Toxicology Digest

Assessment of The Toxicity of Leachate from Municipal Waste Dump Site to Earthworm- *Lumbricus terrestris*

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Abstract

The study characterized leachate from a refuse dump site in Aba, Abia State, Nigeria and assessed its toxicity to *Lumbricus terrestris*. Leachate samples were collected, and the physiochemical properties determined using portable equipment and standard analytical procedures. Ten earth worms with mean weight of 0.23±0.11g and length of 6.92±1.88 cm was exposed for 14 days to 500g of sieved soil spiked with 1%, 25%, 50%, 75% and 100% leachate concentration in triplicates. Mortality was assessed on day 7 and 14 while antioxidant activities were assessed on day 14. The results showed that electrical conductivity (360.333±346.634µs/cm), total dissolved solids (28.00±1.00mg/l), arsenic (0.34±0.03mg/l), nickel (0.13±0.03mg/l), cadmium (0.09±0.03 mg/l), lead (0.45±0.01mg/l), biochemical oxygen demand (32.33±3.06mg/l), chemical oxygen demand (129±3.61mg/l), total organic carbon (156.87±116.11mg/l), nitrate (34.00±0.87mg/l), phosphate, (25.80±3.75mg/l), and sulphate (360±43.41mg/l) were above the FEPA 1991 standard. The 7 day and 14 day LC_{50} was 60.04 (52.56-68.34) % and 52.90 (46.85-58.85) % respectively. Glutathione (17.85±2.54µm/l) and catalase activity (14.87±0.47µm/l) decreased significantly ($p<0.05$) in earthworms exposed to 75 and 100% leachate compared to earthworms exposed to 1, 25 and 50% leachate concentration and the control, while super oxide dismutase activity increased and decreased significantly in earthworms exposed to 1% and 100% concentrations respectively compared to the control. Malondialdehyde increased significantly in earthworms exposed to 50%, 75% and 100% leachate compared to the control and earthworms exposed to 1%, 25% leachate concentration. The study showed that dumpsite leachate contained hazardous substances and was deleterious to *L. terrestris*.

***Keywords**: Lumbricus terrestris, oxidative stress, dumpsite leachate, toxicity, antioxidant*

Introduction

Open dumping of municipal solid waste in shallow unlined excavation is a common disposal method in developing countries like Nigeria (Egun *et al*., 2016). One characteristic of refuse dumpsites is lack of collection and treatment of leachate formed by the percolation of moisture through waste in the dumpsite. In some cases, leachate ponds may be created for treatment via aeration, but such ponds could easily overflow to adjacent land.

Leachate, a heterogeneous liquid may contain substances that may be divided into four groups of pollutants: dissolved organic matter including humic and fulvic acids; inorganic components such as nitrogen; heavy metals like lead, nickel, chromium, and cadmium and xenobiotic organic compounds (Kjeldsen *et al.,* 2002; Wiszniowski *et al.,* 2007; Singh and Mittal 2009). The characteristics and concentration of the leachate depends on the composition of the waste, the amount of water that flows through the waste, the length of time of contact with the refuse, depth of waste, available oxygen, temperature and age of landfill (Adhikari, Bikash, 2014). Over time, the concentration of the pollutants could decrease as the leachate stabilizes. However, ammonia which may be toxic to test organisms may remain high (Kjeldsen *et al.,* 2002). On the other hand, leachate is considered an option for improving soil fertility due to the organic matter, nitrogen, potassium, and magnesium that it contains. The use of treated landfill leachates as a possible source of plant nutrient was studied by Nunes Júnior *et al.,* 2016 and Tuyrki and Bouzid 2017) and the leachate was observed to promote plant growth.

Leachate may be hazardous to earthworms like *Lumbricus terrestris* which are in direct contact with the soil and may affect their ability to perform their ecological functions. Earthworms are ubiquitous, abundant and form the largest part of the invertebrate’s biomass in soils where they play vital ecological roles in transforming chemical element (Lemtiri *et al.,* 2014). They are excellent ‘’bio-indicators’’ of the relative health of the soil ecosystem (Fründ *et al.,* 2011). Thus, the aim of the study was to characterize the leachate from a refuse dumpsite and assess its toxicity in earthworm species *Lumbricus terrestris*.

Materials and Methods

Experimental Design

Earth worm acute toxicity test was carried out following OECD, 1984 procedure with slight modification in which natural soils were used in place of artificial soils. *Lumbricus terrestris* were randomly divided into six groups; I, II, III, IV, V and VI where group I was the control group and groups II to VI were the test groups corresponding to five concentrations of dumpsite leachate. The test duration was 14 days and mortality were assessed at day 7 and 14, while oxidative stress and antioxidant activities were assessed on the 14th day.

