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EVALUATION OF HEAVY METALS UPTAKE AND GROWTH PARAMETERS OF *LYCOPERSICUM ESCULENTUM* AND *AMARANTHUS HYBRIDUS* GROWN ON SOIL POLLUTED WITH SPENT ENGINE OIL

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ABSTRACT

The effect of spent engine oil on growth parameters and heavy metals uptake of *Lycopersicum esculentum* (Tomatoes) and *Amaranthus hybridus* (African Spinach) was investigated. The completely randomized design was used with five treatment levels of spent engine oil at 0.0, 10.00, 20.00, 30.00 and 40.00ml were applied to 1.5kg of soil. The percentage survival germination of the plants was determined after four weeks of planting. Height and stem diameter of the plants were taken weekly after five weeks of planting. The heavy metals (As, Zn, Pb, Hg and Cd) were analyzed using Atomic Absorption Spectrophotometry. An increase in the volume of spent lubricating oil leads to the decrease in the growth parameters of the plants. The percentage survival, stem diameter and the leaves number of *L. esculentum* were significantly ($P<0.05$) higher than *A. hybridus*. The height of *A. hybridus* was significantly ($P<0.05$) higher than the height of *L. esculentum*. There is significant difference ($P<0.05$) in heavy metal concentrations of the plants at different treatment levels compared with the control. It is therefore imperative to inform and enlighten the local farmers and the consumers on the danger of planting on soil and using water polluted with spent lubricating oil.

1. Introduction

The advancement in technology and science coupled with the search and massive demand for energy to cater for the increasing population has increased the number of motorcycles, vehicles, generators railway, aircraft and water vehicles. This technological advancement has caused a rapid rise in petroleum consumption and, as a result, a huge amount of hydrocarbons are being discharged into the environment, either deliberately or accidentally, every year. Consequently, the quantity of engine oil and other engine fluids (cooling liquids, brake oils, gearbox oils, etc.) used is expected to increase (Baladincz *et al.*, 2008). The lubricating oils used by vehicle engines have to be changed at least every 20,000 km also that of other machines that make use of lubricating oil needs to be replaced at the appropriate time (Baladincz *et al.*, 2008). These have posed a great environmental alteration that has become difficult to evaluate and fully understand (Ogbuehi *et al.*, 2010) and also pose significant threat to public health as a result of their unregulated disposal of the used engine oil into the environment.

Spent engine oil is commonly gotten or obtained after checking and consequently drained from automobile and generator engines (Anoliefo and Vwioko, 2001) and much of this oil is poured into the soil. This unregulated discarding of spent engine oil undesirably affects floras, microbes and aquatic organisms (Nwoko *et al.*, 2007; Adenipekun *et al.*, 2008) because of the large quantity of hydrocarbons and highly lethal polycyclic aromatic hydrocarbons contained in the oil (Wang *et al.*, 2000; Vwioko and Fashemi, 2005).

Heavy metals such as aluminium, lead, nickel and iron, which are present in large quantities in used engine oil may be retained in the soil, in the form of carbonates, oxides, hydroxides, exchangeable cation and bound to organic materials in the soil (Ying *et al.*, 2007). These heavy metals may lead to building up of essential organic (magnesium, carbon, phosphorous, calcium) and non-essential (magnesium, lead, zinc, iron, copper, cobalt) elements in soils which are eventually moved into plant tissues (Vwioko *et al.*, 2006). Although, heavy metals in low concentration are crucial micronutrients for plants, at high concentrations, they may cause metabolic complaint and growth inhibition for most of the plant species (Yadav, 2010). According to Nwadinigwe and Onwumere (2003), pollution of soil arising from oil spills affect the growth of plants and causes great adverse impacts on food production (Onwurah *et al.*, 2007). Odjegba and Sadiq (2002) also stated low produce and decreased the growth of plant grown in spent lubricant oil polluted the soil. Researchers such as Wang *et al.* (2000); Odjegba and Sadiq (2002); Agbogidi *et al*. (2006) had worked on the effect of spent lubricant oil pollution on soil properties and crop produce.

