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Microbiological Effects of Gas Flaring on Agricultural Soil at Izombe Flow-station, Imo State, Nigeria

Ezeigbo*, O. R., Okike-Osisiogu, F. U., Ihemanma, C. N. and Agomoh, N. G.
Department of Biology/Microbiology, Abia State Polytechnic, Aba
*E-mail of the corresponding author: obyezeigbotxt1 @ yahoo.com

Abstract
The microbiological effects of gas flaring on the agricultural soil from Izombe Flow-Station in Imo State were investigated. The physical and microbiological properties of the soil samples were analyzed. The microbiological analysis of the soil samples were conducted by serially diluting and then inoculating the soil samples on different growth media. Several microbiological and biochemical methods were applied in order to isolate and identify the microorganisms accustomed to the soil samples. An unpolluted soil sample from a farmland outside the Flow-Station served as control. The results showed that temperature increased with increase in distance towards the flare while moisture contents decreased as distance increased towards the flare, indicating that flaring exerts adverse ecological effects on the soil. The pH value at the flare site was acidic (4.0) compared with the near neutral obtained from the control (pH 6.8). Results also showed a decrease in microbial load of the soil samples as distance approaches the Flow-Stack. The total bacterial counts were low (3.0×10^1 cfu/g) when compared with 2.9×10^5 cfu/g obtained from the control. The total coliform counts were also adversely affected by the flare. A value as low as 2.0×10^1 cfu/g was obtained 10m from the flare stack compared with 6.0×10^4 obtained from the control. The bacteria isolated from the soil samples were species of *Staphylococcus spp, Bacillus spp, Escherichia coli, Pseudomona spps, Norcadia spp, Micrococcus spp, Enterobacter spp* and *Streptomyces spp* while species of fungi identified were *Aspergillus spp, Fusarium spp, Penicillium spp* and *Mucor spp.* Acid deposition and intense heat from the flare likely have deleterious effects on the soil ecosystem and therefore should be discouraged.
Keywords: Gas flaring, microbiological effects, physical properties, Microbial load, Flow-Station.

1. ntroduction

Gas flaring is the unscientific burning of natural gas and other petroleum hydrocarbons in flare stacks by upstream oil companies in the oil fields during operation. Gas flaring is the singular and most common source of global warming and contributes to emissions of carbon monoxide, nitrogen (11) oxide and methane which have the propensity of causing environmental pollution and ecological disturbances or destruction (Ubani and Onyejekwe, 2013). This associated gas, a by-product of the petroleum exploration activities, is separated from the oil at flow-stations and more than 95% of this is flared. The burning of such gases releases huge volumes of greenhouse gases into the atmosphere, while emitted sulphur dioxide returns to the soil as acid rain (Ikoro, 2003).

Nigeria flares more natural gas associated with oil extraction than most countries on the planet (Ezeigbo, 2008). Estimate suggesting about 70% of the gas is wasted via flare (Vidal, 2010). This equals about 25% of the UK's total natural consumption and is equivalent to 40% of the entire African continent's gas consumption in 2001 (Ezeigbo, 2008). Alternative to flaring are gas re-injection, or storage for use as an energy source. If properly stored, the gas could also be utilized for community projects (Vidal, 2010). In Western Europe, 99% of associated gas is used or re-injected into the ground. Although, the British government subsequently acknowledged that flaring was unacceptable, it was allowed to continue, without any real effort to change infrastructure and prevent wasting the gas. This is in contrast to Britain policies on gas flaring in their territory where gas flaring has been reduced to a minimum (http://en.wikipedia.org/wiki, 2007). The reason for this economically and environmentally wasteful flare is to maximize crude oil production, while the associated gas accompanying it is often burnt off to decrease cost (Bronwen, 1999). Though companies in Nigeria also harvest natural gas for commercial purposes, they prefer to extract natural gas from deposits where it is in isolation.

