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ECOTOXICOLOGICAL EFFECTS OF DISCHARGING WASTE ENGINE OIL ON SOIL BIOLOGICAL SENTINELS

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ABSTRACT
The discharge of waste engine oil (WEO) into gutters, water drains, open vacant plots and farms is a common practice in Nigeria especially by motor mechanics. Much of this oil is poured into the soil and its presence is usually harmful to soil living forms. This study examined the ecotoxicological effects of discharging waste engine oil (WEO) on soil biological sentinels. The soil samples were obtained from three (3) different sites (0-1 5cm^2 depth) and merged together to form a composite soil. *Nitrobacter* spp. was isolated using heterotrophic *nitrobacter* medium. Acute and chronic, toxicity tests for *Nitrobacter* spp. and *Apporectoda alonga* (earthworms) were carried out. The physicochemical parameters of the composite soil contaminated with different concentrations of WEO were also determined. Analyses of the samples indicated that the various concentrations of WEO (50%, 100% and 200%) in liquid medium containing *Nitrobacter spp.* were increasingly acidic with a corresponding pH of 5.1 2, 4.80 and 4.56. Dissolved Oxygen (DO) was drastically reduced from 9.20 in the control sample to 4.85 at 200% concentration. With increasing concentrations of WEO, there was a gradual decline in total *Nitrobacter* count in the soil from 1.7 x 10^1cfu/g in the control sample to 0.5x10^1cfu/g at 200% concentration. The percentage inhibition on *Nitrobacter* increased hourly as WEO concentrations increased from 3% inhibition (1st hr) to 13% inhibition (3rd hr), signifying the lethality of WEO on soil microbial activities. Nitrite concentration in the soil decreased across the various times analyzed. Chromium, Cadmium. Nickel, Copper and Iron values decreased weekly across all concentrations (50%, 100% and 200%). In the 28 days span, growth rate of earthworms *(A. longa)* in the soil was drastically reduced as concentrations of WEO increased. At 50% concentration of WEO, 0% death rate was recorded in the first week. However, by the 3rd and 4th weeks, a maximum of 100% death rate respectively was recorded at the highest concentration of WEO; thus highlighting the contributions of WEO to the toxicity and death of free living organisms in the soil.

Keywords: waste engine oil, soil biological sentinels, effects.

INTRODUCTION
By far the largest source of waste engine oil (WEO) in development countries are engine oils from motor vehicles, combustion engines and gear boxes. Apart from this minor amount orginate from hydraulic systems, transformers and other diverse industrial application. As at 1996, Nigeria accounted for about 364,166,000 liters of lubricating oil annually. The majority of WEO is generated in small quantities at a great number of places, eg garages , small workshops and private premises.

Engines or lubricating oils are viscous liquids that are used for lubricating moving parts of engines and machines. These oils are derived from petroleum base feedstock which consists mainly of complex mixtures of hydrocarbon molecules (Udonne, 2011). After undergoing several production processes, additives are usually incorporated to boost some of the oil's properties like viscosity, thermal and oxidative stability, etc (Dauda and Obi, 2000), These additives when exposed to the atmosphere have toxic effects to human, animals and microbes. Lubricating oils help to protect

rubbing surfaces, reduce friction between moving and connecting parts, elimate buildup temperatures on the moving surfaces and keep the engine clean. (Ogbeide, 2011).

The relative large amount of hydrocarbons, highly toxic polycyclic aromatic hydrocarbons (PAH) and heavy metals in waste engine oil are usually below detection (Wang et al., 2000) and generally not given attention (ATSDR, 1997). These heavy metals may be retained in the soils in the form of oxides, hydroxides, carbonates, exchangeable cations and/or bound to organic matter in the soil (Youg *et al.*, 1992), and this strongly inhibits carbon mineralization, primary production, nitrogen transformations and mineralization. The PAHs have been known to impair chemoreceptor functions in the aquatic lives, hence leading to extinction of some species and this may result in cancers and other genetic malfunctioning in man and other higher animals as a results of its bioaccumulation along the food chain (Atuanya and Tudararo-Aherobo, 2015). Nevertheless, this is dependent on the local environmental conditions and on the kind of soil constituents present in the soil-water system.

