African Scientist Vol. 18, No. 4, December 31, 2017
Printed in Nigeria

1595-6881/2016 $10.00 + 0.00

http://www.niseb.org/afs

AFS 2017040/18403

Polycyclic Aromatic Hydrocarbon and Culturable Bacterial Profile of Produced Water Effluent Collected from a Flow Station Waste Pit Located in Edo State, Nigeria

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(Received September 11, 2017; Accepted in revised form October 3, 2017)

ABSTRACT: This study was aimed at monitoring the quality of produced water from a flow station waste pit located in Ologbo in Edo State of Nigeria, in order to ascertain the levels of compliance to environmental standards. Samples were analyzed for physicochemical, polycyclic aromatic hydrocarbons and microbiological qualities adopting standard analytical procedures. The results of the study revealed that with the exception of the level of total dissolved solids (TDS) recorded in February 2017, the other physical parameters were found to be in consonance with the Department of Petroleum Resources (DPR) standard. Results showed that the levels of benzo(a)pyrene, benzo(a) anthracene, chrysene, dibenzo(a,h)anthracene, benzo(b) fluoranthene, benzo(k) fluoranthene, indeno(1,2,3-cd)pyrene and benzo(ghi)perylene were higher than the stipulated Department of Petroleum Resources standard. The concentrations of the heavy metals ranged between 0.41 – 1.49 mg/Lfor iron, 0.011 - 0.087 mg/L for manganese, 0.00 – 0.38 mg/Lfor zinc, 0.0 – 0.052 mg/Lfor copper, 0.0 – 0.028 mg/L for chromium, 0.0 – 0.092 mg/L for cadmium, 0.0 – 0.009 mg/L for nickel, 0.0 – 0.032 mg/L for lead, and 0.0 – 0.008 mg/L for vanadium. *Pseudomonas* spp. had the highest percentage frequency of occurrence (28.6 %), followed by *Bacillus* spp. (26.8 %) and the bacterial isolates with the least percentage frequency were *Staphylococcus* and *Micrococcus* spp. (13.4 %). This study revealed that the quality of produced water from the waste pit does not completely conform to the prescribed environmental standards and thus will require proper effluent treatment and monitoring to improve its quality prior to its discharge into the receiving environment.

Keywords: Produced water, Monitoring, Environmental standards, Heavy metals, PAHs

Introduction

Produced water has been described as water from underground formations that is brought to the surface during oil or gas production (API, 2000; Veil *et al*., 2004). Produced water make-up one of the largest waste stream from petroleum production operations, with chemical composition including oil, grease, and sometimes, naturally occurring radioactive materials which could harm the environment (Perry and Gigliellok, 1990, Sullivan *et al*., 2004). The management of produced water has continues to receive attention because of its high generated volume, cost of treatment and most especially the potential environmental implications. In Nigeria, current estimate of 1:1 water to oil (which is approximately one billion barrels) are disposed annually from oil and gas production operations (Isehunwa and Onovae, 2011).

According to Georgi and Baker (2001), the toxicity of produced waters from gas production is relatively higher than produced waters from oil production, which is due to the higher contents of flow molecular (weight aromatic hydrocarbons such as benzene, toluene, and xylene). Onojake and Abanum (2012) had earlier evaluated trace metal levels of produced water from five selected oil fields in Niger Delta, Nigeria. Data analysis of discharged produced water at nearshore terminals in Portharcourt and Warri indicated that the

receiving aquatic environments were not adversely affected by metal pollution (Obunwo and Chukwudi, 2015). The works of Okoro and Amund (2002) had shown the bioremediation potential of indigenous microbial flora from produced water polluted sites.

The aim of this study was evaluate polycyclic aromatic hydrocarbon and culturable bacterial profile alongside the physicochemical qualities of produced water effluent from the flow station waste pit located at Ologbo Community, Ikpoba-Okha Local Government Area, Edo State; and also to determine the extent of compliance with environmental regulatory standards.

Materials and methods

Location of the study area: The study area is situated at Ologbo Community, Ikpoba-Okha Local Government Area, Edo State (Fig. 1). Samples were collected from a waste pit constructed by an oil producing Company which lies along latitude N06.05698 – N06.06468º; longitude E005.57561º – E005.58394º (Plate 1).

Sampling period: The duplicate samples were collected from the month of February 2017 to June 2017, while the controlled sample was collected from the flow station separator, with the aid of grease-free polystyrene plastic containers (Physico-chemical and polycyclic aromatic hydrocarbon analysis) and 250 ml grease-free sterile glass bottles (for microbiological analysis). Samples were immediately taken to the laboratory for analysis (Ademoroti, 1996; Onyeonwu, 2000).

