EFFICACY OF AMENDMENTS IN TPH REMOVAL DURING BIOREMEDIATION OF AGRICULTURAL CRUDE OIL-POLLUTED SOIL.

Barisiale Baranu[12*]., Chimezie Ogugbue[3*] and Gideon Okpokwasilli[3*]

[1]World Bank Africa Centre of Excellence-Centre for Oil and Chemicals Research, University of Port Harcourt

[2]Department of Microbiology, Rivers State University

[3]Department of Microbiology, University of Port Harcourt

Abstract

This study identified the efficacy of different amendments as biostimulants in bioremediation. This experiment was carried out for 4 weeks in the laboratory. One kilogram of pristine soil was spiked with one liter of crude oil in earthen pots, to each pot 10 grams of amendments were added and mixed thoroughly. The amendments used were poultry dropping (C-PD), cow dung (D-CD), N.P.K (E), and a control (A and B) setup undergoing natural attenuation. The microcosms' initial physicochemical characteristics such as total organic nitrogen, pH, temperature, total organic carbon, total petroleum hydrocarbon (TPH), and polyaromatic hydrocarbon (PAH). The microbial enumeration was done for total heterotrophic bacteria (THB) and hydrocarbon utilizing bacteria (HUB). The molecular characterization of the pristine soil (A) and contaminated soil (B) was also done using the shotgun analysis. The THB of A and B was 1.3 x 10^7 and 2.1 x 10^2 while the HUB was 1.63 x 10^5 and 1.1 x 10^1on day 1 respectively. The THB of treatments during bioremediation at week 2 was 1.75 x 108, 1.89 x 108, 1.5 x 108 and 2.2 x 108 while at week 4, the THB was 1.90 x 108, 2.1 x 108, 2.20 x 108 and 2.25 x 108 while the HUB at week 2 was 1.20 x 10^5, 3.0 x 10^5, 2.5 x 10^5 and 1.98 x 10^5 while at week 4, the HUB was 2.0 x 10^6, 2.19 x 10^6, 2.46 x 10^6 and 2.1 x 10^6 for B, PD, CD ,and N.P.K respectively. The molecular characterization of A and B showed there was a higher microbial diversity in the contaminated soil than in the pristine soil. This study has shown that cow dung is more effective in the bioremediation of total petroleum hydrocarbon, and polyaromatic hydrocarbon in crude oil-contaminated soil.

Keywords: Bioremediation, Biostimulants, Natural attenuation, Poultry dropping, Cow dung, Petroleum hydrocarbon.

Introduction

Soil pollution is the contamination of the soil with pollutants, toxic chemicals, or any contaminant in such a quantity that reduces soil quality and makes it to habitable to organisms such as insects and other microbes [1]. It is the result of human interaction and activities in the ecosystem through physical, chemical, and biological scientific activities, leading to the spread of destructive or harmful substances [2]. Oil spills result from excessive oil exploration and development, resulting in spills into the environment, causing destructive and harmful activities of ecosystems, resulting in national economies [3].

The Niger Delta is a rich oil ecosystem situated in the southern part of Nigeria, which covers an area of approximately 70,000 km^2 and is known as African's largest wetland, also considered as a notable wetland and marine ecosystem in the world. About 95% of the crude oil produced in Nigeria is from the Niger Delta region and is said to have a great impact on the sustenance of Nigeria's economy [4]. Crude oil is a complex mixture of a vast number of individual chemical compounds including asphaltic compounds, heterocyclic, normal alkanes, cyclo- and isoalkanes, aromatics. [5]. The Niger Delta region experiences a high number of oil spill incidents because it is the seat of crude oil activities in Nigeria [6]. These activities that cause oil spills could be mistakes resulting from the operational processes during production, deliberate acts of sabotage during the production process, and accidents involving oil storage facilities oil tankers [7].

Another major cause of the oil spill is the disruption of pipelines in the attempt to steal oil from pipelines [8]. The disruption of the soil with crude oil leads to a loss of agricultural lands, a loss of microbial structure, ecosystem services, and source of livelihood for indigenous citizens of the Niger Deltans due to the health problems associated with exposure to oil such as respiratory damage, decreased immunity and increased cancer risk [9]. It becomes imperative to reduce, remove or completely stop these harmful activities. The ecosystem can be restored using bioremediation.

