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Stress Indicators and Behavioural End-Points of *Lybriodrillus violaceous* Exposed to Petroleum Products Contaminated Soil

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Abstract

The effect of fresh gasoline contaminated soil (GS), fresh diesel contaminated soil (DS) and a 3 years old oil spill soil on indigenous tropical earthworm, *Lybiodrillus violaceous*, was carried out using five acute toxicity tests. This included coiling exhibition, swollen clitellium, 14 days survival test, 48hrs avoidance response test and 56 days juvenile production test. Soil samples from a non-contaminated soil (NCS) were used as control. The exposure of earthworms to GS and DS soils resulted in exhibition of coiling response and swollen clittelium region. For survival test, results showed 60% and 20% survival for GS and DS respectively while 80% and 100% survival were recorded for CS and NCS. Avoidance test showed that there was 8:2 (NCS:DS), 6:4 (NCS:GS) and 5:5 (NCS:CS) ratio of *L.violaceous* present in soil types. Study on Juvenile production showed that there were no juveniles produced in both GS and DS contaminated soil, however, there were juvenile production recorded in CS and NCS. The negative impact of all toxicity tests on *L.violaceous* was more in diesel contaminated soil compared to gasoline contaminated soil and least in the 3 year old spill soil. The results indicated negative effect of diesel and gasoline contamination on *L.violaceous*.

Keywords: Acute toxicity; *Lybriodrillus violaceous;* Diesel; Gasoline; Soil

Introduction

In recent times, a growing number of residential and office area have become polluted with gasoline and diesel owing to the fact that most of Nigeria populace has resorted to the use of diesel and gasoline generators as an alternative source of electricity. The issue of pipeline vandalization had also become common placed which is also a source of petroleum pollution. Pollutants from oil spills have far reaching effect on their environment acutely and long after the incidents have occurred. These pollutants are known to percolate deep into the soils [1] where some remain recalcitrant, posing prolong threats to organisms [2,3].

Earthworms form one of the major macro-fauna among soil biota; they play an essential role in maintaining the structure and fertility of soils, recycling nutrients, improve their habitat and are important decomposers in a food web, sustaining dynamic equilibrium in the soil [4-6]. Earthworms are relevant test-organisms in ecotoxicological tests representing 60-80 % of the total soil animal/invertebrate biomass. Because of their ubiquitous nature and sensitivity, earthworms play a vital role in estimating the effect of pollutants on terrestrial environment [1].

Mortality or reproduction rate tests are important ecological parameters used to determine the consequence of exposure to chemicals in soils. The emigration of earthworms and the subsequent loss of their beneficial functions in soil lead to a degradation of soil qualities. Furthermore, reduction in earthworms' population from an area might also affect the abundance and distribution of their vertebrate predators. This event consequently has an indirect impact on an ecosystem [7]. The regular endpoints evaluations in earthworm ecotoxicology guideline; mortality and reproduction rates alone cannot indicate the influence of pollutants on population since changes in behavior such as substrate avoidance can result in emigration which impinges on soil ecosystems. It is therefore not realistic and would be a wrong prediction to depend on mortality and reproduction results alone for long term effects on organisms exposed to oil spills [1].

Although, the use of earthworm for toxicity testing has been over the last 20 years and is currently used as a basis for international regulatory guidelines in EU for risk assessment [8] there have been with several problems [9]. The most widely used test species is *E. fetida*. Puzzling questions have arisen with the use of *E. fetida* because it has been argued that it is more tolerant than most earthworms to contaminants [10,11] and it is not found in all natural soils [12]. Sometimes field-collected "wild" earthworms could be used for toxicity testing however their genetic background and local subspecies are not well known. Hence, there can be problems extrapolating results from laboratory to field scale. However such research could also be done as they give an insight into the actual situation on site which might be necessary to propose policies within such regions.

