

Bio-accessibility based risk assessment of PAHs in soils from sites of different anthropogenic activities in Lagos, Nigeria using the Fed Organic Estimation Human Simulation Test method

Oluwatoyin T. Adetunde^a, Graham A. Mills^{b*}, Kehinde O. Olayinka^a, Babajide I. Alo^a

^a*Department of Chemistry, Faculty of Science, University of Lagos, Akoka, Yaba, Lagos, Nigeria*

^b*School of Pharmacy and Biomedical Science, University of Portsmouth, Portsmouth, PO1 2DT, United Kingdom*

*Corresponding author:

E-mail: graham.mills@port.ac.uk

Tel.: 00 44(0) 23 9284 2115

Abstract

The aim of this study was to carry out a bio-accessibility based risk assessment of polycyclic aromatic hydrocarbon (PAHs) in soils from sites of different anthropogenic activities in Lagos, Nigeria. Using an *in vitro* gastrointestinal model – Fed Organic Estimation Human Simulation Test method (FORESht), the concentration of bio-accessible 16 priority US Environmental Protection Agency (USEPA) PAHs in soils were determined. Total concentration of 16 priority USEPA PAHs was also determined. The concentration range was 702 to 253,922 ng g⁻¹ and 92-760 ng g⁻¹ for total and bio-accessible PAHs respectively. For persons involved with activities at these sites no health risk were observed, based on bio-accessibility values of PAHs. Mean daily intake of PAHs from these soils were below the oral mean daily intake threshold for PAHs in food. Also, overall estimated theoretical cancer risk (2.5×10^{-09} , 6.5×10^{-07} , 5.5×10^{-10} , 2.7×10^{-09} , 6.5×10^{-10} , 9.5×10^{-10} , 2.0×10^{-09} and 4.1×10^{-07} for the eight sites based on their bio-accessible concentration) for exposure to PAHs in surface soils were below the health guidelines for extreme (1×10^{-04}) and normal (1×10^{-06}) exposures.

Key words: Bio-accessibility, Polycyclic Aromatic Hydrocarbons, Risk assessment, Fed Organic Estimation Human Simulation Test method

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the environment. Human activities such as refining of petroleum, burning of fossil fuels, oil spills and open incineration of waste among others have contributed significantly to the PAHs concentrations in the environment. PAHs are semi-volatile, hydrophobic organic compounds covering a wide range of molecular weights. Hence they can be deposited onto soil, plant surfaces and water bodies (Li et al. 2009; Abdel-Shafy and Mansour, 2016, Lawal & Fantke, 2017). Soil PAHs, when taken up by plants roots bio-accumulate. Plant PAHs are transferred to other organisms by ingestion; often reaching concentrations that can cause toxicological effects (e.g. cellular mutations) (Manoli et al. 2004). Human exposure to PAHs occurs via various routes such as ingestion of contaminated food, inhalation of contaminated air and dusts, or dermal contact with contaminated media (Hussein et al. 2016; Kim et al. 2009; Oomen et al. 2003; USEPA, 2008a; USEPA, 2008b). Concentration of PAHs in soils are significantly associated with their corresponding concentrations in air, household, urban street dusts which have far reaching agricultural, environmental and human health effects. The concentration of PAHs in soil is a good indicator of the overall degree of environmental pollution (Adetunde et al. 2014). Therefore, contaminant levels in soils are of concern to regulatory agencies in most countries.

Exposure to the 'total' concentration of a chemical (estimated using exhaustive extraction procedures such as ultra-sonication or Soxhlet) in soil is often used as the basis of risk assessments e.g. the overall estimated theoretical cancer risk (ER), mean dietary intake (MDI), or benzo(a)pyrene equivalence dose (BaP_{eq}). This approach, however, can lead to overestimation of any risk, since only a fraction of the total concentration of the chemical is absorbed into the systemic circulation (Adetunde et al. 2014; Gomez-Eyles et al. 2010; Turkall et al. 2009). The most important factor in predicting or assessing the health risk of lipophilic organic chemicals, such as PAHs, is the bio-available and bio-accessible fractions (Tao et al. 2009).

There have been attempts to measure both the bio-availability and bio-accessibility of pollutants using *in vivo* procedures, but these have limitations because of ethical issues, disparities between human and animal absorption systems (Intawongse and Dean, 2006). *In-vitro* assessment may overcome some of these limitations. The Fed Organic Estimation Human Simulation Test (FORESHT) a standardized *in vitro* bio-accessibility test for organic pollutants in soils was developed by members of Bio-accessibility Research Group Europe (BARGE) as a follow-on from the Unified Barge Method used for inorganic contaminants (Cave et al. 2010).

Knowledge of the bio-availability/bio-accessibility of a pollutant is important for risk assessments especially in risk based clean-up when a level of remediation is to be determined

(Liptak and Lombardo, 1996; Zia et al. 2011). The degree of any risk-based clean-up of a contaminant in the environment increases with its decreasing bio-availability. That is the limiting values are high if the contaminant is not bio-assessable and low limits are used if the contaminant is bio-accessible (Rostami and Juhasz, 2011; Wan-ling et al. 2011; Zia et al. 2011).

Lagos, the fastest growing city in Nigeria, is one of the largest and most densely populated city in Africa. It is situated on the South-Western Coast of Nigeria and approximately 60% of Nigeria's industrial and commercial activities are situated here (Ajibola et al. 2012; MoELS, 2010). Thus the land of Lagos over the years has been put to many uses including industrial. The increasing population of Lagos state has also led to an increase in the demand for land and to meet this demand reclamation of swamps, former industrial sites and dumpsites are on the rise. Hence, the need for site specific risk assessment of such sites. Bio-accessibility is highly site and source-specific, insufficient data usually makes it difficult to adequately define a value that differs from the default approach of 100%. A site-specific assessment of bio-accessibility can be undertaken to carry out risk assessment (Hansen et al. , 2007).

