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Soil Contamination by Refined Crude Oil Using *Lumbricus terrestris* as Toxicity Indicator at a Petroleum Product Depot, Ibadan, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Refined petroleum products may contain toxic compounds as additives causing environmental pollution, with adverse effects on living organisms. The study assessed the ecotoxicological effects and level of soil contamination of the tank farm area at a petroleum product depot in Ibadan, Nigeria. Surface soils (0-15 cm) and earthworms *(Lumbricus terrestris)* were sampled at four different tank areas; petroleum motor spirit (PMS), regular motor spirit (RMS), automotive gas oil (AGO) and dual purpose kerosene (DPK) as well as control site without prior petrochemical activities. Concentrations of heavy metals and total petroleum hydrocarbons (TPHs) were determined using atomic absorption spectrophotometer (AAS) and gas chromatography mass spectrophotometer (GC-MS). Earthworm's lysosomal membrane stability, a biomarker was also determined using the neutral red retention time (NRRT) assay.
The results showed that soil lead (Pb) and cadmium (Cd) concentrations significantly ($P<.05$) varied from 831.9 to 2055.8 mg/kg and 0.8 to 6.3 mg/kg respectively while Pb and Cd concentrations in

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earthworms varied from 101.3 to 328.8 mg/kg and 0.08 to 0.26 mg/kg respectively. The average NRRT observed at AGO, DPK and RMS tank farm areas were 28.3, 38.3 and 46.7 minutes respectively, implying a possible compromised state of the sampled earthworm's lysosomal membrane. Soil-and earthworm-accumulated Pb significantly correlated with NRRT (r^2=-0.65, P<.05; r^2=-0.94, P<.01 respectively). A naphthalene compound (2.4%) was found in soil samples around the PMS tank area. Generally, soil Pb concentration of the study area exceeded the USEPA permissible concentration (400 mg/kg) and detected naphthalene indicated soil contamination with potential harm to humans and soil organisms. This also, suggests possible importation into Nigeria of petroleum products still containing Pb.

Keywords: Soil contamination; ecotoxicology; lysosomal membrane stability; total petroleum hydrocarbon.

1. INTRODUCTION

Soil contamination from petroleum products remains a major source of environmental problem. This is of public health concern especially in the developing countries of the world.

The ease of access to petroleum products or fuels within cities in Nigeria is enhanced by storage of these products in depots, which are sometimes situated within or around residential housing as a result of increasing urban spread. Altogether, there are about 500 km stretch of pipelines and about 20 oil depots, an industrial facility for the storage of oil and or petrochemical products and from which, they are transported to end users or further storage facilities in Nigeria [1,2].

Environmental pollution has become a global problem affecting both developed and developing countries [3] and it has assumed global proportions since it is a threat to the wellbeing of all life forms including humans. Pollution of the environment by petroleum products is an inevitable consequence of oil production, transportation and distribution activities. Spills or leakages from storage tanks and/or tanker trucks during loading and offloading of petroleum products or washing of oil storage tanks, damage pipelines (old or vandalized) can cause soil environment to be contaminated [4,5]. Many petroleum industries production and processing activities, including crude oil transformation to produce final consumable products such as liquefied petroleum gas (LPG), premium motor spirit (PMS), automotive gas oil (AGO), dual purpose kerosene (DPK), etc, result to the release of heavy metals, among others to the environment such as receiving waters [6].

Metals are persistent in the environment and once it gets into food chain, through plants, animals and water sources leads to biomagnification and bioaccumulation in living cells and tissues [7,8]. The toxicity of crude oil or petroleum products varies widely, depending on their composition, concentration, environmental factors and on the biological state of the organisms at the time of the contamination. Contamination of soils by heavy metals is the most serious environmental problem and has significant implications for human health [9].

According to [10], massive importation of refined petroleum products commenced in 1996 and is still on the increase till date owing to poor state of refineries in the country. Arising from increased number of mushroom companies that flooded the Nigerian petroleum industries, it has become increasingly difficult to regulate the importation of high quality petroleum into the country since more emphasis has been placed on profit making. Consequently the products are imported from some African countries including Algeria and Egypt whose fuel quality is known to still have traces of lead [11]. For this reason, study by [12] showed significantly high lead levels in soils along busy roads relative to less heavily trafficked roads.

