

Research Article

Simulated Leachate of Soil from Petroleum, Diesel and Kerosene Dispensing Sites Induced DNA Damage using Ames Salmonella Test and SOS Chromo Test

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Abstract

Indiscriminate location of petrol filling stations is of public health concern, as these filling stations are located around residential and agricultural areas. This study assessed the mutagenicity of simulated leachate from soil collected from petrol, diesel and kerosene dispensing sites (sites 1, 2 and 3 respectively) in a petrol station by using two standard microbial assays: the *Salmonella* reverse mutation assay (Ames test) and the *E. coli* SOS chromo assay. Physico-chemical parameters of the samples were also analyzed. The result of the Ames test showed mutagenicity of the test samples. The SOS Chromotest results were in agreement with those of the Ames *Salmonella* fluctuation test. The petrol dispensing site soil was the most mutagenic, followed by the diesel dispensing site and then kerosene dispensing site soils. The level of mutagenicity observed correlates with the level of the physico-chemical parameters analyzed. Fe, Cd, Mn, Cu, Ni, Zn and Pb analyzed in the samples were believed to play significant role in the observed mutagenicity in the microbial assays. The results of this study showed that the simulated leachate from soil contaminated with petroleum products showed strong indication of a mutagenic risk. This should foster legislation about the location of petrol filling stations away from residential and agricultural areas.

INTRODUCTION

Petroleum products are among the most valuable natural resources abundantly available in Nigeria. Nigerians and people everywhere use petroleum products as a fuel in their automobiles, generating sets, industrial plants and for cooking purposes, thus making petroleum products an essential commodity that is needed for the daily operations of individual, industrial and national activities. Petroleum products such as Premium Motor Spirit (PMS), Automated Gas Oil (AGO) and Dual Purpose Kerosene (DPK) are products utilized by almost everybody on daily basis at an average of 60 million litres per day. Due to increasing level of standard of living, more Nigerians are increasing the number of cars on the road, the need for constant power supply to aid uninterrupted production of goods and services and also for domestic use, more Nigerians are increasing the demand for fuel. With a population of over one hundred and sixty five million people, growing at an average rate of 2.7% per annum and an economic growth rate of about 5.7% in the past five years, the market for refined petroleum products are growing. This has made refined petroleum product filling station business a very

lucrative business. Indiscriminate location of such filling station is of public health concern, as these filling stations are located around residential and agricultural areas.

Petroleum products are common soil contaminants and often contain potentially toxic compounds, particularly, the polycyclic aromatic hydrocarbons (PAHs). Petroleum hydrocarbons released into the environment can pose risk to ecosystems and human health. Some compounds in petroleum products are known to be mutagenic and carcinogenic. Extensive chemical extraction and analysis of petroleum contaminated soil can provide detailed information about the total contaminant concentration. However, the potential impact on the ecosystem may not be easily predicted using only concentration data.

The use of bioassays for ecotoxicity evaluation of contaminated soil has gained widespread attention over the past twenty years. Bioassays have clearly demonstrated that chemical analysis alone is not adequate to assess the potential ecological impact of contaminated soil. These tests have been shown to be useful particularly when predicting the effect of a complex

mixture of compounds, such as petroleum. Bioassays also can be useful in predicting bioavailability since responses can occur at contaminant levels lower than those easily detectable by chemical assays.

Bioassays of soil may involve direct exposure to the soil or a soil solution. Either method assumes that the organisms are being exposed to readily available contaminants.

Since petroleum is a major contaminant of terrestrial soils world-wide, it is important to use different bioassay species for risk assessment. The goal of this study was to assess the mutagenicity of petroleum contaminated soil using microbial assays.

MATERIALS AND METHODS

Sampling site

The study site is a filling station located in Sagamu, Ogun state, South west Nigeria. It has been in operation for about 15 years. The filling station is divided into three areas, with each selling PMS, AGO and DPK. The sites with the AGO and DSK spillage had a characteristic black color while the site with the PMS spillage had a characteristic brown color. The soil surfaces of the three sites were hard.

Sample collection and simulation of leachate

Soil samples were collected at each of the three sites using sterile trowel after clearing debris from the soil surface and transferred directly into clean, sterile containers. Samples were transported to the lab, air-dried for 12 weeks and subsequently ground to powder. Leachate simulation from the soil was carried out using dimethyl sulfoxide (DMSO) according to the American Society for Testing and Materials category A extraction procedure as modified by Bakare et al. [1]. Soil sample from Babcock University in Ogun State was collected and used as a control for the physic-chemical analysis. The leachate samples were filtered using a 15-cm filter paper (Whatman®, Maidstone, UK), pH measured and stored at 4°C until use.

