Sustinere
Journal of Environment and Sustainability
Volume 8 Number 1 (2024) 91-102

Print ISSN: 2549-1245 Online ISSN: 2549-1253
Website: https://sustinerejes.com E-mail: sustinere.jes@uinsaid.ac.id

RESEARCH PAPER

Ecological risk evaluation of spent engine oil pollution using earthworm and microbial bioassays

Azorji John Paul Nnawuike[1*], Angela Chika Udebuani[2], Nwachukwu Udodi Chibuike[3], Igwe Charles Ekene[4], Dike Kelechi Stanley[5], Nzenwa Peter Odinaka[6], Igbokwe Moses Chukwuebuka[1]

[1]*Dept. of Biological Sciences, Hezekiah University, Umudi, Imo State, Nigeria*
[2]*Dept. of Biotechnology Federal University of Technology, Owerri, Imo State, Nigeria*
[3]*Dept. of Biology, Alvan Ikoku University of Education Owerri, Imo State Nigeria.*
[4]*Dept. of Environmental Resource Management, Michael Okpara University of Agriculture, Abia State, Nigeria*
[5]*Dept. of Microbiology, Imo State University Owerri, Imo State, Nigeria*
[6]*Dept. of Animal & Environmental Biology, Imo State University Owerri, Imo State, Nigeria*

Article history:
Received 1 February 2024 | Accepted 7 April 2024 | Available online 20 May 2024

Abstract. The study aimed to assess the ecotoxicological risk associated with the indiscriminate disposal of spent engine oil on terrestrial ecosystem using earthworm and microbial assays. Soil samples were collected from a depth of 0-20 cm and subjected to standard analytical protocols for analysis. Earthworms (assessed by mortality rate) and microorganisms (evaluated for inhibitory effects) covered a wide range of short-term lethal and sub-lethal endpoints used for risk characterization, analyzed through Probit analysis. The result of acute toxicity assay revealed that microbial absorbance rated depended on the dose and type of organism, ranking in the order: *Acinetobacter* > *Enterobacter* > *Bacillus species* >*Pseudomonas*. Aas oil concentratoin increase, mortality among earthworm was observed. Risk Quotient (RQ) values for *Zea mays, Vigna unguiculata, Glycine max* and earthworm varied from low to very high risk based on estimated Predicted No Effect Concentration (PNEC) values. Microorganisms exhibited differing level of biotolerance to spent engine oil exposure as indicated by respective risk quotient values. Exposure to spent engine oil posed minimal risk to *Pseudomonas sp., Bacillus sp., and Acinetobacter sp.,* with RQ values below unity (< 1). Conversely, *Enterobacter sp.* Showed a high risk with values above unity. Earthworms play a pivotal role in agriculture due to their numerous economic benefots. Soil microorganisms are essential for maintening soil quality by performing vital processes. The antimicrobial properties of spent engine oil on soil may distort microbial activities, potentially inhibiting their growth and leading to alterations in ecological functionality of the soil.

Keywords: Risk; Spent engine oil; Pollution; Earthworm; Microbial; Bioassays

1. Introduction

Ecological risk assessment entails estimating the potential effects of human activities on a natural resource (Nishitha et al., 2022). These activities include various industrial, mining and agricultural practices, pharmaceuticals, and urban runoff (Ozbay et al., 2021). The indiscriminate

*Corresponding author. E-mail: jnazorji17@gmail.com
DOI: https://doi.org/10.22515/sustinere.jes.v8i1.382

disposal of wastes generated from such activities has become a major devastating ecological problem worldwide (Odjegba & Sadiq, 2002; Oh et al., 2014).

The unsustainable use and management of the ecosystems is leading to increased soil degradation and the subsequent loss of vital resource essential for life. Therefore, managing ecosystems for continuity and sustainable health is crucial and requires urgent attention, particularly given the escalating anthropogenic pressure on the soil due to intensive material consumption by humans (Farombi et al., 2013). Recent technological advances have led to an increase in ecological and toxicological issues in both developing and developed countries unregulated release of toxic contaminants to the environment (Ibe et al., 2021). Addressing these challenges is essential to ensure the long-term health and sustainability of our ecosystems and resources.

In recent decades, environmental pollution caused by petroleum and petrochemical products has become a significant global concern (Abioye et al., 2010; Mandri & Lin, 2007). In Nigeria, as in many developing countries, soil pollution resulting from the indiscriminate disposal of petroleum and its related products is considered as a serious ecological issue (Emoyan et al., 2020).

Spent engine oil, characterized by its dark to brown color, has been identifies as harmful to the soil ecosystem (Adedokun & Ataga, 2007). During its use in vehicle, engine oil accumulates additional compounds and dirt from engine wear (Uchendu & Ogwo, 2014). Due to these additives and resulting contamination, spent engine oil is considered more harmful than crude oil (Abioye et al., 2010). Spent engine oil is a complex mixture of various chemicals, including low and high molecular weight aliphatic hydrocarbons (C15-C20), polychlorinated biphenyls, chlorodibenzofurans, lubricate additives, and decomposition of products (Onwuka et al., 2012).