Continuous use of leaded gasoline contributed greatly to the number of cases of childhood lead poisoning in Nigeria [13]. Leaded gasoline in Nigeria contains lead in the concentration range of 0.65 to 0.74 g/L, with proposed initiatives to reduce the concentration to 0.15 g/L and finally to zero level [14]. The consequences have been severe environmental problems.

Earthworms are important organisms of the soil eco-system, mainly because of their favourable contributions to soil structure and function [15,16]. Their burrowing and feeding activities contribute notably to increased water infiltration, soil aeration and the stabilization of soil aggregates [17]. These features, among others, have led to the popularity of earthworms as excellent bioindicators of soil pollution [18,19]. These organisms ingest large amounts of soil, thereby being continuously exposed to contaminants through their alimentary surfaces [20], thus can be used as bioindicators of soil quality [17].

Biomarker can be defined as "any substance, structure or process that can be measured in the body and influence or predict the incidence or outcome of disease [21]." It can be classified as markers of exposure, effects and susceptibility [21]. For reliable hazard identification of environmental contaminants/pollutants, knowledge of the effects on different levels of biological organisation is necessary. Lysosomes are ubiquitous membrane-bound intracellular organelles with an acidic interior for degradation and re-cycling of macromolecules delivered by endocytosis, phagocytosis and autophagy [22]. Lysosomes do not only function as a central site for sequestration and accumulation of toxic metals and organic xenobiotics, but they also play a key role in detoxification processes and further excretion of these compounds [23]. The lysosomal membrane stability test evaluates the lysosomal membrane fragility caused by exposure to and destructive perturbation by pollutants. Destabilization of the lysosomal membrane is a response to organic and inorganic toxic compounds that correlates with the degree of stress induced by the contaminant [17]. One method of determining lysosomal membrane stability is by using a neutral red retention assay which is a reliable, dose-dependent and practical technique used in marine [24] and terrestrial systems [25].

Owing to increasing population and city expansions, the demand of petroleum products has increased remarkably with a corresponding increase in the adverse environmental impacts particularly on the soil, consequently, the need to monitor the levels of contamination. With the possibility of environmental pollution, especially of the soil, occurring within this area, the heavy metals and hydrocarbon contamination of the depot area (NNPC tank farms, Ibadan), which has received little attention was studied. Hence, this study was conducted to determine soil contamination by refined crude oil using *Lumbricus terrestris* as toxicity indicator at Nigerian National Petroleum Corporation (NNPC), Apata Ibadan.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted at Nigerian National Petroleum Corporation (NNPC) depot (Fig. 1), Apata Ibadan, Oyo State located between latitude (07° 23° 26.9') and longitude (03º 49º, 02.3') in Ibadan Metropolis, Oyo state Nigeria.

2.2 Sample Collection

Sampling was carried out for two weeks in May, 2014. Representative soil samples (0–15 cm) were collected from three sampling points at 2 m equidistant around each tank farm and were pooled together to derive composite samples. The tanks were categorised into four groups based on their petroleum contents– Petroleum Motor Spirit (PMS), Regular Motor Spirit (RMS), Dual Purpose Kerosene (DPK) and Automotive Gas Oil (AGO). About 20 kg composite soil samples were collected and earthworms were collected by hand picking. These were stored in appropriately labelled containers and transported to the laboratory for further analyses. Samples from the Botanical Garden (Latitude 07º 26'; Longitude 03º 54') University of Ibadan without any history of spillage or flood were also taken using the same procedure as above. Additionally, soil samples for TPH analysis were purposively taken from a heavily polluted tank area and the control site. These were wrapped in aluminium foil and kept in the freezer, to ensure preservation of the volatile compounds, until analysed.

