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Article

Comparative evaluation of TPHs standards in the analysis of petroleum-contaminated, and remediated soil

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Abstract: Commercially available Total Petroleum Hydrocarbons (TPHs) standards have been widely used for instrumental analysis of TPHs in soils and other environmental matrices. However, there are concerns about the universality of these standards developed in one region of the world to reliably estimate TPHs in environmental matrices in other regions, which prompted this investigation. TPHs standards were prepared from contaminating crude oils at polluted sites at Tibshelf, UK, and Ogoniland, Niger Delta, Nigeria. The prepared standards were used in comparison with some commercially available TPHs standards (TPHs-gasoline diesel range and TPHs C10-C40) for assessment of TPHs levels in the contaminated soil samples treated for phyto and myco-remediation. Results obtained revealed significant differences in the quantification of TPHs between these standards. The TPHs standards prepared from the contaminating crude oils estimated higher levels of TPHs in the soil samples compared to those of the commercially available standards. In assessing the % of TPHs reduction in the remediation experiment, all the standards provided similar estimations of TPHs reduction, with no significant differences. The result revealed that although all the TPHs standards provided consistent evaluation of TPHs remediation in all cases, the commercially available TPHs standards may underestimate the concentration of TPHs in certain environments during pollution incidents. Therefore, with respect to toxicological evaluations, there is a need for TPHs standards specifically developed for a region of interest to be used. This study offers a good insight on how such standards can be prepared.

Keywords: petroleum contaminated soil; TPHs standards; crude oil; TPHs analysis

1. Introduction

Analysis of Total Petroleum Hydrocarbons (TPHs) in petroleum-contaminated soils is achieved by techniques that involve extraction of the TPHs, followed by instrumental analysis [1]. The instrumental analysis employs the use of analytical standards for the calibration and quantification of TPHs [2]. A number of commercial TPHs standards are available. However, these standards are not 'over the counter' reagents and must be ordered when required. Analytical standards are expensive and sometimes difficult to obtain, and therefore may not be readily available, when required [3]. The acquisition and delivery process of these standards can cause delays in the analysis and evaluation of TPHs, even with instrument availability. Thus, analysis and estimation of TPHs are many times unduly delayed, thereby hindering investigations requiring urgent insight.

Occasionally, analysts doubt the reliability of commercially available TPHs standards in the assessment of TPHs levels in certain environmental matrices, especially when such standards were prepared from raw materials obtained from a different region of the world. This is because most commercially available TPHs standards are prepared by specialized industrial laboratory reagent manufacturers using primary materials best available to them. Therefore, the characteristics of such standards will be closely aligned with the source and origin of the feedstock. Michelsen and Boyce [4] reported that commercially available TPHs gasoline-diesel range standard has the advantages of accounting for a wide range of petroleum hydrocarbons in environmental matrices, but many of the commercial TPHs standards were developed for targeted contaminants at particular contaminated sites, and as such, may not be very suitable for other sites.

Okparanma and Mouazen [5], stated that the use of commercially available hydrocarbon standards does not produce a true assessment of contaminant concentrations because the hydrocarbon source used for the standards is often very different from that of the contaminating oil. Okparanma and Mouazen [5], further highlighted that a single hydrocarbon standard may not be suitable as a universal calibration standard for TPHs analysis.

The current debate that TPHs standards developed for the analysis of petroleum and environmental contamination in one region of the world may not provide the needed insight for analysis in another region of the world, requires some attention, and investigation. Harmsen et al. [6] reported that with current TPHs standards as reference points, further developments in analytical standards can be achieved for monitoring of petroleum contaminants in environmental matrices. Hence, investigation for complementary standards for TPHs analysis is highly desirable, especially if they originate from the same region as a pollution incident being investigated.

Although the limitations of the commercially available TPHs standards have been identified, and the need for TPHs standards specific to the region of contamination emphasized, no study to date has demonstrated how the assessment of TPHs levels in soil samples would differ if standards developed from regional contaminating crude oil as opposed to commercially available TPHs standards were used.

The aim of this study was to comparatively evaluate the estimation of TPHs in petroleum-contaminated and remediated soils using different TPHs standards for quality assurance. Therefore, the study specifically investigate the prospect of using TPHs standards prepared from the contaminating crude oil as analytical standards for assessment of their associated petroleum-contaminated soils, in comparison to existing commercially available TPHs standards.

To achieve the set aim, analytical standards were prepared from the contaminating crude oil and used to assess TPHs levels in the associated soil samples. Some commercially available TPHs standards were also purchased and used for the same purpose. The target was to comparatively assess the reliability of such standards and, most importantly, the prospect of having some quick and readily available options for assessment of TPHs levels in soils during pollution and remediation programs.

2. Methodology

2.1. Sample collection and treatment

Petroleum-contaminated soil samples were collected from a site of the British first oil well at Tibshelf, Derbyshire, and Ogoniland, Nigeria. Glasshouse remediation treatments of the soil samples were carried out according to Dickson et al. [7]. The soil sample designations and Glasshouse remediation treatments are listed in (**Table S1**, supplementary material). Treatment times were from the start of the treatment ($T = 0$) to the end of the Treatment (90 days, $T = 3$).