In Nigeria information on the concentration and distribution pattern of PAHs in soils within the country is lacking. This information is important for regulators in order to make valid hazard and risk assessments. We undertook a study to assess occupational exposure to PAHs via involuntary ingestion of soil from different sites around the city of Lagos (Adetunde et al. 2014). Assessment was based on the total 16 PAHs on the USEPA's priority list. The aim of this follow-on study was to measure the bio-accessible PAHs at these locations and use it to evaluate of risk associated with bio-accessible PAHs present. This preliminary information will be beneficial to Nigerian agencies who are tasked with undertaking environmental health assessments.

MATERIALAND METHODS

Sampling

Six sub-samples of surface soil (collected at a depth 0-10 cm) were obtained from eight locations (A-H) associated with different anthropogenic activities in Lagos, Nigeria (Fig. 1). Samples were collected during April, 2011 corresponding to the rainy season with ambient temperatures ranging typically between 18-30°C. A composite sample for analysis from each site was made by thoroughly mixing the sub-samples followed by sieving (2 mm mesh size). The eight soil samples were classified as A = dark grey sandy/ clayey, B = dark grey sandy/clayey, C = dark brown sandy/silty, D = dark brown sandy/ silty, E= oily black sandy/clayey, F = sandy/gravel, G = grey sandy/silty and H= dark black oily sandy/clayey types.

Chemicals and Standards

A mixture of 16 USEPA PAHs (all 2,000 $\mu\text{g mL}^{-1}$) was obtained from Supelco (Bellefonte, PA, USA). The components of the mixture were naphthalene (NAP), 1-methylnaphthalene (1-MNAP), 2-methylnaphthalene (2-MNAP), acenaphthylene (ACY), acenaphthene (ACP), fluorene (FLR), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), benzo[a]anthracene (BaA), chrysene (CHR), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (IcP), dibenzo[a,h]anthracene (DaH) and benzo[g,h,i]perylene (BgP). Deuterated PAHs were used as internal standards. The deuterated internal standard solution (all 2,000 $\mu\text{g mL}^{-1}$ in dichloromethane) contained d_{10} -acenaphthene, d_8 -naphthalene, d_{10} -phenanthrene, d_{12} -chrysene, d_{12} -perylene and d_4 -1,4-dichlorobenzene (Supelco). The certified reference material CRM 172-100G for USEPA PAHs used for method validation was from Supelco. HPLC grade solvents were purchased from Fisher Scientific Ltd. (Loughborough, UK). Gut enzymes (α -amylase (activity: 1,000-3,000 units/mg protein), pepsin (activity: 2,500 units/mg protein), pepsin from porcine gastric mucosa (activity: 3,200-4,500 units/mg protein), pancreatin and lipase) and reagents for the bio-accessibility study were from Supelco Ltd. and Fisher Scientific Ltd.

Extraction, Clean-up and Analysis of PAHs

Extraction, clean up and analysis of PAHs were carried out as in Adetunde et al (2104). Briefly PAHs from 0.5–5 g soil and the CRM 172-100G (1 g) were extracted ultrasonically using three sequential extractions of acetone: *n*-hexane (1:1 v/v). The combined extract (25 mL) was spiked with internal standard solution (25 μL of 10 mg mL^{-1}) and concentrated under nitrogen to 500 μL . C_{18} Bond Elut (200 mg, 5 mL) cartridges were used for clean-up. Cartridges were pre-conditioned, concentrated extracts were loaded on them and eluted with dichloromethane: *n*-hexane (1:1 v/v, 5 mL) at a flow rate of 1 mL min^{-1} . Eluates were evaporated to dryness under nitrogen and reconstituted in *n*-hexane (1 mL). Working standard solutions were prepared daily in *n*-hexane. An Agilent GC/MS (6890N GC) equipped with split/splitless injector, with a HP-5MS UI capillary column (30 m long, 0.25 mm i.d. \times 0.25 μm film thickness) connected to a mass selective detector (Agilent 5975) was used to separate and quantify the PAHs. Samples were injected (2 μL) in the splitless mode at an injection temperature of 290 $^{\circ}\text{C}$. The column oven was held at 50 $^{\circ}\text{C}$ (3.2 min), raised to 150 $^{\circ}\text{C}$ (30 $^{\circ}\text{C min}^{-1}$), then raised to 238 $^{\circ}\text{C}$ (2 $^{\circ}\text{C min}^{-1}$), 272 $^{\circ}\text{C}$ (3 $^{\circ}\text{C min}^{-1}$) and to 300 $^{\circ}\text{C}$ (70 $^{\circ}\text{C min}^{-1}$ and held for 2.73 min). Helium was used as carrier gas at a constant flow rate (1 mL/min). Mass spectra were acquired using electron ionization (EI) at 70 eV. Identification of

PAHs was by confirmation of retention time and abundance of quantification/confirmation ions compared to authentic standards. Compounds were quantified using selective ion monitoring (SIM). The analytical samples series comprised the CRM, one standard treated similarly to the samples (to determine recoveries), a blank and six standards for calibration (Marce and Borruell, 2000; Oluseyi et al. 2011; Silva et al. 2011). Internal standard calibration using the response factors of individual PAHs related to the respective internal standards based on six-point calibration curve were used to quantify individual compounds in the soils.

