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Effect of Spent Lubricant on the Mortality rate of Earthworm (*Lumbricus terrestris*) in Obio/Akpor L.G.A, Rivers State

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Abstract

A 4- week toxicological study was conducted to ascertain the effect of spent lubricant on the mortality rate and physiological function of Earthworm (*Lumbricus terrestris*). A total of 50 Earthworms of weight ranging from 0.3g-0.7g each were collected and acclimated in a test soil for 2 weeks after which they were used for acute and sub-lethal bioassay. The experiment which consisted of five groups and four treatments were laid out in a randomized block design. An acute toxicity test at concentration of 0ml/kg (control); 50ml/kg; 100ml/kg; 200ml/kg and 400ml/kg with an exposure period of 96 hours was carried out. Similarly, sub-lethal toxicity test was also conducted for a period of 30 days at a concentration of 0ml/kg (control), 7ml/kg, 13ml/kg, 20ml/kg and 27ml/kg respectively. Results revealed that in both tests, *Lumbricus terrestris* were sensitive to varying concentrations of used lubricants but were able to survive for a few hours at concentrations higher than 100ml/kg. The study revealed that Earthworm survival could serve as suitable indices to assess engine oil pollution in soil.

Keywords: Earthworm, acute toxicity, sub-lethal toxicity, spent lubricant, concentrations

INTRODUCTION

Nigeria being an oil producing country currently faces the issue of oil pollution. Currently the disposal of used engine oil is not properly covered by legislation. Although much of the world depends on the production, trade or use of oil fuels, their activities can cause damage to the environment; either intentionally or unintentionally. Used engine oils when disposed of inappropriately can disrupt microbial activities, kill most soil organisms, threaten the extinction of several plants, alter the annual and fish life of the region and finally affect humans (Udom *et al*., 2012).

Most soil organisms most affected by this pollution range from larger creatures like earthworms and nematodes, to microscopic organisms including bacteria, fungi, algae and protozoa. These organisms play a vital role in maintaining soil health and fertility. They retain nutrients in the soil, preventing them from leaching, compete with, inhibit and consume diseases as well as decompose plant residue, toxic materials, and pollutants that kill plant roots. The formation of soil aggregates by these organisms improves water infiltration, root penetration and water holding capacity of the soil.

Earthworms are soil organisms which through their actions improve nutrient availability for plants, improve soil drainage through their burrowing activities, soil structure, mineralization of nutrients for plants and dissolution of toxicity in the soil. . Their soft bodies make them sensitive to certain environments and as such, soils with high pH range, pesticides, herbicides and conventional fertilizers with high salt index, petroleum products amongst a host of others, should be stabilized and properly treated for optimal performance from these creatures. Therefore the absence or presence of these organisms in the soil can then be used to assess the health of such soils. Additionally, loss of earthworms from an area might also affect the numbers and distribution of their vertebrate predators; making them good test organisms in terrestrial ecotoxicology. It is on this premise therefore, that this study investigated the effect of used lubricant concentrations from engine oils on the mortality rate of earthworms.

MATERIALS AND METHODS

The experiment was carried out in five different groups consisting of four treatments and one control and was laid in a randomized block design. The earthworms and soil sample for the acute and sub-lethal toxicity test were taken to the environmental laboratory of the Department of Animal and Environmental Biology and were acclimated for a period of 2 weeks. The room temperature during the experiment was maintained at a temperature of 18 ° C.

Samples Collection

Mature earthworms, ranging in weight from 0.3 to 0.7g each, were hand picked at random from Abuja campus, University of Port Harcourt. Uncontaminated soil sample was also collected from a farmland behind Clinical hostel Abuja campus (4^{0} 54'10'N, 6^{0} 55'12"E), using a clean polyethene bag, and a soil core sampler. The soil was air dried with plant residues, stones and other debris excluded, after which it was sieved using a 2mm sieve mesh and basic soil parameters were checked. Similarly, Used lubricant (from diesel engine) was collected in a clean bottle from Abuja campus power house (4^{0} 54'6"N, 6^{0} 55'11"E), University of Port Harcourt, Rivers state.

Physico-chemical Parameters of the Soil

Prior to the introduction of the earthworm into the soil, physico-chemical test was carried on the soil to determine the Water holding capacity (WHC), Total organic carbon (TOC), Soil pH, Total porosity, Electrical conductivity and Particle size distribution using standard methods.

Sub-Lethal Toxicity Test

Test substrates containing 1.5kg of sieved soil (2mm) in 3.2litres plastic containers with perforated lids each were prepared using distilled water of 350m1 to give a saturated soil. The different concentrations of 10ml, 20ml, 30ml, and 40ml of the pollutant was introduced in the different test containers and mixed properly until a homogeneous mixture was obtained, while no pollutant was introduced in the container for control, 10 adult worms were individually weighed and placed in each test container already consisting of the test substrate respectively. Survival rates of the worms were checked using acupuncture after every one week and the number of surviving worms was recorded. The moisture content of the substrate was maintained by adding makeup deionized water when necessary. The samples were monitored for a period of four weeks.

