Journal of Scientific Research & Reports
2(2): 544-558, 2013; Article no. JSRR.2013.006

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Acute and Sub-Lethal Toxic Effects of a Contaminated Dumpsite Soil to the Earthworm, *Eisenia fetida* (Savigny, 1826)

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Authors' contributions

Author ATH initiated the study; author AAO wrote protocol, carried out the experiments, managed the literature searches and analysis of the study and wrote first draft. Both authors read and approved the final manuscript.

Research Article

Received 3rd May 2013
Accepted 16th July 2013
Published 14th August 2013

ABSTRACT

Aims: Bioassays measure environmentally relevant toxicity. Consequently, increasing attention has focused on incorporating toxicity tests in hazard evaluations of contaminated sites. We evaluated the lethal and sub-lethal effects of contaminated dumpsite soils at various depths using the earthworm *Eisenia fetida*.
Study Design: Four contaminated dumpsite soils WDA [Waste Dump Area] 1 & 2 representing top soils (0-15cm depth), WDA 3 (15-30cm depth) WDA 4 (75-100cm depth) and field control (CS- control soil) were inoculated with *E. fetida* (12 worms/500g soil).
Place and Duration of Study: Institute of Applied Ecology, Shenyang, China. March 12-31, 2007.
Methodology: Survival, body weight, and an oxidative stress biomarker (Malondialdehyde levels-MDA) of the earthworms were assessed over a seven-to-fourteen day period. Total and bio-available metal and other physico-chemical parameters of the soil were determined using standard procedures. Correlation coefficients and ANOVA were used for data analysis.
Results: Test-soils WDA 1-2 had the highest levels of cadmium: (Mean±SD-28.17±0.01; 24.77±0.003), zinc (1777.98±0.49; 2883.90±1.48); copper (493.08±0.09; 684.57±0.40), and manganese (1345.53±3.46; 1548.03±0.64)mg/kg soil. WDA 3 had the highest levels

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of lead (356.80±0.18mg/kg). Earthworms showed significant (*P* =.05) weight reduction in test soils WDA 1-3 from 0.353±0.013g, 0.348±0.035g, 0.348±0.035 to 0.215±0.003g, 0.216±0.019g by day 14; and 0.215±0.030g by day 7 respectively. Mortality in WDA 3 was 100% by day 14. Only earthworms in WDA 4 did not differ significantly in weight from the control. MDA levels showed a general pattern of increased levels by the second day and subsequent declines by the seventh day, suggestive of oxidative stress onset and possible role of anti-oxidant defense mechanisms. Total lead, bio-available cadmium and zinc correlated with growth reduction data on day 7 (-0.919;*P*=.05), (-0.924) and 14 (-0.995;*P*=.01) and may partly be responsible for the observed effects. Increased pH in WDA 4 may have through increased metal sorption reduced bio-availability.

Conclusion: These results may explain the absence of earthworms in metal contaminated soils.

Keywords: Earthworms; dumpsite; growth; survival; Malondi-aldehyde levels (MDA).

1. INTRODUCTION

Toxicity bioassays and physico-chemical analysis have been used by a number of authors to assess the environmental impact of contaminants [1-4]. The reason for this is that chemical data alone are insufficient to evaluate the toxic effects resulting from landfill leachate and waste contaminated soils. Consequently, increasing attention has focused on the incorporation of toxicity tests in hazard evaluations of waste dumps. Bioassays constitute a measure for environmentally relevant toxicity. The environmental risk is usually related to the bio-available fraction of contaminants such as heavy metals in soil [5]. However, most of the studies involving solid phase bioassays (soil) are usually performed in artificial soils to which the contaminants/pollutants to be tested are added and only a few use field-contaminated soils [6]. Thus, our aim is to assess the toxic effects of landfill leachate / solid waste field-contaminated soils of a major dumpsite (the Aba-Eku dumpsite) in Ibadan, Nigeria. The tested soils have been shown to have high levels of heavy metals. The results obtained, in addition to chemical data will provide information on the environmental impact of the above contaminants.

The use of annelids in solid phase assays is informed by the fact that they are recognized indicators of environmental quality [7]. Earthworms represent the largest (up to 60-80%) of the total soil biomass [8-9]. They also occupy a key position in the transfer of pollutants towards other trophic levels [9]. They modify organic matter both chemically and physically, mix leaf litter with the soil, and generate soil porosity. They also influence other organisms such as invertebrates, micro-organisms and also plants; and as such are referred to as “ecosystem engineers”. The important role played by earthworms in soils makes them a key indicator for ecosystem health, hence their use in many toxicity assessment studies [6].

