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Full Length Research Paper

Toxicity of Neatex (industrial detergent) and Norust CR 486 (corrosion inhibitors) to earthworms (*Aporrectodea longa*) in naturally spiked soil

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Ecological effects of indiscriminate disposal of industrial chemicals into soils of the Niger Delta environment of Nigeria were assessed using earthworms in spiked natural soil in the laboratory. Populations of indigenous epigeic adult earthworms, *Aporrectodea longa*, were exposed to varying concentrations of two chemicals (industrial detergent and corrosion inhibitor) in natural soil to determine the acute toxicity of the chemicals. Earthworm acute toxicity test (OECD) 207 method was employed. After two weeks of earthworm exposure to Neatex and Norust CR 486 concentrations of 62.5, 125, 250 and 500 mg/kg, percentage mortality was measured as the ecological endpoint in the earthworms. Based on OECD 2003 guidelines, the chemically spiked soil rating showed that both chemicals were slightly toxic. The estimated LC50 concentrations showed that Norust CR 486 (corrosion inhibitor) was more toxic than Neatex (detergent) at $p < 0.05$, $t = 8.213$. Earthworm mean mortality in both chemicals increased with increasing concentrations and exposure duration. Mean mortality observed in Neatex and Norust CR 486 were significantly different from the negative control suggesting that mortality may be attributed to the effect of the chemicals. The results obtained are therefore indications of early warning signs of future soil deteriorations occasioned by indiscriminate disposal of these chemicals in the Niger Delta environment. This calls for regular monitoring and sustainable effluent disposal management.

Key words: Earthworms, chemicals, mortality, and ecotoxicology.

INTRODUCTION

The global environment in recent time has changed radically since the birth of the chemical industry in the early 20th century (Lightowlers, 2004). Chemicals are an integral part of modern life and most products contains chemicals, which can contaminate the environment and pose as public health problems.

The escalating environmental contamination of the environment by chemicals is of growing concern in Nigeria and Worldwide (Ezemonye and Enete, 2004; Ezemonye and Enuneku, 2005; Booth et al., 2002). The apparent human and ecological disorders experienced in industrial settlements as a result of the improper disposal of chemicals such as detergent and corrosion inhibitors calls for careful surveillance on the state of the environment. In Nigeria, only few chemicals have been ecologically tested for safety inspite of their environmental and ecological impact. Lately the Federal government of Nigeria is emphasizing the need for adequate environmental protection in any technological and socio-economic development or endeavours by strictly asking industrial operators to sustainably manage the disposal of chemicals into the environment (DPR, 2002).

Soil constitutes an essential resource of our environ-

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Table 1. Characteristics of *Aporrectodea longa* isolated from Niger Delta soil.

Organism	***A. Longa***
Colour	Red-violet
Anterior segment	Anterior black segment
Prostomium	Prolobous
Length	8-14 cm
Clitellum	Saddle shaped/not flared
Segment	140-159
Tubercula pubertatis (TP)	Bar shaped
Genital tumescences (GT)	Alternates

ment. Many of the organic chemicals produced and emitted end up in the soil, where they impact soil organisms, which are essential to maintain soil fertility and health. Several organic pollutants have influenced the amount and distribution of organisms in the soil. The time of exposure, soil type, temperature, soil pH, soil organic matter, life stage of organisms, species size and organism type are factors, which have been known to influence the toxicity of chemicals in soil (David and Jason, 1997).

The suitability of earthworms as sentinel in soil toxicity is largely due to the fact that they ingest large quantity of the soil and are in full contact with the substrate they consume (Sandoval et al., 2001). Earthworms have been recommended as a critical (suitable) representative of soil organisms and an indicator of soil health (Culy and Berly, 1995). This is because, they consume huge quantities of decomposed litter, manure, and other organic matter deposited on soil - helping to convert it into rich topsoil (Sandoval et al., 2001; Reinecke et al., 1999). Earthworms are superb 'barometers' or 'sentinels' providing an early warning of deterioration in soil quality. This is important for protecting the health of natural environments, and of increasing interest in the context of protecting human health (Beeby, 2001).

Detergents are very widely used in both industrial and domestic premises to wash equipment, installations, heavy-duty machines, and vehicles and oil soiled materials. They are also used in pesticide formulations and for dispersing oil spills at sea. Linear alkylbenzene sulphonate (LAS), a major detergent and corrosion inhibitor ingredient is poorly broken down in rivers and soils and may be toxic to soil organisms (Lightowlers, 2004). All detergents destroy the external mucus layers that protect organisms from bacteria, parasites and can cause severe damage to vital organs. Detergent disturbs the function of the respiratory organ in earthworms. This causes death of the organisms through suffocation.

