Asian Journal of Physical and Chemical Sciences

5(3): 1-9, 2018; Article no.AJOPACS.38035
ISSN: 2456-7779

Evaluation of Level of Petroleum Hydrocarbon in Water, Fishes and Plants from Pond and Well in Oghara Community in Delta State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author EEI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author ANE managed the analyses of the study. Author AEA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJOPACS/2018/38035
Editor(s):
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Complete Peer review History: http://www.sciencedomain.org/review-history/24008

Original Research Article

Received 8th November 2017
Accepted 9th January 2018
Published 5th April 2018

ABSTRACT

Total petroleum hydrocarbon (TPH) is of immense interest to environmental chemist because they are toxic to human system and animals. Some of the TPH compounds are carcinogenic and poses serious health problem to humans and aquatic life. Hence, there is need for continuous check of the level of TPH in the communities in Niger Delta region of Nigeria were crude oil is being exploited. This research was carried out to investigate the level of TPH in water, plant and fishes from the pond and well located in Oghara community in Delta State, Nigeria. The samples were collected, prepared and TPH extracted and purified using standard analytical methods. The extracts were then concentrated and separately analyzed by capillary gas chromatography with flame ionization detector (GC/FID) after silica gel fractionation. The results revealed that the levels of Total Petroleum Hydrocarbons (TPH) and individual aliphatic and aromatic hydrocarbons in well and pond samples examined were below the standards of World Health Organization (WHO) and Standard

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organization of Nigeria (SON) of 0.007 mg/l. Though the samples collected in this community were not polluted by TPH; however there is need of regular monitoring for adequate environmental protection of the water body in this region.

Keywords: Total petroleum hydrocarbon; pond; well; fish; plant and water.

1. INTRODUCTION

Total petroleum hydrocarbon (TPH) is a mixture of hydrocarbons found in crude oil it's comprised of aliphatic and aromatic hydrocarbons as well as other non-polar organic compounds in petroleum [1]. Some of the chemicals found in TPH are hexane, benzene toluene, xylene, naphthalene etc [2]. It has been reported that petroleum products from crude oil contaminate the environment during production and this poses health hazards [3].

TPH can originate from petroleum products ranging from oil to highly refined products and often contain heterocycles [4]. The presence of petroleum hydrocarbons in form of crude oil and grease in domestic and river water is concern to the public. Biologically, they have deleterious impact on aquatic life [5].

There are many sources of TPH contaminants in our environment which include petroleum extraction, transportation, refining and consumption [6]. The amount and types of compounds in a petroleum hydrocarbon release differ widely depending on the product spilled and how it weathered [1].

Oil spills devastate soil and aquatic systems and cause alteration in important microbial process [7]. It is estimated that over ten million tons of crude oil enters the environment each year from accidental spills, associated with routine operations [8]. These petroleum products are the major sources of total petroleum hydrocarbons in our environment.

Studies have shown that TPH especially the polycyclic aromatic hydrocarbons (PAHs) are toxic to human. They cause harmful effect on skin, body fluid and the body's system for fighting disease after both short and long exposures. Some heavier PAHs are shown to exhibit acute water toxicity at levels below solubility due to photo-enhanced toxicity in the presence of UV light or other types of solar radiation [9].

However, high levels, particularly of aromatic and high molecular weight aliphatic hydrocarbons are often indicative of petroleum pollution [10].

Studies of the accidental and intentional releases of petroleum based products to the aquatic environment indicate that aquatic organisms are able to bio-accumulate some total petroleum hydrocarbons (TPH) fractions particularly polycyclic aromatic hydrocarbons (PAHs) [11]. It has been reported that certain PAHs, for example Benzo (a) pyrene do affect egg production in fish [12]. Exposure has been found to reduce primary ocyte numbers and reduce plasma testosterone and estrogen levels [13]. Teratogenic effects and decreased percentage hatch were also observed in the fry and eggs of the fish exposed to anthracene as adults when the eggs were subsequently exposed to solar ultraviolet radiation [12]. This research work aimed at evaluating the level of TPH in water, fish and plants from well and ponds in Oghara Village in Delta State, Nigeria. In order to enlighten the community on the level of TPH in these samples and the need to check the level of this contaminant regularly.