2.3 Laboratory Analysis

All soil samples were air-dried at room temperature and passed through a 2 mm sieve. Sub-sample of 2 mm sieved were crushed and finely ground for metal and organic carbon analyses. The coelomic fluid of earthworm was aspirated for the neutral red retention time assay. Earthworm samples were washed with deionized water, placed in envelopes and oven dried at about 70ºC. Dried earthworm samples were finely ground and stored in polyethylene bags for further analysis.

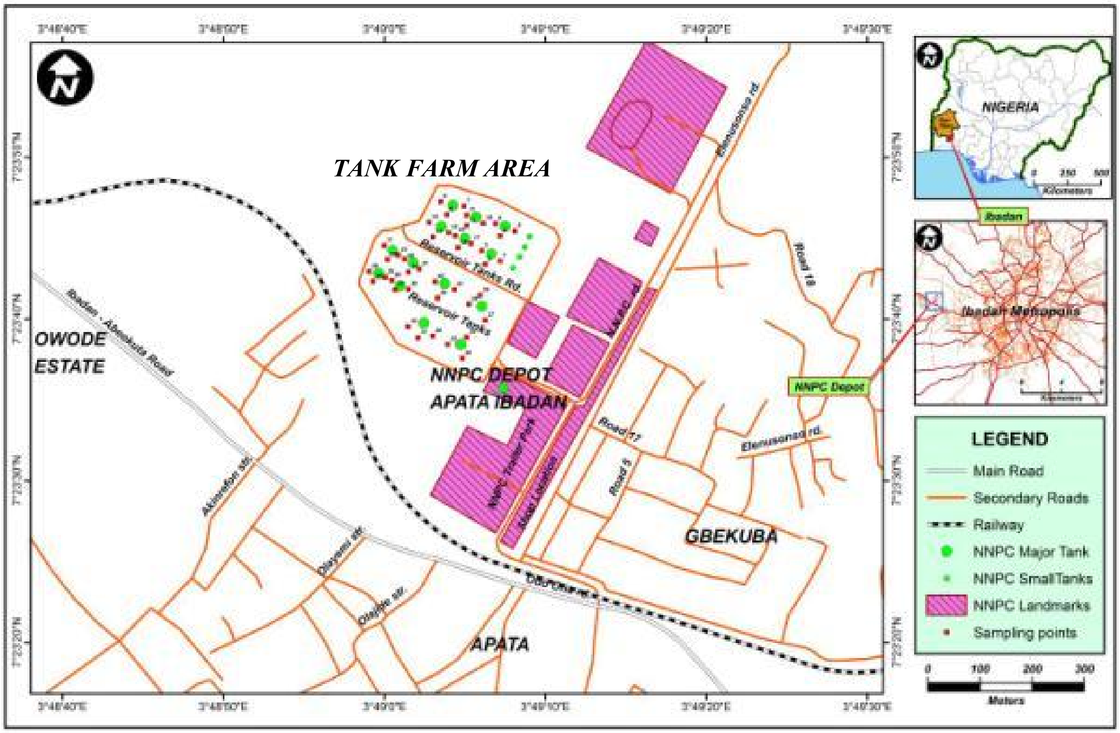

Fig. 1. Map area of the study site

The pH and conductivity were determined on 2 mm sieved air-dried soil at 1:2 soil to water ratio using well calibrated pH (PHS-25, Techmel and Techmel) and conductivity (Jenway 470) meters respectively. Soil temperature was determined in-situ using soil thermometer while soil moisture contents and organic carbon were determined following standard methods [26;27]

Accurately 1.0 g each of the finely ground soil and earthworm samples was transferred into a crucible and 20 ml aqua regia, mixture of concentrated HNO_3 and HCl (ratio 3:1) was added to each sample. The samples were allowed to stand for 20 min and digested on a hot plate in the fume cupboard until the nitric acid brown fumes disappeared. On cooling, digested samples were filtered into 25 ml volumetric flask and made up to the mark with deionised water [28]. The heavy metal contents were determined using atomic absorption spectrophotometer (AAS-Buck Scientific Model 210 VGP).

