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EFFECT OF SPENT OIL CONTAMINATED SOIL ON THE GROWTH OF *AMARANTHUSHYBRIDUS* AND POPULATION OF EARTHWORM *EUDRILUSEUGENIAE*

Kanu Valentine C.[1], Makanjuola Winifred A.[2], Umeobika Valentine C.[3] and Onmonya Yvonne A.[4]

1. Acterra, Action for a Healthy Planet, 3921 E Bayshore Rd, Palo Alto, CA 94303, United States.
2. Department of Zoology, Faculty of Science, University of Lagos, Lagos, Nigeria.
3. Department of Civil Engineering, Federal Polytechnic Kaura Namoda, Zamfara State, Nigeria.
4. Environmental Biotechnology &Bioconservation Department, National Biotechnology DevelopmentAgency, Abuja, Nigeria.

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Abstract

The effect of spent lubricating oil (SLO) on the growth of *Amaranthushybridus*, a staple leafy vegetable; and on the population build-up of earthworm were investigated. The treatment consisted of 0, 10, 20, 30, 40, 50 and 60ml of spent lubricating oil per kilogram of soil in 3 replicates. Two seedlings, each of *A. hybridus* were planted in perforated pots containing soil rich in earthworm in each replicate. Baseline analysis of SLO and soil was carried out. Stem, soil, and earthworm were analyzed for copper, lead, cadmium, zinc, and nickel at 6 weeks post treatment. There was significant growth retardation with corresponding increase in level of SLO. There was no significant difference in the plant parameters among the treatments. There was no population build-up of earthworm in all the treatments except in the soil with lowest contamination of 10ml/kg and control. The *A. hybridus* stem had significantly higher levels of Zn, Cd and Cu than controls; but cadmium was not detected. For soil Zn, Pd and Cd were higher in all treatments; Ni was significantly higher only at the 60ml/kg concentrations with a value of 0.14±0.014mg/kg while Cu was significant in all treatments except in the 10ml/kg and control with values of 0.097±0.002 and 0.087±0.002 respectively. For earthworm, Zn, Ni, Cu, and Cd were detected with the highest concentrations in the 10ml/kg. The level of heavy metals in treated soil exceeded WHO MPL. The bioaccumulation of metals intreatments may lead to biomagnification along the food chain leading to hazardous effects on humans.

***Corresponding Author:-** **Kanu Valentine C.,** *Acterra, Action for a Healthy Planet, 3921 E Bayshore Rd, Palo Alto, CA 94303, United States.*

Introduction

Various products derived from petroleum are common soil pollutants as they often contain potentially dangerous chemical constituents (Sharifiet al., 2007). Lubricating oil, produced by vacuum distillation of crude oil (Kayode et al., 2009), is an essential product of petroleum that aids in reducing frictional forces between contacting metal surfaces of an engine. The use of lubricants minimizes material wear, overheating, and contributes to improved efficiency of equipment or machinery including fuel and energy savings (Awodoyin and Ogbeide, 2010).

Spent oil is obtained after servicing and subsequent draining of oil from vehicles, generators, industrial machines, and other machinery. Spent Lubricating Oil has several synonyms which include used oil, used crankcase oil, waste oil, mineral-based used crankcase oil and used auto lube (Irwin et al., 1997).Spent Lubricating Oil can be defined as mineral or synthetic oil contaminated by physical or chemical impurities through use as a lubricant, or similar function to the point where it is no longer fit for its original purpose (New Zealand Min of Env., 2000). Mineral-based oil are those products obtained from refining petroleum crude; while synthetic oils on the other hand are those products made from petroleum or vegetable feed stock and are often "tailor made" for specific application (Ogbeide, 2010).

Amaranthus species, including other common vegetables, though frequently grown by the urban populace in a bid to augment personal incomes and offset food insecurity occasioned by rural-urban drift, is often consumed by a greater part of the entire population (Shagal et al., 2012).Most mechanic workshops are near vegetable farms which are widely and commonly grown at subsistence level. This makes the land particularly ladened with spent oil. Service stations in most parts of Nigeria find it difficult to properly dispose of the SLO hence large volume is indiscriminately disposed on plots, land, sewage, and drainage ditches (Odjegba and Sadiqi, 2002) thus increased pollution incidents in the environment are more widespread than pollution with crude oil (Atuanya, et al., 2012). Changes in some soil properties resulting from contamination with petroleum derived substances bring about some changes in the biological composition of soil (Njoku, et al, 2009).

Spent oil is a major waste stream in Nigeria; contamination of soil by spent oil is a widespread environmental problem. Bamiro and Osibanjo, (2004), estimated the quantity of spent oil generated in Nigeria to be 150 million litres/annum (mlpa). However, in Nigeria, the alternative power sector, an unfortunate sector borne out of the country's power inadequacies also contributes a significant proportion to the quantity of spent oil generated each year through the millions of electric power generators used all over the country. An estimated 60 million Nigerians now own power generating sets for their electricity, with most of them disposed in nearby water bodies and used oil from one oil change can contaminate 1 million gallons of fresh water – a years' supply for 50 people (Patrick-IwuanyanwuandOnwuka, 2010). Spent lubricating oil has been shown to alter soil biochemistry, which includes alteration in soil microbial properties, $P^{H,}$ oxygen and nutrient availability (Odjeba and Sadiq, 2002; Osubor and Anoliefo, 2003).