2. MATERIALS AND METHODS

2.1 Study Area

The study area was Oghara in Ethiope west local government area of delta state, Nigeria. It is located within latitude 5°55' 28.28"N and longitude 5°39' 53.31"E. It is one of the largest petroleum oil producing communities in Nigeria

2.2 Collection and Preparation of Fish Sample

Fish samples were collected from their natural and semi-natural ecosystems. Two species Tilapia *(oreochromis niloticus)* and cat fish (*Claris gariepinus* and *Heterobranchus bidorsalis*) were sampled from fish ponds in Oghara community in close proximity to Nigerian Naval Base. The fish samples average 200 gm were collected and wrapped in sterile aluminium foil and immediately stored in ice-packed cooler before being taken to the laboratory for pre-treatment and analysis.

TPH extraction mixture was prepared. The mixture contains acetone and dichloromethane (1:1 v/v). 250 ml of acetone and 250 ml of

dichloromethane were measured into a 500 ml standard volumetric flask and mixed properly.

Each of the fish samples was cut into pieces using a stainless steel knife and crushed in a mortar with pestle. 10 g of the crushed sample was weighed into a 100 ml beaker and 60ml of TPH extraction mixture was added. The Fish TPH contents were extracted by a shaking method described by [14]. The beaker with the content was placed on magnetic stirrer/heater and shaken for about 15 minutes at 70°C. The extract was decanted into a clean round-bottom flask. 30 ml fresh solvent was added and the process repeated. The extracts were combined and 5g of anhydrous sodium sulphate to remove water. The extract was concentrated to 3 ml with rotary evaporator maintained at 20ºC [15].

1.5 ml of the concentrated extract was loaded on a silica gel column. The silica gel column was prepared by loading a 2 g glass wool followed by 30 g chromatography silica gel, onto a chromatography column (2 cm internal diameter and 10cm long).

Each of the bed was conditioned with 40 ml HPLC-hexane to remove any organic contaminant. The 1.5 ml concentrated extract was loaded and eluted with 30 ml HPLC hexane into a labeled 100 ml beaker to get the aliphatic hydrocarbon components in the sample. While the hexane was almost getting dried, Hexane was replaced with 30 ml of dichloromethane to elude the aromatic hydrocarbons contents into another labeled 100 ml beaker. 2 g of anhydrous sodium sulphate was added to remove any traces of water left in the extract. These were re-concentrated using a rotary evaporator to about 2 ml. 1 ml of the extract was transferred into a well-labeled chromatography vial ready for gas chromatographic analysis. The samples were stored at 4°C until GC analysis.

2.3 Collection and Preparation Water Sample

Nine water samples were collected from three wells randomly selected within the community, and nine water samples were also collected from three (3) fishponds randomly selected from an individual fish farm in Oghara community. The selected wells were about 200 meters apart. Each of the water sample collected was approximately 500 ml.

All glass sample bottles used were thoroughly cleaned and rinsed with dichloromethane (DCM) prior to use. A piece of sterile aluminum foil was used immediately to cover each bottle so as to prevent any sort of contamination. No space was allowed between the foil and the water samples. The glass bottles were thereafter tightly covered with screw cover. These were kept in an ice-packed cooler and transferred to laboratory for pre-treatment and analysis. 2 ml of 0.2 M H_2SO_4 was added to the water to bring the pH to about 2.

The extraction was carried out using separator funnel using liquid-liquid extraction method [16]. This method measures the collective concentrations of extractable aliphatic and aromatic petroleum hydrocarbons that may be found in a water sample. The method uses a solvent extraction step followed by a silica gel fractionation into two extracts – an aliphatic extract (C_9- C_{18}, C_{19} – C_{36}) and an aromatic extract (C_{11} – C_{22}). The two extracts were then concentrated and separately analyzed by capillary gas chromatography with flame ionization detector (GC/FID).