Total Petroleum Hydrocarbon (TPH) was extracted using the USEPA Ultrasonic Extraction Method 3550B (1996). Briefly, 30 g soil sample was weighed in a conical flask and extraction was done using 30 ml and 20 ml dichloromethane (DCM) respectively inside an ultrasonicator for 10 min each to ensure total extraction of sample constituents. The mixture was then separated by careful filtration into polyethylene terephthalate (PET) bottles which was left open for the extractant (DCM) to evaporate. The extraction was done at room temperature (25°C), with a standard pressure of 760 mmHg. Extracts were analysed using GC-MS Agilent 7890B Gas Chromatograph coupled with MS Agilent technologies 5975 series MSD.

Neutral red retention time assay determination involved insertion of a fine-needled syringe [29] into the coelomic cavity at a site posterior to the clitellum of the earthworm samples and withdrawing of a small volume (20–50 µl) of coelomic fluid with a gentle drawing action [30]. This was placed on a slide, allowed to adhere to the surface for 30 s and 20 µl of 80 µg/ml neutral-red working solution was applied to the slide and covered with a cover-slip. The preparation was scanned under a light microscope (x400 magnification) for 5 min, during which time several fields of view were chosen at random and the number of unstained cells and cells with a stained cytosol (exhibiting dye loss from the lysosomes to the cytosol) were counted. Following each observation period, the slide was returned to a humidity chamber for a further 2 min prior to the next observation. Observation stopped when the number of stained cells exceeded 50% of the total. This time (the midpoint of the 5 minutes time interval of 50%) was taken as the neutral-red retention time.

2.4 Statistical Analysis

The computation was achieved with the use of statistical package for social sciences (SPSS version 20) to determine the mean and standard deviation of the heavy metals and the correlation between the heavy metals and lysosomal membrane stability of the earthworms (P<.05). Analysis of variance (ANOVA) was used to compare the means of all parameters across groups while Tukey's multiple range tests were used to show differences within each parameter.

3. RESULTS AND DISCUSSION

3.1 Physico-chemical Parameters of the Soils

The soil pH at the study site were 6.7, 6.7, 6.9 and 6.9 at the PMS, AGO, RMS and DPK tank areas respectively and were not significantly different from the control site which was 7.3 (Table 1). This shows that the pH of soil within the study area was mildly acidic to mildly alkaline and slightly lower than the value recorded for the control site. The moisture content at the study ranged from 17.3 to 32.5% with the highest (32.5%) measured at the PMS tank while the control site soil contained 7.6%. The high level of moisture content observed at the study site especially around the PMS tank area can be attributed to regular washing of tanks to remove the sediments. Soil conductivities *(μS/cm)* were 5.9, 6.2, 8.9, 9.8 and 31.0 for tank areas PMS, AGO, RMS, DPK and control site respectively. A similar pattern for soil conductivity and pH was observed in the findings of [31]. Organic carbon content ranged from 1.1 to 1.6% for the study site area while the control site had 1.2%.

3.2 Heavy Metal Concentrations in Soil and Earthworms

Soil and earthworm-accumulated heavy metal concentrations are shown in Table 2. The soil Pb concentrations followed the pattern; 831.9<1533.0<1757.0<2055.8 mg/kg at DPK, RMS, AGO and PMS tank areas respectively while the control site had the lowest concentration (43.5 mg/kg). The highest (2055.8 mg/kg) and lowest (831.9 mg/kg) concentrations of soil lead measured at PMS and DPK tank areas, respectively, at the study site differed from the findings of [32], which showed that the metal contents of Nigerian petroleum products vary with viscosity and increase progressively from light to heavy petroleum distillates (Gasoline<Kerosene<Diesel). The soil Pb concentrations were higher than the USEPA permissible limit (400 mg/kg) by magnitudes of 5, 4, 4 and 2 at PMS, RMS, AGO and DPK tank areas respectively, posing threat to both the environment and living organisms. The high Pb level found around the PMS tank area suggests lead additives are still in use to improve the octane rating of petrol in Nigeria, as supported by other studies [33], whose findings showed that petrol in Nigeria still contained lead. Lead (Pb) is a toxic heavy metal, posing health risk to humans and can be absorbed through ingestion by food and water and inhalation [34].