Spent lubricating oils are disposed indiscriminately into the environment, especially by the roadside mechanics and other allied workers (Kayode et al., 2009). The presence of this pollutant in terrestrial and aquatic environments constitutes public health and socio-economic hazards (Edeworet al. 2004). Apart from this, spent oil constitutes a potential threat to humans, animals, and vegetation (ATSDR, 1997). Soil contamination with SLO can lead to water and oxygen deficits as well as shortage of available forms of nitrogen and phosphorus needed for plant growth (Uchida, 2000). Spent oil contaminated soils are unsuitable for agricultural use and are potential sources of surface and ground water contamination. (Nwoko et al., 2007). It enters the ecosystem through run-off following unguided disposal (Gbadebo et al., 2009).Indices of plant growth such as measurements of germination, leaf area, plant height, fresh and dry matter analysis have been used to study the effect of spent oil onplants, and results showed a negative impact on growth and development. (Okonokhua et al., 2007 and Kayode et al., 2009). It is therefore the focus of this study to determine the impact of spent lubricating oil on the growth potential of Amaranthushybridus and the population of earthworm *Eudriluseuginiae*.

Methods and Materials

The Spent Engine Oil was obtained from a motor mechanic workshop within the University of Lagos campus. A soil sample containing earthworm was obtained from an uncontaminated site at the back of the zoological garden; and the plant seedlings were obtained from the National Horticultural Research Institute (NIHORT), Idi-Ishin, Ibadan.

Baseline study of Spent Engine Oil and Soil; Determination of PH, density, and viscosity of spent oil.

The chemical analysis of the spent oil was carried out at the University of Lagos, Department of Chemistry laboratory.The physicochemical properties of the oil analyzed were P^{H}, density, and heavy metals. The soil parameters analyzed were heavy metals.

The PH of spent oil was measured by dipping the electronic P^H meter (Extech electronic P^H meter) directly into the spent oil sample in a 50ml beaker. The reading was taken and recorded. The density was determined by using a graduated beaker. The density was calculated using the simple formula mass divided by volume (m/v).

$$\text{Density} = \frac{Z-X}{V_{oil}}$$

Z = mass of measuring beaker filled with spent oil
X = mass of empty measuring beaker
V = volume of spent oil
The viscosity of the oil was determined using a viscometer 25^0C.
Amaranthushybridus seeds used for this experiment were obtained from the University of Lagos botanical garden.

Preparation of Spent Oil Contaminated Soil
The experiment consisted of six treatments consisting of various concentrations of spent oil and the control in three replicates. Two kilograms of loam soil each were placed in 21 plastic containers measuring 15 by 10cm. Three pots of soil each were respectively treated with 0, 20, 40, 60, 80, 100 and 120ml of spent engine oil per 2kg of soil thoroughly mixed. The spent oil was measured using a measuring cylinder for 20, 40, 60, 80,100, and 120 ml of spent oil which was mixed with the 2 kilograms of soil for each of the triplicates using the weighing balance. Each of the 6 treatments in triplicate was respectively treated with the spent oil while the control was left untreated to obtain a 0, 10, 20, 30, 40, 50 and 60ml/kg.

Preparation of Nursery Plants for Experimentation
The preparation of nursery was carried out in the screen house at the zoological garden, University of Lagos. The Amaranthus seed was initially planted in a nursery pot with rich soil for a period of 2 weeks before been transplanted to the planting pots for growth and development. The setup was examined and watered daily at about 8.00hrs GMT+1 and 5:00hrs GMT+1.

Experimental Setup
After two weeks in the nursery, healthy seedlings were selected from the nursery and planted in the various treatments and control of all replicates. The plants were watered daily up to the 6 weeks post-transplanting and all the parameters measured.

Number of leaves and plant height
The plant height was measured with a meter rule placed at the surface of the soil to the apex (tip) of the highest leaf. The number of leaves were counted and reported at the end of the nursery and growth period.

Earthworm Tissue Analysis
After harvest, the earthworms were pooled together from each triplicate, stored in sample contains containing dilute formalin and washed with de-ionized water. The washed worm samples were digested in 2.5ml concentrated nitric acid and heated to dryness with a hot plate. The dried earthworms after being pooled together from each treatment were crushed and re-dissolved in 1.5 nitric acid and the solution was filtered into a volumetric flask. The filtrate was made up to 50ml with distilled water. Heavy metal determination for the earthworm using AAS method was carried out (Oketola&Olaoye, 2015).

Statistical Analysis
The data obtained were analyzed using SPSS version 20 statistical tool. Means of 3 replicates were quoted with their standard error. One Way Analysis of Variance (ANOVA) was calculated according to Scheffer, multiple range test at $P<0.05$ level of significance.

Result and Discussion

Baseline Sample Analysis of spent oil.
The result from baseline study of spent lubricating oil used in the study shows it contains 0.80mg/kg Copper, 0.64mg/kg Lead, 1.06mg/kg Zinc, 1.52mg/kg Nickel (Table 1). However, Cadmium was not detected. Spent oil was acidic with a pH value of 4.25. The density of the oil and the viscosity are 0.8955g/ml and 336 centistokes at 25^0C respectively.

Table 1:- Concentration of metals in spent oil and soil.

Heavy Metal	Concentration (mg/L)	Soil (mg/kg)
Copper	0.80	1-2
Lead	0.64	0.01
Zinc	1.06	3-5
Nickel	1.52	0.02
Cadmium	ND	0.003

ND= Not Detected

Figure 1 above shows the respective amount of Cu, Pb, Cd, Zn and Ni detected in the stem, soil and earthworm in spent oil treated soil. The variation in the concentration of copper in plant stem, as shown in fig. 1, ranges from a minimum of 0.0085±0.002 mg/kg in control to a maximum of 0.121±0.003* mg/kg in 50mg/kg exposed concentration. One way ANOVA shows a significant variation in the concentration of copper in the exposed plant in relation to control at $p<0.05$.