WEO also produces some drastic changes in the microbial communities that participate in the nitrogen cycle as well as the metabolic activities of the aerobic microbes that oxidize hydrocarbons. Soil contaminated with these pollutants commonly reduces the diversity or evenness of soil bacteria (Van Loosdrecht *et al.*, 2007). A primary effect of the discharge of WEO in the soil is a very important changes in the number and amount of microbes promoting an increase in microorganisms able to biodegrade these substrates. Oxygen access is hampered, a significant number of microzones arise in soil aggregated which have an oxygen deficiency. Denitrifying microbes are also increased due to stimulation by increase in organic substrates as well as the low oxidation reduction potential (Ismailov, 1981). Its presence inhibits plant development and metal content of surviving plants is increase hence destroying soil fertility (Nwachukwu *et al.*, 1999).

Toxicity tests are based on the understanding that under a set of given test conditions, there is a measurable and progressive relationship between dose and effect. Toxicity test measure an endpoint or groups of endpoints (eg. Mortality, reproductive capacity, growth rate) over a range of known concentration of a chemical. The results are then analyzed to determine the nature of the dose response relationship. The contaminant, concentration, soil, test species (fish, microorganism, and earthworm) and test condition chosen should reflect the purpose of the test and use of the results. Toxicologists based their selection of test (sentinels) on several factors; sensitivity to a variety of ecosystem and ease of maintenance and culture under laboratory conditions (Atuanya and Tudararo-Aherobo, 2015).

The genus *Nitrobacter* belong to a variety of nitrite-oxidizing bacterial which are responsible for the first and second steps of the nitrification process (oxidation of ammonia to nitrite and nitrite to nitrate). The bacteria were used to study the acute toxicity test of WEO on nitrogen transformation activities in the soil. Inhibition of these steps under uncontrolled conditions may lead to accumulation of ammonium and nitrite nitrogen which is toxic (Dokaniakis *et al.*, 2005). Sentinel species are biological monitor that accumulate a pollutant in their tissues without significant adverse effects primarily used to measure the amount of pollutant that is biologically available. For the purpose of this research, earthworm (*Aporrectoda longa*) were used. This study examined the ecotoxicological effects of discharging waste engine oil on soil biological sentinels.

MATERIALS AND METHODS

Sample Collection

Soil for this study was obtained from a composite sample of top soil (0-15cm*"depth) and accurately measured into perforated poly bags. The soil was sieved with a mesh to separate the non-

degradable materials out of the soil in order to keep it free of unwanted materials which could disturb the proper functioning of soil micro-organisms, proper stretch of the roots and proper organization of the soil. The sieved soil was air-dried^ before weighing in order to avoid apparent weight of soil which could be brought about by the weight of water. By this procedure, an accurate weight of soil was obtained in relation to the engine oil used. Waste engine oil was obtained as pooled WEO from heavy-duty vehicles from an auto mechanic's workshop.

Source of Earthworms

Earthworms *(Aporrectoda longa)* commonly found in various parts of Nigeria were collected according to the method described by Terhivuo *et al.* (1994) and Spiegel (2002). They were collected by digging and hand sorting from surface litters and taken to the laboratory for identification. They were then washed free of adhering soil particles and left on moist filter paper for voiding. Earthworms were selected based on their maturity (shown by presence of clitellum) liveliness and active response when the anterior segment is prodded).

Sample Preparation

The experiment was laid out as a completely randomized block design. Three; treatments of WEO (5ml 10ml, and 20ml) were applied to a-constant level of 4kg of soil samples. The waste engine oil was measured with the measuring cylinder grade up to 100± 1ml. A geometric series of three concentration were used and two replicates for both treatments and control were used.

The test was carried out in the dark at 25±20^{c}C.The contents of the samples were mixed thoroughly and covered with perforated polythene to prevent excessive evaporation of water and volatile fractions. The duration of the study was 28days. Composite soil sampling was carried out on days 7 and 28 and analysis was carried out for some physicochemical parameters; pH, THC, total organic carbon, PAHs, nitrate and ammonia. Microbiological parameters included enumeration of total heterotrophic bacteria and *Nitrobacter sp.* count using the DSMZ heterotrophic nitrobacter medium.