Measurement of Bio-accessible PAHs

The extraction of bio-accessible PAHs present in soil samples was undertaken by using the FOREhST method developed by BARGE (Cave et al. 2010; Lorenzi et al. 2012) with modifications at the clean-up step. The procedure involved three stages and was carried at 37 °C utilizing an end-to-end rotator to simulate human bowel movements. At the first stage, gastrointestinal fluids namely saliva (pH: 6.8 ± 0.5), gastric (pH: 1.3 ± 0.5), duodenal (pH: 8.1 ± 0.2) and bile fluids (pH: 8.2 ± 0.2) were simulated using gut enzymes, mucin salts and urea. At the second stage gastrointestinal fluids were used to extract the bio-accessible PAHs. Test soil sample (0.3 g), food (organic cream porridge, from HiPP UK Ltd., Newbury, UK), water and oil (sunflower oil, from ASDA Stores Ltd., Leeds, UK) were placed in amber bottles and extracted by adding gastrointestinal fluids one by one. To simulate the mouth, saliva was put in the food mixture for 5 mins. Gastric phase was simulated by adding gastric juice to the mixture from mouth phase and allowing it to rotate for 2 h. The intestinal phase was simulated by adding duodenal and bile fluids to derived gastric phase mixture. The intestinal phase was left to mix for 2 h. Fluid ratio for saliva:gastric:duodenal:bile was 1:2:2:1 v/v/v/v in at the end of the test. Saponification is the last step which is an extra isolation stage that helps to facilitate the complete extraction of PAHs from the complex food and enzymatic juice mixture. The resultant mixture was centrifuged at 3,000 rpm for 5 min. An aliquot (1 mL) of supernatant was removed into a heat resistant glass vial for saponification (3 mL of KOH (5.6 M) in methanol for 1 h at 100 °C).

Extraction and quantification of bio-accessible PAHs was carried out after extracts were left to cool. The extracts were diluted with water (6 mL) and leaned-up on a pre-conditioned C₁₈ Bond Elut SPE cartridge (200 mg, 5 mL). The elution was performed by dichloromethane (5 mL). Eluents were concentrated to dryness under nitrogen, then reconstituted in *n*-hexane (500 µL). Concentration of the bio-accessible PAHs was measured using the GC/MS-SIM method as described above. Extractions were carried out in triplicate. A blank extraction was also carried out. Spiked blanks were used for recovery studies of PAHs in the bio-accessibility study.

Risk Assessment

Health-risk posed by exposure to PAHs was determined using the quantified bio-accessible PAHs. Carcinogenic potency of PAHs relative to BaP was calculated as given by Tsai et al. (2001) using the TEFs developed by Nisbet and LaGoy (1992). Xia et al. (2010) and Boström et al. (2002) suggested these TEF were better risk indicators. BaP_{eq} dose was calculated as follows:

BaP_{eq} dose ($\mu\text{g g}^{-1}$) = TEF x concentration ($\mu\text{g g}^{-1}$) (Huang et al. 2005), Sum BaP_{eq} dose ($\mu\text{g g}^{-1}$) = $\sum(\text{TEF} \times \text{concentration } (\mu\text{g g}^{-1}))$.

Mean daily intake (MDI) concentrations, annual daily exposure dose (D_a) and estimated theoretical cancer risk (ER) from exposure to contaminants were calculated as in Adetunde et al. (2014), Davoli et al. (2010) and Lorenzi et al. (2011) but bio-accessible PAHs fractions were used in this calculations since it is this fraction that causes the actual risk.

Briefly, D_a ($\mu\text{g kg}^{-1} \text{ day}^{-1}$) = $[\text{EC} \times \text{SIR}]/\text{BW}$ based on daily exposure and MDI is like D but without BW taken into account. Where BW= body weight of adult (70 kg) (ATSDR, 2005; USEPA, 2011), SIR = soil ingestion rate for adult (0.10 g day^{-1}) (ATSDR, 2005), EC = exposure concentration of PAHs ($\mu\text{g g}^{-1}$). The annual daily exposure dose (D_a) also called the average life time daily exposure or estimated exposure dose and is calculated from D.

D_a was estimated for workers on these sites based on 246 work days a year. This was arrived at by considering 52 weeks in a year. The 15 days of public holidays per year in Nigeria and 2 weekend days (Saturday and Sunday) a week when workers usually do not go to work were also considered. The nature of work undertaken at these sites is unstructured so leave from work was not considered in this assumption. Working hours were taken as 8 h day^{-1} . It was assumed that a person will work for 40 years (25-65 years of age) at these sites. The default value for bio-accessibility and bio-availability in calculation of D_a is one when the study on availability or accessibility study is not carried out. A value of one assumes that all of the PAHs to which the workers on site are orally exposed to solubilises in the guts (bio-accessibility) or is absorbed from the guts (bio-availability)(ATSDR, 2005) but since bio-accessibility was carried the default value of one was not used. AF = bio-accessibility factor, was used in calculating D_a .

The estimated theoretical cancer risk (ER) from exposure to contaminants was calculated by multiplying the estimated exposure dose by the cancer slope factor (CSF) for a suspected or known carcinogenic substance (ODH, 2011). Where $\text{ER} = \text{CSF} \times \text{estimated exposure dose } (\text{mg kg}^{-1}\text{day}^{-1})$ and $\text{CSF} = (7.3 (\text{mg kg}^{-1}\text{day}^{-1})^{-1})$ (Nyarko et al. 2011, ODH, 2011).