Table 2:- Mean number of leaves and height of plant at various levels of soil contamination at three- and six-weeks post treatment.

Concentration of SLO (ml/kg)	Mean number of leaves post treatment		Height of Plant (cm)	
	3WPT	6WPT	3WPT	6WPT
(Control) 0	9	10	7	29
10	4	6	5	9
20	2	5	5	5.1
30	3	6	4	6.3
40	2	4	3.33	5.6
50	3	5	3.33	3
60	3	5	4	3

Slo-Spent Lubricating Oil; Wpt – Weeks Post Treatment

Table 3:- Level of Heavy metals absorbed by the Earthworm.

Treatment	Concentration (mg/kg)				
	Cu	Pb	Cd	Zn	Ni
Control	0.002±0.001	ND	0.61±0.016	2.10±0.009	0.13±0.008
10ml/kg	0.014±0.005	ND	0.83±0.005	4.95±0.014	0.31±0.018
20 ml/kg	ND	ND	ND	ND	ND
30 ml/kg	ND	ND	ND	ND	ND
40 ml/kg	ND	ND	ND	ND	ND
50 ml/kg	ND	ND	ND	ND	ND
60 ml/kg	ND	ND	ND	ND	ND

* Significant at $P<0.05$; ND- Not Detected.

The table showed that at higher concentrations of treatment, Cu, Cd, Zn and Ni were not detected in the earthworm except in 10kg and control, while no lead was present in all the treatments and control. However, in the control and 10ml/kg, Cu, Cd, Zn and Ni were present in the earthworm. The amounts of the metals were higher in the 10ml/kg than in the control.

Baseline Composition of experimental soil, oil, and earthworm

Figure 1 above shows the respective amount of Cu, Pb, Cd, Zn and Ni detected in the stem, soil and earthworm in spent oil treated soil. The variation in the concentration of copper in plant stem, as shown in fig. 1, ranges from a minimum of 0.0085±0.002 mg/kg in control to a maximum of 0.121±0.003* mg/kg in 50mg/kg exposed concentration. One way ANOVA shows a significant variation in the concentration of copper in the exposed plant in relation to control at $p<0.05$.

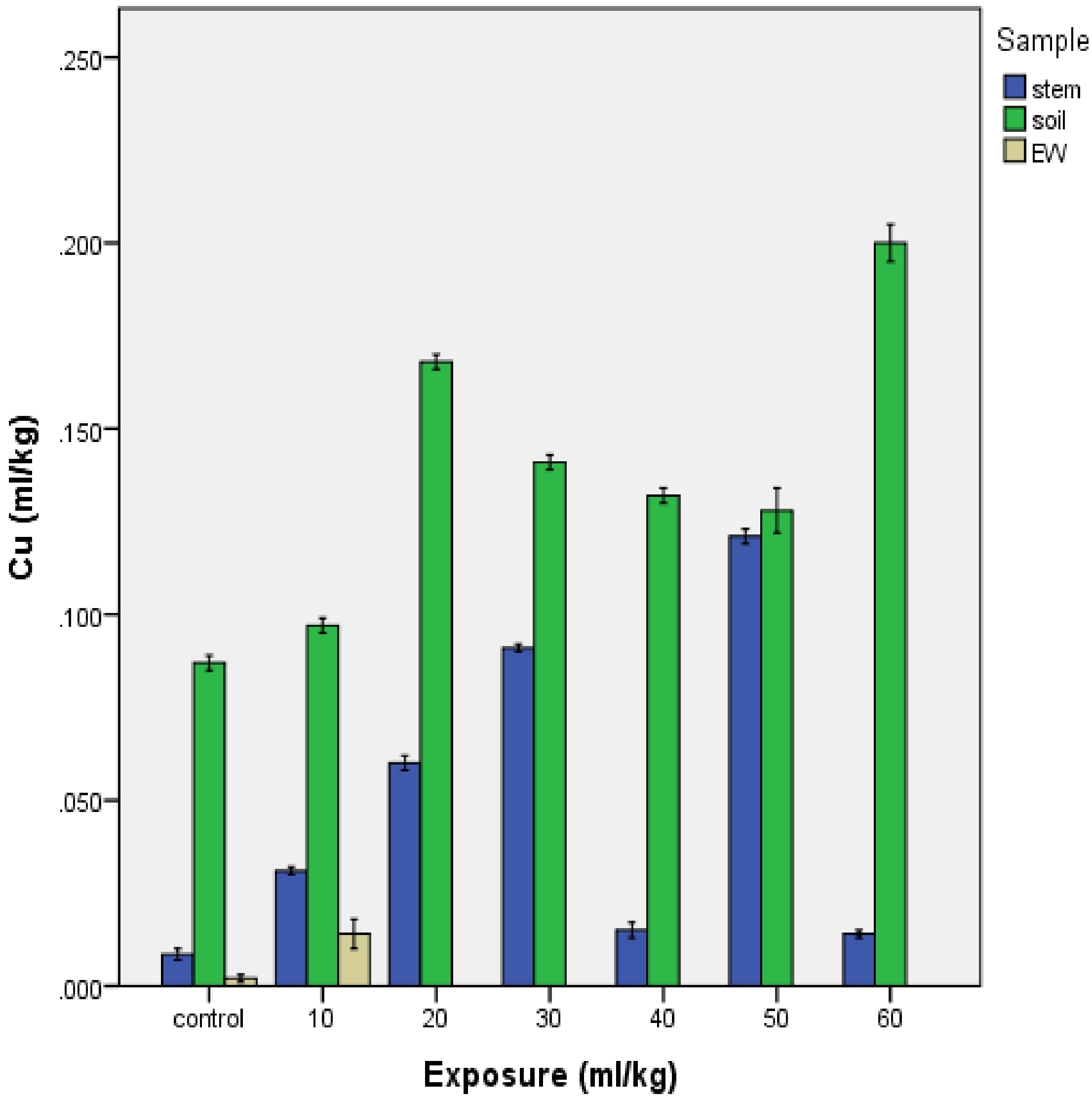

Fig. 1:- Level of Cu present in plant stem, soil and earthworm at various dosages of spent oil.