The contamination of terrestrial ecosystems by indicators of spent engine oil such as heavy metals, Total Petroleum Hydrocarbons (TPH), and polycyclic aromatic hydrocarbons (Okafor & Opuene, 2007) poses a significant environmental problem. The environment serve as a direct receptacle for waste products generated within its own space. Moreover, these contaminants have been documented to possess carcinogenic, mutagenic, and teratogenic properties. Therefore, it is crucial to assess the potential ecological risks organisms are exposed to due to co-contamination in ecosystem. Consequently, it is imperative to take the ecological study of these toxicants seriously to mitigate their potential effect and avoid the costly consequences of unchecked contamination. Proper management and remediation strategies are essential to safeguard the health of ecosystems and human populations affected by these pollutants.

2. Material and method

The study was conducted at the Teaching and Research Laboratory of the Department of Biology, Federal University of Technology Owerri, Imo state situated at latitude 5.3866° N and longitude 6.9916° E.

2.1. Terrestrial acute tests on test organisms

OECD (2006) recommends a series of ecotoxicological bioassays to characterize soil matrices. In this study, the selection of specific ecotoxicological bioassay was based on several factors including sensitivity, response time, cost-effectiveness, reproducibility, ease of measurement, and diagnostic capability. Considering these criteria,, two different species representing trophic levels in the ecosystem were chosen: soil microorganisms and earthworms (OECD, 2006). All acute tests on the selected test species (microorganisms and earthworms) followed International Standard Guidelines (OECD, 2006). Terrestrial ecotoxicity tests were carried out using soil contaminated with various concentrations of spent engine oil in accordance with ISO and OECD guidelines (ISO, 2011; OECD, 2004). To assess the impact on the critical life events of organisms, survival and growth inhibition tests were performed for soil invertebrates (microorganisms and earthworms). The effective/lethal concentration (EC50 or LC50) of the contaminant that inhibited 50% growth in each test organisms was estimated and used for risk characterization in each scenario.

2.2. Collection of spent engine oil

The spent engine oil used for the acute toxicity study was sourced from 5-liter containers obtained from various auto mechanic workshops in Imo State. The oil was collected, bulked, and utilized for the study. Information regarding the location, car type, and age of engine, and duration of oil used is presented in Table 1.

Table 1. Details of spent engine oil used for the study

Details	Owerri zone	Okigwe zone	Orlu zone
Car type	Lexus 350 XL	Toyota	Sienna
Age	5	7	3
Brand of oil	AZ	AZ	AZ
Engine type	Petrol	Petrol	Petrol
Duration of usage	4	6	3

2.3. Microbial growth inhibition bioassay

The microbial growth inhibition assay has gained widespread use in routine ecotoxicological assessment of pollutants in water, sediment and soil due to its short duration, reproducibility, and simplicity (USEPA, 2021). This test was performed in accordance with OECD (2006) protocols.

2.4. Collection and processing of soil samples

This soil samples were collected with sterile containers based following the technique described by Phulpoto et al. (2016) with slight modifications. Specifically, 5 grams of each sample collected from each site was dissolved in 50 mL of sterile distilled water and placed in 250 mL flasks. These sample-containing flasks were then incubated at 37°C for 2 hours. After incubation, approximately 5 mL of each sample was used as an inoculum for the enrichment procedure. Microbial load (Total Heterotrophic Bacteria, THB) was determined using a surface spreading technique. Serial dilutions ranging from 10^{-1} to 10^{-10} of both soil samples were prepared on general purpose medium (Nutrient Agar). Triplicate of each dilution were made and then incubated at 28°C for 36 hours before enumeration. The results were expressed as colony-forming units (CFU) pergram of dry soil.

2.5. Pre-isolation sample enrichment with test pollutant

An enrichment technique utilizing of a mineral salt media (MSM) was prepared according to Guo et al. (2011), containing 0.5 g/L $MgSO_4$, 0.2 g/L $CaCl_2$, 13.6 g/L KH_2PO_4, 5 g/L $(NH_4)2.SO_4$, 0.05 g/L $FeSO_4.7H_2O$, 15 g/L Na_2HPO_4. The components were mixed and autoclaving at 121° C for 15 minutes at 15 psi. To begin the experiment, soil suspension (5 mL) was aseptically added into flasks containing 100 mL of prepared MSM broth enriched with spent engine oil at varying concentrations: 0%, 4%, 8%, 12% and 16% (0 mL, 0.4 mL, 0.8 mL, 1.2 mL and 1.6 mL) as a carbon source, maintaining a concentration of 100 ppm. The experimental setups were then incubated at 37°C for 10 days under agitation (150 rpm). During incubation, the absorbance of microbial growth was measured in every 24 hours at 600 nm and recorded.

2.6. Isolation and identification bacteria isolate

Experimental setups that exhibited the highest absorbance values, indicating higher microbial presence, were selected for microbial isolation. Bacteria were initially isolated using nutrient agar, with 0.1 mL of samples plated onto appropriately prepared culture media. The evaluations encompassed presumptive, confirmatory, comprehensive experiments (Bagherzadeh-Namazi et al., 2008). The bacteria isolates were identified based on colonial morphology, surface characteristics, shape, size, margin, and pigmentation observed on nutrient agar medium. Microscopic examinations, including Gram staining, and biochemical tests such as citrate utilization, starch hydrolysis, methyl red, Voges-Proskauer (MR-VP) test, triple sugar iron (TSI) test for lactose, dextrose, sucrose, glucose, and mannitol fermentation, carbohydrate

fermentation, H_2S production, indole production, urease activity, catalase activity, citrate utilization, and sugar fermentation, were carried out following standard procedures. Identification was based on protocols outlines in Bergey's Manual of Systematic Bacteriology.