2.2. Sample preparation and analysis

Soil samples were prepared for analysis according to Dickson et al. [7]. Extraction of TPHs in samples was carried out according to the methods of USEPA METHOD 3546 [8] and Punt et al. [9] 1999. TPHs standards were prepared as stated in ISO/TS 16558-2: [10] and ISO 18287:2006(E) [11], respectively. Sample extracts and the TPHs standards were all analyzed in a GC-MS according to ISO 16703 [12] and ISO/TS 16558-2 [10].

2.3. TPHs extraction from the samples

TPHs were extracted by microwave-assisted extraction with a Milestone MA182-001 ETHOS UP Microwave system, using a 1:1 acetone—heptane solvent mixture [8]. 10 g of air-dried and sieved (< 2mm) soil samples were weighed into the glass vials of the extraction vessels of the microwave. 25 mL of extracting solvent (1:1 acetone-heptane) was added to the soil samples. Both Teflon heating pads and magnetic stirrer were inserted into the extracting vessel, which was then sealed, placed into the microwave instrument and extracted for 15 min. Conditions of the microwave are as listed in **Table 1**. Method blanks, as well as matrix spikes with the surrogates, 2-flurobiphenyl and 4-terphenyl-d14, were also prepared similarly to the samples and placed along for extraction [13], for determination of extraction efficiency.

After extraction, extracts were allowed to cool in the extractor for 15 min, then removed and filtered into a centrifuge tube. These extracts now contained the TPHs, as well as the acetone and heptane solvents. To remove the acetone, deionized water was added to the extracts, the extracts were centrifuged at 4500 rpm for 10 min, and allowed to settle. The supernatant (heptane containing the TPHs) was carefully pipetted out into a Falcon tube and stored prior to analysis.

Table 1. Operating conditions of microwave assisted extraction instrument.

Temperature:	100–115 ℃
Pressure:	50–150 psi
Time at Temperature:	15 min
Cooling:	To room temperature

Extraction validity

Extraction validity was evaluated by spiking the soil samples with known concentrations of the surrogate compounds. The spiked soils were extracted with the same conditions of microwave, and extracts were analyzed in GC-MS in triplicates. Extraction validity returned $\alpha > 0.95$.

2.4. Preparation of the TPHs standard from the contaminating crude oil samples

Crude oil samples were collected from the 2 locations associated with the contaminated soil sampling points: (1) Tibshelf, Derbyshire, UK and (2) Gio, Ogoniland, Nigeria.

The TPHs standards from the contaminating crude oil samples were prepared as follows: A given mass of each oil was weighed out and dissolved in 10 mL of n-heptane (**Table 2**). The solution obtained was filtered to remove undissolved solids. The mass of the residue was determined and subtracted from the initial mass of the crude oil sample to determine the actual mass of the crude oil in solution. From this, the concentration of the stock solution of crude oil in heptane was determined in parts per million (ppm) (**Table 2**). Calibration standards of 8000, 5000, 2500, 1000, 500, and 100 ppm of the prepared TPHs standards from the contaminating crude oil samples were prepared by serial dilutions of the stock solution (**Table 2**).

Table 2. Preparation of stock, and calibration solutions of the prepared TPHs standards from the contaminating crude oil.

	Tibshelf, Derbyshire (UK) Crude oil	**Ogoniland, Nigeria Crude oil**
Initial mass of crude oil	1.850 g	1.875 g
Mass of residue after dissolution and filtration	0.709 g	0.651 g
Actual mass of crude oil in 10 mL of n-heptane solution	1.152 g	1.225 g
Initial concentrations of crude stock solution in ppm	115,200 ppm	122,500 ppm

Preparation of calibration solutions of the prepared TPHs standards from the contaminating crude oil, from their stock solutions

	Tibshelf, Derbyshire (UK) Crude oil			**Ogoniland, Nigeria Crude oil**		
Calibration solutions (ppm)	**Volume of stock (μL)**	**Volume of solvent(mL)**	**Total volume (mL)**	**Volume of stock (μL)**	**Volume of solvent(mL)**	**Total volume (mL)**
8000	3472.22	46.53	50.00	3238.87	46.76	50.00
5000	2170.14	47.83	50.00	2024.29	47.98	50.00
2500	1085.07	48.91	50.00	1012.15	48.99	50.00
1500	651.04	49.35	50.00	607.29	49.39	50.00
1000	434.03	49.57	50.00	404.86	49.60	50.00
700	303.82	49.70	50.00	283.40	49.72	50.00
500	217.01	49.78	50.00	202.43	49.80	50.00
300	130.21	49.87	50.00	121.46	49.88	50.00
100	43.40	49.96	50.00	40.49	49.96	50.00

2.5. Quantification of TPHs levels in the soil samples using the prepared TPHs standards from the contaminating crude oil samples, and the commercially available TPHs standards

The method ISO/TS 16558-2 [10] was used for the quantification of TPHs using both the standards prepared from the contaminating crude oil samples and commercially available TPHs standards (TPHs-gasoline diesel range and TPHs C10-C40 standards). Both the prepared and commercially available TPHs standards were used for the estimation of TPHs in the soil samples. The standards prepared from the respective crude oil were used to assess TPHs levels in the soils from the same region (i.e., the prepared TPHs standard from crude oil from Tibshelf was used for soil samples from Tibshelf, in addition to the commercially available TPHs standards; while that from Nigeria was used for soil samples from Nigeria, in the same way).