Figure 1 shows that there was a general increase in copper in the plant stem and soil with increasing concentration of spent oil in the soil. The analysis of variance showed the amount of copper detected in the stem, soil and earthworm was significantly different when compared with the control. In the case of the stem, the concentration of copper increased steadily in concentration up to 30ml/kg treatment with a copper level of 0.019mg/kg when compared with the control. Thereafter there was a significantly lower level of copper in the stem at the 40 and 60 treatment which was not significantly different for each other. However, the level of copper in stem at 50ml/kg with a copper level of 0.121ml/kg was the highest of all the treatments.For the soil, copper was present in all the treatments including the control with the highest level occurring in the soil that had the highest concentration of spent oil treatment while the control had the lowest copper present.

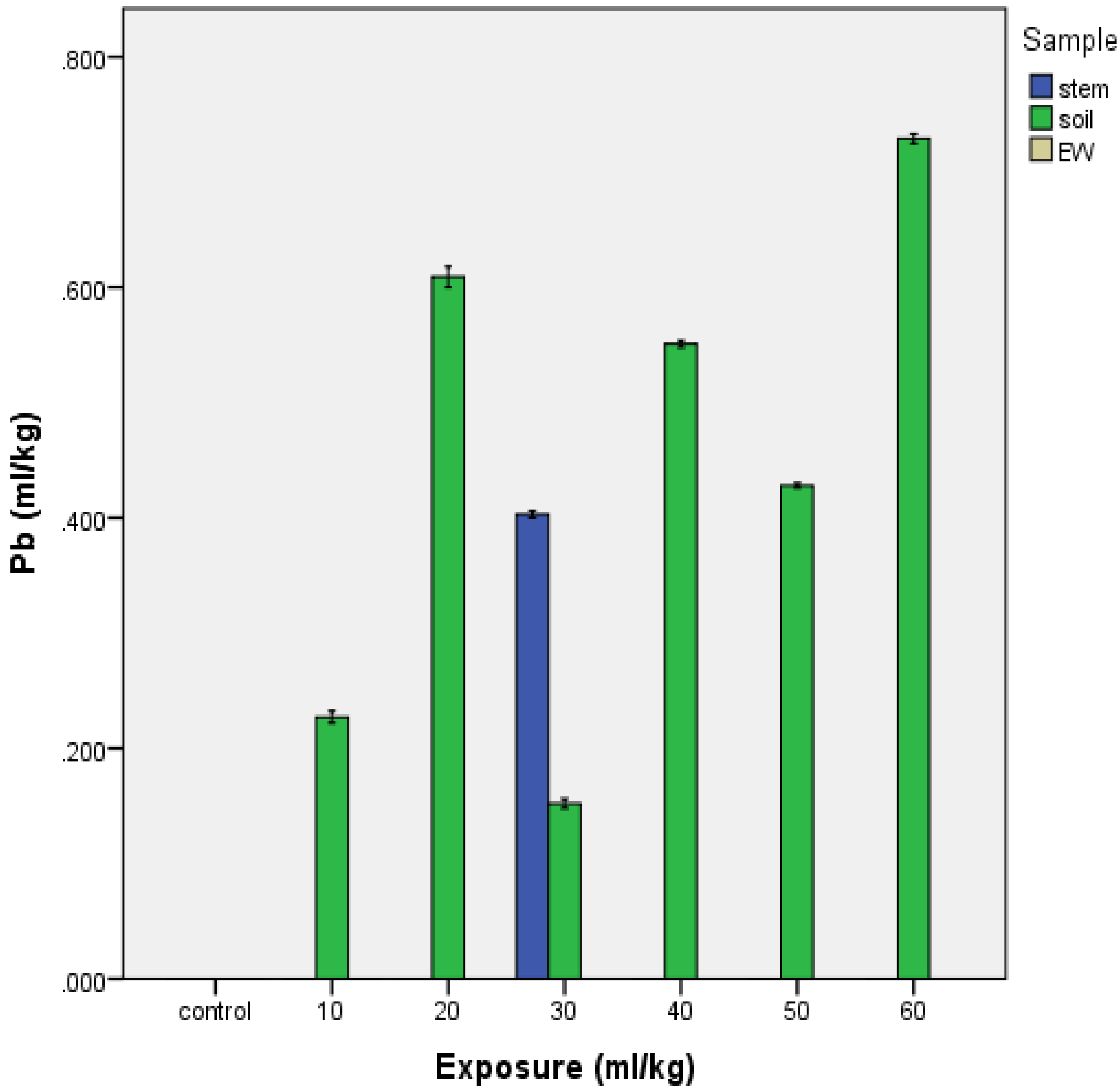

Fig. 2:- Level of Pb absorbed by plant stem, soil and earthworm across the treatments.