Bioremediation

Bioremediation is a process that involves the use of biological processes to return the environment to its original state [10], it is an alternative method to detoxify contaminants, and an effective means of mitigating hydrocarbon, non-chlorinated pesticides and herbicides, nitrogen compounds, and metals [11]. This alternative technique is effective, less hazardous, economically, and environmentally friendly, and versatile in the clean-up of pollutants from the environment [12]. Bioremediation achieves its purpose through biodegradation, a technique that utilizes microorganisms or their enzymes and metabolic activities to restore environmental damage to a suitable state for use [13]. However, bioremediation is a microbial process, and provision among other limiting factors such as nutrients is required for the process by increasing the aeration through small pores is by the addition of organic waste materials such as poultry dropping (C-PD), cow dung (D-CD)which enhances bioremediation. Bioremediation involves the breakdown of hydrocarbon and organic contaminants by soil organisms such as fungi, bacteria, and plants for the improvement and enhancement of life.

The petroleum contaminated sites having less efficient and metabolically poor microbial populations can be remediated significantly by the addition of some of the rate-limiting nutrients or through the process of bio-stimulation [14]. Various researchers have dealt with nutrient applications as a bio-stimulant for bioremediation of petroleum polluted sites and have employed the use of agricultural wastes as nutrient supplements [15, 16]. A study carried out in Ondo; Southwest Nigeria showed that the use of appropriate manure on the soil will not only reduce contaminant concentrations but can increase crop yield for farmers [17]. This study has shown that cow dung is more effective in the bioremediation of total petroleum hydrocarbon, and polyaromatic hydrocarbon in crude oil-contaminated soil.

This work aims to investigate the efficacy of cow dung, poultry dropping, and N.P.K in the reduction of the total petroleum hydrocarbon concentration while improving the proliferation of hydrocarbon-degrading bacteria in crude-oil contaminated soil during bioremediation.

2. Materials and Methods

2.1 Sample Collection

The soil samples were collected from Airforce farm at Obio-Akpor, Rivers State, Nigeria at 4°51'19.5"N 7°01'33.1"E. The soil was collected from a depth of 0-30 cm and 30-60 cm, the samples were mixed to obtain a composite sample. The cow dung and poultry dropping were collected from the Rivers State University, Nigeria, the N.P.K of ratio 20:10:10 was purchased from the Agricultural Development Program (ADP), Rumuodamaya, Port Harcourt, Nigeria.

2.2 Physicochemical characterization of soil.

The physicochemical parameters such as total petroleum hydrocarbon (TPH) and polyaromatic hydrocarbon (PAH) were analyzed using gas chromatography. The pH was carried out using the ASTM D1293B method, electrical conductivity was done using the

ASTMD1125 method, total organic matter using the routine colorimetric method, the temperature was done using a thermometer (H- 19811-5, Romania). It was immersed into the sample in such a way that the mercury bulb was well covered by the sample, the final reading was recorded in ºC after it was allowed to stabilize and was considered the actual reading (APHA, 2005) and phosphorus using the EM Test kit 5934.

2.3 Enumeration of total heterotrophic bacteria counts

The Total Heterotrophic Bacteria (THB) populations of soil samples were determined using the spread plate method on nutrient agar [18]. Serial dilution was carried out and 0.1 ml diluent was cultured using the spread plate method.

2.4 Enumeration of hydrocarbon utilizing bacteria (HUB) counts

One gram (1 g) of soil was homogenized in distilled water, plated out on Bushnell-Haas agar modified with Agar-agar (to aid solidification) using appropriate dilutions of 10^{-5} and 10^{-6}. Hydrocarbon was supplied through the vapor phase transfer technique by placing sterile Whatman No.1 filter paper saturated with 5ml of crude oil in the inside lid of each plate kept in an inverted position and incubated at 37^0C for 48 hours. Bacteria growth in Bushnell-Haas agar becomes visible from 3-7days after inoculation, colonies formed were counted and expressed as colony-forming units per gram (cfu/g) [19, 20, 21]

2.5 Experimental Design

Soil collected from depths of 0-30 cm and 30-60 cm was mixed thoroughly using a sterile hand trowel and placed in 4-liter pots. In each pot, 2 kg of soil was artificially contaminated with 1 liter of Bonny Light crude oil, 10 g of cow dung, poultry droppings, and nitrogen-phosphorus-potassium amendments were added and labeled C, PD, and N.P.K while the controls A (pristine soil) was not polluted and had no amendment and B was artificially contaminated without amendment. The mixture in the pots was kept in a greenhouse and watered periodically (every other day) to enhance the aeration. The statistical method employed was the one-way analysis of variance.

