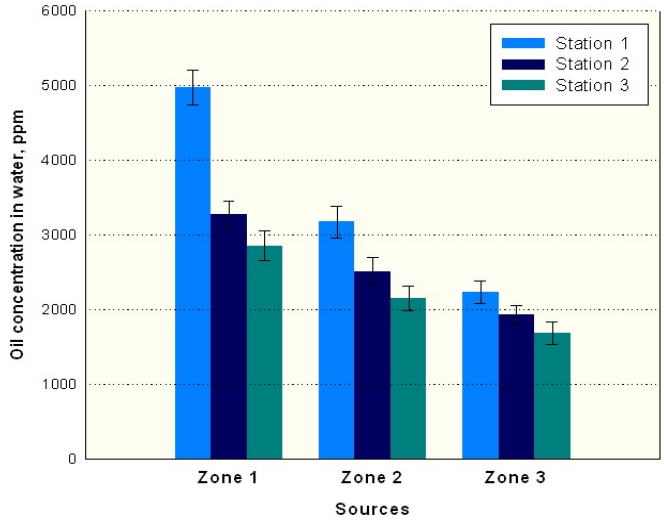

Fig. 2: Oil concentration in water after containment and scooping (September, 2001)

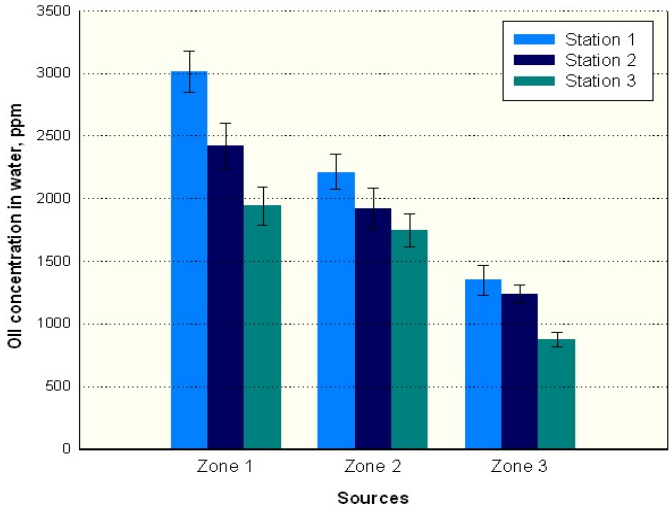

Fig. 3: Oil concentration in water after containment and scooping (January, 2002)

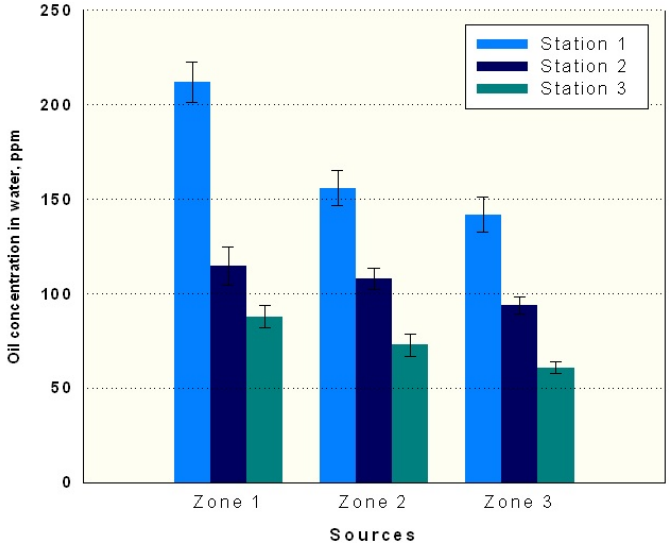

Fig. 4: Oil concentration in water after containment and scooping (July, 2003) (Fingerlings were feasible in Zone 1, Station 2)

because it is one of the least toxic anions. But when sulphate in drinking water exceeds 500 mg/l, WHO suggests an urgent action by health authorities.

The HCO_3^- concentration showed a lot of fluctuation in the different zones sampled, but was unusually high at point of pollution (table 2). [20]reported that the buffering capacity of water is determined by the concentration of bicarbonate ions. In this study, bicarbonate buffer must have been lost during long period of acid inputs, resulting in large fluctuations in pH and probably the fish kills. The resultant impact of the crude oil spill is captured in the photograph shown in Plates 1, 2 and 3.

The polluting crude oil concentration decreased from the site of pollution down stream in accordance with the flow of water in the ecosystem except in station 2 for zone 2 where there was a sudden surge, but the decrease continued downstream after this zone (Fig. 1). The highest recorded concentration of crude oil in the polluted freshwater was in zone 1, station 1, at the point of pollution in July 2001 (Fig.1). Following scooping and containment of the oil in Septmber of the same year, the concentration was observed to have decreased almost twice and thrice to undetectable limits in concentration in September 2001, January 2002 and July 2003 (Fig 1-4) respectively. Observation during sampling of the study area in July, 2003 indicated that fingerlings populations were generally recovering and it could be seen that fingerlings abundances had returned to the pre polluted freshwater in almost all the zones and stations investigated.

The results of the cultural, morphological and biochemical properties revealed that *Pseudomonas aeruginosa* and *Aeromonas* spp. were found in the various parts examined (Table 3). *Pseudomonas* and *Aeromonas* spp. are typical habitants of surface waters. The number of CFU/ml obtained in this research was in the ranges referred by[33].

The presence of *E.coli* may indicate faecal contamination probably through handlers who are most of the time along the roads and use neighbouring bushes as their toilet and do not wash up properly after use, while the presence of *Bacillus subtilis* is not unexpected, since it is present in large numbers in the soil and is able to survive long periods in the environment due to the production of spores, but its presence in food is of great concern since it causes food poisoning by production of an enterotoxin on ingestion of the spores[18]. The bacteria isolates encountered in this study, has previously often been observed in both the aquatic environment and in the internal fish organs[34].

In conclusion, fishery products should factor significantly into the food security agenda because in Nigeria in particular, and in the study area for example the fishery products are significant for domestic consumption because it provides approximately 22 percent of the animal protein.

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