The variation in the concentration of Lead in plant stem, as shown in fig. 2 was not detected in all the treatments to a maximum of 0.403±0.004* mg/kg in 30mg/kg exposed concentration. One way ANOVA shows a significant variation in the concentration of lead in the exposed plant in relation to control at $p<0.05$. The variation in the concentration of Lead in soil, as shown in figure 2 was not detected in control to a maximum of 0.73±0.005 mg/kg in 60mg/kg exposed concentration. One way ANOVA shows a significant variation in the concentration of copper in the exposed soil in relation to control at $p<0.05$. Lead was not detected in Earthworm in all the concentrations. One way ANOVA shows no significant variation in the concentration of lead in the exposed plant in relation to control at $p<0.05$. In general, the variation in the concentration of lead ranges from soil>stem>earthworm (fig.2).

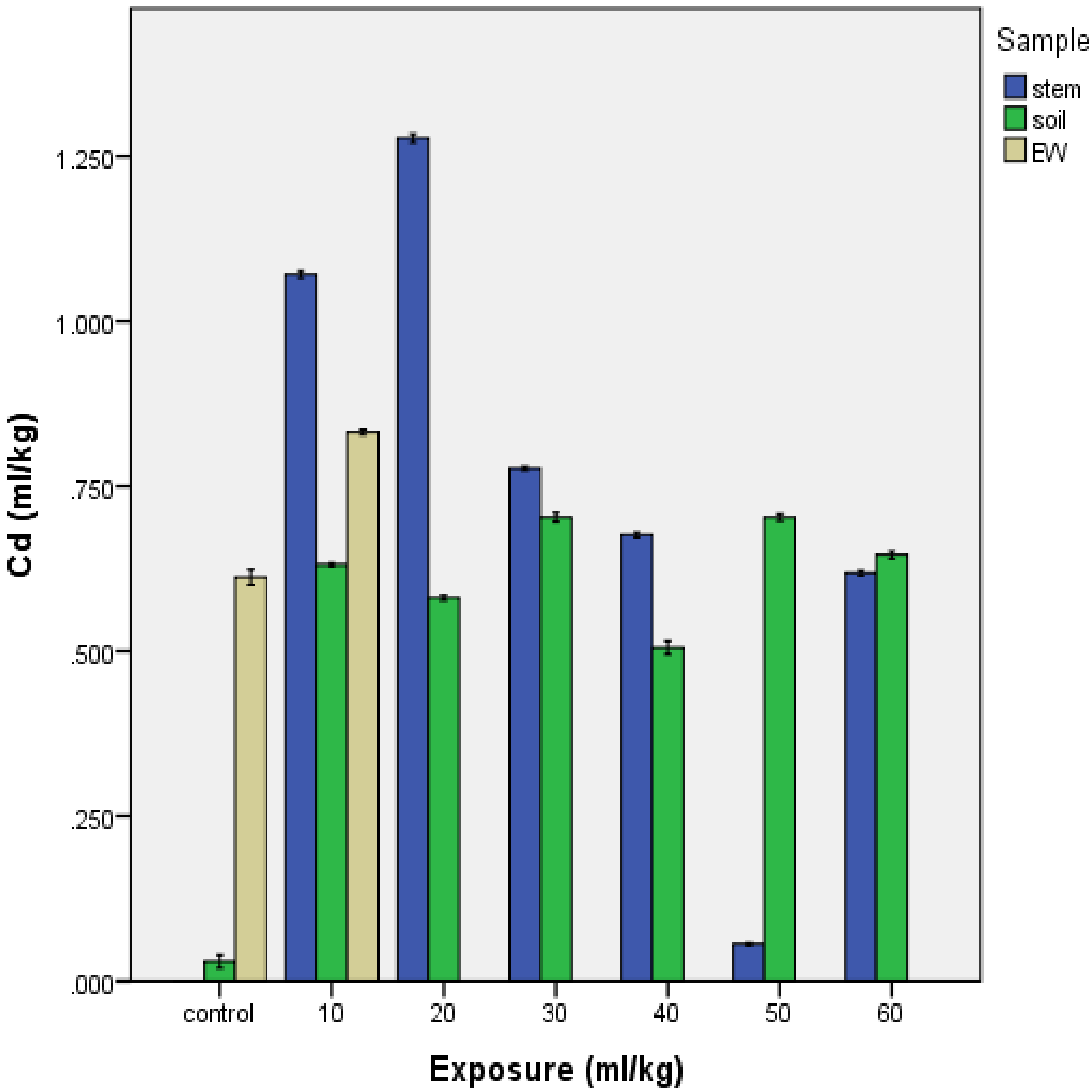

Fig. 3:- Level of Cd absorbed by plant stem, soil and earthworm across the treatments.

The variation in the concentration of Cadmium in plant stem, as shown in fig. 3 was not detected in the control to a maximum of 1.07±0.007*mg/kg in 10mg/kg exposed concentration. One way ANOVA shows a significant variation in the concentration of lead in the exposed plant in relation to control at $p<0.05$. The variation in the concentration of Cadmium in soil, as shown in figure 3 ranges from a minimum of 0.003±0.014 mg/kg in control to a maximum of 0.70±0.007 mg/kg in 50mg/kg, exposed concentration. One way ANOVA shows a significant variation in the concentration of cadmium in the exposed soil in relation to control at $p<0.05$. The variation in the concentration of Cadmium in earthworm, as shown in figure 3, ranges from a minimum of 0.61±0.016 mg/kg in control to a maximum of 0.83±0.005 mg/kg in 10mg/kg, exposed concentration. One way ANOVA shows a significant variation in the concentration of cadmium in the exposed earthworm in relation to control at $p<0.05$. In general, the variation in the concentration of lead ranges from stem>soil>earthworm (fig. 3).