Amaranthus hybridus (African spinach) generally called green leaves are cultivated in numerous parts of the world, including China, South America, India, United States and Africa (He *et al.*, 2003). The properties of amaranth in cholesterol reduction as an anti-oxidant, anti-allergic, anticancer and antihypertensive agent; and as food for patients with celiac disease and immune deficiencies, have been assessed in clinical studies. *Amaranthus hybridus* have been used locally for the management of liver infections and knee pain and for its diuretic, laxative and cicatrisation properties (Dhellot *et al.*, 2006). *Lycopersicom esculentum* (Tomatoes) is an important horticultural crop worldwide, and its fruits vary widely in size, shape, and colour (Dietmar *et al.*, 2014). It is a staple and common vegetables consumed by all classes of Nigerian citizens. Tomatoes are a good source of potassium, calcium, magnesium, and antioxidant that has been shown to lower high blood pressure and reduce the risk of heart disease, preventing acidosis, neutralizing dangerous free radicals that otherwise damage cells and cell membranes.

2. Materials and methods

Study Site

The study was carried out as a potted experiment in a screened house located behind the academic building at the Federal University of Technology Akure, Ondo State, Nigeria.

Soil Sampling

The top soil was collected from the vegetation (fallow ground) behind the new security building at the Federal University of Technology, Akure. The soil sample was air-dried and sieved using 2 mm sieve to remove stones, roots and other materials that may be detrimental to the emergence of the young plants from the seeds.

Experimental Plant and Spent Lubricating Oil (SLO)

Lycopersicon esculentum and *Amaranthus hybridus* seeds were obtained from the seed gene bank of the International Institute for Tropical Agriculture (IITA) in Ibadan, Oyo State. Spent engine oil was obtained from the Mechanic Village, located along Ondo road in Akure Metropolis, Ondo State.

Experimental Design and Treatment Application

The sample of 1.5kg of soil was filled into a plastic pot perforated, 5-8 holes at the base. The soil was treated with the different levels, 0.0, 10, 20, 30 and 40 ml/1.5kg of soil. The engine oil and soil were mixed thoroughly with a trowel to achieve uniformity. The treated soil was allowed to stay for two days, to allow to mix oil very well with the soil and to enable the volatile compounds present to escape into the atmosphere. *Lycopersicon esculentum* seeds were planted at the rate of 10 seeds per pot and 25 seeds per pot for *Amaranthus hybridus* seeds. The experimental pots were watered every three days, and subsequently during the course of the experiment, the weeding of the pots was done by hand. After four weeks (4weeks) of planting, the population of surviving plants was reduced to four plants per pot by thinning. The pots were arranged in a completely randomized design consisting five treatments replicated five times. This makes a total of fifty (50) pots for the two plantations.

Survival counts was taken per pot after 28days of sowing the plants in soil contaminated with the spent oil and the percentage survival were calculated as described by Njoku *et al.*, (2008) with the formula below:

$$\frac{\text{No of seedlings contaminated}}{\text{No of seedling that survived}} \; x\,100$$

Growth and Yield Measurement

The growth parameters of the plants were taken weekly using a metre rule to measure plant height, length and breadth of the leaves; vernier caliper to measure the diameter of the stem, and counting the number of leaves. After 12 weeks of sowing the seeds, the plants were harvested, and the wet mass of the leaves was taken after rinsing the leaves thoroughly with water.

Digestion of Samples

Five (5) gramme each of the dried leaf samples which has been ground into powder using a ceramic mortar and pestle was transferred into digestion flasks, then 4ml of hydrochloric acid and 8ml nitric acid was added to each respective sample. The digestion flasks were put on a hot plate set at 120^0C (gradually increased) until the samples were digested. After digestion, the digested samples were diluted with distilled water appropriately in the range of standards which was prepared from the stock standard solution of the metals.