Several works on the toxicity of contaminants on *Eisenia fetida,* [7,13,14] *Lumbricus sp* [15], *Eudrilus eugeniae* [1] have been reported. However, reports on toxicity of local tropical earthworm species are rare. The objective of this study is to use simple and easy stress indicators including coiling reaction, survival test, avoidance response test and juvenile production to observe in local tropical earthworms exposed to gasoline and diesel contaminated soil and soil from Agaye community. This community had been exposed to repeated oil spill (undefined) due to vandalization of petroleum pipelines, a common occurrence in Nigeria. Soil from a non-contaminated area was used as control. These toxicity parameters give insight into the severity of the different petroleum products to the most abundant and dominant

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earthworm species (*Lybriodrillus violaceous,* [16]) identified in Agaye soil.

Experimental

Test animal

Lybiodrilus violaceous, were collected by hand picking from Agaye soil, which was humus with high organic content. The earthworms were collected from the same site in order to reduce variability in biotype. Earthworms were brought into laboratory in plastic buckets containing soil collected from the same soil and supplemented with half-boiled, ground water-leaf (*Talinium triangulare*) [17] and then moistened with distilled water. They were left to acclimatize for 12 days. Subsequently, earthworms were fed with leaflets of lettuce *Nymphea lotus,* 3 g per 10 worms every 4 days throughout the period of all studies carried out [1]. Adult earthworms of length 18 cm-20 cm and body weight 1.3 g-1.5 g were picked and used for the bioassay tests

Control soil

Control soil was collected from a garden soil in Ibadan which had been void of any crop protection product within the last one year of use and also void of organic fertilizer within the last six months of use for testing [18]. All recommended manipulations for natural soils by ASTM, 2004 was also applied. Analysis of micro pollutants prior to use was also done. The soil had 37% sand, 13% clay and 50% silt. The pH values of control soils range from 7.1 to 7.7.

Soil preparation and contamination

This was done according the method adopted [1] with slight modifications. Soil samples were collected from generator areas using strictly gasoline and diesel respectively from redemption camp, Ogun state, Nigeria. All soil samples were air-dried, macerated and sifted through a 0.30 mm (mesh size) screen so as to standardize the grain size. Diesel and gasoline contaminated soil were properly mixed with control soil at proportion 1:1 (50%) and 1:10 (10%) respectively. Each soil mixture was moistened with 5 ml of distilled water. 1 kg of 100%, 50% and 10% prepared soil (diesel and gasoline soil) were then placed in plastic vessel of size 20 cm in diameter (base) by 35 cm height and 35 cm in diameter (top), the bottom was perforated while the top was covered with mesh and made to stay with elastic bands. 1 kg of fresh soil from Agaye community and control garden soil from a non oil contaminated soil were also prepared as described above.

Experimental set-up

1kg of each type of soil; GS (gasoline contaminated soil), DS (diesel contaminated soil), CS (soil from Agaye community) and NCS (non-contaminated garden soil), were placed in vessels and labelled accordingly. 10 adult earthworms were applied to each vessel and this was done in triplicate. Set-up was arranged in a randomized block design and was used for the following toxicity studies; coiling response, swollen clittelium, 14 days survival test, 48hrs avoidance response test and 56 days juvenile.

Avoidance test

A two chambered test system was used [7]; 1 Kg of each soil, gasoline (GS), diesel (DS) and contaminated soil (CS) were added to one of the chambers. 1 kg of non-contaminated soil (NCS) was then applied to the second chamber of each system; the two chambers were entirely separated so as to prevent mixing up of both soil types. The separator was then removed thereby creating a groove in between both soil types; this was done to allow free movement of earthworms between the two chambers. 10 earthworms each were added to the groove of each set-up. Each chamber was covered with mesh and made to stay with the aid of elastic band and left for 48 hrs. Prior to counting, the separator was put back in place (in groove), each soil type removed separately and the number of earthworms in each chamber was counted after 48hrs for each set-up and recorded.

