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Research Article

Morphological Responses of *Chromolaena odorata*, *Axonopus compressus* and *Aspilia africana* Grown in Used Engine Oil Polluted Soil

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Abstract

Background and Objective: The resultant effect of indiscriminate disposal of spent engine oil is a trending issue in most developing countries of the world. Contamination of agricultural soil by spent engine oil may alter soil physicochemistry and subsequently reduce crop production. The study was carried out to ascertain the morphological responses of *Chromolaena odorata, Axonopus compressus* and *Aspilia africana* grown in used engine polluted oil-polluted soil. **Materials and Methods:** Equal volume of pre-sieved soil (20 kg) was filled in polythene bags and arranged at 0.5 m spacing between polybags and 1 m between replications and perforated at the base to avoid water logging. Concentrations of spent engine oil spiked included 0, 4, 8, 12 and 16% (v/w) and which was allowed to stabilize for 2 weeks to simulate the condition of a natural spill. Parameters recorded at 2 weeks intervals were: Plant height, number of leaves per plant, fresh weight and root number. **Results:** A dose-dependent decrease in plant height, number of leaves per plant, fresh weight and root number. The mean growth parameters of the control (0%) were significantly higher than that of plants exposed with 4, 8, 12 and 16% concentration of spent engine oil at p<0.05%. The trend in a marked reduction of growth parameters was as follows: *A. compressus* > *C. odorata* > *A. africana*. **Conclusion:** Although spent engine oil affected the growth parameters measured, the plant species were able to tolerate and grow in various concentrations. This underscores the need to further probe *Chromolaena odorata, Axonopus compressus* and *Aspilia africana* as possible plant species for remediation of used engine oil-polluted soil in the tropics.

Key words: Chromolaena odorata, Axonopus compressus, Aspilia africana, used oil, polluted, soil, morphological responses

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

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INTRODUCTION

Petroleum and its allied products are a critical part of modern life and a cornerstone of any modern industrial strategy¹. Environmental problems posed by indiscriminate disposal of petroleum products have thus far contradicted their original beneficial use². Contemporary literature is awash with the effects of spent engine oil soil contamination on the ecosystem³⁻⁸. Study by Eapen and D'Souza⁹ observed a significant decrease in soil properties subjected to various concentrations of spent engine oil. The challenge of soil contamination is a major one because it reduces the value of industrial profitability as the sustainable development of environmentally safe practices is threatened through its negative impact on flora and fauna¹⁰. Moreover, Ebong et al.¹¹ reported a significant decrease in height, number of leaves, leaf area and number of flowers, fruits and dry weight of Solanum gilo with an increase in the foliar spray of petroleum hydrocarbon.

Spent engine oil has been documented to affect the moisture content in *Corchorus olitorius* Linn¹². Efe and Okpali¹³ reported a significant decrease in biochemical parameters including fiber and carbohydrate content in cowpea (*Vigna unguiculata*) growing in soil contaminated with spent engine oil. In *Zea mays*, leaf formation was disrupted, stunted growth and a high mortality rate were observed when it was exposed to soils contaminated with used oil^{14,15}. This resulted in low yield and poor quality of grains observed during harvest for *Zea mays* and *Arachis hypogea*¹⁶. This study was specifically undertaken to evaluate the morphological responses of *Chromolaena odorata*, *Axonopus compressus* and *Aspilia africana* grown in used engine oil-polluted soil.

MATERIALS AND METHODS

Study area: The research work was carried out between May to August, 2022 at the Screen House of the Department of Biology, Federal University of Technology, Owerri, Imo State. Owerri is located between Latitude 5°17'N and Longitude 7°54'E. The annual rainfall ranges from 2250 mm to 2500 mm with a temperature range of 26 to 28°C and relative humidity of 80 to 86°F.