Earthworm Collection and Acclimatization

L. terrestris with individual weight of 200 to 400 mg used in the study were sourced from Michael Okpara University of Agriculture Umudike. They were conditioned for 48 hours in the natural soil prior to the start of the experiment.

Dumpsite Leachate, Soil Collection and Spiking of Sediments

Dumpsite leachate samples were collected in triplicates from the solid waste dumpsite located at Aba in Abia State, Nigeria in 20 litre plastic container and transported to the laboratory and stored in the dark under room temperature. Top soil (0-15 cm) was collected using an auger. Prior to the start of the experiment, the soil samples were air dried and sieved using sieve of 1.0 mm mesh size to remove unrepresentative material, such as stones, trash, and twigs and to increase homogeneity. Selected soil properties were analysed in the laboratory following standard procedure. The sediment suspension technique (Landrum *et al.,* 1992) was adopted to spike the sediments with 1%, 25%, 50%, 75% and 100% of leachate stock solution prepared through serial dilution. 500g of sediments was spiked with 200ml of the test leachate solutions.

Determination of Physicochemical Parameters

Temperature, pH, electrical conductivity, dissolve oxygen (DO), and total dissolved solids (TDS) of the leachate were measured in-situ using portable meters, while Arsenic, (As), Nickel (Ni), Cadmium (Cd), Lead, (Pb), Mercury, (Hg) were determined using atomic absorption spectrometer (AAS), while biological Oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), NO_3, PO_4 and SO_4 were analysed in the laboratory using appropriate techniques (APHA, 2005).

Length and Weight Measurements

The length measurement of the earth worm was determined using a meter rule in centimeters (cm), while an electronic balance was used to measure the weight in grams (g).

Toxicity Test

For the acute toxicity test, the spiked sediments were placed in poly-pot and ten earthworms in triplicates, were place in each pot spiked with different concentrations of leachate for 14 days. Mortality was assessed by emptying test medium onto a glass plate, sorting worms from the medium and testing their reaction to a gentle prodding. After the 7 day assessment, worms and medium are replaced in the test poly pot. Worms are classified as dead when they do not respond to a gentle stimulus to the front end.

Antioxidant Analysis

To evaluate the effect of the leachate on antioxidant enzymes of earthworms, only earthworms that were alive after 14 days except for earthworms exposed to 100% leachate were collected. Earthworm samples were homogenized with 9.0 ml 4.0m phosphate buffer pH 8.0 and centrifuged at 3000rpm for 10 minutes. The supernatant was collected and used for the analysis. Superoxide dismutase (SOD) activity was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 at 30 °C (Magwere *et al.*, 1997). Catalase (CAT) was assayed spectrophotometrically based on the measurement of a decrease in absorbance of the test sample by the induced decomposition of H_2O_2 in the presence of an analyte enzyme (Aebi *et al.*, 1983), while reduced glutathione (GSH) was determined by the method of Jollow *et al.*, (1974). Lipid peroxidation was determined spectrophotometrically by measuring the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin *et al.*, (1993).

Data Analysis

The lethal concentrations were determined using the probit method. The mean values of the SOD, CAT, MDA and GLT of the exposed earth worms was compared with control group using one-way ANOVA while turkeys test post hoc test was used to obtain the specific significant difference. Analysis was computed with SPSS version 22. However, the graphs were plotted using Statistica version 10.

Results

Dumpsite Leachate and Soil Physicochemical Properties

The physicochemical characteristics of the leachates and the soil samples are presented in Table 1 and Table 2 respectively.

Table 1: Physicochemical Properties of Dumpsite leachate

Physicochemical Parameters	Mean value	FEPA Standard (1991)	Percentage difference
Odour	Objectionable	N/A	NC
Dissolved Oxygen (mg/l)	0.13±0.58^{+}	5	-97%
pH	6.33±0.75	6-9	
Electrical Conductivity (μs/cm)	360.333±344.634*	125	188%
Temperature (^{o}C)	28.00±1.00^{+}	27-35	NC
Total Dissolved Solids (TDS) (mg/l)	2704.33±855.25	2000	35%
Biological Oxygen Demand (mg/l)	32.33±3.06*	30	8%
Chemical Oxygen Demand (mg/l)	129.00±3.61*	75	72%
Total Organic Content (mg/l)	156.87±116.11^{+}	200	-22%
NO_3 (mg/l)	34.00±0.87*	20	70%
PO_4 (mg/l)	25.80±3.75^{+}	50	-48%
SO_4 (mg/l)	360.00±43.41*	100	260%
Arsenic (mg/l)	0.34±0.03*	0.1	240%
Nickel (mg/l)	0.13±0.03*	0.01	1200%
Cadmium (mg/l)	0.09±0.03*	0.01	800%
Lead (mg/l)	0.45±0.01*	0.05	800%

Values are mean ± standard deviation of three replicates, * above permissible limits, + indicates below permissible limits, N/A-not available, NC- not calculated

Electrical conductivity, biological oxygen demand, chemical oxygen demand, nitrate, sulphate, cadmium, nickel, arsenic, and lead were above the FEPA 1991 standard, by 188, 8, 72, 70, 260, 800, 1200, 240, 800 % respectively, while dissolved oxygen, total organic content, and phosphate were below the standard by 97, 22, and 48% respectively Temperature and pH were within the acceptable limits.

