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Mutagenicity Studies of Potable Water Sources in Okerenkoko community, Gbaramatu, Delta State, Nigeria

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Abstract

Mutagenic testing of portable water is fast becoming an important protocol for assessing water quality. Two potable water samples were selected from both ground and surface water sources within the Okerenkoko community, Gbaramatu Kingdom, Delta State Nigeria. Ame's test was employed in the assessment of potential carcinogens in the potable water available to the community. The study was carried out in triplicate to broaden the reproducibility of the study. Sensitivity of the test organism was evaluated using the *Salmonella* TA98 and *Salmonella* TA100 which was reconstituted for the study. The average revertant colonies from the study was enumerated to indicate that the test conducted on S_3OK (Surface water) had 428.1 and 637.84 revertant colonies using *Salmonella* TA 100 for both +S9 mix and -S9 mix-conditions respectively while similar replica result was for *Salmonella* TA 98 was 629.7 and 66.12 revertant colonies. The samples obtained from S_1OK had slightly lower number of revertant colonies of 209.16 and 115.94 colonies for *Salmonella* T.A 100 while for *Salmonella* T.A 98 had 116.82 and 108.90 colonies for treatments containing S9 mix preparations. The study identified the test organism was *Salmonella* T.A 98 was more sensitive to the carcinogen present in the S_3OK sample being used by a wide population of indigenes of the lower economic class. This study further buttresses the need to develop a more robust monitoring protocol for packaged water sources in Nigeria. Regulatory agencies such as NAFDAC must enforce strict intervention limits for potable water sources.

Keywords: *Mutagenic, Potable water, Sensitivity, Ame's test, Carcinogen, Revertant colonies*

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I. INTRODUCTION

Water is the most essential need of living things and the most abundant natural resource on earth while groundwater and freshwater are the largest source of potable water in the world today (Agbalagba *et al*., 2011). It is a vital component of life's processes as it contributes over 70% of the metabolic processes. It can also be available in groundwater or surface water (Batista *et al*. 2016). In most riverine communities of the Niger Delta region of Nigeria, accessibility of drinking water has remained a persistent challenge to policy makers and health enthusiasts. Proper potable water has to comply to specific international physicochemical and microbiological standards which are indicator proxies of wholesomeness and usability. This is because the presence of contaminants or pollutants can deter a water resource and make it unfit and unsafe for consumption (Bushini *et al*., 2004). The socio-economic importance of potable water to persons living in riverine and rural communities cannot be over-emphasized. Groundwater is the most reliable source of potable water to every socio-economic class in Nigeria. It is believed to serve as a source of potable water for drinking, cooking and for all other domestic purpose. This is because it is believed to be a safe resource for drinking water after spring water. Other studies have also reported that most groundwater sources are packaged in sachet or plastic containers as a retailed commodity for the purpose of drinking, domestic and industrial use (Sheshe and Magashi, 2014).

Ame's test is a simple, rapid and effective assay approach which have gained a wide array of applications in both agricultural and industrial sectors. The test involves the use of *Salmonella typhimurium* or *Escherichia coli* for the purpose of identifying potential mutagenic components in a wide variety of substances. This test was introduced by a Bruce Ames a researcher at UC Berkeley after discovering the test in 1970. The conventional and primitive procedure was more complex and time-consuming. Ames and his co-researchers and students standardized the procedures further re-inovated the procedures by introducing the revertant or mutant test organisms. The new approach further identified the role of histidine and termed it Reversion Assay (Ames,

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1971). These innovations and improvements in the use of the Assay approach, the role of histidine has in the recent times improved the sensitivity of the test. Mutagenic compounds are a range of cancer-causing compounds for which some are organic while a number are inorganic elements. A number of these compounds are associated with crude oil fractions and refining activities. A range of compounds called the Polycyclic Aromatic Hydrocarbons (PAHs) have been reported in a number of peer review articles. These compounds are volatile and the residual compounds can be recalcitrant or persistent in the environment. They may exert some level of stearic hindrance which may account for their performances. One of the major compounds is pyrene. They have been associated with a number of health. Okerenkoko community stream (S_3OK) is a source of potable for the indigenes of the Gbaramatu kingdom. There are a number of indigenes that access water for domestic and cottage food processing activities from this surface water source. Some of the food and food products processed within the community are retailed in and around the Nigerian Maritime University Okerenkoko. The quality of most of these processed food products retailed in the community can be source-tracked to the quality of water available to the populace. Sanitary quality in and around the stream may have been a contributing factor for the quality. The tributaries that connect the Okerenkoko stream water have been impacted by a number of industrial activities including artisanal refineries.