Determination of Heavy Metal Concentration

The digested or processed leaf samples were all analyzed for heavy metals (cadmium, mercury, lead, arsenic and zinc) absorbencies, using Atomic Absorption Spectrophotometers (AAS), Model 210 VGP respectively (AOAC, 1990). The analysis of the digested samples was carried out at the Institute of Agriculture Research and Training Ibadan, Central laboratory. The data obtained was the absorbencies of each heavy metal (Cd, Cr, Pb, and Zn) which became the raw data that was analyzed using ANOVA in SPSS computer programme to get concentrations of each heavy metal in each sample for the study.

Data Analysis

Data obtained in the course of the research were subjected to analysis of variance (ANOVA), and treatment means were separated with the Least Significant Difference (LSD) test at 5% level of probability (Onuh and Igwemma, 2001). The ANOVA was performed with SPSS version 21. The analyzed data were presented using bar charts, graphs and tables that served as a useful means of bringing out various comparisons between the test variables in the data collected.

3. Results

The percentage germination survival of *Lycopersicon esculentum and Amaranthus hybridus* are presented in Figure I and 2. The highest percentage germination survival values of *L. esculentum* were observed at 0.0ml (80.75%) followed 10ml (69.65%) while the least was observed at 40ml (34.54%). Also, the highest percentage germination survival values of *A. hybridus* were observed at 0.0ml (76.25%) followed 10ml (58.65%) while the least were observed at 40ml (24.54%). The percentage germination survival in *L. esculentum* and *A. hybridus* decreases as the volume of spent lubricating oil increases.

Figure 3 showed the comparison of the height of the *L. esculentum* and *A. hybridus* grown in 1.50kg of soil contaminated with different volumes of spent lubricating oil. It was observed that the height of *A. hybridus* were significantly ($p<0.05$) higher than the height of *L. esculentum* except the soil polluted with 10ml where the height of *A. hybridus* is significantly ($p<0.05$) higher than the height of *L. esculentum*. Figure 4 showed the comparison of the stem diameter of plants grown in 1.50kg of soil contaminated with different volumes of spent lubricating oil. It was observed that the stem diameter of *L. esculentum* were significantly ($p<0.05$) higher than the height of *A. hybridus.* Figure 5 showed the comparison of the leaves number of plants grown in soil polluted with different volumes of spent lubricating oil. It was observed that the leaves number of *L. esculentum* were significantly ($p<0.05$) higher than the leaves number of *A. hybridus.*

The mean concentration of the heavy metals in the leaves of *L. esculentum* and *A. hybridus* grown in 1.50kg of soil polluted with different volumes of spent lubricating oil are presented in Table 1 and 2. The mercury concentrations in the leaves of *L. esculentum* range from 0.2±1.0 to 28.78±3.40 while that of *A. hybridus* range from 0.2±1.0 to 23.65±5.0. The highest values of mercury were observed in *L. esculentum* (28.78±3.40) at 40ml. Also, the lead concentrations range from 4.0±1.0 to 417.98±7.23 in *L. esculentum* and 3.6±1.0 to 317.34±6.56 in *A. hybridus.* The highest values of lead were also observed in *L. esculentum* (417.98±7.23) at 40ml. There is significant difference ($p<0.05$) between the treatments compared with the control. Similarly, cadmium concentrations in *L. esculentum* range from 2.0±1.0 to 227.78±4.6 while *A. hybridus* range from 4.3±1.0 to 527.57±4.67. The highest values of cadmium were observed in *A. hybridus* (527.57±4.67) at 40ml. Arsenic was not detected in control (0.0ml), 10ml and 20ml but were observed at 30ml and 40ml of both *L. esculentum* and *A. hybridus*. The highest concentrations of arsenic were observed in *A. hybridus* (17.67±2.45) at 40ml. The mean concentrations of zinc in *L. esculentum* range from 113.78±2.80 to 1667.83±11.66 while that of *A. hybridus* range from 77.98±2.21 to 1467.59±11.26. The highest values of zinc were recorded in *L. esculentum* (1667.83±11.66) at 40ml. There is a significant difference ($p<0.05$) between the treatments when compared with the control. Also, an increase in the volume of spent lubricating oil leads to the increase in the concentrations of the heavy metals in the plants.