Isolation of *Nitrobacter spp.*

Nitrobacter sp were isolated from the soil samples using the method employed by Okpokwasili and Odokuma (1996) and Colwell and Zambrushski (1972). The *Nitrobacter* sp. bacteria was also obtained and isolated using (DSMZ heterotrophic nitrobacter medium isolates that were grayish, mucoid/ilat, Gram negative, pear shaped and aerobic were selected according to the scheme of Colwell and Zambrushki (1972) and Okpokwasili and Odokuma (1996). Subculture were made into slants of DSMZ - nitrobacter agar and stored at 4°C until required for use.

Acute Toxicity of WEO on the bacteria *(Nitrobacter sp.)*

Three geometric concentrations were used. The methods of Duffus (1980), Wang (1984) and APHA (1998) were adapted with some modifications. A fresh dilution and culture was made from the *Nitrobacter sp.* slant. A loopful of the bacteria was collected from the slant and dislodged in 20ml peptone water and allowed to stand for a few hours at 30°C.(The stock culture prepared by inoculating 180ml of sterilized peptone water with 20ml of the activated culture. The test concentrations were prepared and sterilized at 121°C for 15mins. Concentrations of WEO used were 5ml, 10ml and 20ml. One hundred milliliters (100ml) of each test concentration. was then put into a 250ml conical flask and sterilized at 121"C for 15 mins On cooling, 10ml of the cell suspension was added to each flask containing the different WEO concentrations and control (sterile dilution water). This was done in duplicate. The flasks were shaken thoroughly and incubated 30± 2"C to determine the number of viable cells. (0.1 ml of each test concentration and |he control were collected from the (test solution): and

dispersed onto the surface of prepared -DSMZ nitrobacter agar plate and incubated at 30°C for 24h. Viable cells were counted and recorded.
The EC_{50} was determined using the probit method of analysis (Finney, 1978). The following parameters were determined on the test solutions; pH), conductivity, dissolved oxygen, TDS, ammonia, alkalinity and sulphide. The methods used were adapted from APHA (1998).

For the pH, a commercial pH meter was used and read to 0.05 pH units. It was standardized and calibrated according to the manufacturer's instructions. Conductivity was measured between two (spatially fixed and chemically inert electrodes. The conductance of a solution G is inversely proportional to the distance between the electrode surface area, A, cm^2 and inversely proportional to the distance between the electrodes, L, cm. A commercial dissolved oxygen meter was used and calibrated according to manufacturer's instructions. A mixture of the samples were filtered through a standard glass fiber filter with the filtrate evaporated to dryness and dried to a constant weight at 180°C. The increase in weight dish represents the Total Dissolved Solids. Alkalinity was determined based on the end point of pH used. Total ammonia was analysed using the Kjeldahl method while sulphide analyses were carried out by colorimetric procedures.

Chronic Toxicity (sub lethal) effects of WEO on nitrogen transformation activity in the soil
This was carried out using the OECD TG 216 (2000) test method. The effect of WEO on the nitrifying bacteria was determined and the test used to determine long term chronic effects of WEO to the process of nitrogen transformation activity in the soil. Soil for this study was obtained from a composite sample of top soil (0-15cm depth) and accurately measured into perforated poly bags. The soil was dried, sieved and treated with three concentrations (200, 100, and 50ml/kg) of WEO within the course of 28days. Treated control samples were extracted and analyzed for ammonia, nitrate and *Nitrobacter sp.* counts. The rate of ammonia nitrate formation in treated soil was compared to the rate in the controls and the percentage deviation of the treated from control was calculated. Enumeration of *Nitrobacter sp.* was also done to correlate the microbial growth with the transformed nitrogen. Results were analyzed using a regression model (ANOVA) and the median effective and median lethal concentrations (LC_{50}) were calculated. All analyses were done by ASTM method. The control contained only the soil, a geometric series of three concentrations were used. Two replicates for both treatments and control were used.