Initial calibration of the instruments was undertaken, followed by evaluation of the concentration of the TPHs, and then calibration verification. Chromatograms of the commercially available TPHs standards and those of the prepared TPHs standards from the contaminating crude oils, are given in Figure S1 (Supplementary Material). Calibration functions for each of the standards are also given in SF2 (Supplementary Material).

2.5.1. TPHs Analysis in GC-MS with the commercially available TPHs C10-C40, and TPHs gasoline-Diesel range standards

Semi-quantitative standards suitable for quantification of TPHs [10,13–15] were used in this study. This includes the use of commercial TPHs gasoline-diesel range and TPHs C10-C40 standards. Sample extracts and the TPHs standards were all analyzed in a GC-MS (model Agilent Technologies 7000 GC/MS Triple Quad with 7890 GC and 7693 Autosampler [13]. GC-MS conditions are as listed in **Table 3**.

Quantification of the TPHs in the soils was carried out using the Methods of BS EN ISO 16703 [12]. Initial calibration of the instruments and evaluation of the concentration of TPHs, were carried out. Calibration verifications were also carried out, and n-dodecane, n-tetradecane, and deuterated Dodecane were used as internal standards.

Table 3. GC-MS conditions for the TPHs analysis.

Column	SLB-5ms, 30 m × 0.25 mm I.D., 0.25 µm (28471-U)
Oven	45 ℃ (3 min), 20 ℃/min to 360 ℃ (10 min)
Carrier gas	helium, 1.3 mL/min. constant
Injection	1.0 µL, splitless
Liner	2 mm I.D. straight
Injector temp.	250 ℃
Detector	MSD, 300 ℃

2.5.2. Determination of Total Petroleum Hydrocarbons using the commercially available TPHs and prepared standards

The Total Petroleum Hydrocarbon standards (both the TPHs gasoline-diesel range and the C10-C40 standards) were purchased from Sigma-Aldrich, UK. The retention time window (RTW) standard solution was prepared according to the methods of ISO/16558-2 [10] by weighing 30 mg of n-tetracontane into a 1 L volumetric flask and dissolving it completely in an appropriate volume of n-heptane. 30 µL of n-decane was then added; the solutions were mixed by shaking and sonication and then made up to 1 L. The LOD, LOQ, the linear range and the working range of the instrument were established prior to running of samples.

For the TPHs gasoline-diesel range, calibration standard solutions were then prepared according to methods of ISO/16558-2 [10] by diluting the TPHs standard stock solution with appropriate aliquots of the RTW solution to give the lower concentrations of 10, 50, 100, 500, 1000, 1500, and 2000 mg/L of the TPH gasoline-diesel range standard calibration concentrations. Also, upper ranges of 2500, 3500, 5500, 7500, and 8500 mg/L of the standard were also created. Several calibration concentrations were tried, to determine suitable calibrations that can capture the lower and higher range for calibration of the instrument for the TPHs gasoline-diesel range. Finally, the calibration values of 0, 50, 150, 250, 350, 500, 750, and 1500 ppm were determined as having the best regression value (R-square value) and were thus used for the calibration curves for the TPHs gasoline-diesel range standards (**Figure S2a**). The samples extracts to be analysed and quantified using the TPHs gasoline-diesel range standard, were thus appropriately diluted to be within the calibration range, and the final TPHs concentration evaluated by multiplying the values obtained with the dilution factor.

During the calibration for the TPHs C10-C40 standard, different concentrations of the TPHs C10-C40 standard (0, 50, 100, 150, 200, 250, 350, 500, 750, 850, 1000 ppm) were also prepared and run in the instrument. In the creation of the calibration curves, different combinations of the calibration values (e.g., 0, 50, 100, 200, 500, 750, 1000 ppm; 0, 100, 250, 500, 750, 1000 ppm; 0, 50, 150, 250, 500, 750, 850 ppm, etc.) were also tried to see which combination produces the best Regression (R-square values). The combinations with the best R-square values were also used as the final calibration values for the estimation of the TPHs concentrations. Therefore, the final calibration concentrations used for the calibration curve of the TPHs C10-C40 standard were 0, 100, 250, 500, 750, and 1000 ppm (**Figure S2b**). Similarly, the samples that were to be evaluated were also diluted to this calibration range of the TPHs C10-C40 standard.