The water sample was poured into 1000 ml separatory funnel and 30 ml dichloromethane was added into the sample bottle to rinse it. The solvent was poured into the separatory funnel. The separatory funnel was shaken vigorously for 2 minutes and periodically vents to release excess pressure. The mixture was allowed to stand for about 10 minutes to allow separation between the organic phase and the aqueous phase. The organic phase was drained from the separatory funnel through the anhydrous sodium sulphate into a round bottom flask.

(Whatman No. 40 was placed into filter funnel on which 10 g of anhydrous sodium sulphate was placed and rinse with small quantity of dichloromethane to remove any organic contaminant).

The procedure was repeated twice with fresh 30 ml dichloromethane and the extracts combined. This was concentrated to about 3 ml in a rotary evaporator. 1.5 ml of the extract was loaded into a chromatography column and eluted with 30 ml HPLC Hexane and 30 ml dichloromethane into aliphatic and aromatic components respectively. These were re-concentrated to about 2 ml and 1.5 ml of it was transferred into a chromatographic vial and stored at 4°C pending the gas chromatography analysis. The method was repeated for each of the water samples.

2.4 Collection and Preparation of Plant Sample

The most common plant around the well (Fern), ponds (*Scirpus triqueter* linn*)* were uprooted into a clean well-labeled black nylon bag and transferred to the laboratory for pre-treatment and analysis.

Having washed the root part of the plants with water, the roots, stem and the leaves were cut into pieces and crushed using mortar and pestle. 10 g of the crushed sample was weighed into a 100 ml beaker and the above method for fish extraction was repeated for plant samples using acetone/dichloromethane mixture as extraction solvent.

2.5 Gas Chromatography Analysis

Each extract transferred to 1.5 ml vial was loaded into a gas chromatography system 6890 series model G1530A, with Flame Ionization Detector (FID), and cold on-column injection. 1μl portion of the sample was injected and analyzed for TPH (C_9 – C_{36}). An HP-5 (cross slinked PH ME siloxane) column having the dimensions 30 m x 0.25 mm with a stationary phase thickness of 0.25μ was used for analytical separation. The carrier gas was purified nitrogen held at a flow rate of 50 ml/min. The operating temperature program was started at 60°C for 2 mins and then increase at a rate of 10°C per min to 300°C for 10min [17]. The injector and detector temperature were maintained at 250°C and 300°C respectively. The oven temperature was 60°C. Aliphatic hydrocarbons are quantitated within C_9 _ C_{18}, C_{19} – C_{36}. Aromatic hydrocarbons were quantitated with range C_{11} – C_{22}.

3. RESULTS AND DISCUSSION

The results of petroleum hydrocarbon in water, fishes and plants from pond and well in Oghara community in Delta State, Nigeria, are presented in Table 1 – 7.

The levels of Total Petroleum Hydrocarbons (TPH) and individual aliphatic and aromatic hydrocarbons in well and pond samples examined are below WHO and SON standards of 0.007 mg/l [18]. The trace amount of aromatic hydrocarbon can bioaccumulate in the body tissues as people continued to drink from these wells and eat fish from these ponds on the long run [11]. Aromatic hydrocarbons are possibly carcinogenic.

There was no evidence of underground oil movement, however, the variation in the number of aromatic hydrocarbons in Well 2 when compared to wells 1 and 3 (Table 2) may be attributed to the nearest of this well to a major traffic artery and inputs from atmospheric emissions from the numerous automobile exhausts.

It is necessary to sand-fill this well and another constructed in a safe location. Well, 3 has more of aliphatic hydrocarbon fractions (Table 2). This may be due to high anthropogenic activities around this well. It is located in the heart of the village where many people lived.

Ponds 1 and 2 are newly constructed. The marked difference in the number of individual hydrocarbons present in these ponds might have come from lipids and waxes from decaying wood and microbial matters. Burning of organic matters (wood) might have also contributed to the level of TPH in these ponds. However the level of total petroleum hydrocarbon in water from pond and well is lower than that of the River obtained in the same village as reported in our previous work [1] this is because some of these hydrocarbons might have found their ways into the river through erosion thus increase the TPH levels contained in the river.