Among these organisms, species such as *Lumbricus* and *Eisenia spp.* are considered to be of particular interest to evaluate the adverse effects of contaminants [10]. *E. fetida* (Savigny, 1826) in particular, is a reddish brown, small sized, compost-dwelling worm, usually recommended as a toxicity test species [11]. It is currently used to determine the effects of pollutants on soil biological quality [12], and has been the most commonly used species. It is also useful as a sentinel organism to assess the quality of the terrestrial environment [8]. The ease with which this species can be reared and handled in the laboratory and the limited differences in sensitivity between it and other earthworm species to pollutants such as metals, makes these organisms ideal test species [13].

Malon-dialdehyde – (MDA) is a highly toxic by-product of free radicals derived from lipid peroxidation; an exceeding damaging process which occurs via the peroxidation of unsaturated fatty acids in all aerobic biological systems. It is a potential health hazard of exposure to toxicants especially heavy metals [14]. The free radicals generated are reactive oxygen species (ROS) which can cause oxidative damage in a cell, tissue or organ (oxidative stress). Polluted environments can induce oxidative stress [15]. Oxidative stress can cause toxic and adaptive responses within a cell, including damage to cellular components such as lipid peroxidation, altered anti-oxidant enzymes activities and DNA damage. The occurrence of such alterations at the biochemical level has the potential to serve as “biomarkers” [16]. MDA is thus a bio-marker that serves as an index of oxidative stress; and hence is an indicator of the sub-lethal effects of contaminants or pollutants [8]. It has been used in a number of studies [14,17].

There are various bioassays that may be carried out for the eco-toxicological characterization of wastes and soil quality. Of these, we chose the fourteen day mortality of *Eisenia fetida*, and change in weight in earthworms based on the recommendations of [18] and [19] as the toxicity assessment end-points. MDA levels, also used in a number of studies [14,17] were also one of the toxicity assessment end points. The study objective was therefore to determine the survival, growth and MDA levels (sub-lethal effects) in annelids (earthworms) - *Eisenia fetida* exposed to dump site soils.

2. MATERIALS AND METHODS

2.1 Earthworm Culturing and Laboratory Maintenance

Juvenile earthworms of *E. fetida* (Savigny, 1826) (recommended for use in toxicity tests with growth as end-point – [20]) were purchased from a hatchery farm in Shenyang, China. They were supplied in boxes containing loamy soil. Fruits (apples) were cut into small pieces and added to the soil to stimulate decay and ensure consistent levels of an organic rich environment for the worms to feed and reproduce adequately. *E. fetida* is an epigeic, generalist feeder which requires an organic rich, humid environment composed mainly of plant material in various stages of decay [21]. The soil was moistened with water at periodic intervals (1 - 2 times a week) to prevent drying out of the soil, which may be detrimental to the worms. The culture was covered and maintained indoors in the boxes for two weeks to ensure the availability of an adequate number of worms of appropriate size for the toxicity assessment tests.

2.2 Toxicity Assessments: Test Soils

Soils for use in this study were obtained from the Akanran / Aba-Eku refuse landfill site located at Km 13, along Akanran – Ijebu Igbo road in Ona-Ara Local Government Area of Oyo state, Nigeria. It is a major dumpsite in Ibadan, the Oyo state capital. Four soils of 500 g quantity from the sub-site WDA (Waste Dump Area) at specified depths- two top-soils (0-15 cm depth) and two sub-soils (15-30 cm; 75-100 cm) were used for the toxicity assessments. They were designated WDA 1, WDA 2, WDA 3, and WDA 4 respectively. They were compared with composite soil from a control site located 600m away from the dumpsite which represented the field control and was designated CS (Control soil).

2.3 Physico-chemical Parameters of the Selected Soils

Analytical parameters determined on the soils include: pH of the samples which was determined at a ratio of 1:2.5 soil to water [22] using a pH meter PHS-3B Model and soil organic matter (SOM) determined using colorimetric determination. Others included copper, zinc, lead, cadmium, chromium, nickel, iron and manganese. They were determined on an ICP-OES Perkin Elmer Optima 3000 after Hydrofluoric – perchloric - nitric acid digestion according to the method of [23]. Particle / grain size distribution was determined by fractionation using sieve sizes and classification suggested by [24]. The pH of the selected test soils was determined before and after the tests as stated in the methodology adopted from [25]. Soil organic matter, particle size distribution and total metal content of the soils were as determined according to the respective methodologies stated above. Water holding capacity and bio-available metal content for the selected test soils were also determined according to the methods of [26] and [27] respectively.