Similar approaches were used for the calibration and quantification of the TPHs standards prepared from the contaminating crude oils. Thus, for the TPHs standard prepared from the Derby, UK, Crude oil, the calibration values were, 100, 250, 500, 750, 1000, 5000, and 8000 ppm (**Figure S2c**); while those for the TPHs standard prepared from the Ogoniland, Nigeria, Crude oil the calibration values were, 100, 500, 1000, 2500, 5000 ppm (**Figure S2d**). In all the cases, the samples were diluted to fit into the range of the calibration values for the analysis, and final TPHs concentrations obtained by multiplying the values from such, with their respective dilution factors.

Samples analyzed in the GC-MS include blank (n-heptane), sample extracts, calibration standards, control solutions, and retention time standard solution. Three control solutions within the calibration range of each standard were used in each run for checking that calibrations did not shift during the run (checking recoveries).

For the determination of total petroleum hydrocarbons, the total area between the n-decane (C10) and n-tetracontane (C40) peaks of the chromatogram was integrated. The integration started at the retention time just after the end of the n-decane peak and the signal level in front of the solvent peaks and ended at the retention time just before the beginning of the n-tetracontane at the same signal level. N-tetracontane was integrated separately for the recovery check.

2.6. Data treatment, validity, and reliability; and statistical analysis

Data treatments are as reported in Dickson et al. [7]. All samples were analyzed in triplicates, and the results presented here are the mean values. For statistical analysis, the student *t*-test, analysis of variance, the use of Box and whiskers, correlation and regression analysis, and ternary plots have been employed to statistically evaluate the outcomes of the study. The *t*-test, analysis of variance, use of Box and whiskers, correlation, and regression analyses were all carried out using Microsoft Excel spreadsheet on Microsoft Office 365 Apps for enterprise.

2.7. Scope of the study

In the current study, a total of 20 different samples/treatments were used for the respective analysis. All the samples were analyzed in triplicates, giving rise to about 60 samples, and the mean values were used in the results. The quantification of TPHs in each of the samples was carried out, respectively, using each of the TPHs standards, namely, the TPHs gasoline-diesel range, the TPHs C10-C40 standard, and the TPHs standards prepared from the contaminating crude oils.

The range where the TPHs concentrations were evaluated was in the range of C6 to C50. This range covers the gasoline-diesel range (C6 to C12 and C8 to C26), which consists of the gasoline range and the diesel range organics [1,16,17], (and also the range of C15 to C50, which consists of the Mineral Oils [17].

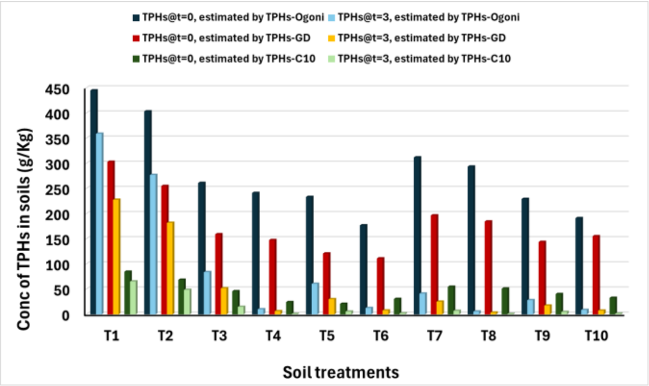
Also, the range of TPHs concentrations analyzed in the present study is from 7.55 to 303.3 g of TPHs per kg of dry soil using the TPHs standard (Gasoline- Diesel range); and 1.58 to 84.8 g of TPHs per kg of dry soil using the TPHs C10-C40. These are also within the range of 30.90 to 338.60 g of TPHs per kg dry soil, using the TPHs standards from Derby, UK crude oil, and 8.90 to 445.19 g of TPHs per kg dry soil, using the TPHs standards from Ogoniland, Nigeria, crude oil (**Tables S2** and **S3**, in Supplementary Material). In all cases these values are all above the soil threshold levels of 1 g TPHs per kg dry soil or > 1% of TPHs concentrations in soils.