2.7. Toxicity assay

The biotolerance of bacteria isolates to varying levels of spent engine oil was assessed using UV spectrophotometer at 600 nm. For each test, 1 mL of the respective bacteria was inoculated into 20 mL of spent engine oil broth, which was prepared using enrichment method described above previously, containing different concentration of spent oil as carbon source. Optical density measurements were recorded at 24-hour intervals over a period of 120 hours, beginning with the initial reading at 0 hours.

2.8. Earthworm bioassay test

The soil used in this study was collected from the Botanical Garden of the Federal University of Technology, Owerri, Imo State, at a depth of 0-20 cm. The samples were combined and transported to the laboratory for routine physicochemical analysis (OECD, 2006, 2008). In the laboratory, the soil samples were air-dried and passed through < 2 mm mesh sieve. Approximately 10 g of sieved soil sample was used for physicochemical analysis. The spent engine of oil utilized in this study was collected from three different auto mechanic workshops within Obinze. The soil samples were homogenized in 5 L container to create a composite sample, which was then stored in the laboratory prior to use.

2.9. Collection and acclimatization of earthworms

The earthworms (species: *Nsukkadrillus mbae*) were collected immediately after rainfall while they were crawling around plant debris seeking shelter. The criterion used for collection included maturity (presence of clitellum) and liveliness (active response when anterior segment was prodded). Subsequently, the earthworms were aseptically placed into transparent bottles and transported to the laboratory for acclimatization to laboratory conditions. They were regularly monitored daily to ascertain their health condition (Ekperusi & Aigbodion, 2015). In the laboratory, the earthworms were fed a diet consisting of 10 % finely ground *sphagnum* peat pellets and soil from the collection source to simulate natural conditions under which earthworms thrive, following OECD protocols (OECD, 2006). The species used in this study was authenticated by earthworm taxonomist using the method described by Yamamoto et al. (2012). All earthworms were allowed to acclimatize in the laboratory for five days before use. Clitellate adult earthworms (ISO, 2007, 2011; OECD, 2008) with an average live weight of 0.8 g were selected for the study.

2.10. Test procedure

The test was carried out following stringent bioassay procedures, with modifications based on previous authors (Abara et al., 2020; Ekperusi & Aigbodion, 2015). Prior to the toxicity assay, a range-finding test was performed to determine the concentration that would result in 100% mortality and 0% mortality of the worm (Abara et al., 2020). The experimental setup was arranged in a completely randomized design with three replicates per treatment and monitored continuously for 72 hours. Throughout this time, the criterion for determining death was the lack of response to external stimuli when prodded (OECD, 2006).

2.11. Definitive test (acute toxicity)

The selected worms were exposed to five concentrations (0, 4%, 8%, 12%, and 16%) of spent engine oil. Ten worms were used in each concentration, and this setup was replicated three times for the earthworm acute toxicity assay (Table 2). Rectangular plastic containers measuring 9 cm in breadth, 15 cm in length, and 8 cm in height, equipped with cover lids and clips on both sides of the edges, were obtained from the local market. Each container was filled with 5 kg of sun-dried soil (ISO, 2011). Using a glass beaker, different concentrations of spent engine oil was thoroughly mixed into each of the containers containing 5 kg of soil, which was moistened with

distilled water to reach approximately 70% of the soil's water holding capacity. The containers were left to stabilize for 24 hours (OECD, 2006). Netting material, cut to size, was placed over each container, and the cover lid frame was securely fastened with clips on both sides (Plate 3.4). This setup prevented the escape of earthworms while allowing adequate oxygen flow to the treatments. The containers were placed in the laboratory and monitored twice daily (morning and evening) basis for 7 and 14 days. The mortality rate was recorded based on the inability of the worms to respond when prodded at both the anterior and posterior ends (Akin-Obasola, 2019).

Table 2. Distribution of the earthworms during acute toxicity test

Treatments (%)	T1	T2	T3	T4	T5	Control	Total
Rep I	10	10	10	10	10	10	60
Rep II	10	10	10	10	10	10	60
Rep III	10	10	10	10	10	10	60
Total no of worms	30	30	30	30	30	30	180

2.12. Assessment of burrowing index

The burrowing and survival responses of the exposed earthworms to soil contamination at various concentrations were evaluated using the protocol outlined by the Organization for Economic Co-operation and Development (OECD, 2008), as reported by Dada et al. (2016) with minor modifications. The time (T) taken for all worms or the last worm to burrow in each concentration level was recorded. A worm was considered to have completely burrowed when no part of its body was visible on the soil surface. Readings were only taken for each container where all worms had completely burrowed. If one or more worms were unable to completely burrow in a container, this was considered evidence of avoidance of that particular toxicant concentration; therefore, the burrowing time was recorded in such cases.

2.13. Assessment of survival responses

Mortality was assessed at 7th and 14th days of exposure (Dada et al., 2016). After 14 days in the laboratory, the soil, along with the worms in each container, was poured into a plastic tray and spread out thinly film to count live worms (OECD, 2006). Worms were considered dead if they did not respond to a mechanical touch stimulus at the front end. The pH of the soil medium used was maintained at 6.8 ± 0.1, and the moisture content was kept at 35 ± 2% (OECD, 2016).