Although, the heavy metal contents of petroleum products from the storage tanks could not be assessed due to company's policy and security reasons at the time of this study. Noteworthy in this study, is the high Pb content measured in soil of the kerosene (DPK) tank area. Kerosene is a popular cooking fuel in most homes in Nigeria, thus, high Pb associated with this product is a serious public health concern. The cadmium concentrations in soil were lower than USEPA limit (70 mg/kg) at the study and control sites (Table 2) thus posing no threat. Generally, heavy metal concentrations at the study site were greater than those from the control site, which might have resulted from spillage of refined oil from tankers and fuel tank washing.

Internal earthworm heavy metal concentrations were assessed to determine the amount of heavy metal available for uptake. At the study site, the highest and lowest earthworm-accumulated Pb (328.8±16.3; 101.3±32.0 mg/kg) and Cd (0.26±0.43; 0.1±0.1 mg/kg) concentrations were at AGO and RMS tank areas respectively. Although, Cd levels were generally low, it was not detected in some earthworms sampled around the tank areas, thus resulting sometimes in higher standard deviation of the means (n=3). There were no earthworms found at the PMS tank areas at the time of sampling which might be due to frequent flooding of the area with gasoline contaminated wastewater commonly released into the environment without treatment.

Additionally, Pb extracted from soil significantly correlated (r=.58; *P<.05*) with Pb in earthworm (Table 4) indicating the Pb originated from the same source.

3.3 Total Petroleum Hydrocarbon (TPH) Content

Table 3 shows the qualitative total petroleum content of the soil samples at the study and control sites respectively. A total of 17, 18, 16 and 12 hydrocarbon compounds were identified at PMS, AGO, DPK tank areas and control sites respectively. Tetracosane was observed to be most abundant at both the AGO (34.6%) and DPK (28.58%) tank areas while 2, 6, 10, 14-tetramethyl-Hexadecane and Heneicosane were most abundant at PMS area (21.1%) and control site (57.4%) respectively. Naphthalene, a toxic polycyclic aromatic hydrocarbon (PAH) was also detected only around the PMS tank area with the implication of environmental pollution especially to the soil ecosystem, an important resource for food production [35,36]. The dominant compound, heneicosane, found at the control site has been known to be one of the important components of *Carthamus tinctorius* flowers essential oil [37]. The possible explanation could be because the control site (Botanical Garden, University of Ibadan) contained diverse vegetative cover comprising grasses, shrubs and trees.

3.4 Earthworms' Neutral Red Retention Time (NRRT)

The neutral red retention time (Fig. 2) was used for the assessment of lysosomal membrane stability of the earthworms (*Lumbricus terrestris*). The average NRRT of *Lumbricus terrestris* at the control site was 58.3 min, compared with those from the study site with the highest (46.7 min) and lowest (28.3 min) NRRT observed at RMS and AGO tank areas, respectively. There was no NRRT value for the PMS tank area because earthworms were not found there. Lesser NRRT for earthworms at the study site compared with the control site indicated exposure to more environmental stress probably from contamination from wash-offs from petroleum product tanks. Also, longer NRRT in the control site of this study corroborates other studies [25] which reported longer NRRTs in *Lumbricus rubellus* at the control site compared with polluted sites.

Table 1. Physico-chemical characteristics of the soils

Parameters	Study site (tank area)				Control site
	PMS	RMS	AGO	DPK	
pH	6.7±0.3[a]	6.9±0.6[a]	6.7±0.2[a]	7.0±0.1[a]	7.3±0.3[a]
Temperature (°C)	22.6±2.1[a]	21.8±1.5[a]	22.9±0.5[a]	22.9±0.8[a]	23.7±0.2[a]
Moisture content (%)	32.5±14.0[c]	27.1±6.4[bc]	17.3±3.2[ab]	17.3±2.3[ab]	7.6±2.1[a]
Conductivity *(µS/cm)*	5.9±3.1[a]	8.9±2.4[a]	6.2±1.9[a]	9.8±2.1[a]	31.0±15.5[b]
OC (%)	1.1±0.6[a]	1.6±0.6[a]	1.1±0.4[a]	1.3±0.3[a]	1.2±0.0[a]
OM (%)	2.0±1.0[a]	2.8±1.0[a]	1.9±0.7[a]	2.2±0.5[a]	2.1±0.1[a]