2.4 Earthworm Bioassays

The test and control soils were inoculated with *E. fetida* (12 worms per 500 g air dried soil) – [28]. Worms of average weight of 0.3g [29] were used for the bioassays. Before inoculation, the worms were placed on moist filter papers in covered petri dishes for 24 hours to void their gut contents [29]. Each of the test soils WDA 1-3 were replicated thrice. The soils were moistened and maintained at 60% of their water holding capacity as suggested by [28]. This was achieved by weighing the bioassay containers, adding water up to 60% of the water holding capacity and maintaining that weight (by adding distilled water if the weight of the container changed) throughout the duration of the experiments. Earthworms were not fed during the test period. For the effects of the pollutants in the soil on earthworm growth to be evident, earthworm survival and body weight were determined at 1, 4, 7 and 14 days after inoculation, almost similar time intervals as those chosen by [28]. Malondi-aldehyde levels were determined in earthworms using the method adopted by [8]. However, MDA levels could not be determined on the 14th day.

2.5 Statistical Analysis of the Obtained Results

ANOVA and Duncan Multiple Range test was used to determine the statistical differences (and similarities) for the toxicity test results on growth between earthworms in the four selected test soils and control. Correlation coefficients were also used to determine the interrelationships between the physico-chemical parameters.

3. RESULTS

3.1 Physico-chemical Parameters of the Test Soils

The physico-chemical parameters of the test soils and field control is presented in Table 1, while the R^2 values for the correlation between the results of the total and bio-available metals are presented in Table 2. The results of the physico-chemical parameters of the soils used in the earthworm bioassay showed that top-soils WDA 1 and WDA 2 had the highest levels of cadmium, while WDA 2 had the highest level of nickel. The sub-soil WDA 3 (16-30cm depth) had the highest levels of lead; and WDA 4 (75-100cm depth) had the highest levels of chromium respectively. The test samples (except control) also showed moderate to high levels of other metals such as iron, manganese, copper and zinc. This has implications for the impact at the various depths.

Table 1. Physico-chemical parameters of the test soils

Physico-Chemical Parameters of The Test Soils	Cs (Control)	Wda 1 (0-15cm)	Wda 2 (0-15cm)	Wda 3 (15-30cm)	Wda 4 (75-100cm)
Water Holding Capacity (%)	26.82	42.82	47.27	25.92	28.92
pH	6.86	7.48	7.62	7.15	7.96
pH (Before Test)	-	7.64	7.48	7.61	7.64
pH (After Test)	8.07	7.41	7.72	7.56	8.23
Soil Org. Matter (SOM)%	1.66	7.32	6.99	8.99	8.61
% Gravel	32.50	40.50	58.00	61.50	33.50
% Sand	64.03	53.81	37.34	37.20	57.60
% Silt/Clay	3.47	5.69	3.41	1.30	8.90
Total Fe (Mg/Kg)	31614.31	29424.81	69641.05	28574.93	27825.61
Bio-Available Fe (Mg/L)	0.000	0.162	0.339	0.183	0.450
Total Mn (Mg/Kg)	818.62	1345.53	1548.03	921.90	1117.22
Bio-Available Mn (Mg/L)	0.739	0.725	0.605	0.344	0.723
Total Cu (Mg/Kg)	44.43	493.08	684.57	398.75	410.59
Bio-Available Cu (Mg/L)	0.004	0.024	0.418	0.030	0.104
Total Zn (Mg/Kg)	37.49	1777.98	2883.90	1280.52	1094.36
Bio-Available Zn (Mg/L)	0.040	0.240	0.215	0.134	0.092
Total Pb (Mg/Kg)	57.66	235.48	280.19	356.80	137.82
Bio-Available Pb (Mg/L)	ND	ND	ND	ND	ND
Total Cd (Mg/Kg)	1.02	28.17	24.77	13.47	10.63
Bio-Available Cd (Mg/L)	0.031	0.104	0.117	0.071	0.056
Total Ni (Mg/Kg)	17.54	31.19	53.43	33.94	35.11
Bio-Available Ni (Mg/L)	0.012	0.023	0.024	0.032	0.032
Total Cr (Mg/Kg)	40.06	80.02	140.98	105.71	222.11
Bio-Available Cr (Mg/L)	0.004	0.005	0.008	0.004	0.005