Gas flaring has potential harmful effects on the health and livelihood of the communities in their vicinity, as they release verities of poisonous chemicals (Adeniye et al., 1983). It was reported to be a major cause of low agricultural productivity in the Niger Delta (Onosode, 1996; Alakpodia, 2000, Daudu, 2001; Aregbeyen and Adeoye, 2001). The free disposal of gas through flaring generates tremendous heat which is felt over an average radius of 0.5 kilometers, thereby causing thermal pollution (Ikelegbo, 1993). The obvious signs of this can be noticed in the poor vegetation growth and scorched soil around gas flare locations (Adenikinju, 1998). Almost no vegetation can grow in the area directly surrounding the flare due to the prevailing heat (Boden and Andres, 2005). This study was therefore designed to assess the impact of flaring on soil ecosystem with its consequent effect on agriculture.

2. Methodology

2.1 Study Area: Izombe, a town in Oguta Local Government Area of Imo State, South Eastern Nigeria falls within the Niger Delta area. The economy is primarily rural agricultural production of cassava, yam, palm oil, maize, plantain etc. Oil exploration started in the area in the early 1960s with its resultant gas flaring. This has resulted to poor yield of crops, respiratory diseases and other health problems due to the release of poisonous chemicals including greenhouse gases. A common feature in the community and especially around the vicinity of the Flow-station is the presence of crops like maize, cassava plants, palm trees, coconut trees that are abnormally very tall without fruiting due to long photoperiodicity.

Sample Collection: Soil samples from the flare sites were collected at different distances of 10m, 50m, and 100m using an auger and kept in sterile plastic bags. The samples were transported to Microbiology Laboratory, Abia State Polytechnic, Aba for analysis in refrigerated coolers to arrest microbial growth. The control sample was collected from a farmland far from the Flow-station but within Izombe community.

2.2 Methods of Soil Analysis

Soil pH and Temperatures: Soil samples were air-dried and sieved with a 2mm mesh according to Allen et al (1974). The temperature of each sample was taken at the site by immersing the bulb of the thermometer in the soil and the reading in ^{o}C taken after 5 minutes.

Soil pH was determined by the method of Bates (1954) using the air –dried samples. To 20g of air-dried soil in 50ml beaker, 20ml distilled water was added, the contents stirred occasionally with a glass rod and then allowed to stand for 30 minutes. The electrodes of the pH meter were inserted into the suspension and the pH reading recorded.

Soil Moisture: The moisture content of each sample was determined by the method of APHA (1985). 5g of each soil sample was weighed and put into a crucible. It was then heated in a hot air oven for a period of 8-12 hours at 80^{o}C till a constant weigh was obtained. The weight of the moisture was determined from the different between the initial and final weights obtained.

Determination of Organic Matter and Hydrocarbon Contents: Organic matter analysis was carried out based on the description of AOAC (1990) while the analysis of hydrocarbon content was carried out using 5g of soil sample which was weighed and poured into a conical flask. Petroleum spirit was added into it in excess to extract the petroleum hydrocarbon in the soil. The soaked soil was filtered with the aid of filter paper and the filtrate was used for further analysis. The filtrate was poured into Petri-dishes which were dried in the oven and cooled before weighing to know the actual weight of the dish and the final weight when the filtrate were poured. It was then placed in a water bath until the filtrate evaporates and later cooled using desiccators and weighed.

Calculation: % HC = W_2-W_1 (100)/ sample (g)

2.3 Microbiological Analysis

Media Preparation: Three different nutrient media were used which were Nutrient agar, MacConkey agar and potato dextrose agar (PDA). All nutrient media were prepared according to manufacturer's procedures.

Serial Dilution an Inoculation: Each sample of soil was vigorously shaken in 10ml of normal saline. An aliquot (1ml) was transferred into the test tubes and diluted serially in ten folds up to 10^5. From the dilutions of 10^3 and 10^4 of each soil sample, 0.1ml each aliquot was transferred aseptically into freshly prepared media plate and spread in duplicate on surface of the solidified media for Nutrient agar, MacConkey agar and Potato dextrose agar. The mixture was evenly spread on each medium. The inoculation plates were incubated at 37^{o}C for 24-48 hours while inoculated plates of Potato dextrose agar were kept at room temperature for 48 hours, after which the plates were examined for growth. Discrete colonies were counted and recorded as total microbial count in the sample and expressed as colony forming units per gram of soil sample (cfu/g).