Table 2: Soil physicochemical properties

Soil physicochemical properties	Value
C/N ratio	12.1±0.07
% Sand	61.4±3.23
% Slit	19.2±1.01
% Clay	19.4±1.02
Texture	Sandy loam
pH (H_2O)	5.5±0.29
pH (KCL)	4.4±0.23
% Nitrogen	0.149±0.01
% Organic Carbon	1.79±0.09
% Organic Matter	3.09±0.16
Ca^{2+} (cmol/kg)	4.8±0.25
Mg^{2+} (cmol/kg)	2.8±0.15
K^{+} (cmol/kg)	0.258±0.01
Na^{+} (cmol/kg)	0.188±0.01
Exchangeable Acidity (EA) (cmol/kg)	1.48±0.07
Effective Cation Exchange Capacity (ECEC) (cmol/kg)	9.53±0.5
Water Holding Capacity (%)	49.8±2.62
Soil Colour	Dark greyish brown 7.5yR (4/2)

Values are mean ± standard deviation of three replicates

Table 3: Nominal heavy metal concentration to which earthworms were exposed

Test solution	Heavy Metals			
	As (mg/l)	Ni (mg/l)	Cd (mg/l)	Pb (mg/l)
100% leachate	0.34	0.13	0.09	0.45
75% leachate	0.255	0.0975	0.0675	0.3375
50% leachate	0.17	0.065	0.045	0.225
25% leachate	0.085	0.0325	0.0225	0.1125
1% leachate	0.0034	0.0013	0.0009	0.0045
0% leachate (Control)	ND	ND	ND	ND

ND- Not Determined

Concentration of heavy metals were calculated using dilution formula $C_1 \times V_1 = C_2 \times V_2$, where C_1 is the initial concentration, V_1 is initial volume, C_2 is the final concentration V_2 is the final volume.

Mortality Rate of Earthworm Exposed to Dumpsite Leachate

Figure 1 shows the mortality pattern of exposed earthworms showing little/no mortality at leachate concentration of 1%, partial mortality at 25, % 50 and 75% and total mortality at 100% leachate concentration after 14 days of exposure.