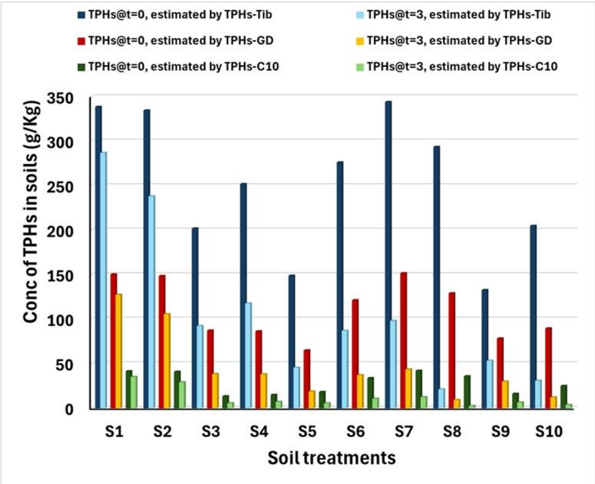
3. Results and discussions

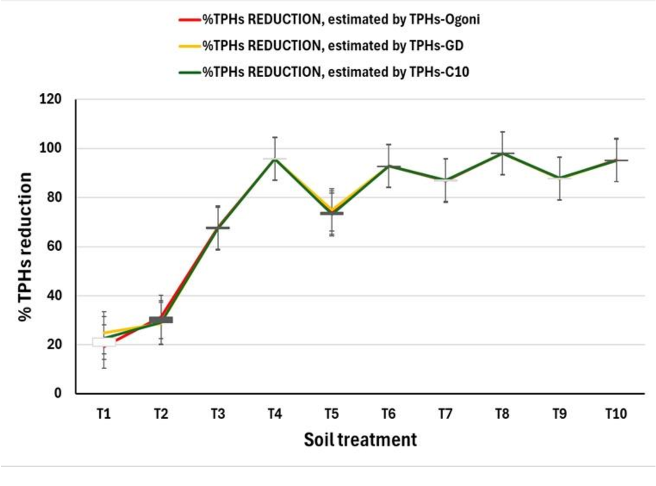
3.1. Results

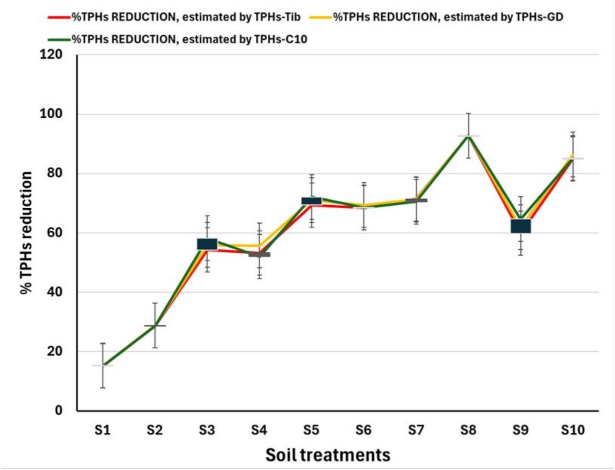
The chromatograms of the commercially available TPHs Gasoline-diesel range standard (TPHs-GD), and the TPHs standards prepared from the contaminating crude oils from Tibshelf, Derbyshire, UK (TPHs-Tib), and Ogoniland, Nigeria (TPHs-Ogoni), all have a similar overall form, despite different retention time (SF1, supplementary material). There were observed marked differences in the chromatogram of the commercially available C10-C40 standard (TPHs-C10), compared to the other chromatograms. For the commercially available TPHs Gasoline-diesel range, the range of peak retention times was from 0 min to 18 min, while those of the prepared TPHs standards from both the Tibshelf, UK crude oil (TPHs-Tib) and Ogoniland, Nigerian crude oil (TPHs-Ogoni) were from about 0 min to 55 min. The commercially available C10-C40 standard showed peaks with retention time from 0 min to a little beyond 30 min (SF1, Supplementary Material).

The values of TPHs estimated for each of the soil samples for each treatment regime revealed lower values of TPHs obtained with the commercially available TPHs C10-C40 standard when compared to the commercially available TPHs gasoline-diesel standard and the prepared TPHs standards from the contaminating crude oils (**Figure 1**). TPHs values obtained by TPHs gasoline-diesel range standard were also lower than the corresponding values of those of the prepared standards from the contaminating crude oil. In all cases, the TPHs standards prepared from the contaminating crude oils produce the highest values in the estimation of TPHs levels in the soil samples.









(a) **(b)**

Figure 1. (a) Comparative evaluation of TPHs in soils of Tibshelf, UK; using TPHs standards prepared from the contaminating crude oil from Tibshelf, UK, and some commercially available TPHs standards (Gasoline-diesel range, & TPHs C10-C40). The figure to the left represents TPHs concentrations in the soil, during soil remediation by the TPHs, while to the right is the remediation efficiency measured by each standard; **(b)** Comparative evaluation of TPHs in soils of Ogoniland, Nigeria; using TPHs standards prepared from the contaminating crude oil from Ogoniland, Nigeria; and some commercially available TPHs standards (Gasoline-diesel range, & TPHs C10-C40). The figure to the left represents TPHs concentrations in the soil, during soil remediation by the TPHs, while to the right is the remediation efficiency measured by each standard.

Analysis of variance (ST4, ST5, in Supplementary Material), revealed significant differences in the estimation of TPHs levels by the different standards (TPHs-Tib, TPHs-GD & TPHs-C10), for the soil samples from Tibshelf, UK ($p = 3.5 \times 10^{-30}$ @T = 0, $p = 7.1 \times 10^{-11}$ @ $T = 3$). Similar differences were highlighted for the soil samples from Ogoniland, Nigeria, using the associated TPHs standards ($p = 7.0 \times 10^{-9}$ @$T = 0$, $p = 4.0 \times 10^{-3}$ @$T = 3$). Paired sample *t*-test conducted on associated pairs of the TPHs standards revealed significant differences in the estimation of the TPHs levels in soils of Tibshelf at both $T = 0$ and $T = 3$, by TPHs-Tib against TPHs-GD, TPHs-Tib against TPHs-C10, and TPHs-GD against TPHs-C10. Similar differences were also obtained for the soil samples from Ogoniland, Nigeria. There were 3 exceptions during the estimation of TPHs from the soil samples from Ogoniland, Nigeria where p-values were >0.05 indicating that the values were not statistically different, in those instances.