3.2 Earthworm Growth and Survival

The results of growth and survival of the earthworm - *Eisenia fetida* in the selected test soils (WDA 1 – 4) and field control (CS) is presented in Figs. 1 & 2, while the results of MDA levels in earthworms over a seven day period is presented in Fig. 3. All earthworms in the respective soils were of similar weight (average weight of 0.3g) at the beginning of the fourteen day experiment. Analysis of Variance (ANOVA) results confirmed that there were no significant differences at p = .05 between earthworms in the test soils and field control on day one. As the experiment progressed, there was a significant reduction (p = .05) in weight in earthworms exposed to the contaminated dump site soils (i.e. WDA 1 – 3) compared with those in the field control (CS). There was however an exception in WDA 4 which did not show any significant differences in weight from the control soil. Mortality occurred in only WDA 3, with 100% mortality of earthworms occurring by the 14th day (Figs. 1-2; Table 3). Mortality was however not observed in WDA 1, 2 and 4, as well as in the field control (Fig. 1). Although, replication could not be carried out with the experiments on the malondialdehyde (MDA) levels, results showed elevated levels of MDA in earthworms exposed to contaminated dump site soil versus field control. The results also showed a trend of slightly elevated MDA levels by day two, followed by a decline by day seven. WDA 2 was however an exception in this regard, as it showed progressive declines in MDA levels up to day seven (Fig. 1). Results of the correlation between earthworm weights on day 7 and 14 and total and bio-available metal concentrations (for only those metals with R^2 values above .7) are presented in Table 3. Although lead was not detected in the soil solution, it was included for reasons explained later.

Table 2. Correlation (R^2 values) between total and bio-available metal

Metals	**R^2**
Fe	0.294
Mn	0.173
Cu	**0.729**
Zn	**0.868***
Cd	**0.970****
Ni	0.500
Cr	0.237

*Correlations above .7 are highlighted in bold. *: significant at P = .05,*
***: significant at P = .01*

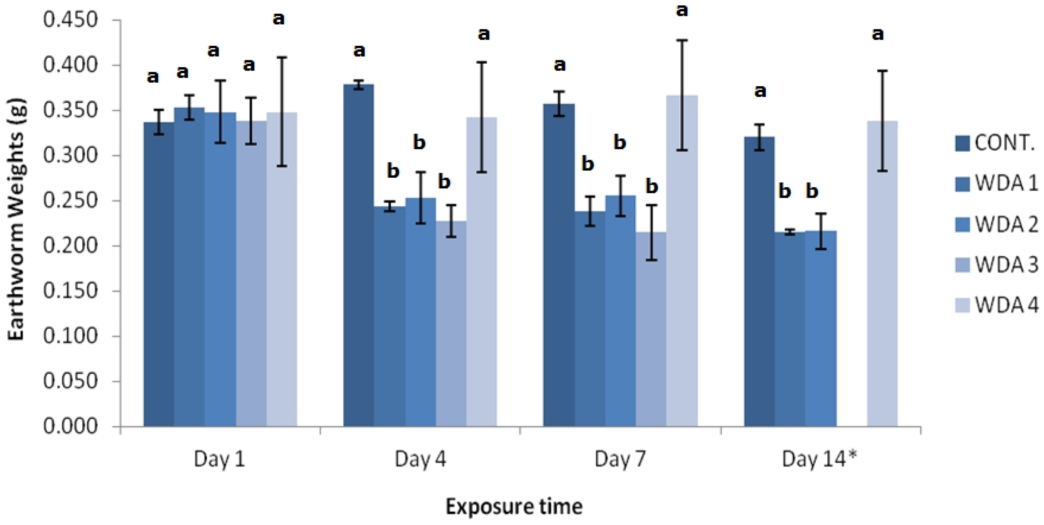

Fig. 1. Weight change in *Eisenia fetida* during 14d exposures

3.3 Correlation Coefficient Matrix

Table 3 shows the correlation coefficient matrix between the physico-chemical parameters of the test soils with day 7 and 14 growth and survival data. The results revealed a number of significant relationships at *P* = .01 and *P* = .05: bio-available cadmium and total cadmium (0.969; *P* = .01) as well as between bio-available zinc and total cadmium (0.995; *P* = .01). Other significant relationships at p = .01 were between bio-available zinc and bio-available cadmium: 0.971 as well as between total copper and total zinc: 0.973. Significant relationships at *P* = .05 were: Total lead and earthworm weights on day 7: -0.919; total lead and % gravel (0.923) as well as total lead and % sand (-0.934). Other relationships significant at *P* = .05 included soil pH (after test), and earthworm weights on day 7- (0.949); bio-available cadmium and:- total zinc (0.958); total copper (0.923) and water holding capacity (0.901 – Table 3). There were also a number of high correlations above 0.7 which were however not significant. These included those between soil pH (after test), and earthworm weights on day 14: 0.938; total cadmium and day 14 data (-0.904), total lead and earthworm weights on day 14 (-0.883), total cadmium and day 7 data (-0.702); bio-available cadmium and day 14 data (-0.924) which was also correlated less strongly with earthworm weights on day 7 (-0.716 – Table 3).