RESULTS AND DISCUSSION

Concentration of PAHs in Soil

The sum of concentrations of the PAHs in the different soils ranged between 702-253,922 ng g⁻¹. Heavily contaminated soils are defined as those where the total concentration of PAHs is > 1,000 ng g⁻¹, contaminated soils 600-1,000 ng g⁻¹, weakly contaminated soils 200-600 ng g⁻¹ and non-contaminated < 200 ng/g (Maliszewska-Kordybach, 2005). Using these definitions, soil from sites A, B, D, E, F and H were classified as heavily contaminated, and sites C and G as contaminated (Table 1). The very high concentrations of PAHs (~254,000 ng g⁻¹) found at site E were associated with its long time use as a kerosene and petrol loading station.

Analytical performance of FOREShT procedure for bio-accessibility test

In order to test the effectiveness C₁₈ Bond Elut SPE cartridges in extracting the PAHs from the saponified extract, recovery experiments were carried out. The standard USEPA PAHs mixture was spiked into the diluted saponification medium (such that each of the individual USEPA PAHs had a concentration of 5,000 ng mL⁻¹), applied to the SPE cartridge and then eluted with dichloromethane (5 mL). The results are shown in Fig. 2. As expected lower recoveries were found for the more volatile compounds (i.e. naphthalene, acenaphthylene, acenaphthene, fluorene) in this multi-step clean-up method; as has been observed by other workers using the FOREShT procedure (Lorenzi et al. 2012). Generally, this overall analytical method was considered acceptable; typically with accuracy (% recoveries between 70% and 130%) and precision (relative standard deviation < 30%) in accordance with USEPA criteria for the quality control and validation of analytical methods (USEPA, 1992).

Bio-accessibility of PAHs in soils

Bio-accessibility (using the *in vitro* FOREShT model) was assessed by measuring the orally accessible PAHs to the human gut due to the potentially involuntary consumption of soil by workers at the eight test sites. Many of the USEPA priority PAHs were not bio-accessible from the soil. The concentration of the bio-accessible priority USEPA PAHs that were measured is shown in Table 2. The bio-accessible fraction expressed as a percentage of the total concentration found is also given in the Table. Naphthalene was the most bio-accessible of 16 USEPA PAHs present for all the soil samples. Total bio-accessible priority USEPA PAHs for the soils studied ranged between 0.1% and 41.2% of the total amount (total amount of PAHs in this study was between 689 and 104,445 ng g⁻¹). In other studies, bio-accessible PAHs varied between 10-60% for soils containing

between 10,000-300,000 ng g⁻¹total PAHs (Cave et al. 2010), 0.1-1.4% (Van de Wiele et al. 2004) and 1-3% in aged crude oil contaminated soil (Kogel-Knabner et al. 2000). Our study, like others (Tao et al. 2009; Cave et al. 2010), showed that only a small fraction of PAHs present in soil is bio-accessible to humans.

Estimation of risk-base on bio-accessible PAHs

Bio-availability/bio-accessibility is site dependant and source-specific. Since insufficient data has been available on bio-accessibility, the default approach of risk assessment is 100% (total concentration of PAHs) in calculation. However, for comprehensive work (e.g. remediation, allotments among others) a site-specific assessment of bio-accessibility is required for accurate risk assessment (Hansen et al. 2007). Sites in this study classified as contaminated or heavily contaminated by PAHs were assessed for the risk posed based on their degree of bio-accessibility.

BaP_{eq} of soils base on the bio-accessibility study

Sum BaP_{eq} dose at different sampling sites in Lagos, Nigeria was between 0.03 (forest soil) and 16.79 µg g⁻¹ (petroleum product handling site) (Adetunde et al. 2014). A sum BaP_{eq} dose of 0.048 µg g⁻¹ was found for rural soil from Delhi, India (Agarwal, 2009), 0.892 µg g⁻¹ for roadside soil in Shanghai, China (Jiang et al. 2009), 1.009 µg g⁻¹ for traffic dust in Delhi India and 0.650 µg g⁻¹ for surface soils in Agra, India (Masih and Taneja, 2006) and 0.124 µg g⁻¹ for soil from Tarragona, Spain (Nadal et al. 2004). The dose values found in this study for sites with similar activities were lower than those reported by other workers. This may have been as a consequence of the approach used. Previous studies used the traditional risk assessment approach which makes use of the total concentration PAHs present in the matrices (usually derived from exhaustive extraction techniques). The sum BaP_{eq} dose calculations in our study were, based on the bio-accessible PAH(s) concentration derived from the FOREShT method, because this is the fraction that poses a health risk. The sum BaP_{eq} dose ranged from zero (sites C, F and G) to 0.637 µg g⁻¹ (site B). The sites were ordered C, F, G < D, E (0.001 µg g⁻¹) < A (0.002 µg g⁻¹) < H (0.275 µg g⁻¹) < B for the test sites (Table 3).

Mean dietary intake of PAHs base on the bio-accessibility study

Lorenzi et al. (2011) estimated risk by comparing the MDI for soil with the oral mean daily intake threshold for PAHs in food (oral MDI). In our study, a comparison of MDI for the soils (base on the concentration of bio-accessible PAHs) with oral MDI for PAHs in food was also undertaken (Table

3). The results showed that all the PAHs in composite samples were less than the oral MDI for PAHs in food. This indicates that there was no risk associated with activities on these sites, based on the MDI risk assessment approach.