Coiling and swollen clitellum, survival/juvenile production tests

10 earthworms were applied to each set-up described above (i.e. DS, GS, NS and NCS with varying concentrations). The vessels were thereafter covered with meshes which were kept in place by elastic band. The number of surviving earthworms, coiled animals and swollen clitellum in various treated soils were observed and recorded 1 day, 2 days, 5 days and 7 day period. Vessels with survivals were left till the 56th day and the number of juveniles were counted and recorded.

Statistics

Means of earthworms numbers in the various toxicity test were done and t-test analysis was using SPSS version16, package.

Results

Survival and coiling reaction

Adult earthworm, *L.violaceous*, exposed to gasoline and diesel soil mixtures were observed for survival and coiling. Result showed that about 14.29-66.67% and 20-100% of the exposed animal exhibited coiling for gasoline and diesel respectively. The highest percentage of coiled earthworms was recorded within the first 24 hours of observation (Tables 1 and 2).

Soil type	Day 1	Day 2	Day 5	Day 7	Day 14
GS (100%)*	6(83.33)	5(60)	5(40)	4(33.33)	4(0)
GS (50%)*	8(87.5)	7(57.14)	7(42.86)	7(33.33)	7(0)
GS (10%)*	9(33.33)	8(25)	7(14.29)	7(14.29)	7(0)
NCS	10(10)	8(12.5)	8(0)	8(0)	8(0)
CS	9(0)	9(0)	9(0)	9 (0)	8 (0)

*=significantly different at P<0.05

The t-test results comparing means of surviving earthworms exposed to gasoline contaminated soil showed that there was significant (P<0.05) difference in all treated media compared to the control media (NCS) but not with the contaminated soil (CS) from Agaye.

Table 1: Mean number of surviving earthworms/(Percentage of coiled ones in Parenthesis) of exposed to gasoline contaminated soil.

Soil type	Day 1	Day 2	Day 5	Day 7	Day 14
DS (100%)*	0(0)	0(0)	0(0)	0(0)	0(0)
DS (50%)*	3(100)	3(66.66)	2(50)	2(0)	1(0)
DS (10%)*	6(83.33)	6(50)	6(33.33)	5(20)	5(0)
NCS	10(10)	8(12.5)	8(0)	8(0)	8(0)
CS	9(0)	9(0)	9(0)	9 (0)	8 (0)

*=significantly different at P<0.05

The t-test results comparing means of surviving earthworms exposed to diesel contaminated soil showed that there was significant (P<0.05) difference in all treated media compared to the control media (NCS) but not with the contaminated soil, NCS (P>0.05) from Agaye

Table 2: Mean number of surviving earthworms and (Percentage of coiled ones in Parenthesis) of exposed to diesel contaminated soil.

Swollen clitellum

Adult earthworm, *L.violaceous*, exposed to gasoline and diesel soil mixtures were observed for clittelium swellings on Day 1, 2, 5 and 7. Results (Tables 3 and 4), shows that mean number of earthworms with swollen clittelium increased with increasing days of exposure. No swelling was observed in animals exposed to lower concentration soil mixture (10%/50% and 10% for gasoline and diesel respectively). There were also no clitella swellings in CS and NCS. Statistical comparisons also showed that there was significant ($P<0.05$) difference in the mean number of earthworms with swollen clitellum in treated media compared to the control media except with CS.

Juvenile production

The mean number of juveniles observed after 52 days showed that there was no juvenile produced for all set-up exposed to the various soil mixture of gasoline and diesel whereas 10 and 18 juveniles were recorded in CS and NCS respectively.

Discussion

A lot of studies on environmental impact of contaminants have been done with both aquatic and terrestrial biota of economic importance without much consideration of other less economic important biota like earthworms. The exposure of soil mixtures of 100%, 50% and 10% of gasoline and diesel respectively resulted in exhibition of coiling response and swollen clitella region in exposed animals.