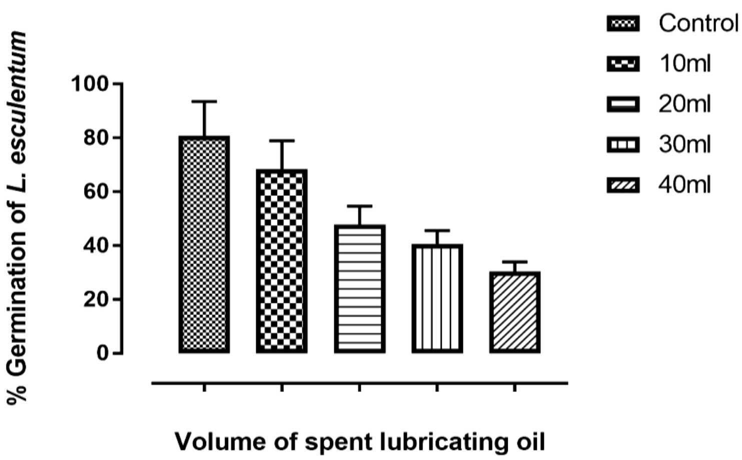

Figure 1: Percentage germination of *Lycopersicon esculentum* after four weeks of planting

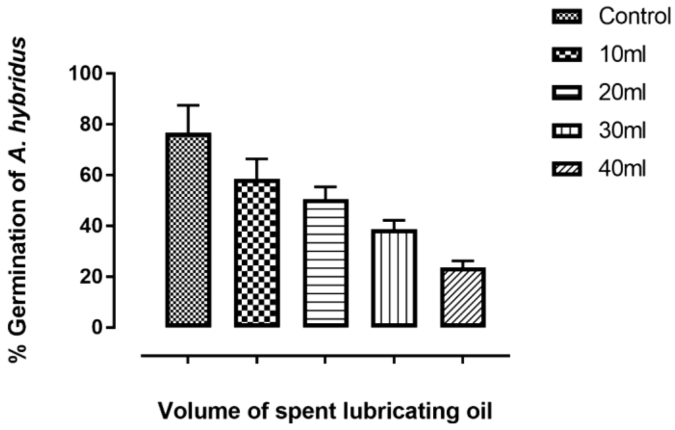

Figure 2: Percentage germination of *Amaranthus hybridus* after four weeks of planting

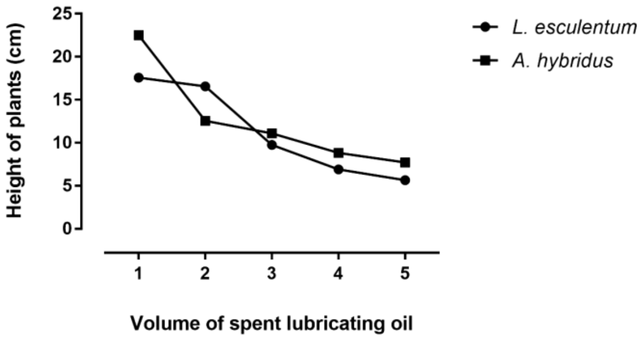

Figure 3: Comparison of the height of *Lycopersicon esculentum and Amaranthus hybridus* grown in 1.5kg of soil polluted with spent lubricating oil