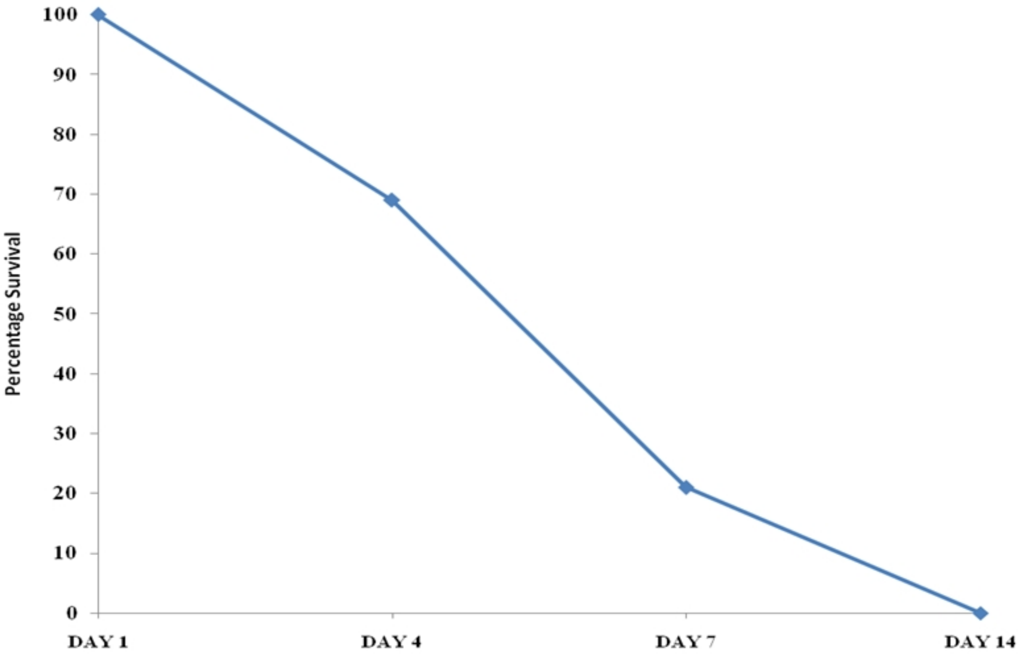

Fig. 2. Mean percentage survival of *Eisenia fetida* in WDA 3

Table 3. Correlation coefficient matrix for physico-chemical parameters and growth data

	CuT	**CuB**	**ZnT**	**ZnB**	**CdT**	**CdB**	**PbT**	**WHC**	**pH**	**pHB4**	**pHAFTER**	**SOM**	**DAY7**	**DAY14**	**GRAVEL**	**SAND**	**SILT/Cl$^-$**
CuT	1	0.729	**0.973****	0.845	0.872	**0.923***	0.689	0.767	0.679	-0.897	-0.469	0.711	-0.576	-0.709	0.595	-0.719	0.082
CuB	0.729	1	0.807	0.446	0.458	0.640	0.296	0.691	0.457	-0.948	-0.013	0.184	-0.159	-0.470	0.471	-0.562	-0.050
ZnT	**0.973****	0.807	1	0.867	0.876	**0.958***	0.666	0.855	0.539	-0.900	-0.516	0.545	-0.606	-0.805	0.636	-0.737	-0.060
ZnB	0.845	0.446	0.867	1	**0.995****	**0.971****	0.650	0.867	0.372	-0.384	-0.793	0.490	-0.752	-0.940	0.486	-0.556	-0.054
CdT	0.872	0.458	0.876	**0.995****	1	**0.969****	0.624	0.867	0.463	-0.363	-0.739	0.536	-0.702	-0.904	0.443	-0.529	0.040
CdB	**0.923***	0.640	**0.958***	**0.971****	**0.969****	1	0.676	**0.901***	0.430	-0.666	-0.699	0.499	-0.716	-0.924	0.582	-0.663	-0.090
PbT	0.689	0.296	0.666	0.650	0.624	0.676	1	0.304	0.112	-0.360	-0.758	0.722	-0.919	-0.883	**0.923***	**-0.934***	-0.498
WHC	0.767	0.691	0.855	0.867	0.867	**0.901***	0.304	1	0.394	-0.611	-0.488	0.147	-0.421	**-0.963***	0.270	-0.351	0.060
pH	0.679	0.457	0.539	0.372	0.463	0.430	0.112	0.394	1	0.017	0.182	0.665	0.113	-0.065	-0.057	-0.126	0.751
pHB4	-0.897	-0.948	-0.900	-0.384	-0.363	-0.666	-0.360	-0.611	0.017	1	0.082	0.574	0.245	0.494	-0.625	0.707	0.446
pHAFTER	-0.469	-0.013	-0.516	-0.793	-0.739	-0.699	-0.758	-0.488	0.182	0.082	1	-0.334	**0.949***	0.938	-0.613	0.576	0.504
SOM	0.711	0.184	0.545	0.490	0.536	0.499	0.722	0.147	0.665	0.574	-0.334	1	-0.491	-0.275	0.494	-0.605	0.198
DAY7	-0.576	-0.159	-0.606	-0.752	-0.702	-0.716	**-0.919***	-0.421	0.113	0.245	**0.949***	-0.491	1	**0.994****	-0.823	0.795	0.595
DAY14	-0.709	-0.470	-0.805	-0.940	-0.904	-0.924	-0.883	**-0.963***	-0.065	0.494	0.938	-0.275	**0.994****	1	-0.783	0.739	0.455
GRAVEL	0.595	0.471	0.636	0.486	0.443	0.582	**0.923***	0.270	-0.057	-0.625	-0.613	0.494	-0.823	-0.783	1	**-0.983****	-0.696
SAND	-0.719	-0.562	-0.737	-0.556	-0.529	-0.663	**-0.934***	-0.351	-0.126	0.707	0.576	-0.605	0.795	0.739	**-0.983****	1	0.556
SILT/Cl$^-$	0.082	-0.050	-0.060	-0.054	0.040	-0.090	-0.498	0.060	0.751	0.446	0.504	0.198	0.595	0.455	-0.696	0.556	1