A comparison of the ratios of TPHs in the soil samples (**Table 4)**, estimated by the different TPHs standards in each of the treatments revealed significance differences in the proportion of TPHs estimated by each standard ($p = 0.0001$). The general observation was that for the soil samples from Ogoniland, the prepared TPHs standard produced TPHs values that were twice those determined when using the TPHs Gasoline diesel. Similar observations were obtained in ratios of the levels of the TPHs estimated by the prepared standards when compared to the commercially available TPHs Gasoline-Diesel range in each of the soil treatments for Tibshelf, Derbyshire, UK. The ratios of TPHs estimated by the prepared TPHs standards were

5 to 11 times greater for Ogoniland, and 4 to 7 times greater for Tibshelf than when using the commercially available TPHs C10-C40 standard (**Table 4**).

Table 4. Ratios of TPHs estimation by the different TPHs standards on the soil samples from Tibshelf, UK and Ogoniland, Nigeria.

	TPHs_Tib to TPHs_GD		**TPHs_Tib to TPHs_C10**		**TPHs_GD to TPHs_C10**			**TPHs_Ogoni to TPHs_GD**		**TPHs_Ogoni to TPHs_C10**		**TPHs_GD to TPHs_C10**	
	***T* = 0**	***T* = 3**	***T* = 0**	***T* = 3**	***T*=0**	***T* = 3**		***T* = 0**	***T* = 3**	***T* = 0**	***T* = 3**	***T* = 0**	***T* = 3**
S1	2	2	4	4	8	8	**T1**	2	2	5	5	4	4
S2	2	2	4	4	8	8	**T2**	2	2	6	6	4	4
S3	2	2	6	7	15	16	**T3**	2	2	6	6	4	4
S4	3	3	6	5	17	16	**T4**	2	2	10	10	6	6
S5	2	2	4	3	8	8	**T5**	2	2	11	11	6	5
S6	2	2	4	4	8	8	**T6**	2	2	6	6	4	4
S7	2	2	4	4	8	8	**T7**	2	2	6	6	4	4
S8	2	2	4	4	8	8	**T8**	2	2	6	6	4	4
S9	2	2	5	5	8	8	**T9**	2	2	6	6	4	4
S10	2	3	4	3	8	8	**T10**	1	1	6	6	5	5

For monitoring of remediation efficiency (% reduction of TPHs) in the treated soil samples, similar percentage reduction in TPHs levels were observed with all the standards (**Table 4**). Analysis of variance revealed no significant differences in % reduction of TPHs obtained by the use of either TPHs C10-C40, TPHs Gasoline-diesel range or the prepared TPHs standards ($p = 1 \times 10^{-6}$ for Tibshelf soils, $p = 1 \times 10^{-6}$ for Ogoniland Soils). The similarity in the values of % TPHs reduction estimated by the different TPHs standards in the different soil treatments during the remediation experiment, are further emphasized by the Box and Whisker plots (**Figure 2**).

Regression analysis (Residual plots, Line fit plots, and Normal probability plots, **Figure S3,** in supplementary material), also confirm that the estimation of % TPHs reduction by all the different standards were of similar values.