Corrosion inhibitors are used in a wide range of applications, such as oil pipelines, domestic central heating systems, industrial water-cooling systems and metal extraction plants. Their toxic effects on soil organisms such as earthworms have necessitated the need for monitoring soils contaminated with effluent containing these chemicals.

This study is an attempt to determine the acute toxicity of Neatex (industrial detergent) and Norust CR 486 (corrosion inhibitors) to earthworms as a predictive assessment tool for chemical contamination in the Niger Delta environment. It will also serve as early warning signs of soil deterioration and safety guide to ecological testing of chemicals in Nigeria.

MATERIALS AND METHODS

Earthworm pre-treatment and identification

Earthworm (*Aporrectodea longa*) samples were collected according to methods previously described (Speigel 2002, Terhivuo et al., 1994) from a pristine environment in a cultured farm in Ekrheranwhen, in the Niger Delta ecological zone of Nigeria. They were collected by gentle digging and hand sorting from sub surface litters. Once organisms are obtained, they were identified and maintained in the laboratory using the procedures described in ASTM Standard E 2172-01 (ASTM, 2001). Acclimatization to laboratory conditions was done for seven days. Earthworms were selected based on sexual maturity, as evidenced by the presence of a clitellum (a-3 mm wide ring around the body), size 0.480 to 0.645 g wet weight and liveliness (active response when prodded).

The identification and nomenclature of earthworm (Table 1) is according to (Sims and Gerrard, 1985). Earthworm species used in this study are ecologically relevant to the Niger Delta of Nigeria. *A. Longa* represents epigeic species (Macrophagons, litter-dwelling).

Experimental procedure

Experimental procedure for this test was conducted in accordance with the procedures detailed in (Sandoval et al., 2001; OECD, 207). Toxicity modifying factors considered were exposure duration and mortality. The test chemicals used were Neatex (industrial detergent) and Norust CR 486 (corrosion inhibitor). Stock solutions of the respective chemicals were prepared and serial dilutions were made to obtain concentrations in the range of 62.5, 125, 250 and 500 mg/l.

The soil samples were prepared by mixing clean dry sand with 20 g of prepared cellulose and 80 ml (water) homogenized in a 900 ml glass container to obtain moisture content of 35%. Cellulose was added to the soil as food for the earthworms. Feeding with cellulose ensures that earthworms were not starved.

The earthworms were acclimated in an unspiked soil for seven days. Earthworms were kept on moist filter paper for few hours to void contents of the stomach and intestinal tract before being placed in test jars. Thereafter, ten voided earthworms were cleaned and weighed. They were transferred from their holding containers with a sterilized platinum wire (Freeman et al., 1999) to the soils spiked with concentrations of Neatex and Norust CR 486. Three replicates per treatment were prepared for four exposure concentrations for both chemicals. Negative control containing cellulose, water and clean soil was also prepared in conjunction with the chemicals. The test organisms were maintained in the laboratory. The containers were covered with perforated transparent cover, to prevent the test medium from drying and kept under the test conditions for 14 days. Experiments were conducted using a total of four hundred and eighty (480) matured earthworms.

The entire test was conducted at 23±2°C in soils of pH 6.14 ± 0.75.

Table 2. Mean mortality of earthworms exposed to different concentrations of Neatex in a natural soil substrate (Day 14).

Conc. (mg/kg)	Number tested	Number dead			Mean mortality (%)	Mean Probit value
		X1	X2	X3		
Control	10	0	0	0	0	0
62.5	10	0	0	0	0	2.91
125	10	1	1	1	10	3.60
250	10	3	4	4	37±6	4.29
500	10	4	5	6	50±10	4.98

Table 3. Mean mortality of earthworms exposed to different concentrations of Norust CR 486 in a natural soil substrate (Day 14).

Conc. (mg/kg)	Number Tested	Number dead			Mean Percentage mortality	Mean Probit value
		X1	X2	X3		
Control	10	0	0	0	0±0	0
62.5	10	2	2	2	20±0	3.93
125	10	3	3	3	30±0	4.55
250	10	6	6	5	57±6	5.16
500	10	9	9	9	90±0	5.78

Table 4. Mean LC50 values for Neatex and Norust exposure to earthworms at the end of 14 days.

Chemical	Time (days)	LC50 (95% CL), mg/kg	Probit Line Equation	DF	Slope
Neatex	14	511.32 (320.96 - 1091)	Y= -1.21+2.29 LOGX	1	2.70
Norust CR 486	14	207.61 (129.93 - 411.49)	Y= 0.25+2.04LOGX	1	3.10

LC50 = Lethal concentration causing 50% death of organisms exposed to chemical for the test duration.
CL = Confidence limit.