Mutagenic compounds comprise a range of carcinogenic compounds either germane or known and may be parts or wholly components of a group of pollutants can have a deleterious effect on biota and humans (Guan *et al*., 2017). In cases of crude oil pollution related events Polycyclic Aromatic Hydrocarbons (PAHs) and Organochlorine related compounds and analogues have been widely reported to induce cancer and in recent times have been neglected due to a number permissible limit concerns on the basis of them concentration values in different medium (Pan *et al*., 2014). The responses from this category of toxic compounds could be acute or chronic depending on the on the target-receptor interaction. Water have remained the widest receptor of pollutants in the world as it receives a number of mutagenic and genotoxic compounds from a number of industrial activities (Ohe *et al*., 2003). Most of the compounds may exhibit a level of recalcitrance in the environmental medium where they are discharged due to their inability to partition then resulting in a number of tissue deformities. In recent times, there has been a technical inadequacy of conventional and analytical procedures to sufficiently to detect these compounds in water especially in rural communities in the Niger Delta (Lv and Wang 2019). Some routine treatment protocols have been implicated as key mutagens in Potable water supplied to a wide population of residents in most rural and urban areas (Bushini *et al*., 2004). The study was aimed at assessing the mutagenic potentials of potable water sources in Okerenkoko, Delta State Nigeria.

II. Materials And Methods

A. *Sample collection*

A total of eighty (80) samples were obtained for the study from Okerenkoko community. The samples were screened and scaled down to two representative samples for Okerenkoko and Krutie drinking sources. The two samples were obtained from the major potable water sources in Okerenkoko community. The water samples were used to rinse the sample bottles and they were carefully collected in sterile and analytical containers. In-situ and Ex-situ Parameters were measured during the study at the sites of sample collection.

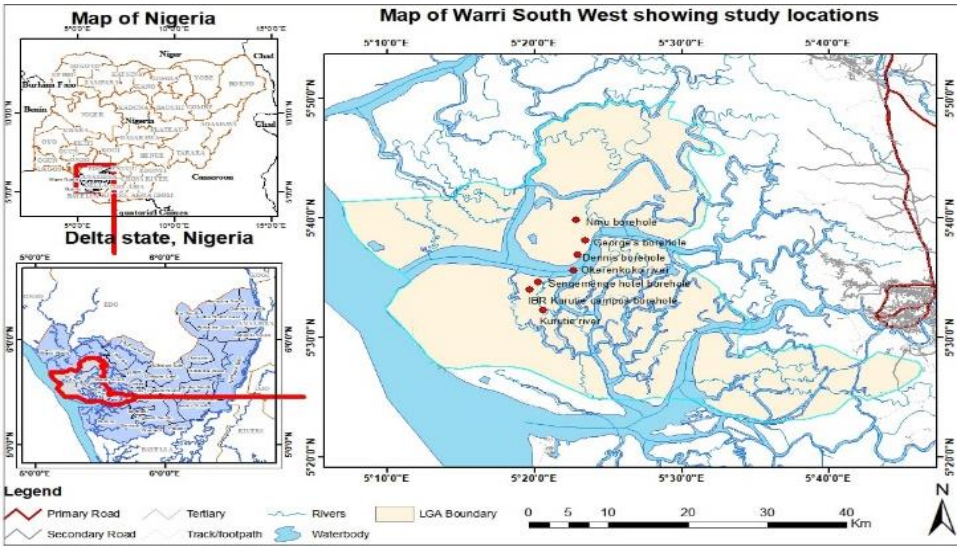

Figure 1. Map of Warri South-West showing the study locations.

B. Mutagenicity test of the water samples

Ames Fluctuation Test Ames was employed for the analysis using the approach as described EBPI (2009). The test isolates *Salmonella typhimurium* (TA 98 and 100) were reconstituted from its lyophilized state without the S9-mix. The test isolates were revived prior to the evaluation by reconstituting and culturing with Moltox Ames II TM media while incubating at 37°C for 16 hours while the cell count was determined using the counting chamber to ascertain the Log phase of the incubation. About 100ml of the water samples were filter-sterilized Whatman filter paper in triplicates designed for vacuum filtration. About 18.0 ml of the filtrate was dispensed into vial bottles and labeled appropriately. The pre-treated water samples were homogenized. Then the reacted solution was left for 25 minutes as instructed by the Moltox Ames guide for users. 1:8 proportion of the reagent mixture and water and were made up to a 20ml mark. A blank solution was prepared using a mixture made up of the reverse osmosis water and the prepared mixture. The *Salmonella* strains (TA 98 and 100) in Liquid suspension were obtained from the incubation process. About 150 µL of the water sample-reagent solution was vortexed vigorously. Negative control used was a background plate containing 17.5 mL of distilled water, 2.5 mL of reaction mixture and 5 µL of bacterial culture. Sodium azide and 2-nitrofluorene solutions were used against the test strains to develop a positive control. The content of each tube (now containing test sample, reaction mixture and bacteria) was poured into the multichannel pipette reagent boat. Using the eight (8) channel multi-pipette, 200 µL proportion of mixture was dispensed into a 96-well microplate wells (200 µl per well). The plates were incubated and the results were read for the possible responses (Agwa *et al*., 2016).