The aforementioned responses are very useful indicators of petroleum related contamination or other forms of stress in the terrestrial ecosystem, they can be very easily detected in the field and their uses in contaminated sites have been reported [1, 19-21]. They can be used for the detection of stress in the soil. Such responses could also be important tools for monitoring effectiveness of remediation/clean-up programme as well as mitigation exercises [1,22,23].

Report indicates that earthworms are generally not sensitive to acute effects of some pesticides like DDT [24]. However, [25] Cook et al. reported that DDT did not have effect on the number of earthworm spp but on cocoon production, hatchability and juvenile production.

The coiling response of earthworm inhabiting contaminated environment is an important parameter for observation as it serves as an early indicator of stress in the environment. Reports of coiling response of the earthworm *Eudrilus Eugenia* showed a negative impact of Forcados crude oil [1] which supports the findings from this study.

For survival test, results showed 60% and 20% survival for GS and DS respectively while 80% and 100% survival was recorded for CS and NCS. The higher percentage of survival in CS compared to NCS (control) could be because the organisms are known to be adapted to their original environment [26]. Avoidance test showed that there was 8:2 (NCS:DS), 6:4 (NCS:GS) 5:5 (NCS:CS) survival ratio of *Lybriodrillus violaceous*. Study on Juvenile production showed that there were no juveniles produced in both GS and DS contaminated soil, however, there were juvenile production recorded in CS and NCS. A report [27] had also shown that soils polluted with petroleum effluent caused 40% death, swelling, body lesions, stiffening, coiling, low reproduction and reduction in populations of *Eudrilus Eugenia*.

Soil type	Day 1	Day 2	Day 5	Day 7	Day 14
GS (100%)*	2 (33.33)	3 (60)	3 (60)	4 (100)	4 (100)
GS (50%)*	0(0)	2 (28.57)	3 (42.86)	3 (42.86)	4 (57.14)
GS (10%)	0(0)	0(0)	1(14.29)	2 (28.57)	3 (42.86)
NCS	0(0)	0(0)	0(0)	0 (0)	0(0)
CS	0(0)	0(0)	0(0)	0 (0)	0 (0)

*=significantly different at P<0.05

The t-test results comparing means of swollen clitellum in surviving earthworms exposed to gasoline contaminated soil showed that there was significant ($P<0.05$) difference in GS with 100% and 50% mixture compared to the control media (NCS) where as there was no significant difference in GS with 10% mixture and CS ($P>0.05$) compared to the control media.

Table 3: Mean number of swollen clitella earthworms (Percentage in Parenthesis) exposed to gasoline contaminated soil.

Soil type/Conc (ml/kg)	Day 1	Day 2	Day 5	Day 7	Day 14
DS (100%)*	4(75)	4(100)	3(100)	0(0)	0(0)
DS (50%)*	6(33.33)	5(66.66)	4(75)	3(100)	1(100)
DS (10%)*	0(0)	1(16.67)	2(66.66)	3(60)	4(80)
NCS	0(0)	0(0)	0(0)	0(0)	0(0)
CS	0(0)	0(0)	0(0)	0 (0)	0 (0)

*=significantly different at P<0.05

Table 4: Mean number of swollen clitella in surviving earthworms (Percentage in Parenthesis) exposed to diesel contaminated soil.

Conclusion

The negative impact of all toxicity tests on *L.violaceous* was more severe with diesel contaminated soil followed by gasoline contaminated soil compared to the 3 year old oil spilled soil, this could be due to the higher density and low volatility of diesel compared to gasoline. *L.violaceous* showed less swollen clitella effect than survival and coiling in all soil mixtures of gasoline and diesel contaminated soil. Also, the soil sample from 3 years old oil spill showed a mild impact on the earthworm species as it would have been void of most of the volatile contaminants, however, the slight negative impact on survival could be because of recalcitrant hydrocarbons present in the soil sample.

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