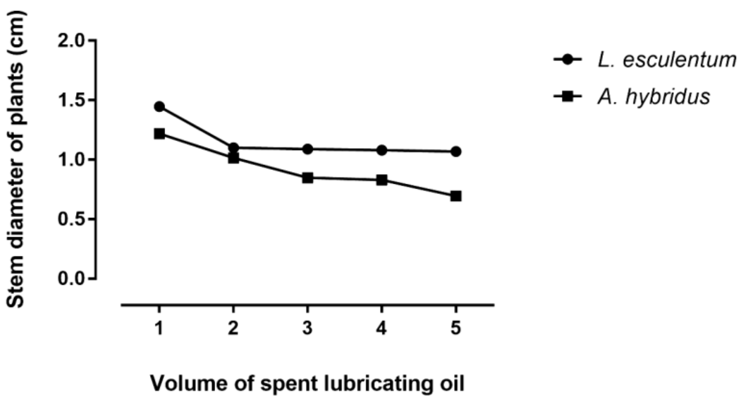

Figure 4: Comparison of stem diameter of *Lycopersicon esculentum and Amaranthus hybridus*

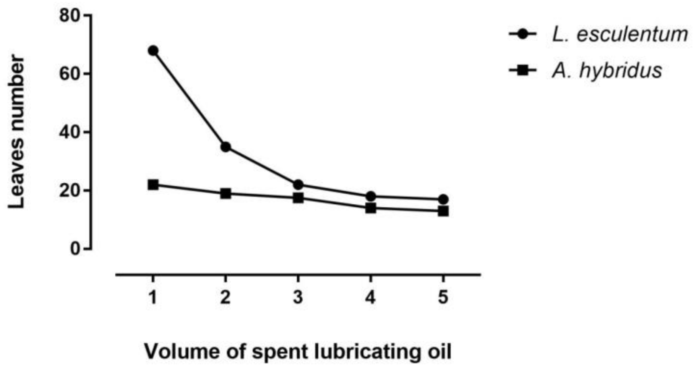

Figure 5: Comparison of leaves number of *Lycopersicon esculentum and Amaranthus hybridus*

Table 1: Heavy Metals Concentration in the leaves of *Lycopersicon esculentum*

Heavy Metals	Volume of spent lubrication oil (ml) used per 1.50 kg of soil samples				
($1x10^{-4}$) mg/kg	0.0	10	20	30	40
Mercury	$0.2±1.0^{a}$	$16.34±2.20^{b}$	$22.45±3.10^{c}$	$25.98±3.50^{cd}$	$28.78±3.40^{d}$
Lead	$4.0±1.0^{a}$	$113.76±5.20^{b}$	$123.78±6.0^{b}$	$303.67±6.30^{c}$	$417.98±7.23^{d}$
Cadmium	$2.0±1.0^{a}$	$92.78±3.5^{b}$	$107.56±4.16^{b}$	$220.87±3.85^{c}$	$227.78±4.6^{c}$
Arsenic	ND	ND	ND	$12.7±1.0^{b}$	$13.9±1.0^{b}$
Zinc	$113.78±2.80^{a}$	$867.75±7.66^{b}$	$1167.89±9.76^{c}$	$1467.56±11.6^{d}$	$1667.83±11.66^{e}$

Note: Means values with the same superscript alphabets in the rows are not significantly different ($p<0.05$) from each other using Duncan's New Multiple Range Test (DNMRT).

ND: Not detected

Table 2: Heavy Metals Concentration in the leaves of *Amaranthus hybridus*

Heavy Metals	Volume of spent lubrication oil (ml) used per 1.50 kg of soil samples				
($1x10^{-4}$) mg/kg	0.0	10	20	30	40
Mercury	$0.2±1.0^{a}$	$4.54±2.0^{b}$	$11.67±3.0^{c}$	$18.89±3.0^{d}$	$23.65±5.0^{e}$
Lead	$3.6±1.0^{a}$	$103.86±3.0^{b}$	$203.98±4.30^{c}$	$303.45±6.50^{d}$	$317.34±6.56^{e}$
Cadmium	$4.3±1.0^{a}$	$120.54±3.15^{b}$	$220.44±3.52^{c}$	$427.56±4.26^{d}$	$527.57±4.67^{e}$
Arsenic	ND	ND	ND	$15.0±2.30^{b}$	$17.67±2.45^{b}$
Zinc	$77.98±2.21^{a}$	$707.67±5.16^{b}$	$1067.56±7.86^{c}$	$1267.67±10.6^{d}$	$1467.59±11.26^{e}$

Note: Means values with the same superscript alphabets in the rows are not significantly different ($p<0.05$) from each other using Duncan's New Multiple Range Test (DNMRT).