2.14. Ecological risk assessment of spent engine oil against test organisms

To assess the potential risks posed by toxic compounds in the ecosystem, conducting risk assessment through exposure assessment and risk characterization are paramount (Kamunda et al., 2016). According to USEPA (2021), an algorithm can be employed to estimate potential ecological risk in soil, dust, air, and water compartments.

In this study, ecotoxicological risk assessment of spent engine oil against different species representing various trophic levels (microbes and earthworms) was determined based on the ratio between the Measured Environmental Concentration (MEC) and the Predicted No Effect Concentration (PNEC). This ratio is denoted as the Risk Quotient (RQ) (Sanderson et al., 2003). The RQ is calculated using Equation 1.

$$RQ = \frac{MEC}{\text{PNEC}} \quad (1)$$

where MEC = Measured Environmental Concentrations, RQ = Risk Quotient, PNEC = Predicted No Environmental Concentration.

The PNECs were obtained from acute toxicity tests using microorganisms and earthworms, based on average estimation of EC50 (effective concentration that killed 50% of the organisms) and LC50 (lethal concentration that killed 50% of the organisms), respectively. The MEC was

derived from average TPH concentration in soil (González-Naranjo & Boltes, 2014). The corresponding PNEC values were calculated by dividing the toxicological dose descriptors (LC50 and EC50) obtained in this study by the appropriate Assessment Factor (AF) of 1000 (for earthworms) and 10 (for microorganisms), as shown in Equation 2 (OECD, 2006; Sanderson et al., 2003).

$$\text{PNEC} = \frac{LC50/EC50}{Assessment\ Factor} \quad (2)$$

The use of an Assessment Factor (dimensionless) highlights the uncertainty inherent in the extrapolation method, as recommended by OECD (2006) and USEPA (2021). In this factor accounts for uncertainties arising from both laboratory and natural environment conditions.

Different risk levels are traditionally determined based on the resulting RQ values, as described by Yamamoto et al. (2012): minimal risk (RQ < 0.1), median risk (0.1 ≤ RQ < 1), and high risk (RQ ≥ 1). However, in this study, the ecological risk level characterization method reported by Sanderson et al. (2003) was adopted: RQ values lower than 0.1 imply "acceptable risk", RQ values between 0.1 and 1 indicate 'needs further survey', and RQ values equal to or higher than 1 signify needs detailed evaluation'.

The approach used in this study involved estimating the incidence of adverse effects resulting from exposure to spent engine oil at measured concentrations, with values measured in mg/L or mg/kg.

3. Result and discussion

3.1. Burrowing responses of earthworm exposed to various concentrations of spent engine oil

The response of earthworms exposed to various concentration of spent engine in soil is displayed in Table 3, while the lethal concentration (LC50) values from the 7-day and 14day acute toxicity assay are depicted in Table 4. The results obtained indicate that burrowing behavior was dependent on the concentration of spent engine oil in all the treatments. During the first 7 days of exposure, the mean burrowing time ranged from 51 to 21.3 minutes. By the end of 14 days, the burrowing time ranged from 46.3 to 20.3 minutes. There was a progressive decrease in burrowing vigor with increasing treatment concentration. Earthworms in the 0 to 8% treatment group burrowed within a relatively shorter time, but burrowing was significantly slowed when concentration reached 12% and 16%, respectively.

Table 3. The time taken for earthworm to burrow in spent engine oil polluted and unpolluted soil

Days	Conc. (%)	Rep 1	Rep 2	Rep 3	Mean
	0	22	20	22	21.3
	4	38	36	37	37
7	8	52	50	51	51
	12	NB	61	63	41.3
	16	NB	NB	62	20.7
	0	22	20	19	20.3
14	4	30	32	34	32
	8	46	45	48	46.3
	12	NB	49	NB	16.3
	16	42	52	50	48

The median lethal concentration (LC50) of earworms exposed to various concentrations of spent engine oil for 7 and 14 days is displayed in Table 4. There was a strong positive correlation (r = 0.89) between 7-day and 14-day LC50 values. Specifically, the 7-day LC50 values for the 4%, 8%, 12%, and 16% treatment were 605, 742, 813 and 942 mg/kg, respectively. By the 14th day of exposure, the LC50 value for 16% treatment decreased slightly from 942 to 912 mg/kg. The corresponding LC50 values for the 14-day exposure were 598, 728, 794 and 912 mg/Kg. The results indicate that the LC50 value 7 days of exposure was the most toxic, with a value of 942 mg/kg. Additionally, the Probit line equation revealed a strong positive correlation between concentration and mortality rate in a dose-dependent manner. This implies that the mortality rate increased with an increase in concentration.

Table 4. The lethal concentration (LC50) of SEO that affected earthworm exposed at 7 and 14 days of exposure

Days	Conc. (%)	LC50 (mg/kg)	95% confidence limit		Slope ± S.E	D.f.	Probit line equation
			Lower	Upper			
7	0	0.00	0.00	0.00	0.00 ± 0.00	2	-
	4	605	116.56	245.44	3.32 ± 0.68	2	Y=3.32+5.47X
	8	742	125.72	250.11	5.65 ± 0.79	2	Y=5.65+6.94X
	12	813	211.78	271.97	6.63 ± 0.93	2	Y=6.63+7.22X
	16	942	316.56	338.22	8.21 ± 1.16	2	Y=8.21+9.46X
14	0	0.00	0.00	0.00	0.00 ± 0.00	2	-
	4	598	126.20	251.82	3.46 ± 0.71	2	Y=3.46+5.73X
	8	728	141.04	263.03	5.71 ± 0.84	2	Y=5.71+7.01X
	12	794	236.23	282.28	6.79 ± 0.99	2	Y=6.79+7.48X
	16	912	343.33	367.07	8.37 ± 1.23	2	Y=8.37+9.73X

Legend: D.F. = degree of freedom; S.E= standard error; LC50=lethal concentration. Conc. = concentration.