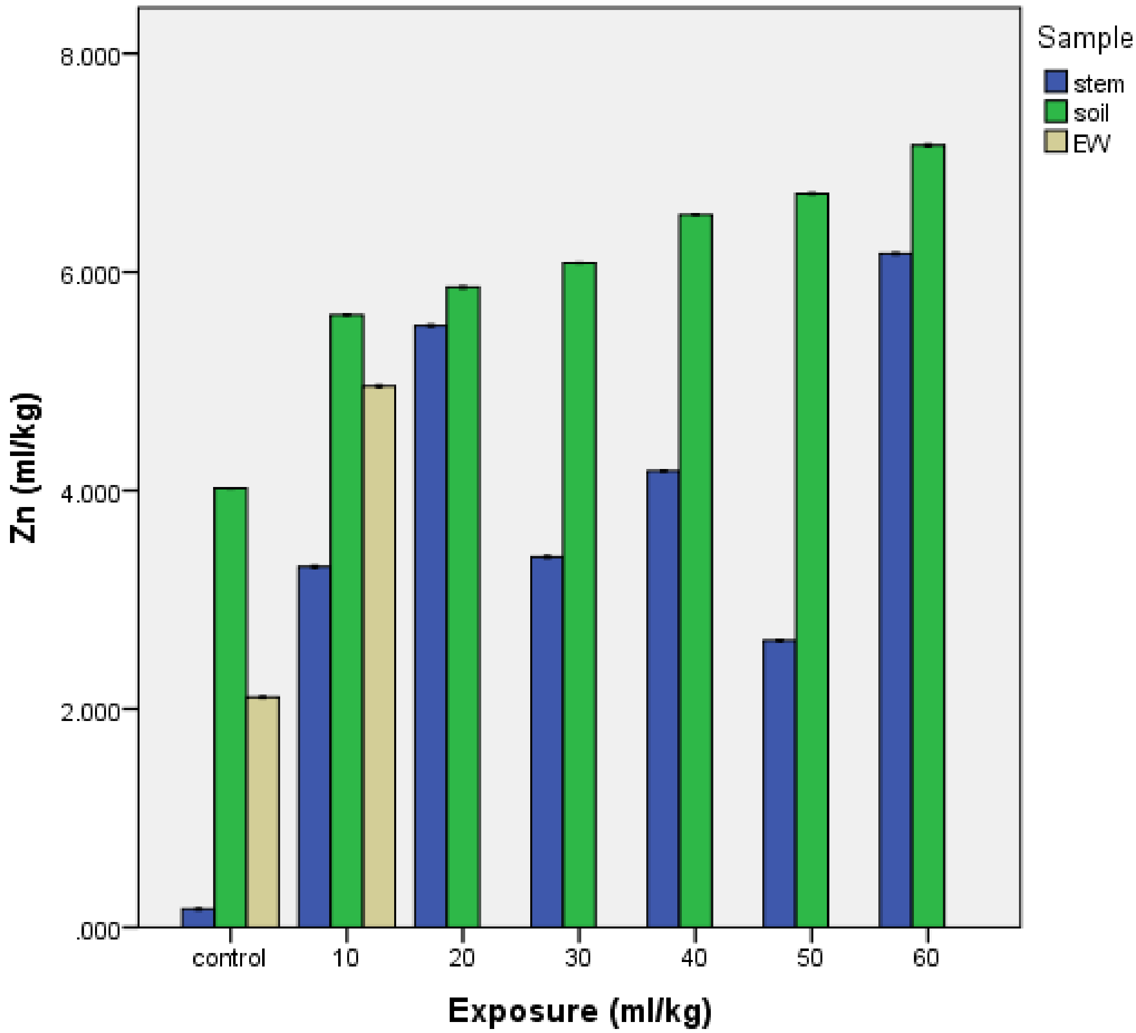

Fig. 4:- Level of Zn absorbed by plant stem, soil and earthworm across the treatments.

The variation in the concentration of zinc in plant, as shown in fig. 4, ranges from a minimum of 0.003±0.014 mg/kg in control to a maximum of 6.16±0.113* mg/kg in 60mg/kg, exposed concentration. One way ANOVA shows a significant variation in the concentration of zinc in the exposed plant stem in relation to control at $p<0.05$. The variation in the concentration of zinc in soil, as shown in figure 4, ranges from a minimum of 4.02±0.002 mg/kg in control to a maximum of 7.16±0.016 mg/kg in 60mg/kg, exposed concentration. One way ANOVA shows a significant variation in the concentration of zinc in the exposed soil in relation to control at $p<0.05$. The variation in the concentration of zinc in earthworm, as shown in figure 4, ranges from a minimum of 2.10±0.009mg/kg in control to a maximum of 4.95±0.014 mg/kg in 10mg/kg, exposed concentration. One way ANOVA shows a significant variation in the concentration of zinc in the exposed soil in relation to control at $p<0.05$. In general, the variation in the concentration of lead ranges from soil>stem>earthworm (fig. 4).