ND: Not detected

4. Discussion

The percentage germination survival in *L. esculentum* and *A. hybridus* decreases as the volume of spent lubricating oil increases. This may be as a result of coating on the seed surface, thereby affecting physiological functions within the seed. It was observed from the result that the percentage germination survival of the seeds of *Lycopersicon esculentum* was higher than that of *Amaranthus hybridus* as the seeds of *L. esculentum* sprouted out more than *A. hybridus.* This could be due to the ability of *L. esculentus* seeds to survive and germinate better as a result of its ability to make use of limited resources in its environment than *A. hybridus.* Also, *L. esculentus* seeds might be embedded with more nutrients that support seeds germination than *A. hybridus*. Low and delayed germination of *L. esculentum* and *A. hybridus* seeds in soil receiving higher spent engine soil treatment indicate the degree of soil degradation. Ekundayo *et al.* (2001) noted that germination of seeds was delayed in oil-polluted soil. This is agreement with similar experiments by Amadi *et al.* (1993) and Ekundayo *et al.* (2001) who reported that spent engine oil reduced germination by coating on seed surfaces, thereby affecting physiological functions of the seed. Onwuka *et al.* (2012) in their study also reported the effect of spent engine oil on seed germination. According to Jaja and Odoemena, (2004), an increase in metal concentration may increase suppression of seed germination. Suppression of seed sprouting and plant growth responses has been ascribed to the establishment of complex toxic effect syndromes owing to the high build-up of the metals within the plant body biomass (Esenowo, 1995).

The increase in the volume of spent lubricating oil significantly affected the height of *L. esculentum and A. hybridus* when compared with the control. The presence of heavy metals in the soil as a result of the treatment contributed to the retarded growth observed in *L. esculentum and A. hybridus*. Spent lubricating oil in the soil could have affected the biological oxygen demand (BOD) level, thereby, interfering with the normal gaseous exchange. The observed increase in the plants grown in the uncontaminated soils (control) could be seen as the unadulterated nature of the soil structure which allowed normal metabolic activities of untreated soil. This is in agreement with Agbogidi and Enujeke (2012) who observed that plots which received spent oil treatment had reduced water infiltration and percolation. Growth reduction and low biomass production could also be interpreted as being due to gross effects of the oil which could have shown up either in the distortion or reduction in the number of stomata per unit area of the leaf, thereby, affecting the photosynthetic process and consequently, the amount of photosynthesis produced. It was reported by Agbogidi (2010) that a reduction in shoot growth is a direct result of the root growths as the roots are input organs for the absorption and translocation of water and mineral nutrients. Dimitrow and Markow (2000) discovered that the occurrence of spent engine oil in the soil knowingly decreased the existing forms of potassium and phosphorus to plants. These nutrients (nitrogen, phosphorus, potassium and oxygen) are essential for plant growth and development; hence the reduction in their bioavailability will lead to reduced plant growth.

The significant decrease ($p<0.05$) in the stem diameter of the treated plants compared with the control groups observed in this study could be as a result of interference with the soil-water-relation as well as nutrient immobilization and the presence of heavy metals could also be responsible for the observed reduction in plant characters as seen in this study. The increasing concentrations of some heavy metals in the polluted soil decrease the diameter of the plants stems as bioaccumulation of one heavy metal in the mixture inhibits or enforce the accumulation of other heavy metals (Peralta-Videa *et al.*, 2002).

