UNDER PEER REVIEW

SOIL CONTAMINATION BY REFINED CRUDE OIL USING *Lumbricus terrestris* AS TOXICITY INDICATOR AT A PETROLEUM PRODUCT DEPOT, IBADAN, NIGERIA

Abstract

Refined petroleum products may contain toxic compounds as additives with the implication of environmental pollution and 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 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) respectively. Earthworm's lysosomal membrane stability, a biomarker was also determined using neutral red retention time (NRRT) assay.

The results showed that soil lead (Pb) and cadmium (Cd) concentrations significantly ($P = .05$) varied from 832.0 to 2055.83 mg/kg and 0.83 to 6.25 mg/kg respectively while Pb and Cd concentrations in earthworms varied from 101.25 to 328.9 mg/kg and 0.083 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 ($r^2 = -0.65$, $P = .05$; $r^2 = -0.94$, $P \leq .01$ respectively). About 2.4% naphthalene compound, a toxic compound was found in soil samples around the PMS tank area. Generally, soil Pb concentration of the study area exceeded USEPA permissible concentration (400 mg/kg), and the presence of 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

Key words: Soil contamination, Ecotoxicology, Lysosomal Membrane Stability, Total Petroleum Hydrocarbon

1. INTRODUCTION

UNDER PEER REVIEW

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 concern 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 of petroleum industries production and processing activities, including the transformation of crude oil 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 parlous 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 place on profit making. Consequently the products are imported from some African countries including Algeria and Egypt whose fuel quality is

UNDER PEER REVIEW

modicum and known to still have traces of lead [11]. For this reason, study by [12] showed a 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. Upon the combustion of the leaded petrol in the engine, the organic lead is oxidized to lead oxide.

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.” 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, andautophagy [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. The lysosomal membrane stability test evaluates the lysosomal membrane fragility caused by exposure to pollutants. It has a great potential as destabilization of the lysosomal membrane is a non-specific phenomenon that appears as a response to organic and inorganic toxic compounds and 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 [23] and terrestrial systems [24].

Owing to increasing population and city expansions, the demand of petroleum products has increased with a corresponding increase in the adverse environmental impacts particularly on

UNDER PEER REVIEW

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 METHOD

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')N and longitude (03° 49°, 02.3')E in Ibadan Metropolis, Oyo state Nigeria.