Naphthalene, 2- Methylnaphthalene, Acenaphthene values were below 0.0001mg/kg in plant sample from Pond 3, none of these compounds were found in the water from the same pond, however, the values of these compounds in fish samples from the pond 1, 2 and 3 were 0.0017 mg/kg, 0.0023 mg/kg and 0.0017 mg/kg respectively (Table 3). This result shows that there is bioaccumulation of these compounds in the fish tissues. This is in line with the results obtained by [19], in their work titled 'Polycyclic aromatic hydrocarbon in edible tissues of fish from the Gulf after the 1991 oil spills. Bioconcentration factor also indicates that there is bioaccumulation in fish samples from Pond 1. The outstanding presence of aliphatic hydrocarbon in fish sample from Pond 3 could be attributed to the species of the fish and their feeding habit.

Table 1. Individual aliphatic and aromatic hydrocarbons content (mg/l) in water from ponds

Component	Pond I	Pond 2	Pond 3
(a) Aliphatic	**$\bar{X}$ ± SD**	**$\bar{X}$ ± SD**	**$\bar{X}$ ± SD**
Nonane	0.0003± 0.0006	0.0003±0.006	0.0003±0.0006
Decane	ND	ND	ND
Dodecane	0.0017± 0.0006	0.0020±0.0000	0.0017±0.0006
Tetradecane	0.0007± 0.0012	ND	ND
Hexadecane	0.0017± 0.0029	0.0003±0.0006	0.0003±0.0006
Octadecane	0.0007± 0.0012	0.0003±0.0006	0.0003±0.0006
Nonadecane	0.0013± 0.0023	0.0003±0.0006	ND
Eicosane	0.0020± 0.0035	0.0003±0.0006	ND
Docosane	0.0017± 0.0029	0.0007±0.0012	0.0007±0.0012
Tetracosane	0.0020±0.0035	ND	0.003±0.0006
Hexacosane	0.0010±0.0017	ND	ND
Octacosane	0.0017± 0.0012	ND	ND
Traicontane	ND	ND	ND
Hexacosane	ND	ND	ND
(b) Polyaromatic			
Naphthalene	0.0007± 0.0006	ND	ND
2-methylenaphthealene	0.0003± 0.0006	0.0003±0.0006	ND
Acenaphthalene	0.0003± 0.0006	0.0003±0.0006	ND
Acenaphthene	0.0003± 0.0006	0.0017±0.0006	ND
Florene	0.0017± 0.0029	0.0003±0.0006	ND
Phenathrene	0.0007± 0.0012	ND	ND
Anthracene	0.0007± 0.0012	ND	ND
Fluoranthrene	0.0010± 0.0017	ND	ND
Pyrene	0.0007± 0.0012	ND	ND
Benzo(a)anthracene	0.0003± 0.0006	ND	ND
Crysene	ND	ND	ND
Benzo(b)fluoranthrene	ND	ND	ND
Benzo(a)pyrene	ND	ND	ND
Benzo(k) fluoranthrene	ND	ND	ND
Indeno (1,2,3) perylene	ND	ND	ND
Benzo(g,h,i)perylene	ND	ND	ND
Benzo (a,h) anthracene	ND	ND	ND

Table 2. Individual aliphatic and aromatic hydrocarbons content (mg/L) in water from wells

Component	Well 1	Well 2	Well 3
(a) Aliphatic	**$\bar{X}$ ± SD**	**$\bar{X}$ ± SD**	**$\bar{X}$ ± SD**
Nonane	0.0003± 0.0006	0.0007±0.0006	0.0003±0.0006
Decane	ND	ND	ND
Dodecane	0.0023± 0.0006	0.0023±0.0006	0.0020±0.0000
Tetradecane	0.0003± 0.0006	ND	0.0003±0.0006
Hexadecane	ND	ND	0.0007±0.0012
Octadecane	ND	ND	ND
Nonadecane	0.0007± 0.0012	ND	0.0003±0.0006
Eicosane	0.0007± 0.0012	ND	0.0003±0.0006
Docosane	ND	ND	0.0040±0.0069
Tetracosane	ND	ND	0.0003±0.0006
Hexacosane	ND	ND	ND
Octacosane	ND	ND	ND
Traicontane	ND	ND	ND
Hexacosane	ND	ND	ND