Mortality rate

Earthworm mortality was evaluated on day 7 and 14 of the experiment in all the triplicates in the natural soil. Direct contact was avoided so as not to induce stress on earthworms. Percentage mortality rate (endpoint of acute toxicity) and physical changes (colour) were estimated (Spurgeon and Hopkin, 1996a). If a worm does not responded to a gentle probing with the platinum wire it was considered dead.

Statistical analysis

The susceptibility of the earthworms to both chemicals was determined using the probit method of analysis (Finney, 1971) for median lethal concentration LC50 at 14 days. Computations of confidence interval of mortality rate were also obtained from the probit analyses used to determinate the LC50 (Probit software). The two-factor ANOVA (analysis of variance) in Microsoft Excel was used to test the variables at $P < 0.05$ level of significance. Multiple bar graphs were also used in this study for the pictorial representation of assessment endpoint.

RESULTS

The results of the acute toxicity of earthworms exposed to varying concentrations of Neatex and Norust CR 486 in spiked soils are presented in Tables 2–4 and further illustrations in Figures 1 and 2.

Negative control

The results showed that no death or morphological changes were observed in the negative controls for day 7 and 14 of the test. Earthworm was defined as dead if it does not respond to a gentle mechanical stimulus to its anterior end (Table 2).

Neatex (industrial detergent)

The test organisms exposed to varying Neatex concentrations at day 7 recorded no mortality in concentrations of 62.5 and 125 mg/kg. However, in same day 7 of the test, concentrations of 250 and 500 mg/kg recorded 20% and 37% mortality, respectively, indicating that mortality increased with increased concentration (Table 2) . In day 14 of the earthworm exposure to 62.5, 125, 250 and 500 mg/kg concentrations, 0%, 10%, 37% and 50% mean mortality, respectively, was recorded indicating that mortality also increased not only with increased concentrations but with exposure duration.

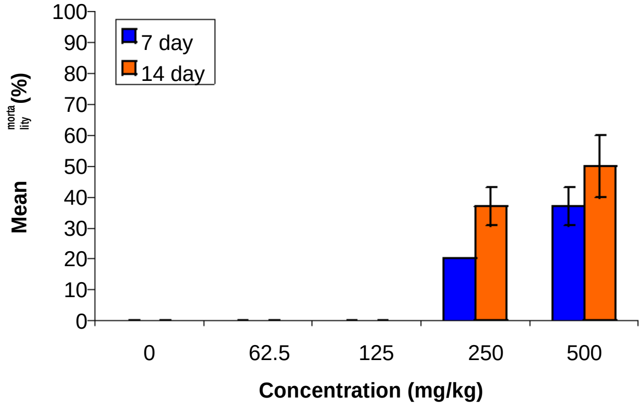

Figure 1. Mortality of earthworm exposed to varying concentration of Neatex.

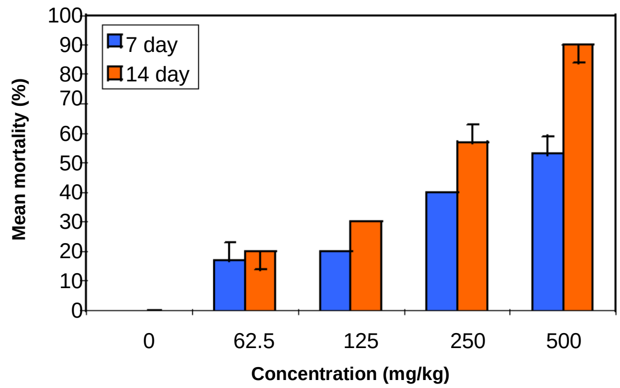

Figure 2. Mean % Mortality of Earthworms exposed to varying concentration of Norust.

Table 5. Earthworm toxicity rating.

Rating	Designation	LC50 (mg/kg)
1	Super toxic	<1.0
2	Extremely toxic	1.0-10
3	Very toxic	10-100
4	Slightly toxic	100-1000
5	Practically non-toxic	>1000

Abbreviations: LC50 median lethal concentration. Data from OECD (2003).

Concentrations, which recorded mortality, also had varying degrees of behavioural alterations in the surviving earthworms, which include lack of burrowing ability, slow and sluggish movement and morphological changes (contraction, rigidity, and elongation). In some cases dicolouration of the earthworms were observed.