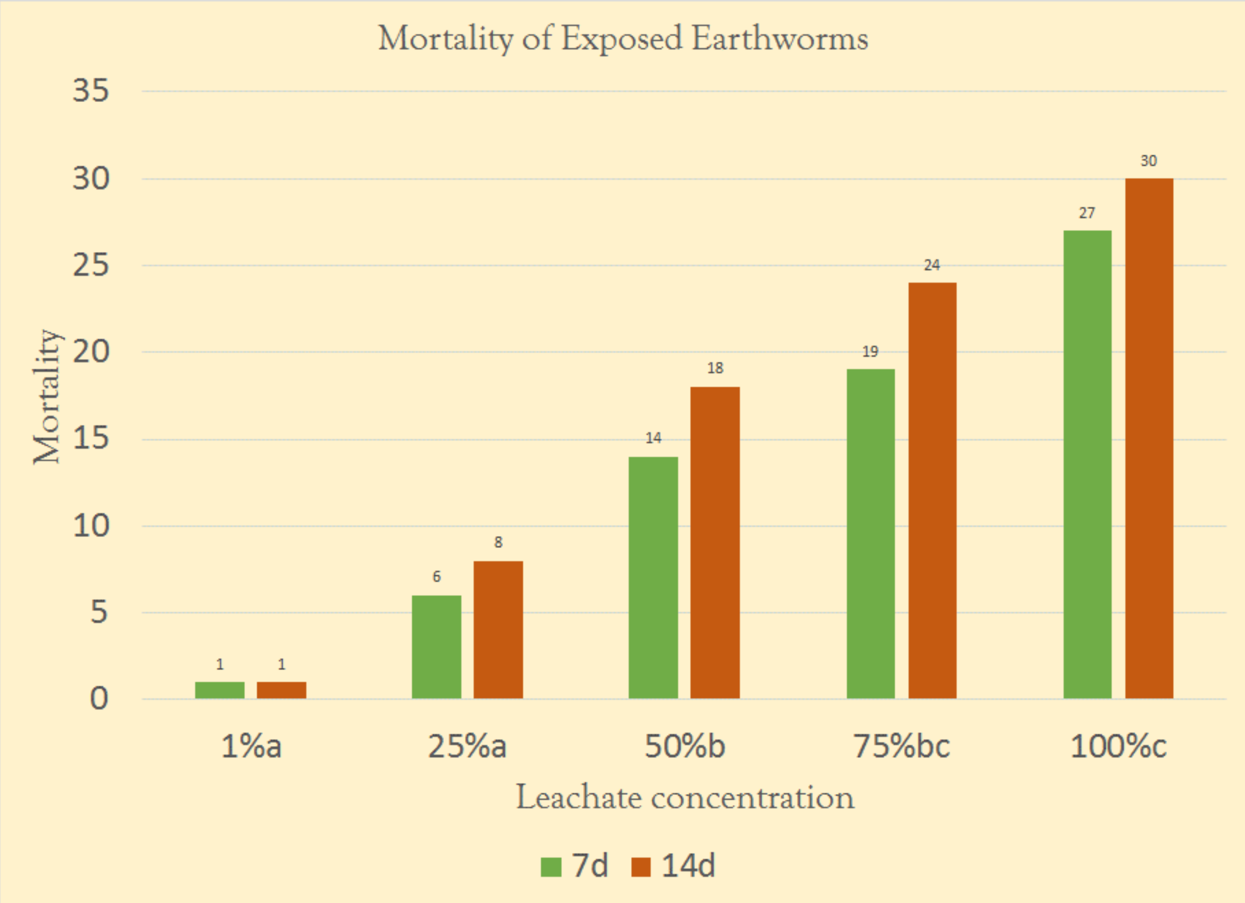

Figure 1. Results of the mortality rate of earthworm exposed to dumpsite leachate, Concentrations with different alphabets are significantly different (p<0.05)

After 7 days of exposure, mortality caused by 1% and 25% leachate concentration were statistically different from mortality caused by 75% and 100% leachate concentration ($p<0.05$) Fig. 1. However, mortality caused by 25% and 50%; 50% and 75% as well as 75% and 100 % were similar ($p>0.05$). After 14 days of exposure, mortality caused by 1% and 25% leachate concentration were statistically different from mortality caused by 50% 75% and 100% leachate concentration ($p<0.05$). However, mortality caused by 1% and 25%; 50% and 75% as well as 75% and 100 % were similar ($p>0.05$).

Acute Lethal Concentration of Dumpsite Leachate

The lethal concentrations of the leachate after 7 days and 14 days of exposure are presented in table 3.