Determination of physical and chemical parameters

The sample was analyzed for a number of standard physical and chemical parameters including chemical oxygen demand, total dissolved solids, alkalinity, biochemical oxygen demand, chlorides, nitrates, ammonia and phosphates, according to the methods described by APHA [2]. A total of eight heavy metals namely Pb, Cd, Cu, Fe, Zn, and Mn were analyzed in the leachate sample according to standard analytical methods [2,3].

Ames (*Salmonella*) fluctuation test

The leachates were subjected to Ames test after sterilization by filtration through a 0.22 µm pore-size cellulose nitrate filter (Millipore). *Salmonella typhimurium* strains TA98 and TA100 obtained from Environmental Bio-Detection Products Inc. (EBPI, Canada) were used in the Ames test conducted according to the method described by Maron and Ames [4]. Tests were conducted under aseptic conditions according to the method described by Rao and Lifshitz [5] and Alabi et al., [6]. Three concentrations of 1, 2.5, 5 and 7.5% (v/v, leachate/DMSO) of each of the simulated leachate were prepared. The first dilution was prepared by

mixing 200 µL of each of the samples with 19.8 ml of the reaction mixture (Davis-Mingioli salts composed of D-glucose [ICN Biomedicals, Aurora, OH, USA; CAS 50-99-7] D-biotin [ICN Biomedicals, Aurora, OH; CAS 22879-79-4], L-histidine [Sigma, St. Louis, MO, USA; CAS 7048-02-4], and bromocresol purple [Fisher Scientific, Nepean, Ont.; CAS 115-40-2]). Reagents were added to sterile culture tubes in the following order: (1) reaction mixture, (2) sample (MSL and BWV) (3) bacteria. The culture tubes were vortexed after each addition and a 200 µL portion was transferred into 96-well flat-bottomed microplates. The microplates were sealed in plastic bags and incubated for five days at 37°C. At the end of this period, the plates were examined for color: all yellow, partially yellow and turbid wells were considered positive, whereas purple wells were deemed negative. The number of positive wells per plate was recorded and compared to the controls. Analysis of variance with Dunnett t-test [7] as post hoc was used for statistical evaluation of the treated plates versus the control plates. A sample is considered mutagenic when there is a significant increase of the number of positive wells in treated plates over the negative control plates (i.e mutagenic index [MI]). The results were expressed as mutagenicity ratio (number of positive wells in treated plates/ number of positive wells in the negative control plates) and are an average of at least three experiments (±standard deviation). Sodium azide (NaN₃) and 2-Nitrofluorene (2-NF) were used as positive controls for TA100 and TA98 respectively, while DMSO was used as negative control.

SOS Chromotest assay

The tester strain *E. coli* PQ37 was obtained from Environmental Bio-Detection Products Inc. (EBPI, Canada). The SOS chromotest was performed without metabolic activation as described by Quillardet and Hofnung [9] with modifications provided by Kevekordes et al. [9] and Alabi et al. [6]. The sample concentrations of 0.625, 1.25, 2.5 and 5% (v/v, sample/DMSO) were considered for each of the simulated leachate in four replicates without metabolic activation. A 600 µL volume of an appropriate overnight culture dilution were added to a tube containing 20 µL sample volume, and incubated with agitation for 2 h at 37°C and subsequently centrifuged at 700 g for 20 min. The supernatant was discarded and the bacterial pellets were resuspended with 200 µL of SOS Chromogen [p-nitrophenyl phosphate (PNPP, Boehringer Mannheim, Laval, Que.; CAS 4264-83-9) for alkaline phosphatase (AP) and 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside (X-gal, Vector Biosystems, Toronto, Ont.; CAS X100) for Beta-galactosidase (bgal). Plates were re-incubated (10 min for AP and 60 min for b-gal), after which optical density readings were taken at 620 (b-gal) and 405 nm (AP) respectively. 4 Nitro-Quinoline Oxide (4 NQO) was used as positive control.

AP reduction factors (RF), b-gal induction factors (IF) and corrected induction factors (CIF-IF/RF) were calculated as described by Legault et al. [10].

$$RF = \frac{XOD_{405t}}{XOD_{405c}}$$

$$IF = \frac{XOD_{620t}}{XOD_{620c}}$$

$$CIF = IF/RF$$

where X is the mean of four OD readings and t and c refer to

test and control dilutions, respectively. As shown above, the RF and IF values account for the background activity of the control. The ratio of IF to RF units yields an estimate of b-gal activity corrected for toxicity. A normalized induction factor of 1.2 or more was considered to represent significant genotoxic activity [10].