*: P = .05; **: P = .01 LEGEND: CuT; ZnT; CdT; PbT = Copper Total; Zinc Total; Cadmium Total; Lead Total; CuB; ZnB; CdB; WHC = Copper Bioavailable; Zinc Bioavailable; Cadmium Bioavailable; Water Holding Capacity; SOM = Soil Organic Matter

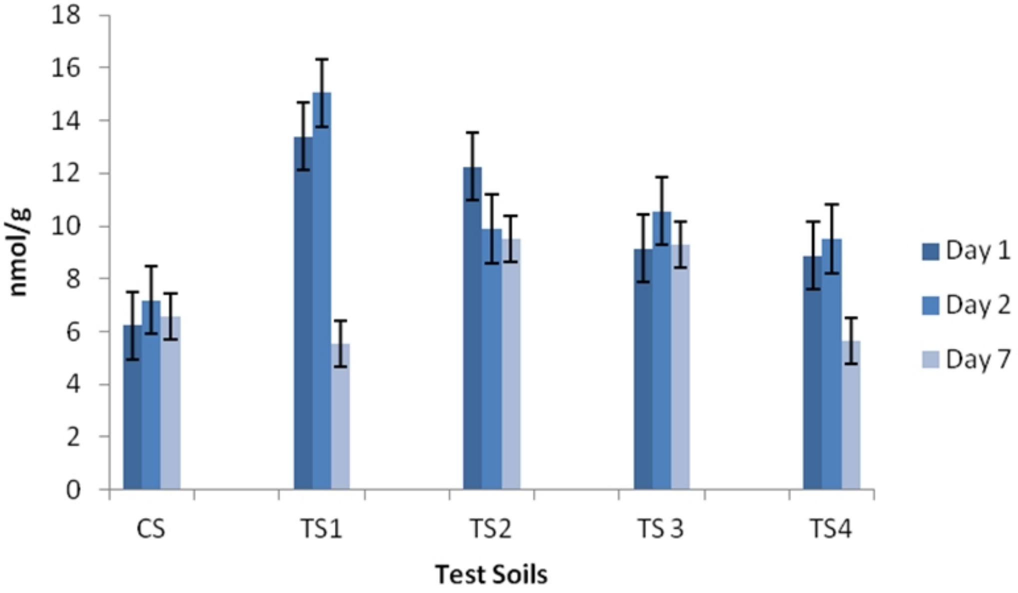

Fig. 3. MDA levels in *Eisenia fetida* after 7d exposures

4. DISCUSSION

4.1 Survival and growth in *E. fetida*

Except for the test soil WDA 3, in which 100% mortality occurred by the 14th day, no mortality was observed in the other tested soils. It was observed that earthworms in WDA 3 stayed on the surface, rather than burrowing into the soil, suggestive of some sort of avoidance response to the presence of pollutants. There was a progressive reduction (significant at P = .05) in earthworm weights over time in all soils except in the control and the test soil WDA 4. Metal contamination and soil properties appeared to influence the response of *E. fetida* in the tested soils. The low mortality and reduced growth observed is in agreement with the works of several authors [28,30-31] on the survival and weight change of *E. fetida* in heavy metal polluted soils. These results suggest that change in weight in earthworms is a more sensitive parameter than survival to assess pollutant effects in soils, particularly those contaminated by heavy metals [18,31]. This is because earthworms are more susceptible to metal pollution than many other groups of soil invertebrates [32]. High concentrations of Cadmium, Copper, Lead and Zinc can affect the density, viability, growth and sexual development of earthworms [13]. The high concentrations of cadmium, copper, lead and zinc in the test soils were significantly higher than the native values in the control soil. This probably explains the avoidance response and mortality in WDA 3, as well as the reduction in earthworm weights overtime in the other test soils- WDA 1 and 2.