Plate 1: Sampling waste pit of the flow station

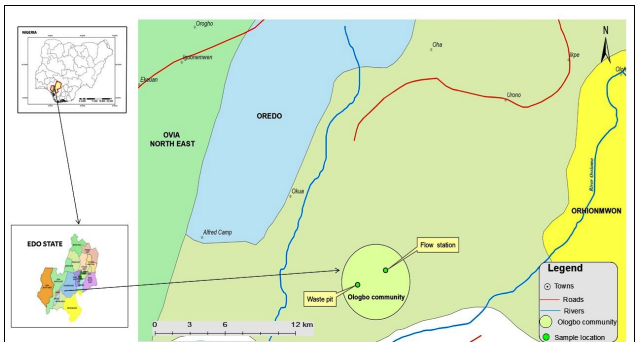

Fig. 1: Map of the studied location showing the sampled flow stations.

Determination of polycyclic aromatic hydrocarbon: One hundred milliliters (100 ml) of the produced water sample was measured into a 500 ml separating funnel and 50 ml of mixed Methanol and DCM, (1:1 of Methanol and DCM) was added and shaken vigorously. The screw cap was opened to remove air, and immediately allowed to stand for 20 mins and extracted into a 250 ml conical flask. Fifty milliliter (50 ml) of mixed methanol and DCM was added into the same sample and shaken vigorously, and it was allowed to stand for 10 mins and

decanted into the same 250 ml flask. Thereafter, 5g of anhydrous sodium sulphate ($NaSO_4$) was added and stir with a stirring rod. The extract was concentrated to less than 10 ml in a rotary evaporator raising the temperature of the water bath. The resultant concentrate was analyzed for polycyclic aromatic hydrocarbon with the aid of a gas chromatograph with frame ionization detector (FID).

Determination of physicochemical parameters:

pH: Hydrogen ion concentration was determined in the laboratory using the pH HANNA HI 8424 Meter, following an initial standardization using buffer 4 and 7.

Electrical conductivity: The electrical conductivity was determined using the HACH conductivity meter. The sample (50 ml) was measured into a 150 ml beaker and the value was taken from the LED screen on the conductivity/TDS meter.

Total Suspended Solids: Total suspended solid was determined using the gravimetric method. A dried 15 cm Whatman Filter Paper was weighed after it was oven dried at 50^{o}C and the weight taken. A 100 ml of the produce water was thereafter filtered through the weighed paper, and oven dried at 50^{o}C with its content.

The Weight of Suspended solids = $(X_2 - X_1)$ g.
Suspended solids (mg/l) = $(X_2 - X_1)$ x1000x10.

Total dissolved solids: Total dissolved solids were determined using the HACH conductivity/TDS meter. The sample was transferred (100) into a 250ml beaker using the probe and the value was taken from the LED screen on the conductivity/TDS meter.

Determination of Total Hydrocarbons: A volume of 50ml of the produce water sample was measured into 150 ml separating funnel, 25ml of n - hexane was added to the funnel. This was thereafter shaken manually for 2 minutes, and allowed to settle for 20mins. The water layer was drained off and the hexane layer collected. The collected residue was read at 460nm using spectronic 20d+ Spectrophotometer (ASTM D3921 infra-red spectrophotometer).

Determination of heavy metals: Atomic absorption spectrometry (AAS) (Model-Solaar 969 Unicam Series) was used for the determination of heavy metals after digesting the samples.

Culturable bacterial isolation: The sample was serially diluted using one in ten serial dilution technique. The diluent was sterile peptone water in test-tube. Following the dilution, 1ml was plated onto duplicate sterile petri dish and molten nutrient and centrmide agar were poured into the plates for heterotrophic bacterial and pseudomonas counts respectively. The plates were incubated for 24 hours after which counts of visible colonies were done to achieve colony forming unit per milliliter (cfu/ml).

Purification and identification of isolates: Purification of the bacterial isolates was done by sub-culturing the discrete colonies onto nutrient agar plates, and they were thereafter Gram-stained. Phenotypic profiling of both Gram-positive and Gram-negative bacteria was undertaken using API 50CHB and API 20E strips (BioMerieux, Marsielle, France). Further tests for spore stain and oxidase were also performed (Cullimore, 2000; Collins *et al*., 2004; Cheesbrough, 2000).