Acute Toxicity (Mortality Test)

Following the same procedure as the previous experiment, Test substrates containing 1kg of sieved soil (2mm) in 3.2 litres plastic containers with perforated lids each were prepared using distilled water of 350m1 to give a saturated soil. The different concentrations of 50ml, 100ml, 200ml, and 400m1 of the pollutant was introduced in the different test containers and mixed properly until a homogeneous mixture was obtained, although no pollutant was introduced in the control. Earthworms were acclimated in test soil for 2 weeks before use for the experiment. The survival rates of the test organisms were observed and recorded within a period of 96hours

RESULTS

The result on Table 1 shows the physico-chemical properties which include soil pH, water holding capacity, porosity, nitrogen content, soil textural class and total petroleum hydrocarbon content in the lubricant after analysis.

Table 1: Physico-chemical properties of sample soil and Total petroleum hydrocarbon of sample lubricant.

Properties	Soil	TPH of Unused lubricant
Nitrogen content (%)	1.07	-
Carbon content (%)	9.10	-
pH	5.20	-
Water holding capacity (%)	28.60	-
Textural class	Sandy loam	-
Total porosity (%)	29.10	-
Total petroleum hydrocarbon (ppm)	-	6369.73

Table 2: Weight loss of live earthworms in each sample (test substrate) after 30 days

Samples	No. of live worms	Initial weights (g)	Final weights (g)	Absolute weight loss (g)
A	10	0.55	0.27	0.28
B	10	0.58	0.38	0.20
C	10	0.55	0.43	0.12
D	10	0.56	0.45	0.11
E	10	0.56	0.54	0.02

Response of Earthworm to Sub-lethal Toxicity

In Table 2;Fig 1, Samples A, B, C, D and E with percentage mortality (%) of 0% respectively were considered to be non- toxic according to the toxicity criterion which stipulates the 50% (LC_{50}) mortality is considered toxic, although there was considerable weight loss in the body mass of worms treated with pollutants. While there might have been weight loss in the body mass of worms in untreated soil, their body mass remained relatively stable.

Table 3: Mortality (percentage of dead earthworms after 96 hours) related to number of individuals at start of the experiment

Samples	Concentration of samples (ml/kg)	Log. conc. of samples (ml/kg)	No. of worms at test start	No. of survival	No. of dead worms	Mortality (%)
A	400	2.6	10	0	10	100
B	200	2.3	10	0	10	100
C	100	2.0	10	2	8	80

D	50	1.7	10	4	6	60
E	0	0.0	10	10	0	0

Response of Earthworm to Acute Toxicity

The acute toxicity test recorded very high mortality rates which are expressed in the recorded values of samples A, B, C and D as 100%, 100%, 80% and 60% respectively. Sample E (control) showed 0% mortality. (Table 3; Fig 2). The logarithmic concentration at which 50% of the populations were killed (LC_{50}) was LC_{50} = 1.5ml/kg

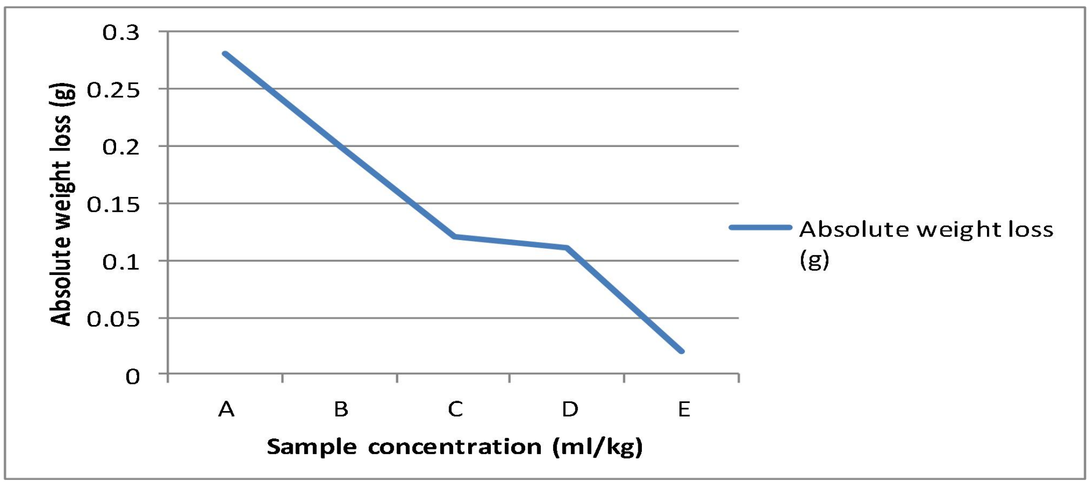

Fig. 1: Weight loss of earthworm against concentration of spent lubricant for a period of 30 days; sub-lethal test