3. Results and Discussion

Physicochemical Characteristics

The texture analysis of soil done using the hydrometer showed sand has a percentage of 40.3 %, clay has 20.08 %, and silt 39.62 % which is a ternary classification as loam soil (Table 1).

The chemical characteristics of the pristine soil were analyzed for parameters like pH, electrical conductivity, temperature, total organic carbon, and total nitrogen (Table.2).

Table 1: Physical characterization of the soil used in the study

Soil texture parameters	Values
Moisture content (wt%)	41.97
Bulk density (g/cm3)	2.596
Water holding capacity	35.91
Sand (%)	40.3
Silt (%)	39.62
Clay (%)	20.08
Porosity (%)	49.45
Soil texture	Loam

The physicochemical characteristics of the pristine soil were analyzed at 25 ^{o}C before pollution. The total hydrocarbon content of the soil was less than 0.1 mg/kg indicating the absence of any hydrocarbon contamination (Table 2a); however, the polluted soil had a total petroleum hydrocarbon (TPH) of 34342 mg/kg and a polyaromatic hydrocarbon (PAH) concentration of 96.45 mg/kg (Table 2b).

Table.2a: Physicochemical Characteristics of pristine soil

Physicochemical characteristics	Value	Methods
pH	6.49	EPA 9045D
Conductivity	35.17	SSST EC (EC METER)
Organic matter	3.01 (%)	Walkley-Black
Total Organic Carbon	1.94 (%)	Walkley-Black
Cation Exchange Capacity	18.64 (meq/100g)	EPA 9081
Total Nitrogen	1.98 (mg/kg)	ASTME258
Total Phosphorus	8.27 (mg/kg)	EPA 365-3
Total hydrocarbon content	0.01 (mg/kg)	ASTM D7066
Total Nitrogen	1.90 (mg/kg)	Kjeldahl Method
Sulphate	1.68 (mg/kg)	APHA 4500-SO3
Chloride	3.67 (mg/kg)	APHA 4500-C1
Nitrate	0.70 (mg/kg)	APHA 4500-NO3
Temperature	25 ^{O}C	APHA 2005

Table.2b: Hydrocarbon fractions in polluted soil.

Physicochemical characteristics	Value	Method
TPH	34342 (mg/kg)	USEPA 8015C
PAH	96.45 (mg/kg)	USEPA 8270D

Total Petroleum Hydrocarbon (TPH)

The TPH of the different microcosms showed a reduction in the level of hydrocarbon in all soils. The bioremediation efficiency was calculated for all amended soils using the equation:

$$B.E\ \% = \frac{\%\ TPHc - \%\ TPHt}{TPHc} \times \frac{100}{1}$$

B.E = Bioremediation efficiency, % TPHc = percentage degradation of residual crude oil (residual TPH) in spiked soil, % TPHt = percentage degradation of residual crude oil (residual TPH) in biostimulated soil [22].

The polluted soil's biostimulation efficiency (B.E %) without amendment B was 9.7 %. and 56.31 %, the microcosms with amendment PD were 31.71 % and 52.70 %, the microcosm with amendment CD was 35.90 % and 85.45 % and the microcosm with amendment N.P.K was 41.70 % and 85.50 % at weeks 2 and 4 respectively. The reduction of hydrocarbon in treatments may be due to biostimulation and an increase in the activity of bacteria in the microcosms [21].