The significant decrease ($p<0.05$) observed between the treated plants, and the untreated plants could be as a result of the accumulation of heavy metals in the leaves of the plants. Cadmium which is one of the heavy metals found in the leaves of the plants reduced chlorophyll content and thus negatively impacted photosynthesis (Kambhampati *et al.*, 2005) and respiration in plants (Liang *et al.*, 2013). The decrease in number of leaves following spent lubricating oil application of soil had been recorded by Agbogidi and Ejemete (2005); Agbogidi and Eshegbeyi (2006) who noted that as hydrocarbons from oil-contaminated soils accumulate the chloroplast of leaves, it makes the photosynthetic ability of the leaves to become reduced, hence, affecting translocation in affected plants as a result of obstruction of the xylem and phloem vessels, hence, reduction in photosynthesis and number of leaves produced by each plants treated with spent lubricating oil.

The observed negative interaction in the germination percentage and the growth parameters (plant height, number of leaves and stem diameter) measured in the cause of this experiment could be attributed to the numerous hydrocarbons and related compounds which is toxic to biological organisms. This is in line with the findings of Adeoye *et al.* (2005) that hydrocarbon oil reduced soil quality and crop yield. This was also corroborated by Rainbow (2007) that spent lubricant oil caused soil degradation and low crop yield. Several other researchers (Odjegba and Sadiq, 2002; Wang *et al.*, 2002) corroborated the earlier report on low yield of crops on spent lubricant oil contaminated soil. Adedokun and Ataga (2007) also showed that treatment of soils with crude oil, automotive gasoline oil and spent engine oil significantly affected the time of germination, percentage germination, plant height, leaf production and biomass of *V. unguiculata* delaying germination and growth rate.

Agreeing to Meinz (1999), spent oil comprises of heavy metals and polycyclic aromatic hydrocarbons and chemical additives including amines, phenols, benzenes, lead, barium, manganese, calcium, zinc, sulphur and phosphorus which are hazardous to live organisms. The high level of toxic heavy metals and polycyclic aromatic hydrocarbon which has been reported to be present in spent oil can also account for the growth inhibition observed in this study. It was observed that the zinc accumulated more in leaves of *L. esculentum* and *A. hybridus* than other heavy metals assessed in this study. The higher accumulation of zinc in the leaves of the plants could be as a result of the more solubility and mobility of zinc than other heavy metal tested even under pollution environment. This agrees with the findings of Baker *et al.* (1994), Ogbuehi and Akonye (2008), Ogbuehi *et al.* (2010) who reported separately that some plant can phytoextract heavy metals from crude oil polluted soils. Adweole *et al.* (2008) reported heavy metals uptake by crops in their work and noted that these heavy metals were stored in crop parts. The implication is that human beings are at the risk of heavy metal toxicity. Anikwe and Nwobodo (2002) and Asadu *et al.* (2008), in their findings, observed that human beings were at risk of heavy metal toxicity if they would utilize crops grown around areas polluted with heavy metals due to heavy metals eco-toxicity. This could be possible through recycling of heavy metals through food chains. Heavy metals lead, and cadmium can cause brain, renal or reproductive disorders in human beings. This suggests that man is at higher risk of lead toxicity when he takes crops grown in spent engine oil treated soils.

5. Conclusion

The study has demonstrated that spent engine oil has a significant effect on the germination, height, a number of leaves and stem diameter of *L. esculentum* and *A. hybridus.* The present of heavy metals in *L. esculentum* and *A. hybridus* grown in soil polluted with spent lubricating oil indicated that *L. esculentum* and *A. hybridus* as a plant absorbed these heavy metals from the polluted environment. Humans and other animals that feed on the plants grown in spent lubricating oil polluted environment stand a risk of gradual accumulation of heavy metals in the body system as this could be detrimental to their health if the bioaccumulation of such heavy metal exceeds the tolerance level in the system. It is therefore pertinent to prevent the dumping and indiscriminate disposal of spent engine oil in the arable land meant for agriculture.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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