(b) Polyaromatic	-	-	-
Naphthalene	ND	0.003±0.006	ND
2-methylenaphthealene	0.0007± 0.0006	0.0007±0.0006	ND
Acenaphthalene	0.0010± 0.0010	0.0003±0.0006	ND
Acenaphthene	ND	0.0013±0.0023	0.0007±0.0006
Fluorene	0.0007± 0.0006	0.0003±0.0006	0.0007±0.0006
Phenathrene	ND	ND	ND
Anthracene	ND	ND	0.0007±0.0006
Fluoranthrene	ND	ND	ND
Pyrene	ND	ND	ND
Benzo(a)anthracene	ND	ND	ND
Crysene	ND	ND	ND
Benzo(b)fluoranthrene	ND	ND	ND
Benzo(a)pyrene	ND	ND	ND
Benzo (k) fluoranthrene	ND	ND	ND
Indeno(1,2,3(perylene	ND	ND	ND
Benzo(g,h,i)perylene	ND	ND	ND
Benzo(a,h) anthracene	ND	ND	ND

ND – Not Detected

Table 3. Individual aliphatic and aromatic hydrocarbons content (mg/kg) in fish from pond

Component	**P_1F**	**P_2F**	**P_3F**
(a) Aliphatic	**$\overline{X}$ ± SD**	**$\overline{X}$ ±SD**	**$\overline{X}$ ± SD**
Nonane	0.0033± 0.0058	0.0120±0.0036	0.0160±0.0036
Decane	0.0013±0.0023	0.0010±0.0017	0.0013±0.0023
Dodecane	0.0113± 0.0118	0.0350±0.0131	0.0283±0.0021
Tetradecane	0.0107± 0.0021	0.0027±0.0046	0.0117±0.0012
Hexadecane	0.0113± 0.0110	0.0107±0.0072	0.0160±0.0113
Octadecane	0.0073± 0.0032	0.0070±0.0010	0.0043±0.0075
Nonadecane	0.0070± 0.0061	0.0040±0.0035	0.0037±0.0032
Eicosane	0.0153± 0.0042	0.0117±0.0038	0.0310±0.0066
Docosane	0.0467± 0.0167	0.0427±0.0174	1.1237±0.9521
Tetracosane	0.0293±0.0309	0.0333±0.0163	0.0260±0.0450
Hexacosane	0.0443±0.0768	0.0353±0.0612	0.0530±0.0918
Octacosane	ND	ND	ND
Triacontane	ND	ND	ND
Hexacosane	ND	ND	ND
(b) Polyaromatic	-	-	-
Naphthalene	0.0043± 0.0006	0.0017±0.0006	0.0017±0.0006
2-methylenaphthealene	0.0003± 0.0006	0.0003±0.0006	0.0023±0.0012
Acenaphthalene	0.0030± 0.0026	0.0003±0.0006	0.0017±0.0006
Acenaphthene	0.0010± 0.0017	0.0003±0.0006	ND
Fluorene	0.0030± 0.0026	0.0017±0.0006	ND
Phenathrene	0.0027± 0.0023	ND	ND
Anthracene	0.0137± 0.0203	ND	ND
Fluoranthrene	ND	ND	ND
Pyrene	ND	ND	ND
Benzo(a)anthracene	ND	ND	ND
Crysene	ND	ND	ND
Benzo(b)fluoranthrene	ND	ND	ND
Benzo(a)pyrene	ND	ND	ND
Benzo (k) fluoranthrene	ND	ND	ND
Indeno (1,2,3) perylene	ND	ND	ND
Benzo(g,h,i)perylene	ND	ND	ND
Benzo (a,h) anthracene	ND	ND	ND

KEY: P_1F – Fish sample from Pond 1; P_2F – Fish sample from Pond 2; P_3F – Fish sample from Pond 3
ND – Not Detected