Characterization and Identification of Microbial Isolates: Discrete colonies were purified by sub-culturing into appropriate agar media. All organisms isolated were subjected to microscopic and biochemical analysis for characterization and identification according to Cheesbrough (2005).

3. Results

The analysis of the soil samples which included the physical factors were shown on Table 1. The soil pH revealed that soil samples were more acidic as distance increased towards the flare site. The soil sample from the flare site showed a pH of 4.0 while the control sample had a pH of 6.8. Temperature and percentage hydrocarbon content also increased as the distance increased towards the flare while the percentage moisture content and organic matter decreased as distance approaches the flare site.

Table1: Physical Properties of Soil Samples

Distance from the Flare (m)	pH	Temperature (^oC)	Moisture Content (%)	Organic matter (%)	Hydrocarbon Content (%)
10	4.0	48.0	16.84	5.32	4.8
50	4.3	45.0	22.66	5.24	2.2
100	5.2	42.4	29.32	6.12	1.4
Control	6.8	32.0	43.50	8.62	0.2

The total viable microbial count of the soil samples were shown on Table 2.There were decreases in total bacterial , total Fungal and total coliform counts as distances increased towards the flare.

Table 2: Total Viable Microbial Counts of Soil Samples

Distance from the Flare(m)	Total Bacterial Count (cfu/g)	Total Fungal Count (cfu/g)	Total Coliform Count (cfu/g)
10	3.0×10^1	2.3×10^1	2.0×10^1
50	5.0×10^2	5.0×10^2	5.0×10^3
100	1.9×10^5	6.3×10^2	5.7×10^4
Control	2.9×10^5	7.7×10^2	6.0×10^4

Table 3 shows the morphological and biochemical characteristics of the various isolated bacteria. The results revealed that the isolated organisms include *E. coli, Pseudomonas spp, Staphylococcus spp, Bacillus spp, Norcadia spp, Micrococcus spp, Enterobacter spp* and *Streptomyces spp*.

Table 3: Morphological and Biochemical characteristics of Bacterial Isolates

Isolates	**Colony Feature**	**Microscopy**						**Biochemical Reactions**						**Carbohydrate Utilization**			
E. coli	Small circle pink colored colonies, moderate growth on MSA.	-	-	+	-	-	Short rods	-	-	+	-	-	+	+	+	+	-
Pseudomonas spp	Small colonies, creamy with green pigmentation, with slimy consistency.	-	-	+	-	-	Short rods	+	+	-	+	-	-	+	--	-	-
Staphylococcus spp	Circular colonies with entire edge creamy on NA.	+	-	-	-	-	Oval cells in Shape	+	-	-	+	-	+	+	+	+	+
Bacillus spp	Large irregular translucent colonies	+	+	-	-	-	Short rods	+	-	-	-	-	+	+	+	+	-
Norcadia spp	Slightly raised colonies, dull with an irregular margin and whitish grey.	+	+	-	-	-	Small rods	+	-	+	-	-	+	+	+	+	-
Micrococcus spp	Yellow colonies, cocci in cluster and chain.	+	-	-	-	-	Cocci	+	-	-	-	+	-	+	+	-	+
Enterobacter spp	Straight rods with peritrichous structure	-	-	+	-	+	Rods	-	-	-	+	+	-	+	+	+	+
Streptomyces spp	Profused growth with visible well developed mycelia on NA.	+	+	-	-	-	Branched mycelia	+	-	+	--	+	+	+	+	+	-

Table 4: Occurrence of Bacterial Isolates in the Different Soil Samples

Distance from the Flare(m)	*Straphylococcus spp*	*Bacillus spp*	*E. coli*	*Pseudomonas spp*	*Norcadia spp*	*Micrococcus spp*	*Enterobacter spp*	*Streptomyces spp*
10	-	+	-	+	-	-	-	-
50	+	+	-	+	-	-	+	-
100	+	+	-	+	-	+	+	-
Control	+	+	+	+	+	+	+	+
Total % Occurrence.	3- 75%	4- 100%	1- 25 %	4- 100%	1- 25%	2- 50%	3- 75%	1- 25%

The morphological characteristics of the fungi isolated were shown on Table 5, which include *Aspergillus sp, Fusarium spp, Penicillium spp and Mucor spp* . The isolated species of fungi showed that *Fusarium spp* and *Penicillium spp* occurred in all the samples irrespective of the distance while *Aspergillus spp* only occurred from 50m away from the flare. All the fungi isolates were present in the control sample (Table 6).