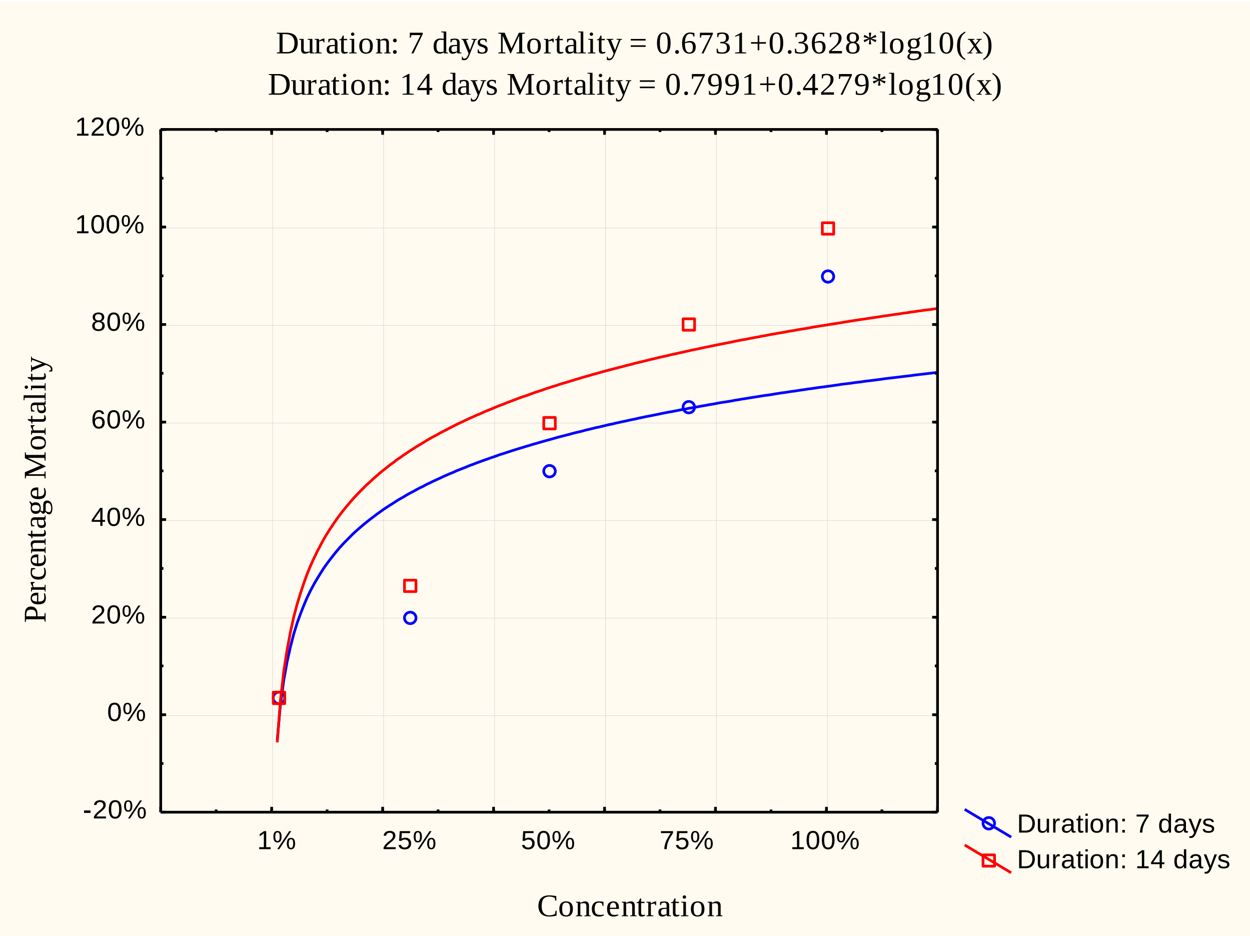

Figure 2: Dose response relationship of earthworm exposed to leachate

Figure 2 is a plot of % mortality vs concentration showing the probit regression equations for both 7 and 14 days exposure time. The graph depicts increase in mortality as concentration and time increases.

Table 4: Lethal concentrations of dumpsite leachate`

Duration of Exposure	LC_5 (95% C.I) (%)	LC_{25} (95% C.I) (%)	LC_{50} (95% C.I) (%)	LC_{75} (95% C.I) (%)	LC_{95} (95% C.I) (%)
7 days	25.22 (16.74-31.84)	42.07 (33.84-48.54)	60.04 (52.56-68.34)	85.68 (74.59-105.31)	142.93 (114.05-212.28)
14 days	26.99 (19.65-32.67)	40.15 (33.32-45.52)	52.90 (46.85-58.85)	69.71 (62.54-80.13)	103.68(88.50-103.79)

Results indicates lethal concentration ± 95% confidence interval

The 7 and 14 days LC_{50} is estimate to be 60.04 % and 52.90 % of the raw leachate concentration respectively.

Antioxidant Activity and Oxidative Stress in *Lumbricus terrestris* Exposed to Dumpsite Leachate

Figures 3-6: Mean plot of Antioxidant Activity and Oxidative Stress of control and exposed *Lumbricus terrestris*

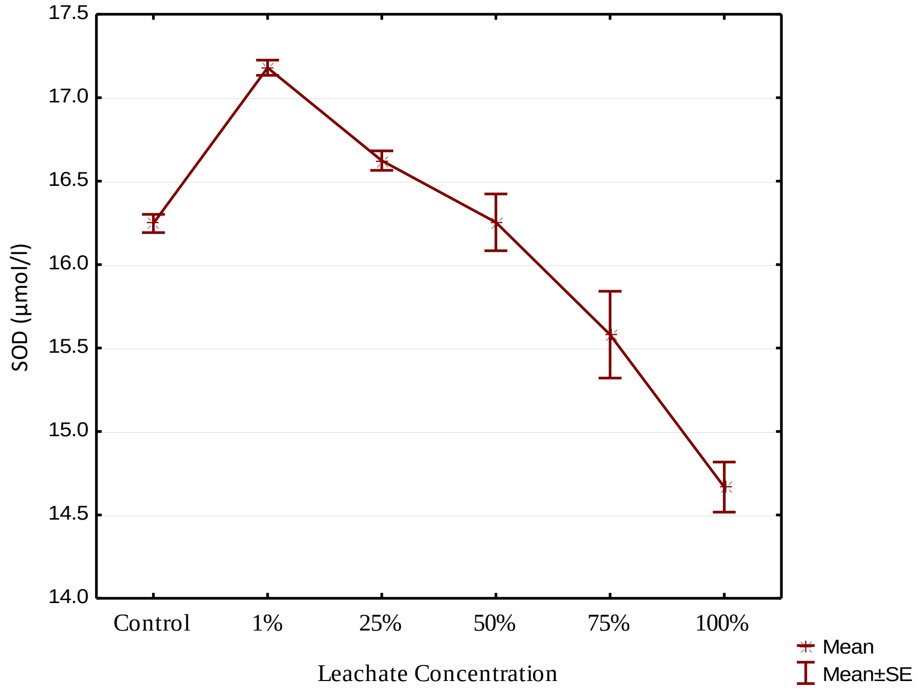

Figure 3: Mean plot of SOD activity of exposed earthworms compared to the control