RESULTS AND DISCUSSION

Physico-chemical analysis

Physical and chemical characteristics of the three sites of the petrol filling station soil simulated leachates are shown in Table 1. The pH was basic (>7.0) for the three sites. Pb, Cd, NH₃, Cu, Fe, Zn, Bo and Mn levels in the three sites were higher than the control soil. Our data of high heavy metals thus showed the potential environmental contamination of soil by petroleum products and constituents.

Ames (*Salmonella*) fluctuation test

Ames test is a simple and reliable biological assay to evaluate the mutagenic potential of chemicals which has been extensively used for screening of mutagenicity. In this study two bacterial strains were used to test mutagenicity of petrol, diesel and kerosene dispensing sites at a petrol station in the absence of the

S9 mixture. Strain *S. typhimurium* TA98 carries frameshift type mutations whereas; *S. typhimurium* TA100 carries a base-pair substitution type mutation [4].

The results of the Ames test conducted in the absence of S9 mix at the concentrations of 1, 2.5, 5 and 7.5% for the three sites simulated leachate is summarized in Table 2. Mutagenicity was observed, which was concentration-dependent and statistically significant (p<0.05) at the three sites, in the two bacteria strains utilized. At the highest concentration of 7.5%, there was complete cytotoxicity in site 1 in the two strains utilized. The result further showed that site 1 (petrol dispensing site) is more mutagenic than site 2 (diesel dispensing site) which was more mutagenic than site 3 (kerosene dispensing site). The MI ranging from 1-25 was observed in the tested samples in both bacteria strains with the highest induction recorded in the highest concentrations of the three sites (Figures 1 and 2). The mutagenicity thus reported is an indication of the ability of the petroleum products and constituents to induce both frameshift and base-pair substitution types of mutation in exposed organisms.

SOS Chromotest assay

Mutagenicity was further confirmed at the three sites using the SOS chromotest. The SOS chromotest employs the error-prone DNA repair pathway of *E. coli* PQ37, also known as the

Table 1: Physico-chemical and heavy metals characteristics of petrol, diesel and kerosene dispensing sites's soil simulated leachate at a petrol filling station.

| Parameters* | Control soil | Site 1 | Simulated leachate Site 2 | Site 3 |
|-----------------|--------------|--------|---------------------------|--------|
| pH | 7.1 | 9.5 | 8.6 | 8.2 |
| Nitrate | 0.16 | 4.2 | 2.8 | 1.9 |
| Phosphorus | 0.20 | 10.8 | 5.6 | 7.2 |
| NH ₃ | ND | 6.4 | 5.3 | 4.3 |
| Sulfate | 1.8 | 218 | 192 | 167 |
| Cd | ND | 8.0 | 11.2 | 7.1 |
| Pb | ND | 26.3 | 18.9 | 11.7 |
| Cu | ND | 1.8 | 0.7 | 0.6 |
| Fe | ND | 52.9 | 40.5 | 23.9 |
| Zn | 0.001 | 5.9 | 3.9 | 3.2 |
| Bo | ND | 1.1 | 0.7 | 0.5 |
| Mn | ND | 22.1 | 18.4 | 15.1 |
| Ca | 250 | 1981 | 1210 | 983 |

ND=Not detected.

*Units of the parameters are in mg/kg except for salinity in parts per thousand and pH which has no unit.

Table 2: Ames *Salmonella typhimurium* mutagenicity test results for petrol, diesel and kerosene dispensing sites soil simulated leachate at a petrol filling station.

| Treatment | Site 1 | Mean±SD ^a TA 98 Site 2 | Site 3 | Site 1 | Mean±SD ^a TA 100 Site 2 | Site 3 |
|---|-------------|---|-------------|-------------|--|-------------|
| Negative (DMSO) | 5.4±1.02 | 5.4±1.02 | 5.4±1.02 | 31.7±0.56 | 31.7±0.56 | 31.7±0.56 |
| 1% | 42.3±0.76* | 26.8±1.48* | 6.0±1.21 | 74.2±0.13* | 60.9±0.84* | 41.5±2.01 |
| Simulated leachate 2.5% | 72.8±1.41* | 43.5±0.82* | 10.8±0.62* | 112.4±0.61* | 91.7±1.06* | 67.9±0.63* |
| 5.0% | 138.2±0.81* | 66.0±2.10* | 18.5±1.41* | 181.7±0.37* | 127.6±0.98* | 88.6±1.12* |
| 7.5% | - | 92.4±0.94* | 16.7±0.65* | - | 158.2±1.43* | 106.1±0.95* |
| Positive (2-NF for TA98 and NaN ₃ for TA100) | 216.8±1.12* | 216.8±1.12* | 216.8±1.12* | 306.5±0.52* | 306.5±0.52* | 306.5±0.52* |

*significant for mutagenicity at p<0.05.