The significant reduction in weight observed in earthworms kept in the polluted soils may also be explained by the fact that in a polluted environment, changes to individual energy budgets will occur, as the organisms expends energy resisting the pollutant by avoidance, exclusion, removal, or complexing [13]. This additional energy requirement will decrease the “scope for growth” of the exposed animals, ultimately resulting in reduced growth and other parameters such as cocoon production [13]. It follows that in the long run, decreased biomass and reproduction will lead to decrease in population size [29]. The reduction of population size due to mortality or reduced reproduction is an ecological consequence of exposure to pollutants in soil [33]. This probably explains why in previous and recent studies on the macro-faunal diversity of the Aba-Eku landfill site, no earthworms were observed [34-37]. Contaminants present at polluted sites occur as mixtures, therefore interaction between individual compounds are of importance [38]. In addition, the possible impact of other contaminants not included in analysis cannot be ruled out. Metals rarely analysed for, such as silver and thallium, may impact on earthworm life cycle parameters such as survival and weight change [28]. Thus, the influence of other contaminants not analysed for, may also perhaps explain the observed mortality in WDA 3.

Out of the four soils tested, only WDA 4 showed similarities with the control soil in terms of earthworm growth and survival. Metal contaminant levels in this soil were however were well elevated above the control soil, although contaminant levels were generally lower than the other test soils WDA 1-3, with a corresponding reduction in bio-availability except for iron and copper. This may also have played a role in the overall reduced toxicity in this test soil. However, it was also observed that the test soil WDA 4 had higher pH at the end of the test period similar to that of the control soil. This suggests that the pH of the soil plays an important role in mitigating the toxicity of metal contaminated soils. Metal adsorption increases with increasing pH, thus reducing bioavailability. It has been stated that soil organic matter and pH play an important role in the mitigation of metal contaminated soils [31]. Furthermore, there was also a higher level of silt and clay content in this soil and mean values at this depth (75-100cm: WDA 4) has earlier been shown to be significant at $p < 0.05$. This may also be suggestive of increased adsorption of metal contaminants by the silt and clay fraction of the soil, thus contributing to the reduced bioavailability of contaminants in this test soil. Heavy metals are usually strongly adsorbed to soil particles, particularly the colloidal fraction, of which clay is a part [39].

The results of this study clearly showed that the metal polluted test soils had an overall significant effect on earthworm growth and survival, which has implications on the ecosystem as a whole. Emigration of earthworms and the subsequent loss of their beneficial functions in soil (aeration, drainage, enrichment of organic material etc) can lead to a degradation of soil qualities. Additionally, the loss of earthworms from an area might also affect the numbers and distribution of their vertebrate predators. Thus the loss of earthworms from an area can impact an ecosystem [33].

4.2 Relationships with the Observed Toxic Responses

Total heavy metal content may not be directly related to soil organism toxicity due to a number of modifying factors like pH, organic matter content and clay content [5]. In an attempt to relate cause and effect, soil samples were subjected to extraction tests to determine the amounts of bioavailable metals. Metal bioavailability represents the relevant exposure concentration for soil organisms [31]. The environmental risk is often related to the bioavailability of heavy metals in the soil [5]. It has however been suggested that the bioavailable heavy metals refers to availability for crops, rather than earthworms and that the available fraction for earthworms is still a matter of debate [40]. The results of the total and bioavailable metals were then subjected to correlation coefficient analysis. Results of the correlation (R^2 value) for the total and soil-solution heavy metal content were: Cd: - 0.970; (P = .01); Zn – 0.868; Cu – 0.729; Ni – 0.500; Fe – 0.294; Cr – 0.237; Mn – 0.173. Pb was below detection limits in the bio-available soil solution. The high correlation for cadmium is indicative of a very high bio-availability of this metal.

Total and bioavailable fractions for parameters showing a good correlation as highlighted above - Cd, Cu and Zn, in addition to total Pb, and the above mentioned soil parameters (pH, organic matter, water holding capacity and texture) were then subjected to correlation coefficients. They were correlated along with day 7 and day 14 growth data in an attempt to relate cause and effect. Total lead was also included in spite of its low bio-availability. The total lead content is an important factor influencing body weight change in *E. fetida* [26]. Iron, Nickel and chromium were however excluded due to their low bioavailability as shown by the earlier correlation results. It has been suggested that Cd, Cu, Zn and Pb are the metals of utmost significance in earthworm metal toxicity studies [13,41-42].