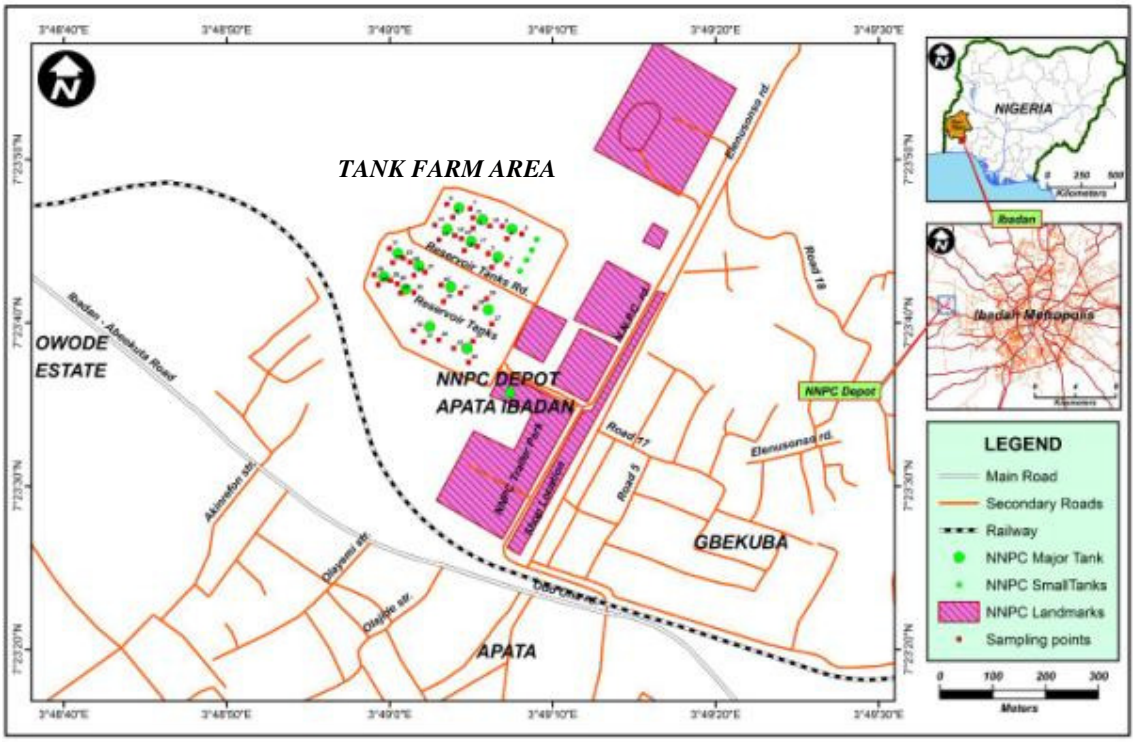

Fig.1: Map area of the study site

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

UNDER PEER REVIEW

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, 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 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 70°C. Dried earthworm samples were finely ground and stored in polythene bags for further analysis.

The pH and conductivity were determined on 2mm 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 [25; 26]

Accurately 1.0g each of the finely ground soil and earthworm samples was transferred into a crucible and 20ml aqua regia, mixture of concentrated $HN0_3$ and HCl (ratio 3:1) was added to each sample. The samples were allowed to stand for 20min and digested on a hot plate the fume cupboard until the nitric acid brown fumes disappeared. On cooling, digested samples were filtered into 25ml volumetric flask and made up to the mark with deionised water [27]. 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, 30g soil sample was weighed in a conical flask and extraction was done using 30ml and 20ml dichloromethane (DCM) respectively inside an ultrasonicator

UNDER PEER REVIEW

for 10min 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 [28] into the coelomic cavity at a site posterior to the clitellum of the earthworm samples and withdrawing of a small volume (20–50µl) of coelomic fluid with a gentle drawing action [29]. 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 (95% confidence level). 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 basic

UNDER PEER REVIEW

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 conductivity *(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. The same observed trend pattern followed by soil conductivity and pH is in accordance with the findings of [30]. Organic carbon content ranged from 1.1 to 1.6% for the study site area while the control site had 1.2%.

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 (%)	1.95±1.0[a]	2.8±1.0[a]	1.9±0.7[a]	2.2±0.5[a]	2.1±0.1[a]

Means followed with different lowercase letters 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

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 disagree with the findings of [31] that the metal contents of Nigerian petroleum products vary with the viscosity and

UNDER PEER REVIEW

increase on proceeding from light to heavy petroleum distillates (Gasoline < Kerosene < Diesel). The concentrations of Pb were all greater than USEPA permissible limit (400 mg/kg) approximately by factors of five, four, four and two at PMS, RMS, AGO and DPK tank areas of the study site respectively, posing threat to both the environment and living organisms. The high Pb level found around the PMS tank area is an indication that lead additives are still in use to improve the octane rating of petrol in Nigeria and is in accordance with study of [32] whose findings showed that petrol in Nigeria still contained lead. Despite the lower concentration of Pb (831.9 mg/kg) measured around the DPK tank area, it was still higher than the USEPA limit (400 mg/kg) by a factor of two. Pb is known to be the most significant toxin of the heavy metals, and the inorganic forms are absorbed through ingestion by food and water, and inhalation [33].This poses risk to the health of the public as kerosene is used as fuel for cooking in most homes especially in Nigeria. However, heavy metal contents of petroleum products from the storage tanks of study site could not be assessed due to company's policy and security reasons. 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 be as a result of spillage of refined oil from tankers, damaged pipes 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 and Cd concentrations were at AGO and RMS tank areas respectively, Pb (328.9 ± 16.3, 101.3 ± 32.0 mg/kg); Cd (0.26 ± 0.43, 0.1 ± 0.1 mg/kg). 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. Despite the pH of the study area which was between mild acidity and mild basicity and an insignificant difference in organic carbon the control site, a high bioavailability of the heavy metal concentrations was observed at the study site with the approximate ratio of soil to earthworm-accumulated heavy metal concentrations ranging from five to fifteen for Pb and three to twelve for Cd.

Table 2: Heavy metal concentrations in soil and earthworms

Heavy	Study site (tank area)	Control‡	UPL†

UNDER PEER REVIEW

metal	PMS	RMS	AGO	DPK		
			Soil (mg/kg)			
Pb	2055.8±286.2[a]	1533.0±951.61[ab]	1757.0±362.9[ab]	831.9±513.8[b]	43.5±4.4	400
Cd	6.25±2.17[a]	0.83±0.72[b]	0.83±0.72[b]	2.08±0.72[b]	0.083±0.14	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 followed with different lowercase letters 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

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 site 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 [34, 35]. 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 [36]. The possible explanation could be because the control site is the Botanical Garden in University of Ibadan containing abundant vegetative cover from preserved plant materials.