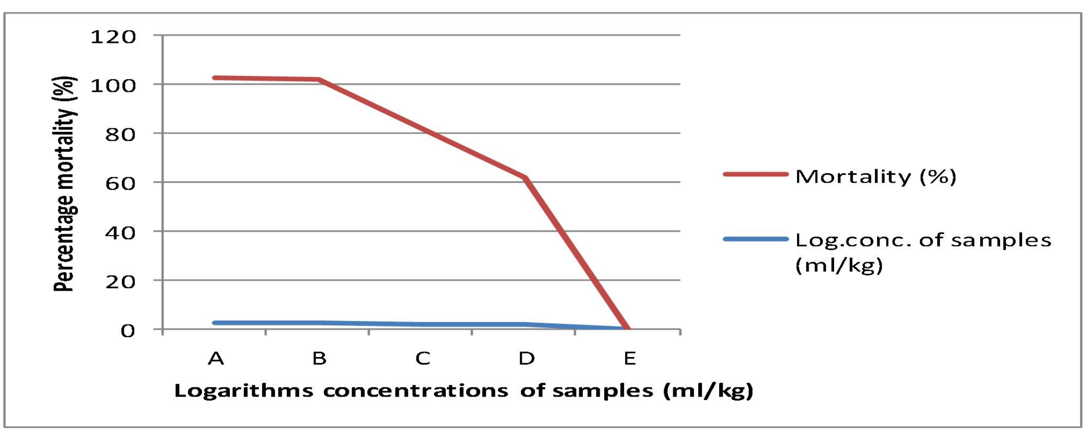

Fig. 2: Percentage mortality of earthworm against concentration of spent lubricant for 96 hours; acute toxicity test

DISCUSSION

Earthworm biomass measurement has clearly shown that used lubricants from generators (diesel engine) have a pronounced sub-lethal effect, i.e. weight loss on earthworms. This may have occurred due to the worms not ingesting soil during the exposure period. Eijsackers and Doelman, (1987) and Curry, (1994) stated that biological adaptation niche problems encouraged earthworms to avoid feeding on contaminated soils. These may be responsible for the eventual weight loss as the worms chose to starve rather than feed on the soils. This is also confirmed by Shin and Jung (2005) who found that diesel had a significant effect on an average weight of earthworms (*Eisenia* spp.).

The behavioural endpoints of earthworms due to their exposure to chemical substances represent the indirect effects, which have consequences for soil ecosystem functions. Since the physicochemical parameters of the soil showed that the soil provided a suitable habitat for the worms, then the behavioural impairments like swelling, curling, and no feeding, observed during the experiment may be due to the toxic nature of used engine oil. This is in agreement with the report of Curry, (1994) who stated that the earthworms avoided the contaminated feed and expressed abnormalities when tolerable concentrations were exceeded.

It was observed that for all concentrations (up to 27m1/kg soil which is 2.7%), 100% of the earthworms survived for 30 days. Cast formation as a result of earthworm activities was also observed at low soil contamination levels. The observed quantity of casts was found to decrease significantly with increase in soil contamination levels. At very high soil contamination concentration (>150m1/kg which is 15%), no cast formation was observed, earthworms were found to lie on or around the soil and not enter into it, wasting of body tissue was also observed. This result suggest that used engine oil contaminated soil is not as toxic as crude oil to earthworms in comparison with the findings of Saterbak and Toy (1999) and Safwat *et al*., (2002) for which earthworm mortality was 100% for concentration < 2.5%. For the purposes of bioremediation therefore, a higher concentration of petroleum hydrocarbon contaminated soil (as high as 15%) may be treated using earthworms whereas for crude oil, only 0.4% petroleum hydrocarbon contaminated soil may be treated with earthworms as reported by Maike and Filser, (2007).

Although there was weight loss in biomass of earthworms as a result of toxicity of used lubricant contaminated soil, the Ftab value (2.51), which happened to be less than the Fcai (3.06), showed no significant cause of toxicity to the worms, hence their continued survival over a long period of exposure to toxicant. On the other hand, the acute toxicity test with value (31.41), which was greater than the Fcal value (3.48), showed very high significance which accounts for the high mortality rate even at a short duration of exposure to toxicant. Contamination of soil by heavy metals is one of the most serious environmental problems and has significant implications for human health. Ezeronye and Ubalua, (2005) in their work also reported that metals are persistent in the environment and once it gets into food chain, through plants, animals and water sources leads to bio magnification and bioaccumulation in living cells and tissues.

CONCLUSION

The study has revealed that earthworm can survive in used engine oil of concentrations up to 100ml/kg, whereas concentrations of up to 200m1/kg of soil were found to be lethal to earthworm. Earthworms can be used for bioremediation as they are useful test organisms to assess toxicity of contaminated soil; they ingest substantial amounts of soil which exposes them to contaminants, thereby making them good bio-indicators of soil health and quality.

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