Table 1. Ranges of revertant colonies obtained from control samples /known mutagens

Control samples	Number of Revertant colonies			
	+S9	*-S9*	*+S9* (Duplicate)	*-S9* (Duplicate)
Salmonella TA 100				
DMSO	138.7-181.4	116.5-141.6	162.4	138.1
SODIUM AZIDE	521.9-562.0	416.6-498.6	459.6	471.6
Salmonella TA 98				
DMSO	39.1-48.6	22.1-26.7	37.8	25.4
2-AMINO ANTHRACENE	664.1-687.1	52.1-71.4	36.1	57.15

Note: The Toxic Standard here were 2-Amino Anthracene and Sodium Azide

III. RESULTS

Table 1 and 2 shows the mutagenicity responses for the water samples obtained from Okerenkoko community below. The Ames test employed the S9 mix as a measure to enumerate the revertant colonies on the basis of presence or absence of the S9 mix in the Ames test. The study also reported a range of revertant colonies for control conditions and their ranges. The Test organism during the study varied between *Salmonella* TA 100 and *Salmonella* TA 98 for which the responses elicited from the isolates revealed the sensitivity of the isolates to the test substances being the potable water sources in Okerenkoko community.

Table 2. Number of revertant colonies with water samples on *Salmonella* TA 100, and *Salmonella* TA 98

Water samples	Batch 1		Batch 2		Batch 3	
	+S9	*-S9*	*+S9*	*-S9*	*+S9*	*-S9*
	Salmonella TA 100					
S_1OK	32.11	32.18	66.84	32.18	76.01	46.95
S_1KR	75.06	57.20	79.15	50.94	78.16	52.06
S_3OK	482.1#	637.84#	671.11#	451.16#	431.16#	441.86#
S_4KR	209.16	115.94	211.14	110.16	221.10	127.06
	Salmonella TA 98					
	+S9	*-S9*	*+S9*	*-S9*	*+S9*	*-S9*
S_1OK	29.01	26.01	26.70	21.00	23.01	21.01
S_1 KR	66.01	41.16	66.71	47.06	71.09	48.70
S_3OK	629.70#	66.12	621.16#	66.81#	621.75#	69.25
S_4KR	116.82	108.90	104.01	124.01	128.01	98.16

Values with # superscript showed significant positive mutagenic responses, batches are triplicates

The study observed the ratio of the revertant colonies for the water samples and identified that the samples obtained from S_3Ok were observed to show presence of mutagenic substances. The water sample S_3OK was surface water and had a history of serving as a route and receptacle for the oil and gas industries such as the Escravos platform. The responses observed that the number of revertant colonies using the *Salmonella* TA 98 were more compared to the *Salmonella* TA 100.

IV. DISCUSSIONS

Mutagenic assessment of the water samples obtained from the study area revealed that the range of revertant colonies using the control samples against and known mutagens (Sodium Azide) had 521.9-562.0 colonies while replicate had 459.6 colonies and 471.6 colonies for both +S9 mix and -S9 mix respectively. while 2-Amino Anthracene range of colonies were 664.1 – 687.1 and 52.1- 71.4 colonies while the colony count was 36.1 and 57.2 respectively. The test conducted on S_3OK (Batch 1) had 428.1 and 637.84 colonies revertant colonies on the *Salmonella* TA 100 for presence of the S9 mix and S9 mix- respectively while similar study obtained for the same sample using *Salmonella* TA 98 for S+ and S- was 629.7 and 66.12; for Batch 2 it had 671 and 451.16 colonies using the *Salmonella* TA 100 while the Batch 3 had 431.16 and 441.86 colonies. The report of the present study identified the water samples S_3OK induced a significant mutagenic effect on the biota in Okerenkoko. This further suggest that there is an emergency and urgent need to source-track the trajectory of the pollutants in the surface water. *Salmonella* TA98 was

more sensitive to the test than *Salmonella* TA100 during the study. This study corroborates the previous findings of Umbuzeiro *et al*. (2001) whose study also identified possible mutagen from the surface water from possible industrial sources. Their study also was able to identify the role of *Salmonella* TA98 in the monitoring of the long-term pollutant sources. Furthermore, Agwa *et al*. (2017) identified mutagenic responses from locally available ground water sources for packaged water companies in Port Harcourt metropolis which in their report suggested that possible in-process of Chlorination accounts for the non-volatile carcinogens in water. Previous studies have identified other carcinogens from hydrocarbon related activities and fraction such as PAHs and TPHs which are predominantly discharged into receiving water in the Niger Delta. In the case of Wang *et al*. (2013) they also made a case for the ground water sources as being endangered from most industrial activities in China. There suggesting that the wholesomeness of water sources must be made to go through genotoxic and mutagenic analysis. Atubi (2011) reported that the impact of the oil companies in the Niger Delta region had in the past affected the quality of the ground water. The Long-term effects of these exposures cannot be overemphasized due to increasing seepages and run-offs.

V. CONCLUSIONS

This present study underscores the decline in the quality of potable water in rural and oil-bearing communities of Niger Delta region of Nigeria. There is need for regulatory agencies in Nigeria to develop more stringent and robust routine monitoring scheme for the most drinking water sources; especially for packaged water. Environmental and health agencies must be encouraged to monitor waste disposal practices by international oil companies and sanitary practices as possible routes to some of the mutagenic agents that could induce the responses observed in the current study.

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