SOD activity increased by 5.74%, 2.32%, and 0.04% but decreased by 4.10%, and 9.73% in earthworms exposed to 1, 25, 50, 75, and 100% leachate concentration respectively compared to the control. The increase was significant in earthworms exposed to 1% compared the control and earthworms exposed to 75 and 100% leachate ($p<0.05$), while the decrease was significant in earthworms exposed to 100% concentration compared to control ($p<0.05$) and between earthworms exposed to 25, 50 and 75% ($p<0.05$) Fig 3.

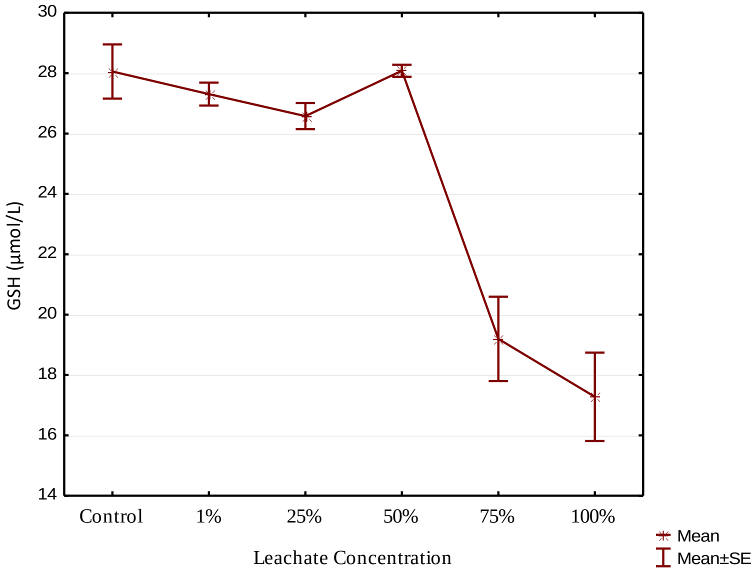

Figure 4: Mean plot of GSH concentration of exposed earthworms compared to the control

GSH level decreased by 2.67%, 5.28%, but increased slightly by 0.08% and then decreased by 31.58%, and 38.42% in earthworms exposed to 1, 25, 50, 75, and 100% leachate concentration respectively compared to the control. The decrease was significant in earthworms exposed to 75 and 100% leachate compared to the control and earthworms exposed to 1, 25 and 50% ($p<0.05$). However, GSH level in the control was not significantly different compared to earthworms exposed to 1, 25 and 50% ($p>0.05$) Fig 4.

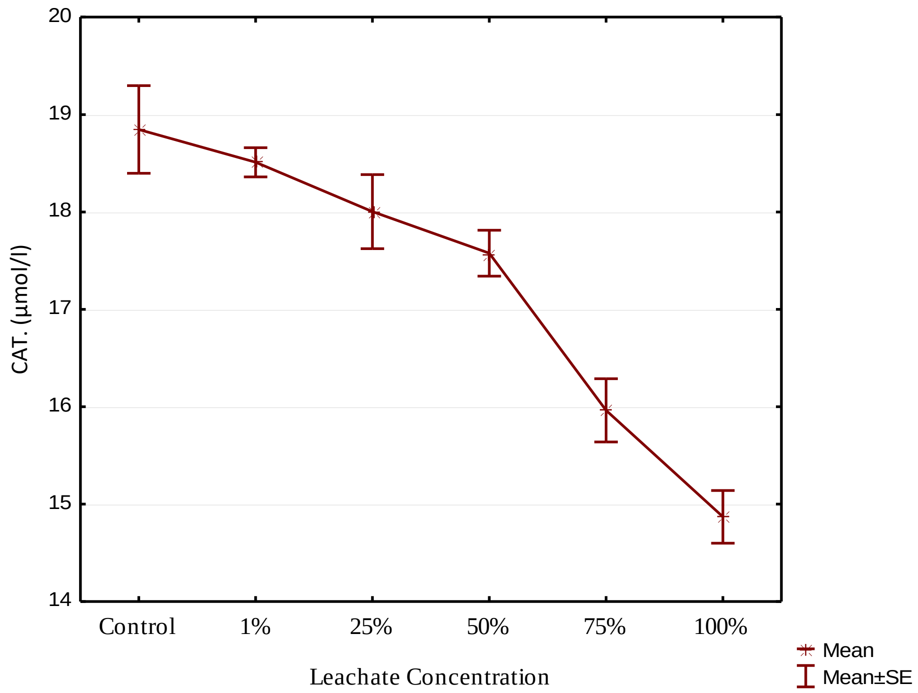

Figure 5: Mean plot of Catalase activity of exposed earthworms compared to the control