Results and discussion

The concentrations of naphthalene, phenanthrene and indeno (1,2,3-cd) pyrene were not detectable in the produce water (Table 1). The concentrations of acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene pyrene were undetectable by the fourth month of their characterization while that of 1, 2-benzothracene, chysene, benzo (b) fluoranthene became undetectable by fifth. This gives their approximate period of auto degradation under the prevailing condition where the samples were collected. The appearance of benzo (b) fluoranthene, benzo (k) fluoranthene, benzo (a) pyrene, dibenzo (a,h) anthracene, benzo (g,h,i) perylene, indeno (1,2,3-cd) pyrene by the third monthl are indications that they are byproduct of other process in this ecosystem. The concentration of PAHs which include benzo(a)pyrene, benzo(a)anthracene, chrysene, dibenz(a,h)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, indeno (1,2,3-cd) pyrene and benzo (ghi) perylene having defined health implication were higher than the stipulated DPR standard in the various period they appeared.

The contamination of the environment by polycyclic aromatic hydrocarbons (PAHs) is becoming a rising environmental concern. They have a widespread distribution in the environment and the carcinogenicity and mutagenicity of several of these compounds have been proven (Alonge, 1988; Koyano *et al*., 2001). PAHs contained in produce water are receiving much attention due to their potential for causing adverse effects in the aquatic environment (Sundt *et al*., 2011). According to the World Health Organization (WHO, 1998), the concentration of individual PAHs in surface and coastal waters are generally in the neighborhood of 0.05 µg/L

and concentration above this point indicates contamination. Also Benzo (a) pyrene concentration of 0.7 µg/L corresponds to an excess lifetime cancer risk of 10^{-5} (WHO, 1998).

The results of physicochemical analysis of the produced water are presented in Table 2. The pH recorded in the study ranged between 7.02 and 7.56. The electrical conductivity values ranged from 3060 µS/cm to 3274 µS/cm were recorded in the month of March and February 2017 respectively. Total suspended solids (TSS) values ranged from 2.9 mg/L to 9.4 mg/L and Total dissolved solids (TDS) values recorded ranged from 1421 mg/L to 5580 mg/l. The highest total hydrocarbon (THC) concentration (6.9 mg/l) was obtained in the month of February. Throughout the duration of this study, the pH appeared to be alkaline in nature and also found to be in consonance with the DPR stipulated range. The electrical conductivity likewise the total dissolved solids recorded a gradual decline from February to June. The total hydrocarbon content also maintained similar pattern of variations as in electrical conductivity. Physico-chemically, it was observed that the value of total suspended solid ranged from 2.9-9.4 mg/L, which was less than the DPR standard (30 mg/L). This trend probably resulted from the non-discharge of fresh produced water into the waste pit as well as possible natural attenuation process of the system.

Table 1: Monthly evaluation of polycyclic aromatic hydrocarbons (PAHs) content of sample

CODE	Control	February	March	April	May	June	DPR Standard
Naphthalene	0	0	0	0	0	0	30µg/l
Acenaphthylene	1941.73	2515.88	0	0	0	0	60µg/l
Acenaphthene	0	0	0	0	0	0	60µg/l
Fluorene	242.10	0	27.30	0	0	0	300µg/l
Phenanthrene	0	0	0	0	0	0	2000 µg/l
Anthracene	105.93	256.99	0	0	0	0	60µg/l
Fluoranthene	0	177.15	3.37	0	0	0	300 µg/l
Pyrene	185.18	364.91	176.96	0	0	0	0.2 µg/l
1,2-Benzothracene	610.50	286.74	0	149.45	0	0	200µg/l
Chysene	2125.93	1005.92	0	0	0	0	0.1µg/l
Benzo(b)fluoranthene	0	0	10.86	92.56	0	0	0.2µg/l
Benzo(K)fluoranthene	0	0	25.14	0	151.75	0	0.2µg/l
Benzo (a)Pyrene	0	0	0	166.99	92.56	71.51	0.2µg/l
Dibenzo(a,h) anthracene	0	0	464.03	0	60.86	42.72	0.3µg/l
Benzo (g,h,i) perylene	0	0	0	252.22	97.30	82.19	200µg/l2011)
Indeno (1,2,3-cd) pyrene	0	0	0	0	0	0	0.4µg/l
Total (ug/L)	5211.36	4607.60	707.67	661.23	427.98	196.43	
Total (mg/L)	5.211	4.61	0.71	0.66	0.43	0.19	