Cancer estimate of PAHs based on the bio-accessibility data

The ER results given in Table 3 were based on the bio-accessible PAHs. In this study, the estimated total annual daily intake of PAHs will be associated with an ER of 2.5×10^{-09} , 6.5×10^{-07} , 5.5×10^{-10} , 2.7×10^{-09} , 6.5×10^{-10} , 9.5×10^{-10} , 2.0×10^{-09} and 4.1×10^{-07} for soils A to H respectively; being based on a 70 kg adult, exposed at work for 40 years (aged between 25-65 years). The overall estimated theoretical cancer risks from occupational exposure to surface soil based on bio-accessible oral ingestion were all lower than both the target risk of 1×10^{-06} for normal exposure and the 1×10^{-04} for extreme exposure at all the test sites. The total ER combining the childhood (5 years) and adult (40 years) exposure periods was 2.3×10^{-05} based on sum PAHs (ODH 2011). ER values for an adult working at the on these sites were between 7.3×10^{-07} and 1.2×10^{-04} according to Adetunde et al. (2014). However, these values were based on the concentration of total 16 priority PAHs in soil. Using the concentrations of bio-accessible PAHs, the fraction that causes the actual harm, the risk associated with sites in this study were lower (between 4.1×10^{-07} and 9.5×10^{-10} as shown in Table 3).

CONCLUSION

This study assessed potential health risk associated with the bio-assessable PAHs of soils impacted by different anthropogenic activities in the Lagos region of Nigeria. Concentrations of the 16 priority USEPA PAHs were measured and FOREShT an *in vitro* gastrointestinal model was used to extract the bio-accessible PAHs in soils for quantification. Results indicated that though soils from sites were classified as heavily contaminated or contaminated, based on their total concentration of PAHs, only a percentage (0.7-41.2%) was bio-accessible for uptake by humans. MDI results showed that no risk was associated with the bio-accessible fraction of PAHs. The overall cancer risk from exposure to surface soil based on oral ingestion was not above the approved health guidelines of 1 in 10,000 for extreme exposures. The concentration of bio-accessible PAHs found in our study was used to carry out a risk assessment of some soils in the Lagos region of Nigeria. This type of approach should now always be used by regulatory agencies when undertaking any comprehensive risk assessment of any contaminated land. It must be noted, however, that values derived by this

approach only provide a theoretical estimate of risk. Since the actual risk of cancer is unknown and it could be as low as zero. PAHs are lipophilic and can accumulate in the body, care should be exercised at all times to ensure minimal exposure from all routes to these to these potentially harmful substances.

References

- Ajibola, MO, Adewale, BA, Ijasan, KC. 2012. Effects of urbanisation on Lagos Wetlands. *Intern. J. Buss. Soc. Sci.* **3**: 310-318.
- Abdel-Shafy, HI and Mansour, SM. 2016. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egypt. J. Petrol.* **25**, 107–123.
- Adetunde, OT, Mills, G.A, Olayinka, KO, Alo, BI, (2014). Assessment of occupational exposure to polycyclic aromatic hydrocarbons via involuntary ingestion of soil from contaminated soils in Lagos, Nigeria. *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.* **49**: 1661–1671.
- ATSDR (2005). *Public Health Assessment Guidance Manual Appendix G: Calculating Exposure Doses (2005 Update)*. In: registry Aftsad, editor. ATSDR, USA.
- Bakker, MI, Casado, UB, Koerselman, JW, Tolls, J, Kolloffel, C. 2000. Polycyclic aromatic hydrocarbons in soil and plant samples from the vicinity of an oil refinery. *Sci. Total Environ.* **263**: 91-100.
- Cave, MR, Wragg, J, Harrison, I, Vane, CH, Wiele, TV, Groeve ED, 2010. Comparison of batch mode and dynamic physiologically based bioaccessibility tests for PAHs in soil samples. *Environ. Sci. Technol.* **44**: 2654-2660.
- Environment Agency. 2009. Updated technical background to the CLEA model. In: Jefferies J, Martin I, eds. *Using Science to create a better place*. Environment Agency of England and Wales, United Kingdom, pp. 166.
- Gomez-Eyles, JL, Collins, CD, Hodson, ME, 2010. Relative proportions of polycyclic aromatic hydrocarbons differ between accumulation bioassays and chemical methods to predict bioavailability. *Environ. Pollut.* **158**: 278-284.

- Hansen, JB, Oomen, A, Edelgaard, I, Gron, C, 2007. Oral bioaccessibility and leaching: tests for soil risk assessment. *Engin. Life Sci.* **7**: 170-176.
- Hong, L, Ghosh, U, Taniamahaj, AN, Zare, R, Luthy, R. 2003. PAH sorption mechanism and partitioning behavior in lampblack-impacted soils from former oil-gas plant sites. *Environ. Sci. Technol.* **32**: 3267-3276.
- Intawongse, M, Dean, JR. 2006. Uptake of heavy metals by vegetable plants grown on contaminated soil and their bioavailability in the human gastrointestinal tract. *Food Addit. Contamin.* **23**: 36-48.
- Kim, DW, Kim, SK, Lee, DS. 2009. Relationship of pyrogenic polycyclic aromatic hydrocarbons contamination among environmental solid media. *J. Environ. Monit.* **11**: 1244–1252.
- Kogel-Knabner, I, Totsche, KU, Raber, BJ. 2000. Desorption of polycyclic aromatic hydrocarbons from soil in the presence of physiologi. *J. Environ. Qual.* **29**: 906–916.
- Latawiec, A. E., Simmons, P. & Reid, B. J. 2010 Decision-makers' perspectives on the use of bioaccessibility for risk-based regulation of contaminated land. *Environment International*, **36**, 383 – 389.
- Lawal, AT, Fantke, P. 2017. Polycyclic aromatic hydrocarbons. A review, *Cogent Environ. Sci.* **3**, 1-89. DOI: [10.1080/23311843.2017.1339841](https://doi.org/10.1080/23311843.2017.1339841)
- Liptak, J. F. & Lombardo, G. 1996. The Development of Chemical-Specific, Risk-Based Soil Cleanup Guidelines Results in Timely and Cost-Effective Remediation. *Journal of Soil Contamination*, **5**, 1-12.
- Li, P, Li, X, Stagnitti, F, Zhanga, H, Lin, X, Zang, S. 2009. Studies on the sources of benzo[a]pyrene in grain and above ground tissues of rice plants. *J. Hazard. Mater.* **162**: 463–468.