***Nitrobacter* acute toxicity test**
Ninety milliliters OF the test soil was put in a 250ml volumetric flask. Ten millitres 10mls of the bacteria standard inoculum was aseptically treated. Nitrite concentration was determined and plates of the Winogradsky media were immediately inoculated by spread plate techniques (Okpokwasili and Odokuma, 1996). This was followed by nitrite determinations and spread plate inoculation from the various soil concentrations after Ih, 2h and 3h. Plates were incubated at room temperature for 24H. The percentage nitrite inhibitions of *Nitrobacter sp* were determined using the formula below; (Grunditz and Dalhammar, 2001):

$$\text{Inhibition (\%)} = \frac{C_{ref} - C_{sample}}{C_{ref}} \times 100$$

Where C_{ref} is the concentration of nitrate formed in control, $C_{sample.}$.

Chronic toxicity effects of WEO on the growth and survival of earthworm *(A. longa)*
The earthworms were obtained identified and maintained in the laboratory using the procedures described in ASTM standard E2172 - 01 (ASTM, 2001). The selected worms were acclimatized for

1-7 days in the soil from their habitat. They were fed with cellulose and test conditions used were: temperature 20±2^{0}C, light-dark circles 16h and 8h. They possessed the following characteristics; (red-violet colour, anterior black segment, a prolobous postonium, 8 -14cm in length and bar-shaped tubercular. Three concentrations of WEO (50, 100 and 200 ml/kg) were prepared for the test, ,4kg of soil was mixed with the various test concentrations and 1g of cellulose. These were manually homogenized and distilled water was added. The control (blank) containing only cellulose water and soil was also prepared. Test samples were duplicated per concentration and prior to use for tests, earthworms were placed on damp filter papers. Three earthworms selected were placed on the surface of the control and test soil samples and then allowed to ingest and burrow in the test medium. Distribution of individual earthworm were, randomized. The test medium and control were analyzed for pH, TPH content, TOC, metals (chromium, cadmium, nickel, copper and iron) at the_ start of the experiment and weekly for 28 days
Mortality was the primary criterion for evaluation of the toxicity of the sentinels. The earthworms in the test and controls were observed weekly for 28 days and the number alive were recorded and the dead, removed. Symptoms were also recorded, adverse effects noted and recorded. The sub- lethal effects and growth data were used to determine the EC_{50} using the probit software. ANOVA was used to test for significant differences between treatment means and the control. At the end of the test, observations were made such as motility, morphology, light sensitivity and physical qualities and documented to provide indication of toxic response.

Statistical Analysis
Results were presented as mean ± standard error. One way ANOVA was used to find significant differences between means of various treatments and control.

RESULTS AND DISCUSSION
Spills occur at all stages of production, transportation and handling of the product. This could be as a result of pipeline rupture, accidents and dumping of waste engine oil (Oyibo and Agboola, 1983). The existing mode of indiscriminate disposal of this waste oil increases pollution incidents in the environment. Soil and surface water contamination by waste engine oil is a common occurrence in most developing countries. This has been shown to have harmful effects on the environment and human beings at large (Abioye *et al.*, 2012).

The study evaluated the ecotoxicological effects of discharging waste engine oil (WEO) on soil biological sentinels *(Nitrobacter* sp. and Earthworms). The pH of the various concentrations of liquid medium containing *Nitrobacter* (50%, 100% and 200%) was found to be 5.12, 4. 80 and 4. 56 respectively (Table 1). This is in consonance with the report of Odjegba and Atebe (2007). The gradual increase in acidity could be due to the fact that engine oil picks up a number of impurities and additional components from engines (Hamad *et al*, 2005) like metal particles and other compounds of barium, sulphur, water, dirt and burnt carbon. Some of these metals in WEO can dissolve in water and move through the soil easily and may be found in surface water and ground water. This is in agreement with the report of Udonne and Onwuma (2014) who observed a slight decrease in the pH from *6.5* to 6.0 in the presence of WEO. The significant decrease in the final pH of oil treated soil could also be related to oil degradation process by soil microorganisms, making carbon available for chemical reactions in the presence of water that could lead to a reduction in pH. The conductivity also increased correspondingly with increased concentration of WEO from 773 to 796 and 952. A drastic reduction in dissolved oxygen (DO) was also observed. It decreased from 9.20 in the control sample to 6.06 at 50% concentration and then 4.85% at 200% concentration. (Table 1). This is in agreement with the report of Rafael (1989) who stated that WEO fills the pores between the soil particles "and hampers oxygen access; a significant number of micro/ones arise in the soil aggregates which have an oxygen deficiency. Same

was observed for total dissolved solids (TDS). Alkalinity and ammonia content increased with increasing concentrations of WEO.