Key: Overall mean values

Table 2: Physicochemical parameters of produce water from the waste pit

Parameters	Control	February	March	April	May	June	DPR Standard
pH	7.64	7.28	7.33	7.02	7.56	7.47	6.5-8-5
EC (*µS/cm*)	4760	3274	3060	2890	2620	2600	NS
TSS (mg/L)	2.9	3.6	2.9	9.4	3.2	3.0	30
TDS (mg/L)	2380	5580	1810	1440	1817	1421	<2000
THC (mg/L)	8.4	6.9	1.27	0.9	0.71	0.42	10

Key: NS - Not Stated, over all mean values

Heavy metals are among the main inorganic constituents thought to be of environmental concern. The toxicity of heavy metals are known to be influenced by their solubility in water. After absorption, these metals can bind to vital cellular components such as structural proteins, enzymes, and nucleic acids, and interfere with their functioning. In humans, some of these metals, even in small amounts, can cause severe physiological and health effects (Wayne and Ming-Ho, 2005). The most commonly studied metals are iron, cadmium, chromium, copper, lead, mercury, nickel, arsenic and zinc (Onojake and Abanum, 2012).

From this study, it was shown that with the exception of iron and zinc concentrations, the other heavy metals were relatively less than that of control. The rank of heavy concentration in samples were Fe> Zn> Cd> Mn>

Cu> Cr> Pb> Ni> V (Table 3). Iron had the highest concentration among the heavy metals that were analyzed (ranging from 0.41 - 1.49 mg/L), while lead and chromium had the least concentration. Vanadium was not detected in this study. The dominance of iron over other heavy metals as observed in this study is contrary to the earlier findings of Isehunwa and Onovae (2011) and Onojake and Abanum (2012).

Significant and positive correlations were observed between polycyclic aromatic hydrocarbons (PAHs) and physicochemical parameters ($P<0.05$), which indicate that these components are from the same source.

The heterotrophic bacterial and Pseudomonas counts were in the order of 10^5 and 10^2 cfu/ml respectively for produced water from waste pit; this was however contrary for control sample that was nil (Table 4a). Table 4b, reveals *Pseudomonas* spp. had the highest percentage frequency of occurrence (28.6 %), followed by *Bacillu*s spp. (26.8 %) and the bacterial isolates with the least percentage frequency were *Staphylococcus* and *Micrococcus* spp. (13.4 %). This observation is not out of place considering the sample type. The high occurrence of *Pseudomonas* spp and other bacterial isolates to utilize produced water as a carbon source and as such the discharged produced water into this waste pit can undergo the process of natural attenuation. Species of *Pseudomonas* and several bacterial isolates have been identified with high potential for biodegradation (Chikere and Ekwuabu, 2014) and also been employed in bioremediation process (Atlas, 1988).

Table 3: Monthly evaluations for the heavy metal content (mg/l) of produced water from waste pit

Sample code	Control	February	March	April	May	June	DPR standard
Iron (Fe)	1.03	0.41	0.73	1.49	1.17	0.5	1.00
Manganese (Mn)	0.020	0.011	0.087	0.072	0.057	0.02	NS
Zinc (Zn)	0.119	0.046	0.14	0.38	0.22	0.0	1.0
Copper (Cu)	0.058	0.031	0.052	0.033	0.026	0.0	1.5
Chromium (Cr)	0.017	0.012	0.028	0.013	0.010	0.0	0.03
Cadmium (Cd)	0.137	0.092	0.039	0.025	0.019	0.0	0.05
Nickel (Ni)	0.0	0.0	0.0	0.009	0.0	0.0	NS
Lead (Pb)	0.021	0.0	0.032	0.016	0.010	0.0	0.3
Vanadium (V)	0.0	0.0	0.0	0.008	0.0	0.0	NS

Key: NS - Not Stated, over all mean values

Table 4a: Monthly evaluations of microbial counts (cfu/ml)

Sample code	Control	February	March	April	May	June	DPR standard
THBC (10^5 (cfu/ml)	Nil	3.7	5.4	5.0	6.8	6.2	NS
Pseudomonas counts (10^2 (cfu/ml)	Nil	1.2	1.7	3.0	3.0	3.1	NS

Key: NS - Not Stated, over all mean values, Total Heterotrophic Bacterial Counts (THBC),

Table 4b: Percentage frequency of occurrence of bacterial isolates

Isolate	Number of isolates	Percentage frequency of occurrence (%)
Pseudomonas spp.	32	28.6
Bacillus spp.	30	26.8
Staphylococcus spp.	15	13.4
Enterobacter sp.	20	17.9
Micrococcus sp.	15	13.4
Total	**112**	**100**