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

Tank farm area							
PMS		AGO		DPK		CONTROL	
Compound name	%	Compound name	%	Compound name	%	Compound name	%
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)	2.4	Z-12-Pentacosene	0.4	Hexacosane	2.7	Tricosane	1.7

UNDER PEER REVIEW

cyclohexyl] tet rahydrofuran							
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

239

UNDER PEER REVIEW

UNDER PEER REVIEW

3.4 Earthworms' Neutral Red Retention Time (NRRT)

The neutral red retention time (Fig. 2) used for the assessment of lysosomal membrane stability of the earthworms (*Lumbricus terrestris*). The result shows that the average NRRT of *Lumbricus terrestris* maintained in the control site (58.3min), a relatively less polluted site, were longer than those from the study site with the highest (46.7mins) and lowest (28.3mins) NRRT observed at RMS and AGO tank areas respectively. There was no NRRT value around the PMS tank area because earthworms were not found there. This could be due to the fact that the earthworms at the study site were exposed to more environmental stress as a result of frequent and prolonged flooding of the area with wash-offs from tanks compared to those at the control site . In a similar field experiment with *Lumbricus rubellus* on a sandy soil performed by [24], longer NRRTs at the control site than polluted sites was observed

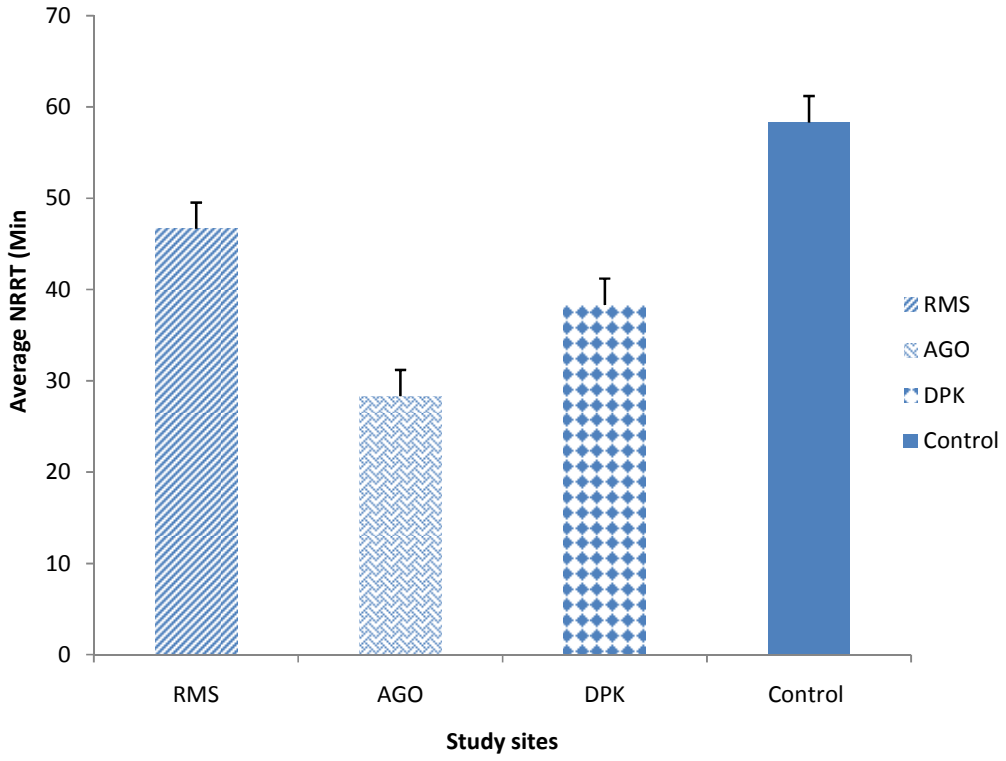

Fig. 2: Average NRRT (min) of earthworms at the study and control sites

UNDER PEER REVIEW

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 5). This implies that increase in Pb concentrations in both conditions 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 [37] 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. Cd concentrations in both soil and earthworm show no significant relationship with NRRT. This could be due to a generally low Cd concentration at the study site. Linear association between soil and earthworm-accumulated lead and NRRT can be found in Figs. 3 and 4 respectively.