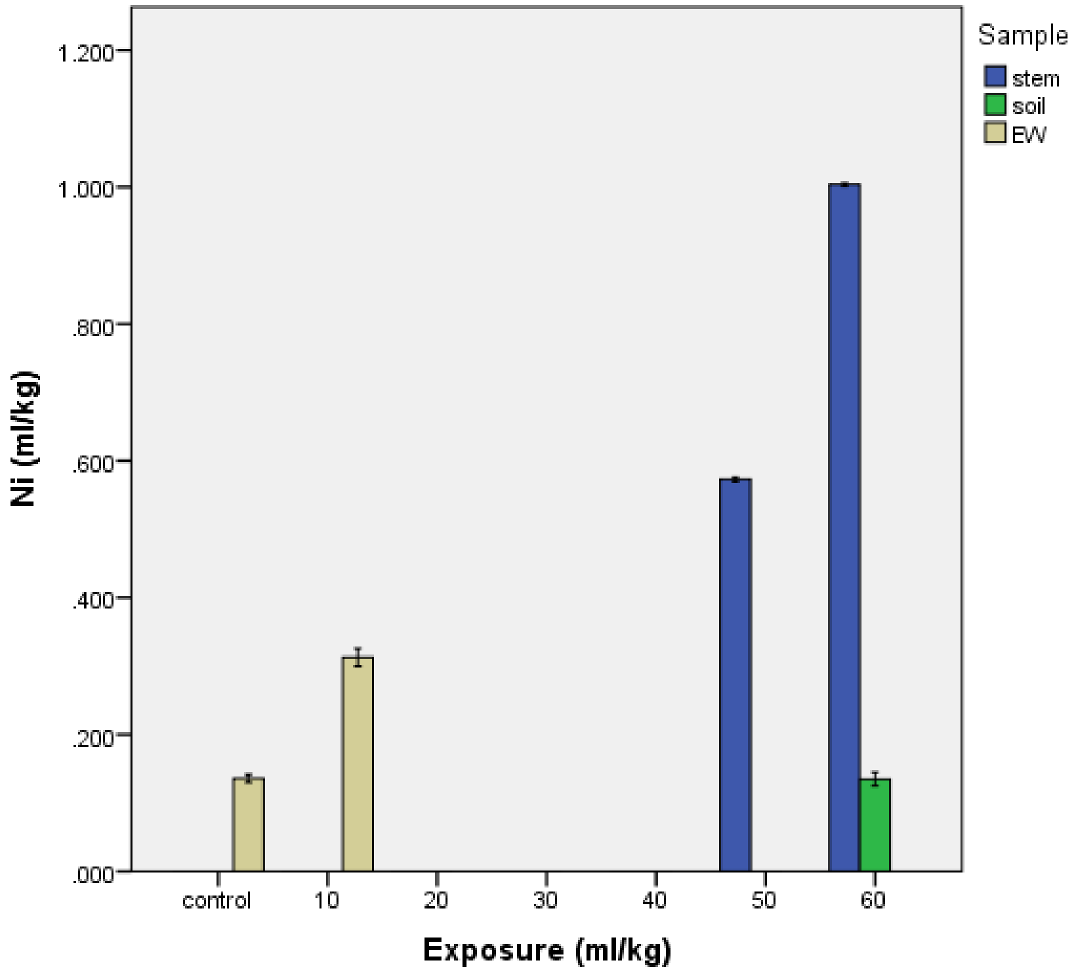

Fig. 5:- Level of Ni absorbed by plant stem, soil, and earthworm across the treatments.

The variation in the concentration of nickel in plant stem, in fig. 5 shows that nickel was not detected in all the concentrations except at 50mg/kg (0.57±0.004*) and 60mg/kg (1.003±003*) concentrations. One way ANOVA shows a significant variation in the concentration of nickel in the exposed plant in relation to control at $p<0.05$. The variation in the concentration of nickel in soil, as shown in figure 5, shows that nickel was not detected in all the concentrations except at 60ml/kg at a concentration of 0.14±0.014 mg/kg. One way ANOVA shows a significant variation in the concentration of nickel in the exposed soil in relation to control at $p<0.05$. The variation in the concentration of nickel in earthworm, as shown in figure 5, shows that nickel was not detected in all the concentrations except in control at 0.13±0.008, and at 10ml/kg at a concentration of 0.31±0.018mg/kg. One way ANOVA shows a significant variation in the concentration of nickel in the exposed earthworm in relation to control at $p<0.05$.

Effect on Plant Growth of Spent Oil at Various Levels of Spent Oil Contamination at 3- and 6-Weeks Post Treatment.

The effect of spent oil on the growth parameters 3- and 6-weeks post treatment is shown in table 5. At 60ml/kg, plant development was stunted. There was a significant difference in the number of Amaranth leaves in the control, 10, 20, 30, 40, 50 and 60ml/kg spent oil treatment. However, the number of leaves was higher in the 30ml/kg and 60ml/kg treatment than in the 50ml/kg treatment. The plant height was higher in the control which significantly different from all the other treatments. There was a significant difference in the 10, 20, 30 and 40ml/kg treatment. The 50ml/kg treatment was significantly the shortest out of all the plants grown under various treatments.

The analysis of variance showed that there was a significant difference in number of leaves between the treatment and control. The highest number of leaves was obtained in the control followed by the 10ml/kg treatment. However, the 10ml/kg was not significantly different from all the other treatments. At the 6 weeks post treatment, there was no significant difference in the number of leaves between the 3- and 6-weeks post treatment. However, for the other treatments the number of leaves on average increased by 50%. The number of leaves in the control was generally twice as high as the treatments. In the case of plant height, the control had the highest height when compared with the treatment. The plant height generally decreased with increase in concentration. By the 6th week, the stem had shrunk in the 50 and 60ml/kg treatments with height of 3cm for each treatment when compared with the 3rd week which had a height of 3.3 and 4cm respectively. For the other treatments, the increase in height from 3rd to 6th week was not significant. However, in the control, the height of plant had increased from 7 to 29cm which was 4 times higher.

Soil Biota Studies: Effect of soil contamination on earthworm.
The activity of earthworm was reduced in all treatment except in the control. Plates 1 to 7 show the soil surface with various levels of earthworm cast. Plate 1 which is the control had the soil surface covered with earthworm casts while other treatments had few or no cast on soil surface.