SD- Standard deviation,

^aNumber of His+ per plate: mean values of at least three experiments ± S.D.

Table 3: SOS-Chromo mutagenicity test results for petrol, diesel and kerosene dispensing sites soil simulated leachate at a petrol filling station.

| Conc. (%) | Positive(4-Nitro-Quionoline Oxide) | IF=Mean±SD Site 1 | Simulated leachate Site 2 | Site 3 |
|-----------|------------------------------------|-------------------|---------------------------|-----------|
| 0.31 | 0.1±0.40 | - | - | - |
| 0.63 | 0.2±0.81 | 1.1±0.67 | 0.9±0.22 | 0.9±0.82 |
| 1.25 | 0.45±0.11 | 1.5±1.01* | 1.3±0.41* | 1.1±0.54 |
| 2.50 | 0.7±0.02 | 2.1±0.83* | 1.8±0.08* | 1.6±1.51* |
| 5.00 | 1.2±0.31 | 2.9±1.18* | 2.3±0.06* | 1.9±0.09* |
| 10.00 | 1.4±0.63 | - | - | - |

*IF≥1.2 is significant for mutagenicity
 -=Not applicable

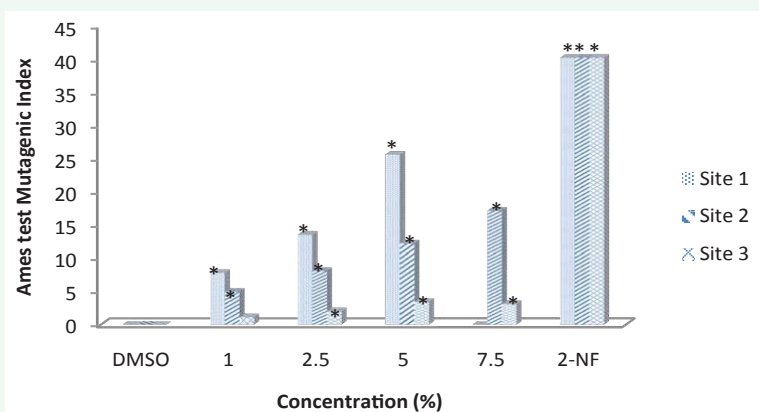


Figure 1 The Ames test mutagenic index (MI) induced by different concentrations of petrol, diesel and kerosene dispensing soil simulated leachate on Salmonella TA 98 strain.
 Site 1= Petrol dispensing site; Site 2= Diesel dispensing site; Site 3= Kerosene dispensing site.
 *significant at p<0.05.

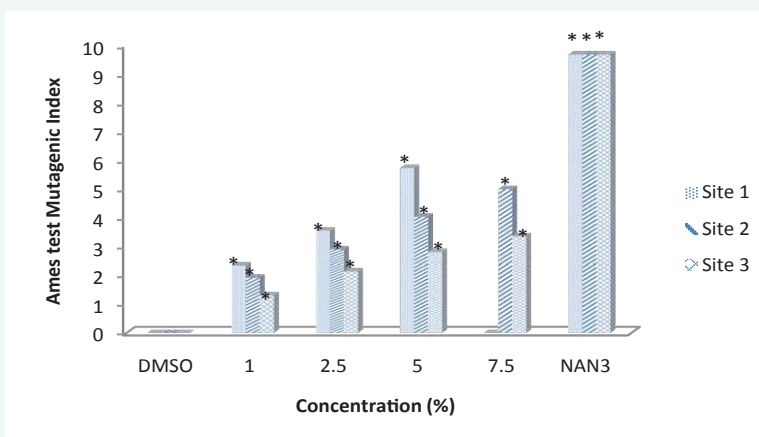


Figure 2 The Ames test mutagenic index (MI) induced by different concentrations of petrol, diesel and kerosene dispensing soil simulated leachate on Salmonella TA 100 strain.
 Site 1= Petrol dispensing site; Site 2= Diesel dispensing site; Site 3= Kerosene dispensing site.
 *significant at p<0.05.