Means (n=3) followed with different lowercase letters along a row show significant different at P<.05; OC-Organic Carbon; OM - organic matter; PMS - premium motor spirit; RMS – regular motor spirit; AGO-automotive gas oil; DPK- dual purpose kerosene

Table 2. Heavy metal concentrations in soil and earthworms

Heavy metal	Study site (tank area)				Control‡	UPL†
	PMS	RMS	AGO	DPK		
			Soil (mg/kg)			
Pb	2055.8±286.2[a]	1533.0±951.6[ab]	1757.0±362.9[ab]	831.9±513.8[b]	43.5±4.4	400
Cd	6.3±2.2[a]	0.8±0.7[b]	0.8±0.7[b]	2.1±0.7[b]	0.1±0.1	70
			Earthworm (mg/kg)			
Pb	NIL	101.3±32.0[a]	328.8±16.3[b]	160.2±20.9[c]	15.8±19.4	-
Cd	NIL	0.1±0.1[a]	0.3±0.4[a]	0.2±0.3[a]	0.0±0.0	-

Means (n= 3) followed with different lowercase letters along a row (comparison limited to tank area only) show significant different at P <.05; Pb – lead; Cd-cadmium; PMS -premium motor spirit; RMS –regular motor spirit; AGO-automobile gas oil; DPK- dual purpose kerosene; UPL-USEPA permissible limits; ‡ control was generally below permissible limits, hence not compared with other groups; NIL- areas where earthworms were not found

Table 3. Percentage composition (%) of total petroleum hydrocarbon (TPH) in soils around tank and control site

Tank farms						Control	
PMS		AGO		DPK			
Compound	%	Compound	%	Compound	%	Compound	%
Pentamethyl decahydronaphthalene	2.4	Thymol	1.2	3-Eicosene	1.1	1-Nonadecene	3.2
1-bromo- Octadecane	3.0	Benzofuran	0.4	Heptadecyl ester Dichloroacetic acid	2.6	1-Docosene	3.4
photocitral B	2.1	8-Heptadecene	0.4	Oleic Acid	1.1	Octacosyl trifluoroacetate	3.2
Dodecane	5.3	Heptadecane	0.9	Octadecanoic acid	25.2	17-Pentatriacontene	2.1
2-methyl-Decane	17.2	1-Octadecene	0.6	1-Docosene	4.0	1-Iodo-Octadecane	4.8
Nonadecyl pentafluoropropionate	4.8	1-Nonadecene	0.6	2-hexyloctyl- Cyclopentane,	2.7	Octacosane	9.3
2,6,10,14-tetramethyl-Hexadecane	21.1	Hexacosane	0.4	Octadecanal	2.8	Tetracosane	11.3
Cytotetradecane	7.4	Octadecanoic acid	0.5	1-ethenyloxy- Hexadecane	8.5	Pentatriacontane	2.1
E-8-Methyl-7-dodecen-1-ol acetate	5.6	9-Hexacosene	0.6	1-Nonadecene	5.1	Heneicosane	57.4
2-Methyl-3-[(1S,2S)-1,3,3-trimethy l-2-(2-hydroxyethyl) cyclohexyl] tet rahydrofuran	2.4	Z-12-Pentacosene	0.4	Hexacosane	2.7	Tricosane	1.7
Hexacosyl pentafluoropropionate	3.4	1-Bromo-11-iodoundecane	1.3	Nonadecane	8.3	Octatriacontyl pentafluoropropionate	1.6
Tritriacontane	2.5	Tritriacontane	2.9	Triacontane	2.3		
Hexacosane	4.9	Octacosane	13.1	Heneicosane	1.7		
Nonacosane	6.7	Triacontane	13.0	Tetracosane	28.6		
Docosane	4.0	Tetracosane	34.6	17-Pentatriacontene	1.7		
Cyclotriacontane	3.4	1-chloro Heptacosane	18.0	1-Hexacosene	2.1		
Hexatriacontane	3.6	5-Nitrothiophene-2-carboxylic acid	0.8				
		Nonacosane	10.1				
Total	100		100		100		100