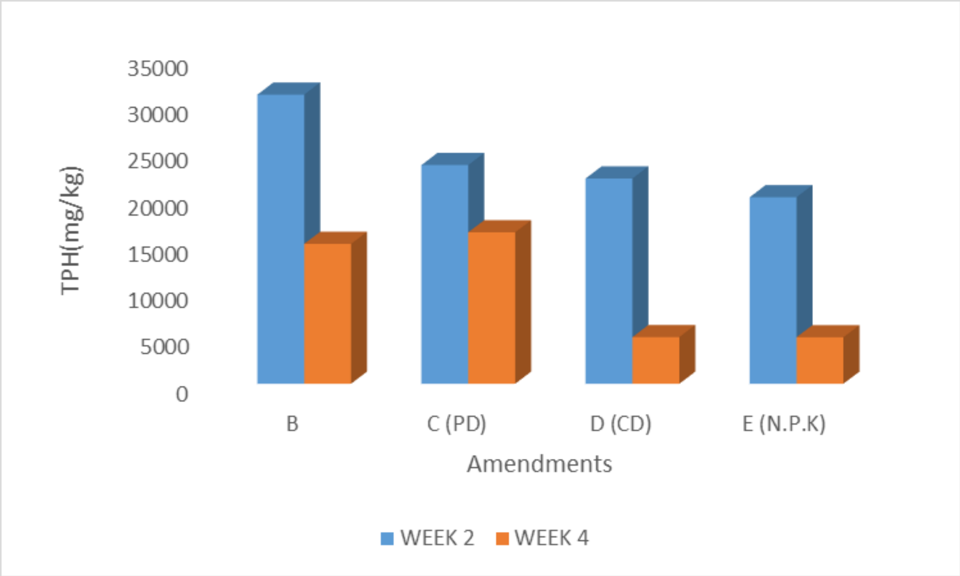

Fig 1: Hydrocarbon fractions observed in microcosms, where B is spiked soil, C(PD) is poultry-dropping treated soil, D(CD) is cow-dung treated soil, and E(NPK) treated soil.

Polyaromatic hydrocarbon (PAH)

The PAH of the treatments was significantly reduced at p = 0.05. The reduction of the treatments at weeks 2 and 4 were 36.6 % and 83.4 %, 43.5 %, and 96.8 %, 43.8 % and 96.9 %, 50.2 % and 97.1 % for B, PD, CD and N.P.K respectively. Polyaromatic hydrocarbon fractions were effectively removed by all the stimulated microcosms. The removal of aromatic fractions was very effective after week 2, this may be attributed to the volatile nature of polyaromatic hydrocarbons. Although all microcosms reduced PAH effectively, cow dung and N.P.K were the highest at week at 96.9 % and 97.1 %. Cow dung is the most efficient organic manure, however, the inorganic manure N.P.K was slightly higher at week 4 with 0.2 % indicating that its efficacy in polluted soil may reduce with time.

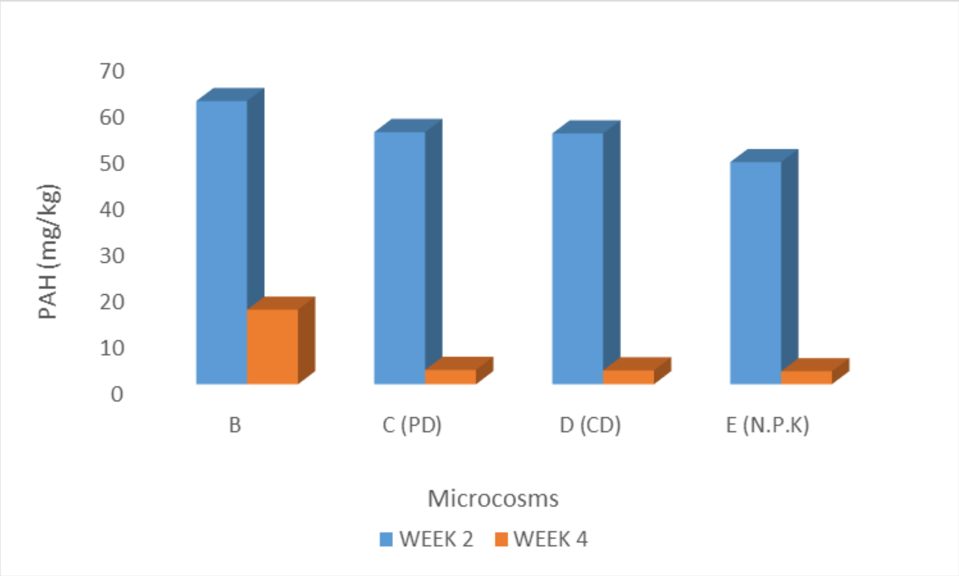

Fig 2: Polyaromatic fraction removal by microcosms at weeks 2 and 4.