Table 4. Individual aliphatic and aromatic hydrocarbons content (mg/kg) in plant from pond, and well

Component	PLP_O	PL_W
(a) Aliphatic	-	-
Nonane	0.017	0.013
Decane	0.000	0.007
Dodecane	0.004	0.047
Tetradecane	0.008	0.005
Hexadecane	0.008	0.005
Octadecane	0.000	0.000
Nonadecane	0.005	0.000
Eicosane	0.005	0.000
Docosane	0.031	0.000
Tetracosane	0.000	0.000
Hexacosane	0.000	0.000
Octacosane	0.000	0.000
Traicontane	0.000	0.000
Hexacosane	0.000	0.000
(b) Polyaromatic	-	-
Naphthalene	< 0.001	0.000
2- methylnaphthalene	< 0.001	0.000
Acenaphthalene	< 0.001	0.000
Acenaphthene	< 0.001	0.000
Florene	< 0.001	0.000
Phenathrene	< 0.001	0.000
Anthracene	< 0.001	0.000
Fluoranthrene	< 0.001	0.000
Pyrene	< 0.001	0.000
Benzo(a)anthracene	< 0.001	0.000
Crysene	< 0.001	0.000
Benzo(b)fluoranthrene	< 0.001	0.000
Benzo(a)pyrene	< 0.001	0.000
Benzo (k) fluoranthrene	< 0.001	0.000
Indeno (1,2,3(perylene	< 0.001	0.000
Benzo(g,h,i)perylene	< 0.001	0.000
Benzo (a,h) anthracene	< 0.001	0.000

Key: PLP_o - Plant from Pond; PL_w - Plant from Well

Table 5. Total petroleum hydrocarbons in plant from pond and well

Parameter	Aliphatic	Aromatic
Plant in Ponds	0.079±0.003	0.001
Plant in Well	0.051± 0.002	0.003
Chi Square	1.529	17.50
P Value	P > 0.05	P < 0.001

Table 6. Total petroleum hydrocarbons in fish from ponds

Parameter	Fish pond 1 $\overline{X} \pm SD$	Fish pond 2 $\overline{X} \pm SD$	Fish pond 3 $\overline{X} \pm SD$	P -Value
Aliphatic Hydrocarbons	0.188^{b}± 0.122	0.196^{b}± 0.059	1.315± 0.808	P = 0.05
Aromatic Hydrocarbons	0.0038^{a}± 0.025	0.003^{b}± 0.002	0.005^{b}± 0.004	P= 0.05

Note: P > 0.05 Not Significant; P < 0.05 Highly Significant

Table 7. Total petroleum hydrocarbons in the well water

Parameter	Well 1	Well 2	Well 3	P. Value
Aliphatic Hydrocarbons	0.004 ±0.002	0.009 ± 0.010	0.008 ± 0.009	P = 0.05
Aromatic Hydrocarbons	0.002 ± 0.002	0.004 ± 0.003	0.002 ± 0.002	P = 0.05

Note: P > 0.05 Not Significant; P < 0.05 Highly Significant

P-value calculated from the one-way analysis of variance (ANOVA) was greater than 0.05 for well and ponds samples at 95% confidence level. Statistically this indicates that there was no significant difference in TPH concentration from sample sites, however, studies have shown that even low levels of petroleum hydrocarbons in the aquatic environment can have acute toxic effects on various forms of zooplankton because of bio-accumulations.

4. CONCLUSION

Since the level of TPH and individual aromatic and aliphatic hydrocarbon are under the SON and WHO standard, Water, Fish and Plants from ponds and well of Oghara community is not polluted in spite of being contaminated by TPH. However, the levels of aliphatic hydrocarbon were higher in all samples than aromatic hydrocarbon. As a result of bioaccumulation, Fish samples contain a greater percentage of TPH than water and plant samples, since they feed on water, sediment and plankton. Although samples studied have moderate to low level of TPH, there is a need for adequate monitoring and checking of this contaminant in the water, fish and plants in this community since the community is located in the oil exploration area and there are also adequate anthropogenic activities in this community.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24008