SOS response, a complex regulatory network that is induced by DNA-damaging substances [11]. The SOS chromotest allows the detection of primary DNA-damaging agents on *E. coli*. The SOS chromo test results of the three sites of the petrol station soil simulated leachate are summarized in Table 3. The results

showed that the three samples contained agents capable of inducing SOS response in *E. coli* PQ37. The IF of ≥ 1.2 was taking as mutagenic which is an equivalent of 5% concentration of 4-Nitro-Quionoline Oxide used as the positive control. There was significant induction of mutagenic response which is

concentration-dependent from 1.25% in sites 1 and 2; and 2.5% in site 3. Similar to the result of Ames test, mutagenicity of the sites were in the ratio: site 1>site 2>site 3.

The observed genotoxicity of the samples were believed to have been caused by high physico-chemical parameters some of which were analyzed in this study. The results of this study are in accordance with previous studies where petroleum and petroleum products have been found to be mutagenic and/or genotoxic in spirotox test [12], earthworm lethality assay [13] and plant germination tests [12,14].

CONCLUSION

In conclusion, mutagenicity of simulated leachate from soil collected from petrol station. The data of this study further confirm the potential mutagenic effect of petroleum products and the need to take precautions in the usage so as to prevent environmental contamination and public health effects.

REFERENCES

1. Bakare AA, Pandey AK, Bajpayee M, Bhargav D, Chowdhuri DK, Singh KP. DNA damage induced in human peripheral blood lymphocyte by industrial solid waste and municipal sludge leachates. *Environ Mol Mutagen*. 2007; 48: 30-37.
2. APHA, AWWA, WPCF. *Standard Methods for the Examination of Water and Wastewater*. 20th Ed. Washington, DC. 1998.
3. United State Environmental Protection Agency (USEPA). *Acid Digestion of Sediments, Sludges and Soil Method 3050B*. Washington, DC: USEPA. 1996.
4. Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. *Mutat Res*. 1983; 113: 173-215.
5. Rao SS, Lifshitz R. The Muta-ChromoPlate method for measuring mutagenicity of environmental samples and pure chemicals. *Environ Toxicol Water Quality*. 1995; 10: 307-313.
6. Alabi AO, Esan EB, Duru C, Oyedele P, Salihu GR. Mutagenicity of automobile workshop soil leachate and tobacco industry wastewater using the Ames Salmonella fluctuation and the SOS chromotests. *Toxicol Ind Health*. 2014.
7. Quillardet P, Hofnung M. The SOS Chromotest, a colorimetric bacterial assay for genotoxins: procedures. *Mutation Research*. 1985; 147: 65-78.
8. Kevekordes S, Mersch-Sundermann V, Burghaus CM, Spielberger J, Schmeiser HH, Arlt VM, Dunkelberg H. SOS induction of selected naturally occurring substances in *Escherichia coli* (SOS chromotest). *Mutat Res*. 1999; 445: 81-91.
9. Legault R, Blaise C, Trottier S, White PA. Detecting genotoxic activity in industrial effluents using the SOS Chromotest microplate assay. *Environmental Toxicology and Water Quality*. 1996; 11: 151-165.
10. Walker GC. The SOS Response of *Escherichia coli*. In: Neidhardt FC, Ingraham JL, Magasanik B, Low KB, Schaechter M, Umberger HE, editors. *Escherichia coli and Salmonella typhimurium, cellular and molecular biology*. American Society for Microbiology, Washington, DC, 1987: 1346-1357.
11. Płaza G, Nałecz-Jawecki G, Ulfig K, Brigmon RL. The application of bioassays as indicators of petroleum-contaminated soil remediation. *Chemosphere*. 2005; 59: 289-296.
12. Dorn PB, Salanitro JP. Temporal ecological assessment of oil contaminated soils before and after bioremediation. *Chemosphere*. 2000; 40: 419-426.
13. Banks MK, Schwab P, Liu B, Kulakow PA, Smith JS, Kim R. The Effect of Plants on the Degradation and Toxicity of Petroleum Contaminants in Soil: A Field Assessment. *Adv Biochem Engineering/Biotechnol*. 2003; 78: 75-95.
14. Banks MK, Schultz KE. Comparison of Plants for Germination Toxicity Tests in Petroleum-Contaminated Soils. *Water, Air, and Soil Pollution*. 2005; 167: 211-219.

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