Total Heterotrophic Bacteria (THB)

The treatment without amendment (B) had 1.75 X 10^8 and 1.90 x 10^8 cfu/g, amendment C with poultry dropping (PD) had 1.89 x 10^8 and 2.10 x10^8 cfu/g, the amendment with cow dung had 1.5 x $10^8$and 2.20 x 10^8 cfu/g, and the amendment with N.P.K had 2.20 x 10^8 and 2.25 x 10^8 cfu/g for weeks 2 and 4 respectively. The bacterial count in treatment B increased slightly from day 1 to week 4, this may be because the bacterial population in this microcosm were competing for the available nutrient for growth and acclimatization [17]. The treatment with PD encouraged a steady growth of bacteria within the 4-week study, this may be attributed to the high phosphorus content of poultry dropping [11]. The treatment with cow dung recorded the highest heterotrophic bacterial count amongst the organic manure used in this study, this may be attributed to the rich carbon nature of cow dung enriched soils, the bacteria in this microcosm had more carbon source for growth than available in PD [23]. The inorganic manure N.P.K had the highest bacterial count at week 2, this may be attributed to the nitrogen, phosphorus, and potassium that was readily absorbed in the N.P.K (20:10:10) amendment used. The amendment provided a quick source of limiting nutrients for growth at week 2, however, only a slight increase was observed at week 4 indicating possible depletion of the available nutrients during competition [15].

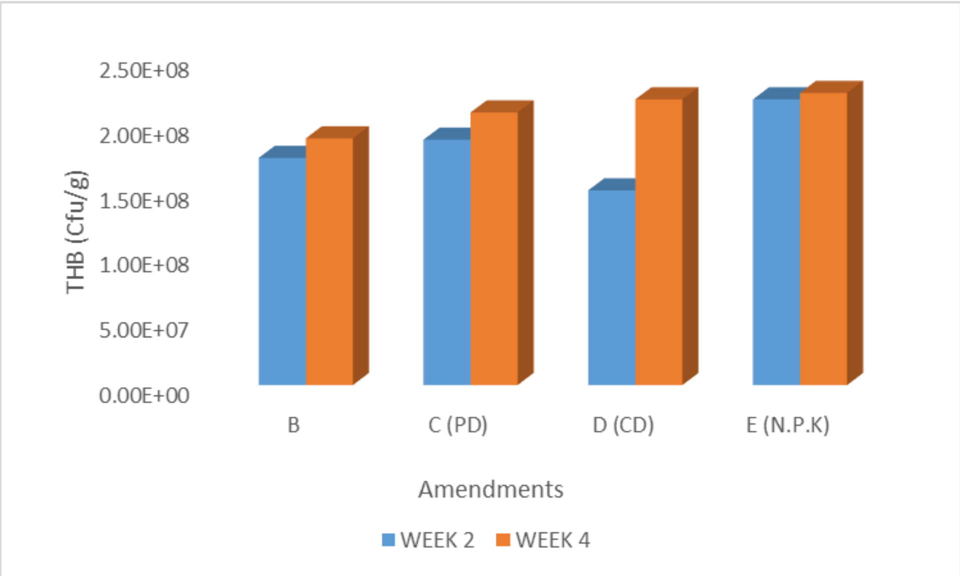

Fig 3: Total Heterotrophic Bacteria from microcosms at weeks 2 and 4 where B is spiked soil, C(PD) is poultry-dropping treated soil, D(CD) is cow-dung treated soil, and E(NPK) treated soil.

Hydrocarbon utilizing bacteria (HUB)

The treatment without amendment (B) had 1.20 X 10^5 and 2.0 x 10^6 cfu/g, amendment C with poultry dropping (PD) had 3.0 x 10^5 and 2.19 x10^6 cfu/g, the amendment with cow dung had 2.50 x 10^5 and 2.46 x 10^6 cfu/g, and the amendment with N.P.K had 1.98 x 10^5 and 2.10 x 10^6 cfu/g for weeks 2 and 4 respectively. The presence of hydrocarbon pollutants in the soil encouraged the proliferation of hydrocarbonoclastic bacteria in B. The addition of amendments further stimulated these hydrocarbon-degrading bacteria at weeks 2 and 4 in treatments with poultry manure, cow dung manure, and N.P.K respectively. However, the treatment with cow dung had the highest hydrocarbon-degrading bacteria amongst all the treatments used in this study. This may be attributed to the high carbon content of cow dung and the presence of rich novel bacteria Pseudomonas and Bacillus species which have been constantly reported to have the ability to degrade hydrocarbons [24].