The total *nitrobacter* counts from *nitrobacter* medium containing different concentrations of waste engine oil was also determined (Table 2). There was a gradual decrease in the count as the concentration increased, signifying the toxicological effect of WEO on *nitrobacter* population. At 50% concentration, a count of $0.09x10^2$ cfu/ml was recorded and $0.05x10^2$ cfu/ml at 200% concentration.

The effects of WEO on nitrogen transformation activity in the soil was also determined (Table 3). Ammonia content decreased gradually as the concentration of WEO increased with the highest value (0.026) recorded at 50% WEO, then 0.022 (100%) and 0.021 (200%). Nitrate value (0.002) was maintained at the first two concentrations (50% and 100%) and a value of 0.005 was recorded for the 200% concentration. The *Nitrobacter* content in the soil decreased drastically from 1.7×10^1cfu/g for the control to 0.6×10^1cfu/g, followed by 0.2×10^1cfu/g and an increase was observed for the 200% concentration (0.5×10^1cfu/g).

Acute determination of nitrite from soil contaminated with different concentrations of waste engine oil (Table 4) was analysed. The nitrite content of the 50% concentration increased hourly from 0.07 at the 1st hour, 0.09 (2nd hour) and 0.16 (3rd hour). The 100% concentration was at variance with the 50% concentration. Nitrite concentration decreased across the various times analysed. Nitrate serves as an important source of inorganic nitrogen for plants. It also evokes rapid changes in metabolism that include the induction of the synthesis of nitrate assimilatory

Table 1: Changes in physicochemical parameters observed during the growth of *Nitrobacter* spp. in liquid medium contaminated with different concentrations of waste engine oil

Parameters	Cone. (%)				*P-value*	EC_{50}
pH	50	100	200	Control		
	5.12±0.12	4.80±0.10	4.56±0.02	6.72±0.19	0.00	
Conductivity(u.S/cm)	773.00±3.00	796.00±3.00	952.00±2.00	890.50±0.50	0.00	
DO (mg/1)	6.06±0.06	5.80±0.10	4.85±0.15	9.20±0.30	0.00	
TDS (mg/1)	384.45±3.55	396.75±0.25	476.00±1.00	445.70±0.20	0.00	
Alkalinity	9.10±0.10	9.35±0.25	10.36±0.15	9.00±.00	0.00	
Sulphide (mg/1)	.23±0.01	0.26±0.01	0.19±0.010	0.14±0.04	0.00	
Ammonia (mg/1)	.028±0.00	0.04±0.01	0.04±0.01	0.01±0.00	0.00	
Nitrobacter ($xl0^2$cfu/ml)	0.09±1.0	0.07±0.5	0.05±0.5	0.15±0.5	0.00	1.125

N.B: where $p < 0.05$ significant difference is observed, where $p>0.05$ significant difference not observed

Table 2: Effects of WEO on nitrogen transformation activity in the soil

Parameters	Cone. (%)				*p-value*	EC_{50}	LC_{50}
	50	100	200	Control			
Ammonia(mg/l)	0.026±0.001	0.022±0.001	0.021±0.002	0.034±0.002	0.06	0.22	0.89
Nitrate(mg/l)	0.002±0.001	0.002±0.0005	0.0005±0.0005	0.007±0.001	0.015	0.22	0.89
Nitrobacter (xlO'cfu/g)	0.6.±0.1.00	0.2±0.050	0.500±0.500	1.7±2.500	0.003	1.00	2.00

N.B: where p < 0.05 significant difference is observed, where p>0.05 significant difference not observed

Table 3: Acute toxicity determination of nitrite from soil contaminated with different concentrations of waste engine oil