The results of the correlation suggests that total Pb and bio-available Cd, to a lesser extent bio-available Zn are partly responsible for the observed effects. It also suggests the influence of parameters other than metal content on the observed toxicity. Increased amounts of coarse sediments rather than fine sediments impacts on toxicity and probably contributes to the toxic effects observed in WDA 3. In contrast WDA 4 had the highest amounts of sand, clay and silt and lowest of gravel, which may have had a positive effect on its response.

The influence of other factors on the observed responses can also be seen in the increased amounts of bio-available cadmium with increasing water holding capacity and the resultant effects on observed responses. Increased levels of free bio-available Cd along with increased soil pore water were observed in studies of [43]. *E. fetida* is more tolerant to metals than other earthworm species [29], while other authors [12] state that there is no significant difference in observed responses between different species. This suggests native earthworm species like *Lumbricus spp*. may demonstrate similar or more pronounced effects. In a study on the macro-faunal diversity of the Aba-Eku landfill site [34] and confirmed by studies of [35], no earthworm species were encountered suggesting their possible emigration to less contaminated areas as stated by [29] and [33]. Furthermore, in recent studies by [36] and [37], earthworms were not observed on the Aba-Eku dump site, but were observed at a distance of about 300 m away from the dump site further supporting the fact that earthworms may have emigrated from the site to less polluted areas.

The results of the lipid peroxidation activity in the exposed worms versus the field control indicated elevated malon-dialdehyde (MDA) levels in the test soils compared with the control (Fig. 2.). The MDA is an endpoint (or index) of lipid peroxidation [14,17]. Lipid peroxidation in living tissues has received considerable attention as a potential health hazard of exposure to heavy metals [14]. These metals can lead to the generation of excessive free radicals or Reactive Oxygen Species – ROS, which leads to the accumulation of oxygen (O^{2-}) and hydrogen peroxide (H_2O_2) radicals [16]. An increase in ROS results in oxidative stress, which is defined as a disruption in the pro-oxidant – anti-oxidant balance in favour of the former leading to potential damage [44]. ROS are also continuously being formed during normal aerobic respiration metabolism. However toxic forms of activated oxygen react with cellular components, resulting in lipid peroxidation, which ultimately results in cell death [45].

MDA levels in test soils rose by day 2 (except in WDA 2 where it rose on the first day), following the introduction of the test organisms indicative of oxidative stress onset. The damaging effects of ROS are counteracted by the anti-oxidant systems (enzymes) which remove the ROS, thereby restoring the balance and protecting the organism from the stress

[15,44]. This probably explains the decline in MDA levels by day 7 after its' initial increase. This is probably suggestive of the activities of the anti-oxidant defense systems in restoring pro-oxidant and anti-oxidant balance. It was not clear why MDA levels in WDA 3 were reduced compared to other test soils, considering that mortality occurred in this soil. It may therefore be necessary to repeat the test for MDA levels with additional replicates to further assess the effects of the field contaminated soils, as well as test for anti-oxidant parameters. The above results however support the earlier observations on survival and weight change and clearly demonstrates that the high levels of metals in the test soils induced oxidative stress in *E. fetida,* an organism considered as a key indicator for ecosystem health [26].

5. CONCLUSION

The toxicity of field contaminated dumpsite soils from the Aba-Eku dumpsite, Ibadan, Nigeria have been assessed using the earthworm species: *E. fetida*. Earthworms showed reduced growth in three out of the four tested dumpsite soils, while mortality was observed in only one of the four test soils. This attests to the fact that growth is a more sensitive parameter than survival to test for the effects of metal contaminated field soils. The elevated MDA levels in the earthworms are suggestive of oxidative stress onset as a result of the exposure to the contaminated dumpsite soils. Additional tests with more replicates may however be necessary to confirm the elevated MDA levels. Anti-oxidant levels may also be assessed in future studies. However, the study clearly showed the effects of field contaminated dumpsite soils on *E. fetida* and may explain the absence of earthworms from metal polluted dumpsites.

6. ACKNOWLEDGEMENTS

AAO acknowledges funding support from the Academy of Sciences for the developing world (TWAS) in association with the Chinese Academy of Sciences (CAS) for a fellowship tenable in China. We also acknowledge the support of Prof Li Peijun and the entire Pollution Ecology group of the Shenyang Institute of Applied Ecology, Shenyang, China. Zhang Hairong, Xu Huaxia and Yao Deming in particular, are also acknowledged for assistance with laboratory analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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