3.2. Total microbial load from spent engine oil polluted and unpolluted soils

The THB loads from spent engine oil-polluted and unpolluted soil samples are displayed in Table 5. An average of 4.35 × 10^6 CFU/g was obtained from the spent engine oil-polluted site, with a corresponding value of 3.04 × 10^5 CFU/g for the reference site (control). There were notable variations in the values between the two sampling sites, although no significant difference (p < 0.05) was observed between polluted and unpolluted soil samples. A total of 7 bacterial species were isolated from the spent engine oil-polluted soil, while 5 bacterial species were isolated from the unpolluted site. These species were identified as belonging to the genera Pseudomonas, Bacillus, Acinetobacter, Enterobacter, Neisseria sp., E. coli, Streptococcus sp., and Klebsiella sp., respectively. The cellular shapes and biochemical characteristics of the isolates were consistent with these identifications. The probable identity of bacterial isolates and their corresponding domiciled microorganisms in polluted and unpolluted soil samples are displayed in Table 5.

Table 5. Total heterotrophic bacteria count from spent engine polluted and unpolluted soils

Sample ID	Sample type	GPS location	THC (CFU/g)
PS	Soil	05º17'39.3" N 007º05'03.0" E	4.37 × 106
US	Soil	0050.6531'33' N 0070.13116'99" E	3.04 × 105

Legend: PS= Polluted soil; UP= Unpolluted soil

3.3. Biotolerance of bacteria isolates exposed to different concentrations of spent engine oil

The bio tolerance of bacterial species exposed to different concentrations of spent engine oil is illustrated in Figures 1 to 5, respectively. From these figures, it is evident that optimum growth of the microbes, as indicated by average optical density values, was recorded at the 4% treatment (lowest contaminant level), suggesting that the microbes may have utilized the spent engine oil as the sole carbon source in the medium. Significant difference ($p < 0.05$) in absorbance rate were observed among all the concentrations relative to the control. For instance, *Pseudomonas species* (Figure 1) exhibited a logarithmic increase in cell numbers within the first 72 hours of incubation but sharply decreased with higher concentrations, with optical density ranging from 0.9 to 2.1. Similarly, the absorbance of *Bacillus species* exposed to varying levels of spent engine oil (Figure 2) showed a stepwise decreased with increasing treatment levels, optical density ranging between 2.0 to 2.06. *Acinetobacter* and *Enterobacter* species (Figure 3 and followed a similar pattern in absorbance under spent engine exposure as shown in Figures 4, followed a similar pattern in absorbance under spent oil exposure, exhibiting a logarithmic increase in absorbance rate that was concentration dependent. In general, these organisms showed higher absorbance rates at lower contaminant levels.

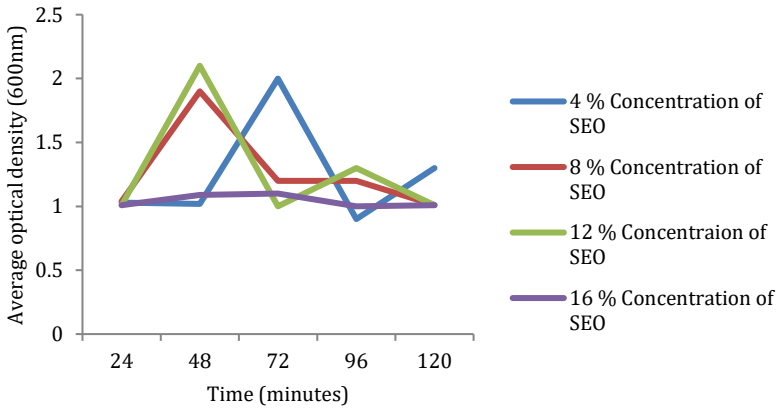

Figure 1. Biotolerance of *Pseudomonas species* exposed to different concentrations of spent engine oil polluted oil

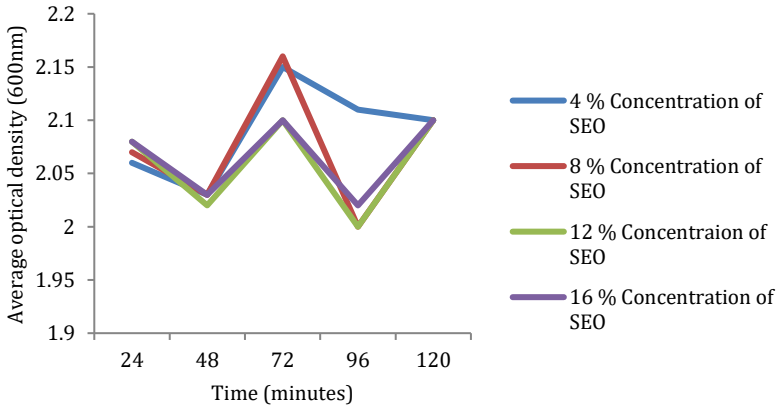

Figure 2. Biotolerance of *Bacillus species* exposed to different concentrations of spent engine oil polluted soil