- Lorenzi, D, Entwistle, J, Cave, M, Wragg, J, Dean, JR. 2012. The application of an in vitro gastrointestinal extraction to assess the oral bioaccessibility of polycyclic aromatic hydrocarbons in soils from a former industrial site. *Anal. Chim. Acta* **735**: 54–61.
- Lorenzi, D, Entwistle, JA, Cave, M, Dean JR. 2011. Determination of polycyclic aromatic hydrocarbons in urban street dust: Implications for human health. *Chemosphere* **83**: 970-977.
- Maliszewska-Kordybach, B. 2005. Dissipation of polycyclic aromatic hydrocarbons in freshly contaminated soils – the effect of soil physicochemical properties and ageing. *Water Air Soil Pollut.* **168**: 113-128.
- Man, BY, Kang, Y, Wang, HS, Lau, W, Li, H, Sun, XL, Giesy, JP, Chow, KL, Wong, MH. 2013. Cancer risk assessments of Hong Kong soils contaminated by polycyclic aromatic hydrocarbons. *J. Hazard. Mater.* **261**, 770–776.
- Manoli, E, Kouras, A, Samara, C. 2004. Profile analysis of ambient and source emitted particle-bound polycyclic aromatic hydrocarbons from three sites in northern Greece. *Chemosphere* **56**: 867–878.
- Marce, RM, Borrull, F. 2000. Solid-phase extraction of polycyclic aromatic compounds. *J. Chromatogr.* **885**: 273-290.
- MoELS. 2010. State of the Environment Report. In, (Lagos: Ministry of the Environment, Lagos State)
- ODH. 2011. Evaluation of Ohio EPA soil sampling in support of the Clyde and Eastern Sandusky County childhood cancer investigation. Clyde Sandusky County, Ohio. Ohio Department of Health, US Ohio. pp. 32.
- Oluseyi, T, Olayinka, K, Alo, B, Smith, RM. 2011. Improved analytical extraction and clean-up techniques for the determination of PAHs in contaminated soil samples. *Intern. J. Environ. Resour.* **5**: 681-690.

- Oomen, AG, Rompelberg, CJM, Bruil, MA, Dobbe, CJG, Pereboom, DPKH, Sips, AJAM. 2003. Development of an in vitro digestion model for estimating the bioaccessibility of soil contaminants. *Arch. Environ. Contaminat. Toxicol.* **44**: 281–287.
- Rostami, I, Juhasz, A. 2001. Assessment of persistent organic pollutant (POP) bioavailability and bioaccessibility for human health exposure assessment: A Critical Review. *Crit. Rev. Environ. Sci. Technol.* **41**: 623-656.
- Silva, BO, Adetunde, OT, Oluseyi, TO, Olayinka, KO, Alo, BI. 2011. Comparison of some extraction methods and clean-up procedures for the 16 priority US EPA PAHs. *J. Sci. Res. Develop.* **13**: 129–143.
- Stokes, JD, Paton, GI, Semple, KT. 2006. Behaviour and assessment of bioavailability of organic contaminants in soil: relevance for risk assessment and remediation. *Soil Use Manage.* **21**: 475-486.
- Tao, Y, Zhang, S, Wang, Z, Christie, P. 2009. Predicting bioavailability of PAHs in field-contaminated soils by passive sampling with triolein embedded cellulose acetate membranes. *Environ. Pollut.* **157**: 545-551.
- Tsai, PJ, Shieh, HY, Lee, WJ, Lai, SO. 2001. Health-risk assessment for workers exposed to polycyclic aromatic hydrocarbons (PAHs) in a carbon black manufacturing industry. *Sci. Total Environ.* **278**: 137-150.
- Turkall, RM, Skowronski, GA, Abdel-Rahman, MS. 2009. Effects of soil matrix and aging on the dermal bioavailability of polycyclic aromatic hydrocarbons in the soil. *Intern. J. Soil, Sediment Water.* **2**: 1-9.
- USEPA. 1992. Guidance for methods development and methods validation for the Resource Conservation and Recovery Act (RCRA) programme In: methods TOoRCaRsOmoE-aa, editor. 2014. US Environmental Protection Agency, Washington, D.C., pp. 32.
- USEPA. 2008a. Child-specific exposure factors handbook. EPA/600/R-06/096F.

USEPA. 2008b. Standard Operating Procedure for an In Vitro Bioaccessibility Assay for Lead in Soil In: Agency USEP, editor, USA.

Van de Wiele, TR, Verstraete, W, Siciliano, SD. 2004. Polycyclic aromatic hydrocarbon release from a soil matrix in the in vitro gastrointestinal tract. *J. Environ. Qual.* **33**: 1343-1353.

Wan-ling, H, Xiao-li, L, Jia-li, S, Xiao,Y, Ji-liang, Z. 2011. Effects of cadmium pollution in soil on cadmium accumulation of cabbage and its biological effects on human intestinal cells line. *Procedia Eng.* **18**: 157-161.