Table 5: 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**	*.18*	*.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*

UNDER PEER REVIEW

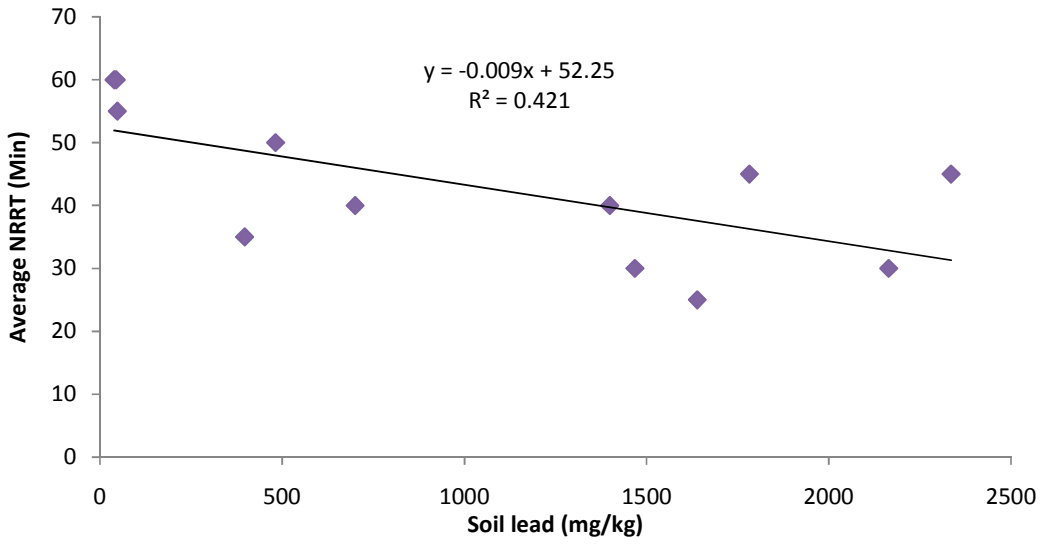

Fig. 3: Relationship between NRRT and soil lead concentration

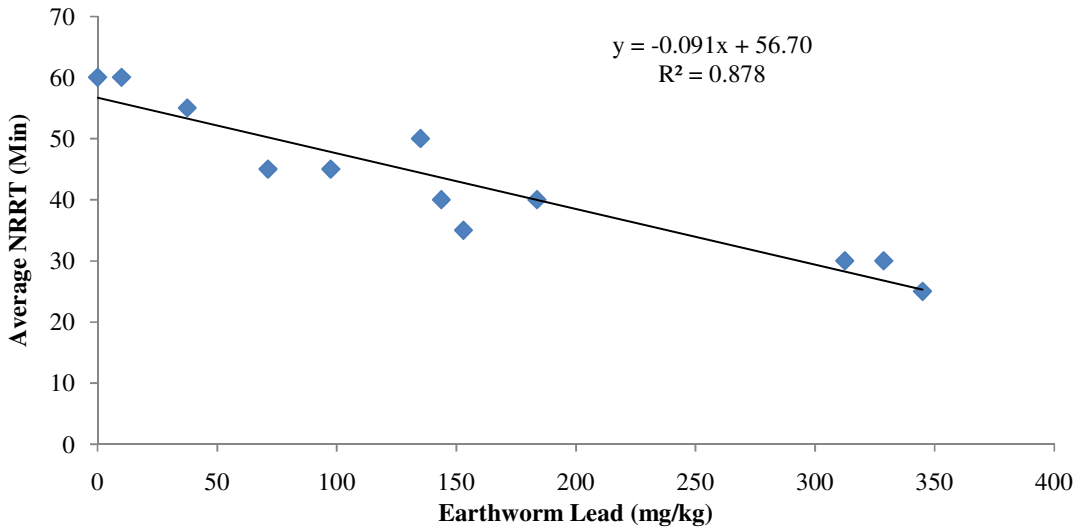

Fig. 4: Relationship between earthworm-accumulated lead and NRRT

UNDER PEER REVIEW

4 CONCLUSIONS

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 (Pb) which poses a serious adverse effect to humans and environment since all Pb concentrations were significantly higher than the USEPA permissible limits. The study site was also contaminated with naphthalene, a known toxic polycyclic aromatic hydrocarbon (PAH) in some areas. The heavy metals investigated in this study were bioavailable due to its high accumulation in earthworm that led to a compromised lysosomal membrane stability of the earthworm coeloms, which might affect the survival of not only the earthworm but other soil biota. Policies that will ensure proper scrutiny of all imported petroleum products and certify the products lead free should be enforced

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UNDER PEER REVIEW

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UNDER PEER REVIEW

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UNDER PEER REVIEW

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