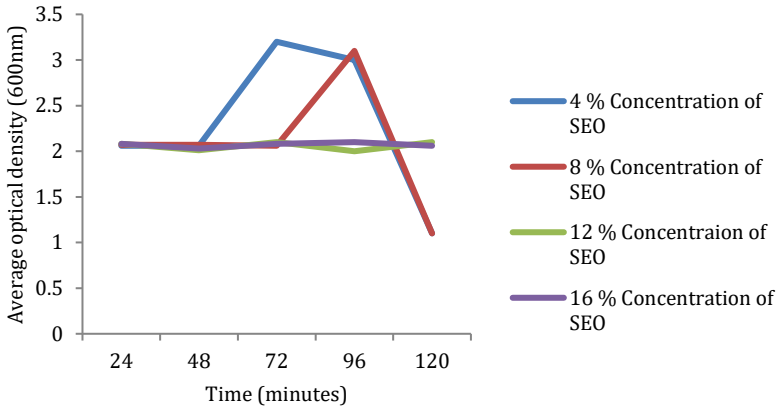

Figure 3. Biotolerance of *Acinetobacter species* exposed to different concentrations of spent engine oil polluted soil

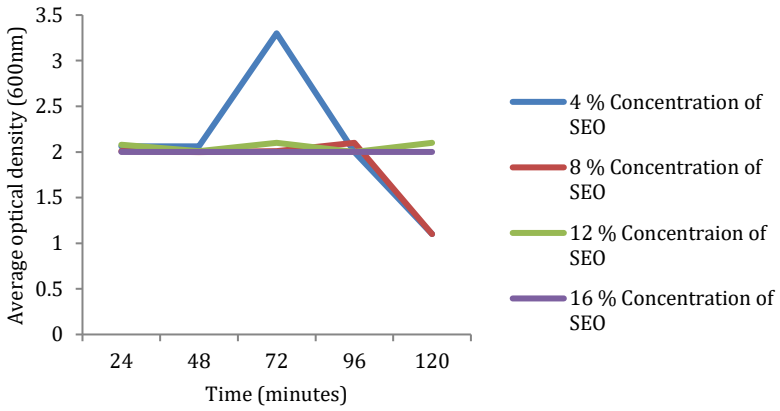

Figure 4. Biotolerance of *Enterobacter species* exposed to different concentrations of spent engine oil polluted soil

3.4. Ecological risk characterization

The Ecological risk characterization based on acute toxicity tests, MEC, and their corresponding RQ for plants, earthworms, and microorganisms is presented in Table 6. To assess the actual risk posed to test organisms in the ecosystem, an estimate of the incidence of adverse effects resulting from spent engine oil exposure was determined at the MEC. Environmental effects were characterized by calculating the PNEC based on average EC50 (Effective Concentration that inhibited 50% growth of the organisms) and LC50 (Lethal Concentration that inhibited 50% growth in the organisms) values obtained during ecotoxicity tests. In this study, the ecotoxicological risk was estimated using the ratio between MEC and PNEC to obtain the corresponding RQ. By convention, RQ < 0.1 indicates that no adverse effect is expected for the evaluated organism in its environment. If 0.1 < RQ < 1, a potentially adverse effect should be considered, but at 1 < RQ < 10, a moderate hazard is probable. Additionally, RQ > 10 indicates a high risk (EMEA, 2006).

The microorganisms exhibited varying degrees of bio tolerance to spent engine oil exposure based on estimated risk quotients. Exposure to spent engine oil posed minimal risk to *Pseudomonas sp., Bacillus sp., and Acinetobacter sp.,* as indicated by PNECs values below unity (< 1). However, *Enterobacter sp.* Showed a high-risk quotient value above 1. For organisms with risk quotient values less than 1, the implication is that the expected ecological risk would be minimal

or neglectable. Conversely, when the risk quotient exceeds 1, it suggests that the studied organisms could be exposed to some level of risks in the ecosystem.

Table 6. Ecological risk characterization based on acute toxicity testing and Measured Environmental Concentration for plants, earthworm and microorganisms.

Species exposed	A.F.	PNEC (%)	MEC (mg/kg)	MEC/PNEC	RQ
Earthworm	1000	48.03	50	1.04*	<1RQ>1
Pseudomonas sp.	10	97.02	50	0.15	<1
Bacillus sp.	10	83.01	50	0.60	<1
Acinobacter sp.	10	73.10	50	0.68	<1
Enterobacter sp.	10	30.42	50	1.64	<1

Legend: AF = assessment factor, MEC = measured environmental concentration, RQ = risk quotient.

In this study, the ecological risk poses by spent engine oil was evaluated, since these pollutants are often disposed directly on soil, potentially seeping into nearby aquatic environment. The ecological risk characterization, assessed through risk quotient analysis (MEC/PNECs), indicates that spent engine oil could have significant impacts on both human health and biological components of the food chain studied (microorganisms, plants, and earthworms). The potential risk was found to be high for plants and earthworms, with risk quotient values exceeding unity (> 1). For microorganisms, the risk quotient suggested a moderate risk, with values approaching unity (1), except for Enterobacter species, which exhibited a risk quotient greater than 1. This suggests a higher likelihood of ecological impact at the measured PNECs. This observation aligns with the findings reported by Guo et al. (2011). Few studies have reported on the ecotoxicological risk of spent engine oil in Nigeria. Valcárcel et al. (2011) reported risk quotient values above 1, and Essien et al. (2012) documented risk quotient values greater than 1 for hydrocarbon contamination in the Niger Delta Region of Nigeria, consistent with the findings in this study.