CAT activity decreased by 1.79%, 4.47%, 6.74%, 15.30%, and 21.10% in earthworms exposed to 1, 25, 50, 75, and 100% leachate concentration compared to the control. The decrease was significantly lower in earthworms exposed to 75% and 100% compared to the control and earthworms exposed to 1, 25 and 50% leachate ($p<0.05$). However, CAT activity in the control was not significantly different compared to earthworms exposed to 1, 25 and 50% ($p>0.05$) Fig 5.

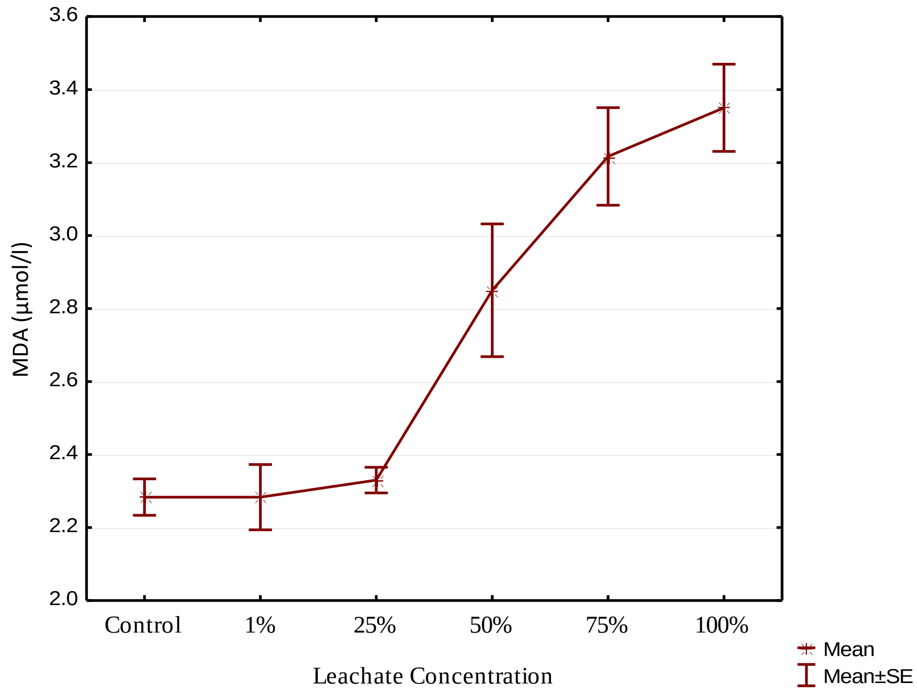

Figure 6: Mean plot of MDA level of exposed earthworms compared to the control

MDA level was unchanged in earthworms exposed to 1% leachate concentration but increased by 2.04%, 24.82%, 40.88%, 46.72% in earthworms exposed to 25, 50, 75, and 100 leachate concentrations compared to the control. The increase was significant in earthworms exposed to 50, 75 and 100% compared to the control and earthworms exposed to 1, and 25% leachate concentration (p<0.05). It was however not significant in earthworms exposed to 1, and 25% compared to the control (p>0.05) fig 6.

Discussion

Leachate contains a cocktail of substances with varying physical and chemical characteristics. The city of Aba is a major commercial and industrial centre and the nature of the waste generated and disposed in dumpsites consists mainly of domestic and industrial waste (Ukpong *et al.,* 2015). Consequently, the physiochemical characteristics of the leachate observed in this study depicted this trend.

In this study, electrical conductivity, heavy metals, BOD and COD (estimates of the degree of organic and inorganic pollution) (), nitrate, and sulphate level of the leachate exceeded the FEPA (1991) permissible limits while dissolved oxygen, total organic content and phosphate were below the standard. The high electrical conductivity may be attributed to the presence of ions from heavy metals. Metals have high electrical conductivity, as they freely lose their electrons to form cations (Jaishankar, *et al.,* 2014). The presence of heavy metals in waste is considered a characteristic of waste from industrial solid waste. Heavy metal may be released from solid wastes from textiles, plastics, and cosmetics, shoe manufacturing industries present in the city. The BOD and COD values suggests that the constituents of the leachate may be mainly inorganic in nature, more so when SO_4 and to a less degree NO_3 are high. The values of TDS, BOD, COD, and NO_3 in this study are consistent with those reported by Asibor *et al.,* (2016) for leachate obtained from dumpsite in Okuvo, Delta State but higher that the values quoted by Ojoawa *et al.,* (2012) for leachate analysed in Ogbomoso Southwest, Nigeria except NO_3 which was lower. In all, dumpsite leachate could be a significant source of heavy metals in soil and lead to soil pollution (Ojoawa *et al.,* 2012; Akujieze *et al.,* 2014; Asibor *et al.,* 2016).