Yunker, MB, Macdonald, RW, Vingarzan, R, Mitchell, R, Goyette, HD, Sylvestre, S. 2002. PAHs in the Fraser river basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. *Org. Geochem.* **33**: 489–515.

Zia, MH, Codling, EE, Scheckel, KG, Chaney, RL. 2011. In vitro and in vivo approaches for the measurement of oral bioavailability of lead (Pb) in contaminated soils: A review. *Environ. Pollut.* **159**: 2320-2327.

Table 1. Concentration (total, ng g⁻¹) of sixteen USEPA priority PAHs found in soil samples collected from the Lagos region, Nigeria

Location	A	B	C	D	E	F	G	H
PAHs								
NAP	6,531	675	105	733	1,070	161	308	2,274
ACY	79.4	2,540	25.3	47.0	8,353	108	2.9	3,843
ACP	51.1	130	43.1	31.4	640	125	4.3	122
FLR	142	750	58.4	67.6	1,855	40.9	22.6	652
PHE	962	17,970	83.5	181.7	86,179	408	107	17,078
ANT	935	2900	4.8	34.08	84,837	111	56.1	3,945
FLT	63	4020	13.7	54.2	2,585	320	28.7	5,663
PYR	242	10,700	30.7	50.7	44,834	246	23.2	6,927
BaA	216	6,840	36.0	2500	2,054	232	7.4	7,053
CHR	320	20,110	77.9	15119	11,217	353	35.2	3,982
BbF	21.0	8,040	28.8	20.5	0	381	17.8	7,453
BkF	28.5	10,320	35.1	28.9	0	492	23.0	10,202
BaP	903	3,300	8.2	1,184	0	143	12.3	1,295
DaH	209	10,040	76.6	425	10,049	330	28.5	10,993
IcP	34.4	5,790	68.7	87.5	0	657	10.4	4,655
BgP	1,113	320	6.1	870	249	0	1.3	236
Sum PAHs	11,850	104,445	702	21,435	253,922	4,108	689	86,373

* Where 0 values correspond to: $\leq 0.03 \mu\text{g g}^{-1}$ for ACY, $\leq 0.04 \mu\text{g g}^{-1}$ for FLR, $\leq 0.02 \mu\text{g g}^{-1}$ for PHE, $\leq 0.03 \mu\text{g g}^{-1}$ for ANT, $\leq 0.04 \mu\text{g g}^{-1}$ for FLT, $\leq 0.03 \mu\text{g g}^{-1}$ for PYR, $\leq 0.01 \mu\text{g g}^{-1}$ for BaA, $\leq 0.01 \mu\text{g g}^{-1}$ for CHR, $\leq 0.02 \mu\text{g g}^{-1}$ for BbF, $\leq 0.02 \mu\text{g g}^{-1}$ for BkF, $\leq 0.02 \mu\text{g g}^{-1}$ for BaP, $\leq 0.04 \mu\text{g g}^{-1}$ for DaH, $\leq 0.01 \mu\text{g g}^{-1}$ for IcP and $\leq 0.01 \mu\text{g g}^{-1}$ for BgP. For abbreviations of individual PAHs see Materials and Methods section.

Table 2. Concentration (ng g⁻¹) and percentage* of the bio-accessible priority USEPA PAHs found in eight contaminated soil samples (n = 3) collected in the Lagos area, Nigeria.

Soil sample										LOD
Com pound	A	B	C	D	E	F	G	H		
NAP	299±2.3 (5%)	92.7±9.15 (14%)	90.5±34.5 (87%)	327 ± 24.6 (45%)	316 ± 132 (30%)	100 ± 34.5 (62%)	267 ±34.5 (87%)	171 ± 8.0 (8%)	11.6	
ANT	17.6±4.0 (2%)	29.7±1.2 (1%)	Nd	28.5 ± 9.8 (25%)	40.1 ± 10.0 (0.1%)	28.5± 5.4 (26%)	17.0 ± 34.5 (30%)	26.2 ± 9.0 (0.7%)	1.6	
BaP	nd	549 ± 3.0 (17%)	Nd	nd	nd	nd	Nd	273 ± 1.56 (21%)	11.4	
DaH	nd	88.6 ± 55.2 (0.9%)	Nd	nd	nd	nd	D	nd	20.0	
BgP	194 ± 6.4 (17%)	nd	Nd	nd	nd	nd	Nd	101 ± 2.8 (43%)	18.8	
Sum bio-accessible PAH	511 ± 4.2 (4.3%)	760 ± 23.0 (0.7%)	91.5 ±35.0 (12.9%)	356 ± 15.0 (1.7%)	356 ± 100 (0.1%)	129 ± 12.9 (3.1%)	284 ± 30.0 (41.2%)	570 ± 18.0 (0.9%)		

*Percentage = Concentration of bio-accessible PAH/Total concentration PAH in soil x 100, LOD = limit of detection, nd = not detected. For abbreviations of individual PAHs see Materials and Methods section.

Table 3. Bio-accessibility data for test soils and associated MDI (mg day⁻¹), Da_(BaP_{eq}) (mg kg⁻¹ day⁻¹) and ER based on bio-accessible PAHs, BaP_{eq} dose (μg g⁻¹).