Earthworm was only found in the control and at 10ml/kg. The 20ml/kg, 30ml/kg, 40ml/kg, 50ml/kg and 60ml/kg had no earthworms at the end of the experiment. Table 5 shows the various concentration levels of heavy metals in the earthworm tissue. The earthworm in the control had the lowest concentration of the metals analyzed while the earthworms in the treated soil had higher levels of the metals. While lead showed no specific trend, copper, cadmium, zinc and nickel showed presence in the earthworms at 10ml/kg.

Discussion

The result obtained from analysis of spent oil in the study confirmed the presence of heavy metals in the oil. Deductions can be made that increasing soil contamination resulted in corresponding increase in levels of Cu, Pb, Cd, Zn and Ni in the experimental soil. Okonofua et al., (2007) reported similar findings from studies carried out to determine the effects of spent engine oil on soil properties. The authors reported that increasing concentrations of spent oil in treated soils resulted in higher concentrations of Cu and Fe in the treated soils than in the control, which was similar to the findings in this study. Yong, (2001) stated that this may be due to a bonding relationship between contaminants and soil surfaces due to sorption forces. Yong, (2001) further explained that heavy metals, being positively charged, are electrostatically attracted to the negative charges on the clay particles.

The baseline study showed that the soil used in the study was suitable for plant growth. Oil contamination is known to alter physical, chemical, and biological properties of soil. Merkl et al. (2005) reported that oil contamination alters soil moisture conditions and can lead to non-homogenous distribution of water in soil due to the hydrophobic nature of oil. Oil contamination leads to poor wetness and aeration of soil which leads to water and air deficiency (Njoku et al.,2008). Anoliefo and Edagbai (2000) further explained that the physical property of spent oil affects water uptake and gaseous exchange in the soil leading to physiological drought in soil organisms.

Soils that were treated with 40ml/kg, 50ml/kg and 60ml/kg concentration of spent oil had visible darker coloration. Okhonokua et al., (2007) stated that dark soils absorb more heat than light ones and may increase temperatures up to 65^{0}C to 70^{0}C which are lethal to many plants and soil microorganisms. Studies have demonstrated that the dynamics of solubilization and ionic exchange in soils are negatively affected under the influence of spent oil and could be responsible for noticeable negative effects on plant growth indices (Odjegba and Sadiq, 2002).The mean number of leaves on the plant observed during the study showed differences compared to the control at 3 WAP and 6 WAP. The control had the highest values for the number of leaves observed at 3 WAP and 6 WAP. Reduction in leaf number of *Amaranthusspp*. exposed at different concentrations of spent oil had been earlier reported by Kayode et al., (2009), while Nwoko et al., (2007) reported reduction in leaf number of *Phaeseolusvulgaris* exposed to the same contaminant. Ekpo and Ebeagwu (2009) attributed reduction in leaf numbers with increasing concentration of hydrocarbon pollution to the interference of oil in the soil physical and chemical properties and subsequent transpiration and photosynthesis in the plant. This study observed a decrease in mean plant height at 3 WAP and 6 WAP.

Wang,(2002) had earlier reported a decrease in dry matter yield of maize grown in soil contaminated with Cadmium (Cd), Lead (Pb), Chromium (Cr), Zinc (Zn). The results obtained from this study indicated that spent oil contamination affected the growth indices of *Amaranthusspp*. negatively. The effect of the contamination was however noticed to be the same at 3 WAP and 6 WAP. Statistical analysis revealed that significant differences exist between the mean number of leaves and height of plant between the control and all other treatments at 3 WAP. Likewise at 6 WAP, significant differences were observed between the control and other treatments for both parameters measured.

This study also revealed that the population of earthworms was reduced in treated soils in a contamination dependent manner. The result obtained in this present study showed significant reduction in the population of earthworms between untreated and treated soil, including where no earthworm in concentrations above 20ml/kg. This may be due to anaerobic conditions caused by spent oil covering cuticle of earthworms leading to oxygen and moisture impairment and/or may be connected to the reduction in soil PH because of increasing concentration of spent oil. Peijnenburg and Vijver, (2009) stated that soil P^H is a limiting factor on earthworm distributions. They all stated that soil PH, moisture and temperature all dictate how well earthworm will thrive in each soil. As mentioned earlier, spent oil contamination of the soil in this study may lead to increased temperature of the soil; this may contribute to unfavorable conditions of the earthworm. Earthworms play key roles in maintaining a healthy soil. Adverse effects of contaminants on earthworms may lead to a reduction in soil fertility. Oboh et al., (2006) reported that exposure of earthworms to hydrocarbons and heavy metals had detrimental effects such as reduction in weights, reduced reproductive ability and accumulation of heavy metals in their tissue which may eventually have long term consequences on earthworm populations and subsequently the food chain. However, the authors stated that earthworms do not suffer significant morbidity responses from direct exposures to heavy metals and organochlorides.

Conclusion

Spent oil contamination in this study significantly affected the growth and development of Amaranthusspp. as indicated by the effect of the contamination on indices of plant growth studied in this experiment. The contamination also significantly affected the population of earthworms, it is therefore important that strict regulation and monitoring is enforced to prevent the indiscriminate disposal of spent oil to safeguard the environment from the negative consequences of spent lubricating oil. Amaranthusspp. is a staple leafy vegetable for a large population of Nigerians, and it is planted almost everywhere in the country. Sanctions should ensure against indiscriminate disposal of spent oil practiced most especially by roadside mechanics. There is a need for the populace to be educated so that they appreciate the impact of such action. Existing legislation on spent oil management should be enforced while new legislation should be enacted where necessary.

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