Correlation analysis was used to determine the nature of relationships between the samples physicochemical, heavy metals and poly cyclic aromatic hydrocarbon parameters. A significant and inverse relationship was obtained between the pH and benzo (b) fluoranthene concentrations in the sampled system. The electrical conductivity exhibited significant and positive correlations with the concentrations of acenaphthylene, anthracene, fluoranthene and pyrene of the system. The levels of total suspended solids in this system exhibited a significant and positive correlation of benzo (g,h,i) perylene concentration. The total dissolved solids in this system showed significant and positive correlations with the levels of acenaphthylene and pyrene. Between the total hydrocarbon and acenaphthylene, anthracene, fluoranthene and pyrene, significant and positive

relationships were observed. The concentrations of zinc and benzo (b) fluoranthene were observed to exhibit a positive and significant correlation. Between the cadmium concentrations in this system and acenaphthylene, fluoranthene and pyrene, significant and positive correlations were observed. Nickel and vanadium concentration in this system exhibited significant and positive correlation with benzo (g,h,i) perylene concentration in this system. The significant and positive correlations observed between the polycyclic aromatic hydrocarbons, physicochemical parameters and heavy metals are indication that these components sources are from the same point. Thus the input of one is the same input of the other.

Table 5: Section of Correlation Matrix showing the Relationships between the Variations in Physicochemical parameters and Polycyclic Aromatic Hydrocarbons

	pH	**EC**	**TSS**	**TDS**	**THC**	**Fe**	**Mn**	**Zn**	**Cu**	**Cr**	**Cd**	**Ni**	**Pb**	**V**
ACENAPHTHYLENE	0.183	**0.881**	-0.259	**0.854**	**0.955**	-0.383	-0.713	-0.412	0.350	0.071	**0.877**	-0.312	-0.246	-0.312
FLUORENE	0.558	0.013	-0.272	-0.030	0.711	0.148	-0.309	-0.121	0.646	0.289	0.800	-0.226	0.397	-0.226
ANTHRACENE	0.005	**0.983**	-0.206	**0.970**	**0.820**	-0.493	-0.671	-0.425	0.186	0.010	0.705	-0.282	-0.389	-0.282
FLUORANTHENE	-0.229	**0.970**	-0.113	**0.976**	0.519	-0.569	-0.506	-0.380	-0.047	-0.057	0.376	-0.205	-0.504	-0.205
PYRENE	0.002	**0.887**	-0.347	**0.883**	0.770	-0.570	-0.391	-0.456	0.457	0.409	0.725	-0.399	-0.002	-0.399
1,2-BENZOTHRACENE	0.268	0.446	-0.049	0.387	**0.923**	0.078	-0.531	-0.093	0.573	0.163	**0.940**	-0.050	0.094	-0.050
CHYSENE	0.447	0.488	-0.287	0.445	**0.958**	-0.098	-0.612	-0.289	0.550	0.160	**0.963**	-0.290	0.062	-0.290
BENZO(b)FLUORANTHENE	**-0.823**	-0.267	**0.983**	-0.325	-0.337	0.698	0.510	**0.836**	0.045	0.076	-0.273	**0.993**	0.201	0.993
BENZO(K)FLUORANTHENE	0.375	-0.319	-0.228	-0.217	-0.377	0.307	0.308	0.248	-0.103	-0.049	-0.338	-0.238	-0.002	-0.238
BENZO(a)PYRENE	-0.499	-0.510	0.797	-0.528	-0.641	0.695	0.369	0.731	-0.443	-0.422	-0.636	0.802	-0.179	0.802
DIBENZO(a,h)ANTHRACENE	-0.050	-0.285	-0.296	-0.244	-0.340	-0.192	0.669	-0.059	0.360	0.721	-0.216	-0.253	0.699	-0.253
BENZO(g,h,i)PERYLENE	-0.610	-0.454	**0.888**	-0.487	-0.572	0.716	0.391	0.778	-0.347	-0.333	-0.557	**0.894**	-0.109	**0.894**

Critical level of correlation coefficient ($p<0.05$; df_4) = 0.811. Bold figures – significant correlation.

Conclusion

The need for proper management of produced water cannot be over emphasized, especially when its source and environmental impact is put into consideration. This study revealed that the quality of produced water from this waste pit does not completely conform to the prescribed Department of Petroleum Resources (DPR) environmental standards and thus will require proper effluent treatment and monitoring to improve its quality prior to its discharge into the receiving environment.

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