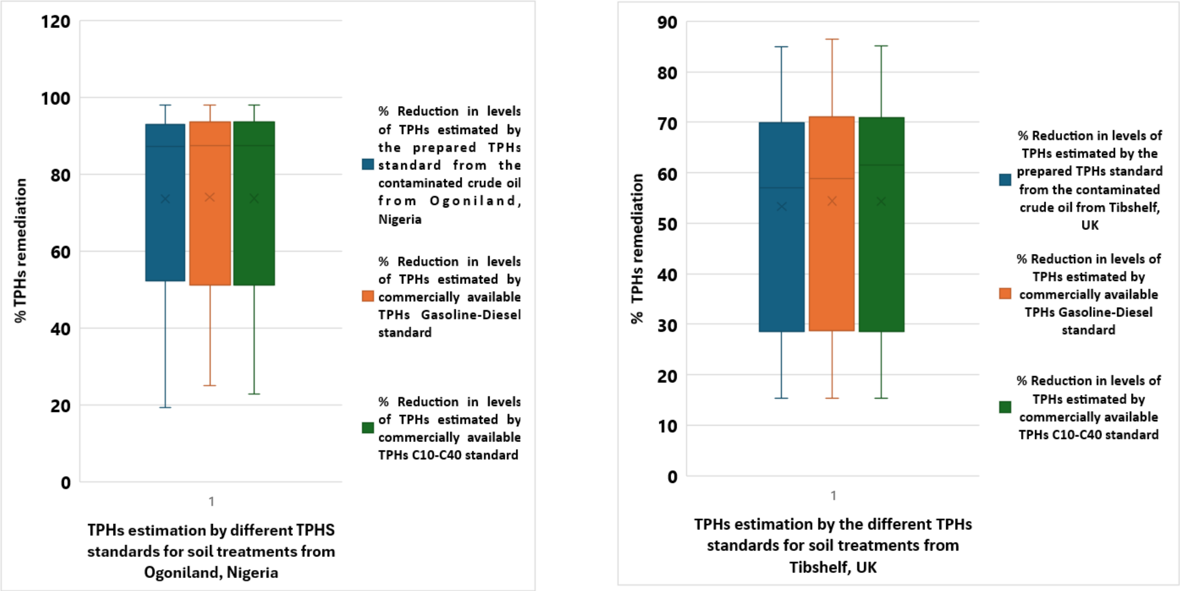


Figure 2. Box and Whisker plots for % reduction in TPHs levels in the different soil treatment for Ogoniland, Nigeria; and Tibshelf, Derbyshire, UK, using the different TPHs standards.

3.2. Discussion

The observed similarity in the chromatograms of the TPHs-GD, TPHs-Tib, and TPHs-Ogoni indicated that these standards are identifying similar TPHs compounds in the soil samples. Similar compounds are expected to have similar retention times in the GC when run under similar conditions and this is what helps in the identification of such compounds [1,18]. The usual practice in identification of unknown compounds in the GC, is a run of a known compound with known retention times and comparing such retention times with those of an unknown, for the identification of such compounds [19,20]. Therefore, the appearance of peaks with a similar shape, especially as expected for TPHs of the gasoline diesel range in GC TPHs analysis (SF1 in supplementary material), indicates the capability of each of these standards in estimating the TPHs levels in the soil samples. The observed marked differences in the chromatogram of the commercially available TPHs C10-C40 standard (TPHs-C10), compared to the other chromatograms (SF1 in supplementary material), is basically due to the fact that the TPHs C10-C40 standard is designed to detect individual hydrocarbons within the C10 to C40 range [21], and will not pick up the peaks of other compounds. The observed narrower range of retention times in the chromatogram of TPHs-GD compared to TPHs-Tib, and TPHs-Ogoni, indicates that some fractions of the contaminating crude oils in the soil samples (those falling outside these retention times), can be overlooked when TPHs-GD is used as the analytical standard in the analysis of TPHs levels in the soil samples. More of such underestimations of TPHs levels in samples will be expected with respect to the use of TPHs-C10.

In general, the different standards produced different TPHs values in the order TPHs-C10-C40 < TPHs-GD < (TPHs_Tib, or TPHs_Ogoni, respectively). TPHs C10-C40 standard consists more of individual hydrocarbon components in the range of C10-C40, therefore, many of the components’ contaminants of the contaminating crude oil may be overlooked using this standard [21]. This accounts for the relatively lower quantification values of TPHs observed with the TPHs C10-C40 standard compared to the prepared standards and the TPHs gasoline-diesel standard. As crude oil is a mixture of a wide range of hydrocarbons[22], and the fact that even the hydrocarbons that are out of the range of the retention times of the TPHs_C10 standards will not be detected, the lower values in the estimation of the TPHs levels in the soil samples obtained for the TPHs-C10, should be expected. A further reference to the chromatogram of the TPHs-GD had revealed a lower retention time range when compared to those of the TPHs-Tib & TPHs-Ogoni, indicating also that some compounds outside the retention time range of TPHs_GD would have been picked up by the prepared standards. This again explains the observed trend in the different TPHs levels estimated for the soil samples by the individual standards.

The above observations corroborate the perceptions hypothesized by Michelsen and Boyce [4] and Okparanma and Mouazen [5] that different TPHs standards developed for different regions will produce different estimates of TPHs levels when used for evaluation of TPHs in samples obtained from other regions. The statistical analysis carried out in the evaluation of the TPHs levels with the different standards in this study further revealed that the differences in the valuation of TPHs levels in the soil samples were significant. This confirmed that the commercially available TPHs standard can under-evaluate TPHs levels in soil samples in certain circumstances. In the present study, plausible explanations and justifications have been deduced for such disparity. There is therefore a need for TPHs standards that are specific to an area of interest when investigating TPHs levels in such environmental matrices to be developed and used for such purposes. This will allow for better evaluation and quantification of such TPHs levels, which will be particularly useful for risk-based assessment associated with such environmental pollution [23–25].

A comparison of the ratios of TPHs levels estimated by each of the TPHs standards (**Table 2**) revealed that the commercial TPHs standards can underestimate the TPHs concentrations by at least a factor of 2, in some cases at least up to 10 times less than those of the standards prepared from the contaminated crude oil. Consequently, it will also be helpful to bear in mind the possibility of the underestimation of TPHs levels by any commercially available TPHs standards if such were not specifically developed from the contaminating crude oil.