In a related study, Yamamoto et al. (2012) applied two cumulative risk evaluation approaches to estimate the risk associated with the presence of seven parabens in water. However, their hazard evaluation was based on toxicity data obtained for each compound individually and did not account for synergistic or antagonistic effects. Although their study employed a different approach, the objective was similar: to estimate the risk quotient in a real-world scenario where organisms are exposed to a mixture of pollutants.

4. Conclusion

Spent engine oil contains pollutants that can have harmful effects on quality of life and may disrupt ecosystem integrity. This further reaffirms the important for comprehensive environmental assessments to estimate ecosystem health in response to unregulated anthropogenic activities. Bioassays involving earthworms and microorganism serve as direct contact tests to measures the impact of a pollutants on these organisms. Earthworms are a key species with multiple economic benefits in agriculture, while soil microorganisms play in maintaining soil quality. The antimicrobial properties of spent engine oil in soil can disrupt the activities of these organisms and selectively inhibit their growth, leading to alterations in soil ecological functionality. The adverse effects of spent engine oil disposal may distort soil function, potentially resulting in biodiversity loss, ultimately affecting humans as the top of the food chain.

References

Abara, P. N., Madu, C. P., Nwachukwu, M. ., Ubah, V. C., Udebuani, A. C., & Adjeroh, L. A. (2020). Toxicity of Chlorpyrifos-Based Pesticide Formulation Termicot ® on Non- target Organism (Clarias gariepinus). *International Journal of Zoological Investigations*, *6*(2), 321–329.

https://doi.org/10.33745/ijzi.2020.v06i02.011
Abioye, P. O., Abdul Aziz, A., & Agamuthu, P. (2010). Enhanced Biodegradation of Used Engine Oil in Soil Amended with Organic Wastes. *Water, Air, & Soil Pollution*, *209*(1), 173–179. https://doi.org/10.1007/s11270-009-0189-3
Adedokun, O. M., & Ataga, A. E. (2007). Effects of amendments and bioaugumentation of soil polluted with crude oil , automotive gasoline oil , and spent engine oil on the growth of cowpea (Vigna ungiculata L . Walp). *Scientific Research and Essay*, *2*(5), 147–149.
Akin-Obasola, B. (2019). Range finding , acute and sublethal test of petrol and engine oil mixture on African catfis, Clarias gariepinus (Burchell, 1822). *International Journal of Fisheries and Aquatic Studies*, *7*(6), 16–20.
Bagherzadeh-Namazi, A., Shojaosadati, S. A., & Hashemi-Najafabadi, S. (2008). Biodegradation of Used Engine Oil Using Mixed and Isolated Cultures. *International Journal of Environmental Research*, *2*(4), 431–440.
Dada, E. O., Njoku, K. L., Osuntoki, A. A., & Akinola, M. O. (2016). Physiological responses of a tropical earthworm: A potential tool for biomonitoring heavy metal pollution in tropical wetlands. *Nigerian Journal of Ecology*, *15*(1), 11–18.
Ekperusi, O. A., & Aigbodion, F. I. (2015). Bioremediation of petroleum hydrocarbons from crude oil-contaminated soil with the earthworm: Hyperiodrilus africanus. *3 Biotech*, *5*(6), 957–965. https://doi.org/10.1007/s13205-015-0298-1
Emoyan, O. O., Onocha, E. O., & Tesi, G. O. (2020). Concentration assessment and source evaluation of 16 priority polycyclic aromatic hydrocarbons in soils from selected vehicle-parks in southern Nigeria. *Scientific African*, *7*, e00296. https://doi.org/10.1016/j.sciaf.2020.e00296
Essien, J. P., Ebong, G. A., Asuquo, J. E., & Olajire, A. A. (2012). Hydrocarbons contamination and microbial degradation in mangrove sediments of the Niger Delta region (Nigeria). *Chemistry and Ecology*, *28*(5), 421–434. https://doi.org/10.1080/02757540.2012.686607
Farombi, A. G., Adebayo, O. R., & Oyekanmi, A. M. (2013). Impact of petroleum product on the soil around automobile workshops in Osun state. *IOSR J Appl Chem (IOSR-JAC)*, *4*(1), 13–15.
González-Naranjo, V., & Boltes, K. (2014). Toxicity of ibuprofen and perfluorooctanoic acid for risk assessment of mixtures in aquatic and terrestrial environments. *International Journal of Environmental Science and Technology*, *11*(6), 1743–1750. https://doi.org/10.1007/s13762-013-0379-9
Guo, Y., Wu, K., Huo, X., & Xu, X. (2011). Sources, distribution, and toxicity of Polycyclic Aromatic Hydrocarbons. *Journal of Environmental Health*, *73*(9), 22–25. http://www.jstor.org/stable/26329217
Ibe, F. C., Duru, C. E., Isiuku, B. O., & Akalazu, J. N. (2021). Ecological risk assessment of the levels of polycyclic aromatic hydrocarbons in soils of the abandoned sections of Orji Mechanic Village, Owerri, Imo State, Nigeria. *Bulletin of the National Research Centre*, *45*(1), 18. https://doi.org/10.1186/s42269-021-00485-2
ISO. (2007). *ISO 11348-3:2007 Water quality Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) Part 3: Method using freeze-dried bacteria*. https://www.iso.org/standard/40518.html
ISO. (2011). *ISO 17512-2: 2011 Soil quality. Avoidance test for determining the quality of soils and effects of chemicals on behaviour Part 2: Test with collembolans (Folsomia candida)*. https://www.iso.org/standard/50779.html
Kamunda, C., Mathuthu, M., & Madhuku, M. (2016). Health Risk Assessment of Heavy Metals in Soils from Witwatersrand Gold Mining Basin, South Africa. In *International Journal of Environmental Research and Public Health* (Vol. 13, Issue 7, p. 663). https://doi.org/10.3390/ijerph13070663
Mandri, T., & Lin, J. (2007). Isolation and characterization of engine oil degrading indigenous microrganisms in Kwazulu-Natal , South Africa. *African Journal of Biotechnology*, *6*(1), 23–27.
Nishitha, D., Amrish, V. N., Arun, K., Warrier, A. K., Udayashankar, H. N., & Balakrishna, K. (2022). Study of trace metal contamination and ecological risk assessment in the sediments of a tropical river estuary, Southwestern India. *Environmental Monitoring and Assessment*, *194*(2), 94. https://doi.org/10.1007/s10661-021-09728-1
Odjegba, V. J., & Sadiq, A. O. (2002). Effects of spent engine oil on the growth parameters, chlorophyll and protein levels of Amaranthus hybridus L. *Environmentalist*, *22*(1), 23–28. https://doi.org/10.1023/A:1014515924037