Table 5: Morphological Characteristics of Fungi Isolates

Characteristics of Isolates	**Isolates**			
	1	**2**	**3**	**4**
Colony Description	Gray fluffy colonies.	Cream colonies with extensive mycelia.	Greenish large colonies.	Grayish-brown woolly colonies.
Morphological Characteristics	Conidiophores terminate in vesicles, conidia in chains.	Conidia borne singly or in pairs, bear conidiophores.	Smooth or rough walled conidiophores, conidia in long chain.	Sporangiophore without rhizoids.
Name of the Isolate	*Aspergillus spp*	*Fusarium spp*	*Penicillium spp*	*Mucor spp*

Table 6: Isolation Frequency of Fungal Isolates

Distance from the Flare (m)	*Aspergillus spp*	*Fusarium spp*	*Penicillium spp*	*Mucor spp*
10	-	-	+	-
50	+	+	+	-
100	+	+	+	-
Control	+	+	+	+
Total % Occurrence	3- 75%	4- 100%	4- 100%	1- 25%

4. Discussions

The results of this study showed that gas flaring has adverse effects on the soil physical and microbiological properties of the soil. The pH of the soil samples from izombe Flow-Station revealed a high level of acidity (4.0 - 5.2) when compared with the control value (6.8). This agrees with the findings of some authors like Nwaugo *et al* (2006), Nwaogu and Onyeze (2010) and Ubani and Onyejekwe (2013) that gas flaring increases the acidity of the soil. The acidity of the soil samples in Izombe could be attributed to the flaring which produces acidic oxides of nitrogen, carbon, sulphur, which dissolves in rain water to form acid rain. High soil acidity creates chemical and biological conditions which may be harmful to plants and soil microorganisms (Nwaogu and Onyeze, 2010). One of such conditions is the reduction in the capacity of plants to absorb cations.

The soil temperatures increased as distance approaches the flare. This excessive and continuous heating, not only reduces the soil moisture (Botkin and Keller, 1998) but also affects the photoperiodicity required for plant flowering and fruiting. The percentage soil moisture content obtained from this study ranged from 16.84 - 22.66% from 10m to 50m distance while the control sample has 43.5% moisture content.

The results also revealed that the organic matter around the Flow-Station was lower when compared with the control. Organic matter from the soil is derived from residual plant and animal materials synthesized by microorganisms and decomposed under the influence of temperature, moisture and optimum soil conditions. These prevailing conditions in this study deprive the soil of the necessary fertility for effective agriculture.

A decrease in total microbial counts was obtained as distance increased towards the flare. The total bacterial counts was low (3.0×10^1 cfu/g) compared to 2.9×10^5 cfu/g obtain for the control sample. The fungal counts was 2.3×10^1 cfu/g at 10m distance compared to 7.7×10^2 cfu/g for the control sample. The coliform counts were

also adversely affected with 2.0 x 10^1 obtained at 10m distance compared to 6.4 x 10^4 cfu/g obtained for the control. The bacterial isolated within 10m distance from the flare were species of *Pseudomonas and Bacillus,* which were hydrocarbon degraders while fungal isolates at this distance were *Fusarium* and *Penicillium.* Consequent to these hazardous effects, it is recommended that alternative measures be taken to avoid gas flaring. Such measures may include:

- Using the gas to power micro-turbine generators for electricity production.
- Re-injecting the gas underground to maintain reservoir pressure during production

This study concludes that gas flaring has adverse effects on the agricultural soil of Izombe, a food producing area in Imo State. This creates a bottleneck in achieving the Millennium Development Goals 1and 7- "Eradicating extreme poverty and hunger" and "Ensuring environmental sustainability".

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