Cone.(%)	1	Time(hr) 2	3
50	0.07±0.001	0.09±0.001	0.16±0.00
100	0.06±0.002	0.03±0.001	0.02±0.010
200	0.04±0.002	0.04±0.003	0.03±0.001
Control	0.03±0.001	0.02±0.002	0.02±0.002
p-value	0.000	0.000	0.000

N.B: where p < 0.05 significant difference is observed, where p>0.05 significant difference not observed

Table 4: Acute toxicity determination of *Nitrobacter spp.* (x 10_1cfu/g) from soil contaminated with different concentrations of waste engine oil

Cone. (%)	1	Time(hr) 2	3
50	3.35±1.50	3.20±1.00	3.20±2.00
100	2.90±1.00	2.35±1.50	1.90±2.00
200	.1.90±2.00	1.55±1.50	1.15±1.50
Control	1.1 Oil. 00	0.60±1.00	0.40±1.00
p-value	0.001	0.001	0.001
%inhibition	3	6	13

N.B: where p < 0.05 significant difference is observed, where p>0.05 significant difference not observed

Table 5: Changes in physicochemical parameters observed during growth and survival of earthworm *(A. longa)* in soil contaminated with different concentrations of waste engine oil (28day period)

Cone.	Weeks	PH	TPH	TOC	Cr(mg/g)	cd(mg/g)	Ni(mg/g)	Cu(mg/g)	Fe(mg/g)
50%	1	4.48±.38	891.69±356.89	7.48±1.132	10.33±1.01	7.02±0.47	24.24±1.64	15.07±1.98	86.05±1.51
	2	4.51±.I	640.13±91.0	6.91±1.	10.01±0.	6.97±0.2	23.18±2.	11.15±1.	80.11±1.22
	3	4.90±,4	510.21±19.1	5.99±2.	9.85±0.	6.10±0.2	21.99±1	9.04±0.7	76.01±2.10
	4	5.10±.1	399.77±12.0	5.10±1.	9.80±0.	5.93±0.2	18.24±2	7.23±1.2	70.21±1.33
	p-value	0.01	0.00	0.00	0.19	0.30	0.06	0.04	0.02
100%	1	4.23±0.	1354.05±462	12.59±1	20.07±1	8.57±1.0	45.66±3	26.30±2.	153.48±2.55
	2	4.38±.9	1018.65±15.	10.22*1	19.99±1.	7.95±0.9	42.32±2	24.01±1.	148.11±1.66
	3	4.85±1	921.33±22	8.99±2.	19.90±0	7.45±1.1	39.22±0	20.22±2.	140.23±0.99
	4	5.23±1	510.44±15.6	7.10±1.	19.10±0	6.77±0.8	35.11±2	18.77±2.	131.12±2.13
	p-value	0.00	0.00	0.01	0.98	0.08	0.02	0.03	0.01
200%	1	3.63±0.	2027.56±138	15.91*2	27.34±1	22.73±2.	77.71±6	59.47±3.	205.86±2.30
	2	3.94±.2	1912.24±132	14.00±1	26.91±0.	21.10±1.	74.88±1	57.11±1.	192.28±1.13
	3	4.10±.2	1600.099±23	12.88±2	26.00±1.	20.99±0.	72.32±1	51.17±2.	185.22±1.21
	4	4.99±.l	1008.211±61	11.10±1	25.51±1	18.77±1.	69.11±2	47.23±1.	179.77±2.14
	p-value	0.00	0.00	0.02	0.07	0.01	0.02	0.01	0.009
Control	1	5.49±0.	9.96±0.21	0.61±0.	0.17±0.0	0.05±0.0	0.65±0.	1.53±0.0	3.88±0.007
	2	5.42±,2	9.7I±0.10	0.60±0.	0.18±0.	0.06±0.0	0.63±0.	1.52±0.	3.89±0.006
	3	5.50±.0	9.83±0.09	0.59±0.	0.17±0.1	0.05±0.0	0.64±0.	1.54±0.0	3.85±0.001
	4	5.48±.2	9.79±0.8	0.58±0.	0.16±0.1	0.05±00	0.66±0.	1.53±0.0	3.79±0.010
	p-value	0.091	0.327	0.40	0.70	0.900	1.02	1.90	0.90