MDI	A	B	C	D	E	F	G	H	^a Oral MDI food
NAP	0.030	0.009	0.009	0.033	0.032	0.010	0.027	0.017	7
ACY	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.14
ACP	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.98
FLR	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.59
PHE	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.54
ANT	0.002	0.003	0.000	0.003	0.004	0.003	0.002	0.003	0.08
FLT	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.35
PYR	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.35
BaA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.06(0.05) ^b
CHR	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.11
BbF	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.11
BkF	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09
BaP	0.000	0.055	0.000	0.000	0.000	0.000	0.000	0.027	0.11
DaH	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.1
BgP	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.04
IcP	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.06
MDI of sum bio-accessible PAHs	0.051	0.076	0.009	0.036	0.036	0.013	0.028	0.057	
Sum BaP _{eq} dose	0.002	0.637	0.000	0.001	0.001	0.000	0.000	0.275	
Da _(BaP_{eq}) sum bio-accessible PAHs	3.39 × 10 ⁻⁰⁷	8.83 × 10 ⁻⁰⁵	7.5 × 10 ⁻⁰⁸	3.67 × 10 ⁻⁰⁷	8.96 × 10 ⁻⁰⁸	1.3 × 10 ⁻⁰⁷	2.7 × 10 ⁻⁰⁷	5.59 × 10 ⁻⁰⁵	
ER based on bio-accessible PAHs	2.5 × 10 ⁻⁰⁹	6.5 × 10 ⁻⁰⁷	5.5 × 10 ⁻¹⁰	2.7 × 10 ⁻⁰⁹	6.5 × 10 ⁻¹⁰	9.5 × 10 ⁻¹⁰	2.0 × 10 ⁻⁰⁹	4.1 × 10 ⁻⁰⁷	

^aOral mean daily intake threshold for PAHs in food (oral MDI) (Nathaniel et al. 2009: cited by Lorenzi et al. [22],

^bAlternative measure of oral MDI (Falco et al. 2003). A value of 0.000 μg g⁻¹ MDI for individual PAH, means that the concentration of the bio-accessible PAH less than or equal to the LOD value in Table 3. Benzo(a)pyrene equivalence dose (BaP_{eq}), mean daily intake (MDI), Annual daily exposure dose base on benzo(a)pyrene equivalence dose Da_(BaP_{eq}). For abbreviations of individual PAHs see Materials and Methods section.

Figure 1: Map of soil sampling locations in Lagos, Nigeria. Key to locations: A = Dump site near Onike canal, Mainland area, B = Depot/loading point for black oil, Iganmu/Oribe, Apapa, C = Motor spirit/kerosene depot, Apapa, D = Dump site, Akoka, Mainland area, E = Motor spirit/kerosene depot, Coconut Island, F = Roadside, central Lagos, G = Trailer park/mechanics workshop, Ibafo, Obafemi Owode and H = Mechanics workshop, Mainland area.

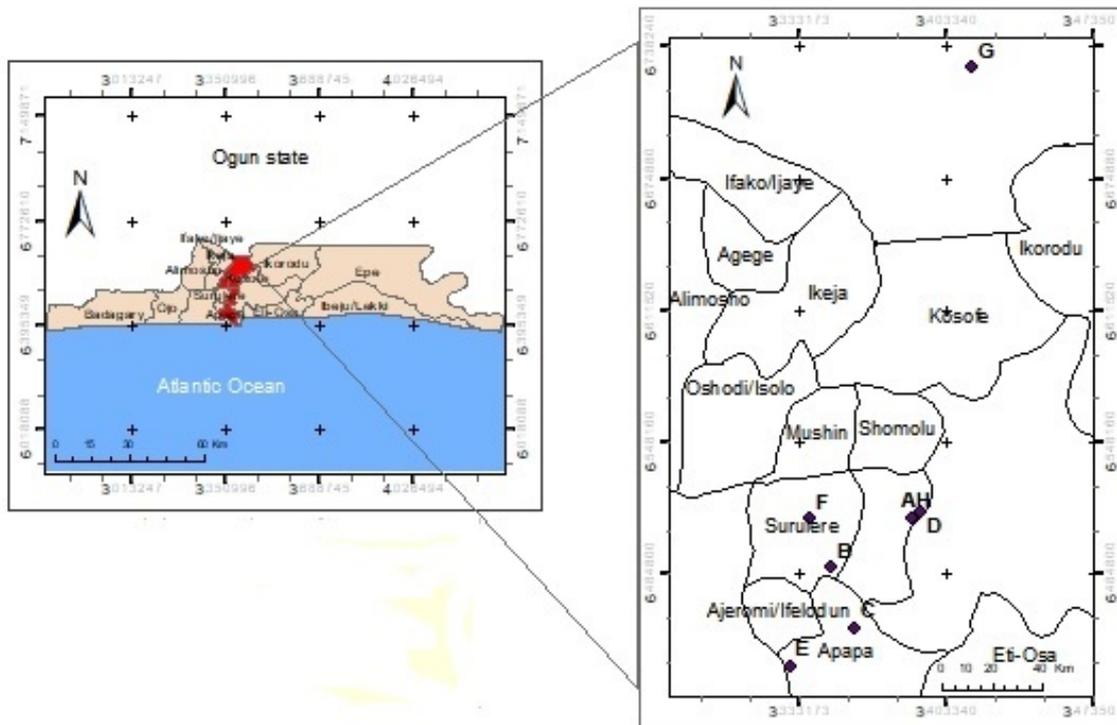


Figure 2: Percentage recovery (n = 4) of the 16 USEPA priority PAHs spiked (5,000 ng/mL) into the FOREShT saponification extract (1 mL) and subsequently extracted on a C18 Bond Elut solid-phase extraction cartridge and analysed by the definitive GC/MS method.