In the current study, the pattern of the mortality as well as the 7 days LC_{50} (60.04 %) and 14 days LC_{50} (51.34 %) showed that undiluted (100%) and diluted (50% and 75%) leachate concentrations were lethal to *Lumbricus terrestris*. The concentration of the toxicants in the diluted leachate were high enough to be deleterious to the earthwormEarthworms are vulnerable to metal pollution (Bengtsson and Rundgren, 1992). LC_{50} of 60.04 % implies exposure to heavy metal mixtures of As (0.204 mg/l), Ni (0.078 mg/l), Cd (0.054 mg/l) and Pb (0.270 mg/l), while LC_{50} of 51.34 % implies exposure to heavy mixture concentrations of As (0.175 mg/l), Ni (0.067 mg/l), Cd (0.046 mg/l) and Pb (0.231 mg/l). Oni and Hassan (2013) observed 100% mortality in *E. fetida* after 14 days of exposure to soils from waste dumpsite in Oyo State, Nigeria polluted with heavy metals. The bioavailable Ni and Cd concentration at which 100 % mortality occurred was 0.032mgl and 0.071mg/l respectively but the values are lower than the estimated concentration of these metals in 100, 75 and 50% leachate to which *L. terrestris* were exposed. This could explain why high mortality still occurred at 50 and & 75% leachate concentrations as the heavy metals level were still high.

In this study, *Lumbricus terrestris* exposed to 75 and 100 % leachate concentration showed significant decrease in glutathione, superoxide dismutase and catalase activity after 7 and 14 days of exposure. However, the increase in SOD activity in earthworms exposed to l and 25% concentrations and subsequent decrease with increased leachate concentration depicts a biphasic response to contaminants in the leachate i.e. induction of the enzyme at lower concentration and reduction in enzyme activity at higher concentrations (75% and 100%) (Calabrese and Baldwin, 2002).Decrease in antioxidants activities in exposed earthworms may be linked to the heavy metals present in the leachate which can inhibit the activities of antioxidants. The mechanism for inhibition for redox inactive metals like lead, cadmium, arsenic and nickel involve inhibition of catalase activity (Singh and Sivalingam 1982), depletion of glutathione and binding to sulfhydryl groups of proteins (Valko *et al.,* 2016). Depletion of GSH, SOD and CAT activity was reported by Maity *et al.*, (2018) in *E. fetida* exposed cadmium. accumulation of free radicals particularly H_2O_2 sustained by metal stress in earthworms exposed to higher concentration of the leachate may be responsible for the inhibition of SOD and CAT enzymes.

Disruption of the antioxidant activities enhances the production of free radicals or reactive oxygen species (ROS) such as hydroxyl radical ($HO^{.}$), superoxide radical ($O^{2.-}$) or hydrogen peroxide (H_2O_2) and ultimately result in oxidative stress (Ercal *et al.,* 2001). Cells under oxidative stress manifest several biochemical markers due to attack by ROS on important macromolecules like lipids, proteins and DNA. Malondialdehyde (MDA) is commonly used indicator of lipid peroxidation because it is a product of the attack by ROS on polyunsaturated fatty acid residues of phospholipids (Rehman *et al.*, 1995; Valko *et al.*, 2005). In the current study, the high MDA levels recorded in earthworms exposed to 50, 75 and 100% leachate after 7 and 14 days of exposure indicates that at these concentrations antioxidant defences of the earthworms were overwhelmed leading to increased rate of ROS generation and inducement of oxidative stress as evidenced by the elevated MDA levels. However, MDA levels was high in earthworms exposed to 50% leachate even though the activity of glutathione and catalase was not significantly reduced in the affected earthworms. It may be possible that glutathione and catalase activity in the earthworms were not statistically different from the control group, but biologically, they were inhibited enough for oxidative stress to occur. Oxidative stress reported in the current study is corroborated the report by Oni and Hassan (2013) in earthworms exposed to heavy metals in field contaminated dumpsite soil.

Conclusion

The study showed that dumpsite leachate which contained hazardous elements including heavy metals was toxic to *Lumbricus terrestris.* High leachate concentrations (100% and 75%) accounted for over 65% of earthworm mortality, inhibited the activities of antioxidant enzymes, and induced oxidative stress. Exposed *L. terrestris* showed concentration dependent decrease in antioxidant activity and increase in oxidative stress. It is possible that metal-induced oxidative stress in cells may to an extent be responsible for the mortality of *L. terrestris* exposed to the leachate.

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