OECD. (2004). *Test No. 202: Daphnia sp. Acute Immobilisation Test, OECD Guidelines for the Testing of Chemicals, Section 2*. OECD Publishing. https://doi.org/10.1787/9789264069947-en

OECD. (2006). *Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, OECD Guidelines for the Testing of Chemicals, Section 2*. OECD Publishing. https://doi.org/10.1787/9789264070066-en

OECD. (2008). *Test No. 222: Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei). Guideline for testing of chemicals. Organization for Economic Cooperation and Development.*

OECD. (2016). *OECD Guidelines for the Testing of Chemicals, Section 2 Effects on Biotic Systemsest No. 222: Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei), OECD Guidelines for the Testing of Chemicals, Section 2*. OECD Publishing. https://doi.org/10.1787/9789264264496-en.

Oh, K., Cao, T., Li, T., & Cheng, H. (2014). Study on Application of Phytoremediation Technology in Management and Remediation of Contaminated Soils. *Journal of Clean Energy Technologies*, *2*(3), 216–220. https://doi.org/10.7763/JOCET.2014.V2.126

Okafor, E. C., & Opuene, K. (2007). Preliminary assessment of trace metals and polycyclic aromatic hydrocarbons in the sediments. *International Journal of Environmental Science & Technology*, *4*(2), 233–240. https://doi.org/10.1007/BF03326279

Onwuka, M. I., Chude, V. O., & Ogwuegbu, G. C. (2012). Remediation of spent engine oil polluted soil using two types of organic manure and their effects on maize growth. *Nigerian Journal of Soil Science*, *22*(2), 245.

Ozbay, G., Jones, M., Gadde, M., Isah, S., & Attarwala, T. (2021). Design and Operation of Effective Landfills with Minimal Effects on the Environment and Human Health. *Journal of Environmental and Public Health*, *2021*, 6921607. https://doi.org/10.1155/2021/6921607

Phulpoto, A. H., Qazi, M. A., Mangi, S., Ahmed, S., & Kanhar, N. A. (2016). Biodegradation of oil-based paint by Bacillus species monocultures isolated from the paint warehouses. *International Journal of Environmental Science and Technology*, *13*(1), 125–134. https://doi.org/10.1007/s13762-015-0851-9

Sanderson, H., Johnson, D. J., Wilson, C. J., Brain, R. A., & Solomon, K. R. (2003). Probabilistic hazard assessment of environmentally occurring pharmaceuticals toxicity to fish, daphnids and algae by ECOSAR screening. *Toxicology Letters*, *144*(3), 383–395. https://doi.org/10.1016/S0378-4274(03)00257-1

Uchendu, U. I., & Ogwo, P. A. (2014). The Effect of Spent Engine Oil Discharge on Soil Properties in an Automobile Mechanic Village in Nekede , Imo State , Nigeria. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, *8*(11), 28–32.

USEPA. (2021). *Office of the Solid Waste, EPA, 530-F-94-008.Ministry of Environment and Forest (MEF) (2008) India Report 378 00RT*.

Valcárcel, Y., González Alonso, S., Rodríguez-Gil, J. L., Gil, A., & Catalá, M. (2011). Detection of pharmaceutically active compounds in the rivers and tap water of the Madrid Region (Spain) and potential ecotoxicological risk. *Chemosphere*, *84*(10), 1336–1348. https://doi.org/10.1016/j.chemosphere.2011.05.014

Yamamoto, N. S., Pereira, C. D. S., Cortez, F. S., Pusceddu, F. H., Santos, A. R., & Guimaraes, L. L. (2012). Avaliação dos efeitos biológicos do farmaco Losartan em microcrustaceos Daphnia similis e Ceriodaphnia dubia (Crustacea, Cladocera). *Unisanta BioSci*, *1*, 49–53.