Norust CR 486 (corrosion inhibitor)

In Norust CR 486, mortality was observed in all concentrations while mean percentage mortality increased with increase concentrations and exposure duration (Table 3). In the 7-day test, mortality were 17, 20, 40 and 53% while the 14-day test mean mortality were 20, 30, 57 and 90% for 62.5, 125, 250 and 500 mg/kg concentrations respectively.

Earthworms decayed rapidly in soils treated with Norust CR 486 and the surviving earthworms showed intense physiological alterations, which is suggestive of higher toxic effect of Norust CR 486.

14-day LC50

Estimated LC50 for the 14-day test showed that Norust CR 486 (207.61 mg/kg) was more toxic than Neatex (511.32 mg/kg) (Table 3). The statistical analysis also showed significant difference between the estimated LC50 for Norust CR 486 and Neatex at $p < 0.05$ and $t = 8.312$. However, the difference between the mean mortality rate for Norust CR 486 and Neatex were not significantly different. The mean mortality for the control experiment was significantly different from the results obtained for Norust CR 486 and Neatex at $p < 0.05$.

DISCUSSION

This study was conducted according to approved OECD 207 guideline procedures for a 14-day acute toxicity testing of chemical to earthworm in natural soils. This is with a view of providing a platform for the assessment and regular monitoring of the Niger Delta environment, which is constantly exposed to chemical contaminations.

Indiscriminate discharge of industrial chemicals into soils is bond to expose organisms living and breeding there to multiple stressors of varying sources and intensity. The study observed the vulnerability of earthworms to chemical contaminants (Norust CR 486 and Neatex). Inspite of the slightly toxic ratings of the 14 day LC50, 511.32mg/kg (Neatex) and 207.61mg/kg (Norust CR 486), the earthworms experienced considerable level of mortality and morphological distortions (Table 5). The toxicity of the industrial detergent (Neatex) and corrosion inhibitor (Norust CR 486) was first manifested in sluggish movement and dicolouration of earthworms on the seventh day.

Subsequently death occurred with increasing concentrations and exposure duration for both chemicals. In concentrations of 50 -100 ppm surfactants will not only denature the cells proteins, but also totally inactivate the enzyme and actually alter the cell wall permeability. Hazardous effects of detergents on terrestrial organisms have been reported (Edwards and Bohlen, 1992).

Soil contamination with organic pollutants (e.g. corrosion inhibitors detergents), heavy metals, and acid precipitation can be detrimental to earthworm populations. Most organic soil pollutants are highly toxic to earthworms. In addition to acute negative affects of some chemicals on earthworms, sublethal concentrations can cause declines in earthworm growth and reproduc- tion. These can accumulate in earthworm tissues, which are potentially problematic for movement into higher trophic levels, because of the wide array of animals that prey on earthworms. Earthworm species vary in their tolerance and reports have shown a decline in earthworm populations in response to large amounts of organic chemical deposition (Bayer and Foy, 1982).

Both chemicals in this study caused a sharp increase in mortality over a large concentration range. This was more with Norust CR 486. The implication of this observation is that increased concentrations of both chemicals either through accumulations over time or by increased point source discharges will likely be detrimental to soil organisms especially earthworms. The differential acute toxicity levels of both chemicals as shown in the different LC50 values may be attributed to the toxic constituents of the chemicals and corresponding regulatory response by earthworm. Organic chemicals have varying effects on earthworm populations, with many chemicals having little or no toxicity to earthworms and others exhibiting acute toxicity (Edwards and Bohlen, 1992). This is because organisms are known to react differently to varying stressors depending on their toxicity profile. It was also observed that exposure of the chemicals over time not only facilitate persistence but also encourage bioavailability of toxic substances or chemicals.

The significant difference between the negative control and the test concentrations showed that the forensic threshold was exceeded, an indication that death may have been induced by the chemicals. Linear alkylbenzene sulphonate (LAS), a major constituent of detergent and corrosion inhibitor have been known to be persistent in rivers and soils leading to their accumulation in soil organisms (Lightowlers, 2004) . This observation is quiet instructive and requires that the discharge of these chemicals be channeled through effluent treatment procedures before they are released into the environ-ment. This study observed signs of possible soil deterioration as well as depletion of vital terrestrial organisms especially earthworms from Neatex and Norust CR 486 contaminations. Through inappropriate disposal of detergent, the quality of the topsoil may be altered with the likely consequence of reduction in soil fertility and poor plant growth (Egharevba, 2002).

It is therefore imperative that sustainable chemical assessment and monitoring programmes should be put in place to protect the delicate biodiversity rich Niger Delta environment.

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