N.B: where $p < 0.05$ significant difference is observed, where $p>0.05$ significant difference not observed

Table 6: Survival rate of earthworm *(A. longa)* in soil contaminated with different concentrations of waste engine oil

	Number of deaths [n(%)]			
Cone (%)	Wkl	Wk2	Wk3	Wk4
50	0(0)	1(33.3)	1(33.3)	2(66.7)
100	0(0)	1(33.3)	2(66.7)	3(100)

200	0(0)	2(66.7)	3(100)	3(100)
Control	0(0)	0(0)	0(0)	0(0)

enzymes and shifting from starch biosynthesis to the production of organic acids to assimilate ammonium (Foyer *et al.*, 2003). Nitrate absorbed by plants must be reduced to ammonium before incorporation into amino acids (Fan *et al.*, 2002).

A primary effect of the dispersion of waste engine oil (WEO) in the soil is a very important change in the amount and species of microorganisms. The spillage of engine oil on soil produces some important changes in the microorganism communities that participate in the nitrogen cycle (Rafael, 1989). Acute determination of *Nitrobacter* ($x10^1$cfu/g) from soil contaminated with different concentrations of waste engine oil was investigated (Table 5). A general decrease was recorded hourly for *Nitrobacter* count as the concentration of WEO increased. The percentage inhibition increased as time increased; 3% inhibition was observed at the 1st hour, 6% (2nd hour) and 13% (3rd hour).

The changes in physicochemical parameters observed during growth and survival of earthworm *{A. longd)* in soil contaminated with different concentrations of waste engine oil (period of 28days) was observed. From the results, a pH range of 4.48-5.10 was recorded from the 1st week to the 4th week. The lowest was recorded in the 1st week (4.48), followed by 4.51 (2nd week), 4.90 (3rd week) and 5.10 (4th week). This is also true for the TPH analysis. Chromium, Cadmium, Nickel, Copper, and Iron values decreased weekly. This pattern was also observed in other WEO concentrations (100% and 200%). The presence of heavy metals in WEO can strongly inhibit primary production, carbon mineralization, nitrogen transformation and mineralization of sulphur and phosphorous. Inhibition of nitrogen transformation is due to a high concentration of metal cations that inhibit microbial activities by inactivating one or more critical enzymes resulting in the formation of an inactive complex between the metal cations and an active enzyme (Wang and Reed, 1984).

Survival rate of earthworm *(A. longa)* in soil contaminated with different concentrations of waste engine oil was also observed. The earthworm *(A. longa)* concentration in the soil decreased rapidly for a long period upon application of the respective concentrations of WEO, as shown in Table 6. At 50% concentration, 0% death was recorded in the first week. However, 33.3% death rate was observed in weeks 2 and 3. The highest was reported in the 4th week (66.7%). At 100% concentration of WEO, the first recorded death occurred in the second week (33.3%), 66.7% in the 3rd week and a maximum of 100% in the 4th week. There was a general increase in death rate when the soil was contaminated with 200% WEO. A rate of 66.7% was recorded for week 2 and a maximum 100% rate was observed in weeks 3 and 4; highlighting the contributions of WEO content to the toxicity and death of free living organisms in the environment. Concentrations of petroleum hydrocarbons determines to a great extent the rate of breakdown hydrocarbons in the environment. The concentration of hydrocarbons can affect biodegradability and toxicity to the degrading organisms and can be inhibitory to the growth and survival of soil biological sentinels.

CONCLUSION

The findings of this research show that the survival and functional niche displayed by biological sentinels in the soil are often influenced by contaminants, notably waste engine oil and if not treated properly before disposal into the environment could pose serious threats to the survival of these organisms. This is of great importance especially in agricultural lands, as the nitrogen fixing activity of *Nitrobacter* spp. was highly affected by waste engine oil. This results in soil remaining unsuitable for crop growth and depending on the degree of contamination, the soil may remain so until the oil is degraded to tolerable

levels. Consequently, the need to encourage the protection of farmlands and its surroundings against indiscriminate disposal of the waste engine oil cannot be overemphasized.

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