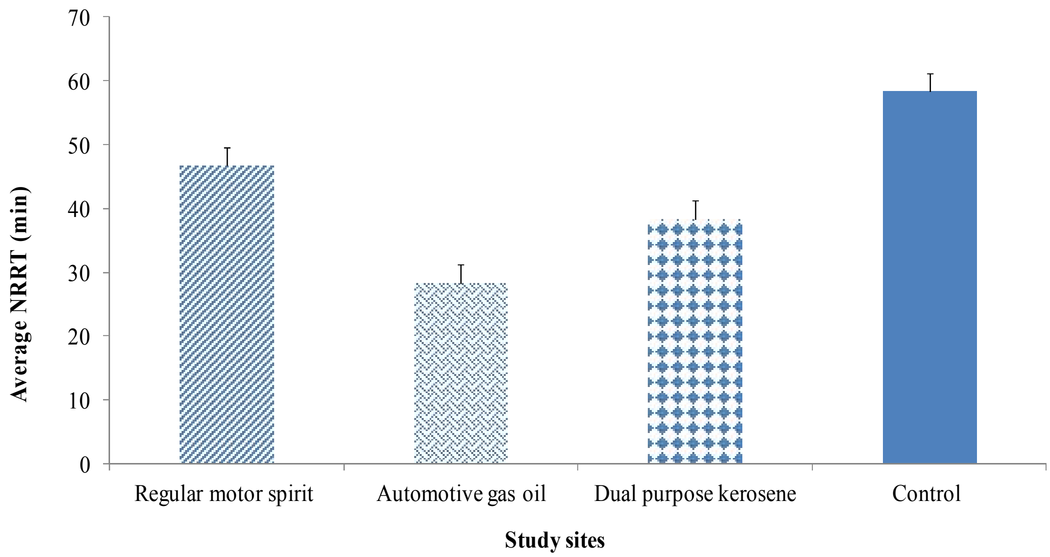

Fig. 2. Average NRRT (min) of earthworms at the study and control sites

Table 4. Correlation between heavy metal concentration and earthworm's neutral red retention time (NRRT)

	Soil Pb	Earthworm Pb	Soil Cd	Earthworm Cd	NRRT (min)
Soil Pb	1	.58*	.46	.37	-.65*
Earthworm Pb		1	.26	.40	-.94**
Soil Cd			1	.05	-.51
Earthworm Cd				1	-.34
NRRT					1

**. Correlation is significant at the .05 level (2-tailed). **. Correlation is significant at the .01 level (2-tailed).*
Pb – lead; Cd – cadmium; NRRT – neutral red retention time

Both soil (r=-0.65, *P*<.05) and earthworm-accumulated (r=-0.94, *P*<.01) lead concentrations show strong negative correlation with the earthworm's NRRT (Table 4 above). This implies that increase in Pb concentrations might have altered the integrity of the lysosomal membranes of the earthworms leading to a decrease in the time at which the cytosol was stained with neutral red. The results correspond with the work of [38] who reported that reduced values for the biomarker neutral red retention time (NRRT) could be explained mainly by high metal concentrations in the soil and the resulting high internal metal concentrations in the earthworms. On the other hand, Cd concentrations in both soil and earthworm showed no significant relationship with NRRT. This could be due to the generally low Cd concentrations at the study site.

4. CONCLUSION

The main purpose of this research was to determine the contamination level at a petroleum depot in Ibadan, Oyo state. The study site was found to be contaminated with lead (>USEPA permissible limits) which poses a serious adverse effect to humans and environment. The study site was also contaminated with naphthalene, a known toxic polycyclic aromatic hydrocarbon (PAH) in some areas. Furthermore, the heavy metals investigated in this study were bioavailable due to high accumulation in earthworm that led to a compromised lysosomal membrane stability of the earthworm coeloms as revealed by the lower NRRT. This might affect the survival of not only the earthworm but other soil biota. The study also, suggests refined petroleum products imported into Nigeria still contains some Pb additives.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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