Fig 4: Hydrocarbon degrading bacteria from microcosms at weeks 2 and 4.

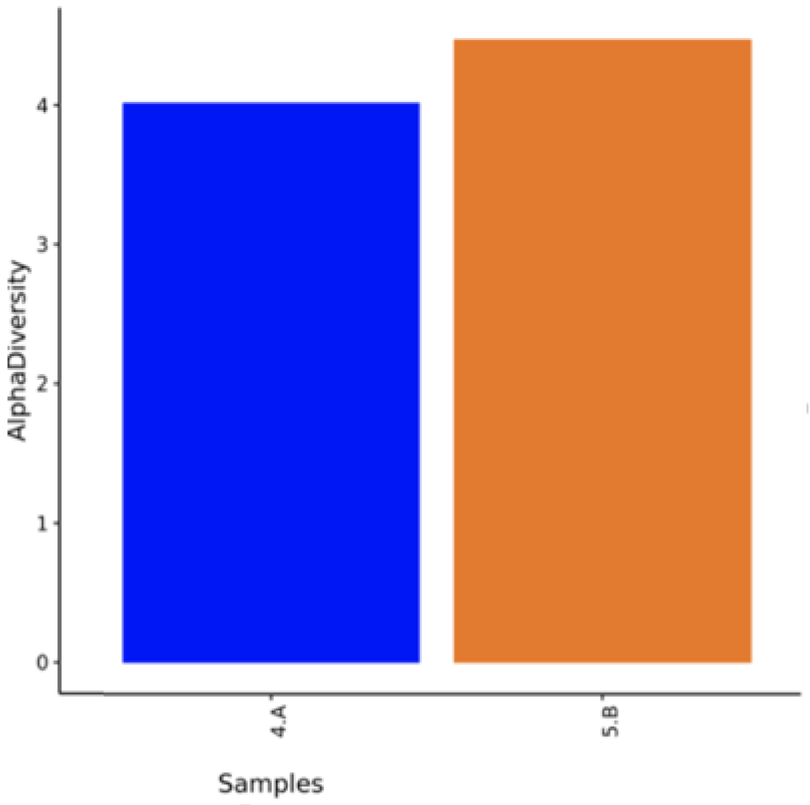

Fig 5: The alpha diversity between the pristine and spiked soil (A and B).

The metagenomic analysis of the pristine and spiked soils (A and B) shows the alpha diversity which is the measurement of microbial diversity in the samples. The Shannon bar plot (Fig 5) shows the number of observed strains increased after spiking with crude oil (5. B). This result could be because the microorganisms in contaminated soil were able to break down the hydrocarbon using it as their nutrient source for proliferation. These hydrocarbonoclastic microorganisms mineralize the hydrocarbon pollutants for growth. The addition of amendments to the polluted soil further increased the proliferation of these hydrocarbonoclastic organisms thereby effectively reducing the TPH concentration. This agrees with the results obtained in the culture-dependent enumeration of hydrocarbon utilizing bacteria [24].

4 Conclusion

The toxic and carcinogenic substances in crude oil-polluted soils can be removed during bioremediation via the use of nutrient amendments as shown in the results obtained in this study. During the bioremediation study, there was an increase in total heterotrophic bacteria and hydrocarbon utilizing bacteria in the microcosms showing the ability of microbes to break down the pollutants and use them as their sole source of energy. The bacterial isolates identified after biochemical characterization in this study were Pseudomonas sp, Escherichia spp, Bacillus spp, Micrococcus, and Staphylococcus. Although there was an increase in the total heterotrophic bacteria, the hydrocarbon-degrading bacteria were highest in the treatment with cow dung manure. These hydrocarbonoclastic bacteria can be cultivated and used to bioremediate crude-oil polluted soils, the proliferation of these bacterial species will enhance many ecosystems service functions. It is evident from findings that cow dung is the most effective amendment for hydrocarbon-degradation in this study.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

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