Since both the commercially available and the prepared standards have similar components from the GC chromatograms (SF1 in Supplementary Material), the prepared standards can pick up signals of TPHs components within same range which the commercial TPHs standard does, and even more, which explains the higher TPHs estimates by the prepared standards. The commercial TPHs-gasoline diesel range standard is prepared by a 1:1 w:w mixture of neat diesel and mineral oil in 95 % n-hexane[26,27]. These are typical components of crude oil, hence the observed quantification in TPHs measurements obtained by both the prepared and the commercially available TPHs gasoline-diesel range standard. The use of TPHs standards prepared from the contaminated sources for evaluation of levels of TPHs in such soils would offer the advantage of specificity in addition to ready availability.

This study has demonstrated how such standards can be prepared, for use in the TPHs analysis.

There were 3 exceptions during the estimation of TPHs from the soil samples from Ogoniland, Nigeria, where *p*-values were >0.05 indicating that the values were not statistically different. These scenarios were particularly observed at low concentrations of the TPHs in the soil samples. The concentrations reported at these instances were 8.94, 3.61, and 1.01 g/kg. It is worth noting that these were the lowest of the TPHs concentrations in the soils used in this study. Hence, this observation reveals that at low concentrations, each of these standards can reliably evaluate TPHs levels in environmental matrices, without much disparity.

In the present study, similarity in levels of % TPHs reduction in the soil samples during the remediation treatment with all the standards (TPHs_Tib or TPHs_Ogoni, TPHs_GD, and TPHs_C10), at every instance, with no significant differences, has been reported (ST4 & ST5 and SF3 in Supplementary Material). Further statistical analysis (F3 in supplementary material) has also revealed that the % reduction in TPHs levels in the soil samples can reasonably be evaluated by each of the TPHs standards. Yang et al. [28] demonstrated that the use of the commercially available TPHs standards can also account for a risk assessment of petroleum contaminated sites. The observed similarity in the remediation efficiency obtained by all the standards in this study indicates that the prepared TPHs standards can also give a good measure of toxicity index and health risk assessment during crude oil soil pollution and the remediation process. Accordingly, the higher values of TPHs estimated by the prepared standards mean a better risk assessment with respect to TPHs concentrations in the associated petroleum contaminated sites by the prepared standards.

TPHs soil background levels and remediation targets have been set at between 1000 mg/kg to 10,000 mg/kg, or 10,000 to 40,000 mg/kg [29,30]. The disparity in soil TPHs estimation by the various TPHs standards implies some uncertainties can arise with respect to toxicological evaluations, especially when using the commercially available standards. This reemphasizes the importance of using TPHs standards specifically developed for an area of interest during toxicological evaluation.

4. Conclusion

The present investigation reveals that TPHs standards prepared from the contaminating crude oil can be used to reliably evaluate the levels of TPHs in petroleum-contaminated soils. Such standards provide a more reliable and site-specific estimate of TPHs levels and risk assessment compared to the commercially available TPHs standards. The TPHs standards can readily be prepared as demonstrated in this study, and the process can aid in a timelier assessment of TPHs in contaminated soils. Such, development will provide readily available alternatives for monitoring petroleum-contaminated soils as well as the remediation process for quick evaluation of TPHs contents in soils in the event of unavailability of the commercial TPHs standards.

Both the commercially available and the prepared TPHs standards can provide consistent evaluation of % TPHs reduction in the contaminated soils in every instance. This validates the reliability of the commercially available standards and reveals the potential of such prepared TPHs standards in the estimation of % TPHs reduction during soil remediation programs. This study has further revealed that although the TPHs levels estimated by the different TPHs standards are significantly different at high concentrations, values were similar at low concentrations (<9 g TPH/kg dry soil). However, because most petroleum-contaminated soils are associated with high concentrations of TPHs, it is highly recommended that, for effective risk-based evaluation of the TPHs levels in petroleum-contaminated soils, the use of TPHs standards specifically developed for an area of interest is desirable.

Supplementary materials: The supplementary material contains information on the following:

- Table ST1:Soil samples designation in the Glasshouse remediation treatments used in the study
- Table ST2: Data for Comparative analysis of TPHs in soils of Tibshelf, UK using the prepared TPHs standard from the contaminated crude oil (from Tibshelf, Derbyshire, UK), and commercial TPHs standard (gasoline-diesel range & C10-C40).
- Table ST3: Data for Comparative analysis of TPHs in soils of Ogoniland, Nigeria using the prepared TPHs standard from the contaminated crude oil (from Ogoniland, Nigeria), and commercial TPHs standard (gasoline-diesel range & C10-C40).
- Table ST4: Test of significance in measurement of TPHs in the soil samples from Tibshelf, UK, using TPHs-Tib, TPHs-GD, and TPHs-C10.
- Table ST5: Test of significance in measurement of TPHs in the soil samples from Ogoniland, Nigeria, using TPHs-Ogoni, TPHs-GD, and TPHs-C10.
- Figure SF1: Chromatograms of the TPHs standards
- Figure SF2: Calibration graphs for the TPHs standards
- Figure SF3: Residual plots, line fit plots, and normal distribution plots for estimation odf TPHs levels in the soil treatments using the different TPHs standards.

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