Collection and processing of soil samples: Soil samples with no history of oil pollution were collected from a depth of 0-20 cm within the Federal University of Technology Owerri using an improvised soil auger of approximately 7.5 cm

diameter and taken to the laboratory for pre-planting soil analysis. The soil samples were air-dried and pre-sieved with <2 mm sieve and used for physicochemical determination.

Nursery establishment of seedlings: Vegetative parts of the plant species (stem cuttings of *Chromolaena odorata, Axonopus compressus* and *Aspilia africana*) were collected from an area with no trace of spent oil pollution and stabilized in the nursery for 2 weeks by which time an average of 4 fully expanded leaves per plant had been developed before been used for the study.

Experimental design and treatment application: An equal volume of pre-sieved soil (20 kg) was filled in polythene bags and arranged at 0.5 m spacing between polybags and 1 m between replications and perforated at the base to avoid water logging. Concentrations of spent engine oil spiked included 0, 4, 8, 12 and 16% (v/w) and which was allowed to stabilize for 2 weeks to simulate the condition of the natural spill. For the treatment, the control soil was not spiked with spent engine oil. Each treatment was replicated thrice and arranged in a complete randomized design.

Transplanting of seedlings into plant pots: Two weeks after nursery establishment of the seedlings, plants with three to four fully expanded leaves were selected for transplanting into the polythene bags containing the respective treatments/control, one plant per pot. Each of the polythene was watered with 200 mL of water a day before transplanting. Overall, three plant species (*Chromolaena odorata, Axonopus compressus* and *Aspilia africana*) were tested in five treatment soils each having three replicates.

Data collection: The observations on the growth of the plant species were recorded at 2 weeks intervals using plant height which was measured using the meter rule, The number of leaves per plant (NL) which was estimated using the total number of leaves found in the plants counted from three randomly selected plants and the average data was recorded.

Plant harvesting: Plants were carefully harvested after 12 weeks of treatment and washed with distilled water to rid them of soil particles. The plants were sorted according to treatment and separated into roots, shoots and leaves and placed in paper envelopes before oven drying. The three replicates from each treatment were pooled together to give a composite sample from which data from the following parameters were taken:

- Fresh weight (FW) (g): The weight of the above-ground fresh biomass of the three randomly selected shoots was recorded
- Root number (RL) (cm): The number of roots was counted from the three randomly selected plants and the average data was recorded
- Fresh weight (FW) (g): The three randomly selected roots were washed and allowed to air dry and the fresh weight was recorded

Statistical analysis: Data collected were presented in charts and tables. Mean separation was done using Duncan's Multiple Range Test at a 0.05% probability level.

RESULTS

Effects of spent engine oil on the growth of *C. odorata, A. africana* and *A. compressus*

Effects on plant height: The results of the plant heights for *C. odorata, A. africana* and *A. compressus* exposed to different concentrations of spent engine oil were presented in Table 1. There was an observable significant decrease in mean plant height with a corresponding increase in spent engine oil levels across the weeks. The mean height of the control (0%) was significantly higher than that of plants exposed to 4, 8, 12 and 16% concentrations of spent engine oil at p<0.05%. Although mean plant height increased as the week progressed from 4 WAP to 12 WAP as recorded in treatment levels. A marked decrease in plant height was observed with an increase in treatment in all the test plants. However, a significant reduction was observed from the 10th to 12th WAP with the 16% treatment level having the highest negative effect on the plant height followed by 12% treatment whereas the

highest plant height was recorded in the control followed by treatment at 4% level. There was no significant difference (p<0.05) between 4 and 8% treatment levels for *C. odorata* and *A. africana*, while *A. compresses* showed no significant difference (p<0.05) between 8 and 12% treatment levels, respectively.

Effects on the number of leaves: Concerning the mean number of leaves, the highest number of leave was recorded in the control (unpolluted soil) and this was statistically (p<0.05) different in comparison with other treatments as shown in Table 2. A significant marked reduction was observed in soils treated with 16% concentration compared to other treatments. There was a significant difference (p>0.05) between the various treatments and the control. The trend in marked leaf reduction was as follows: *A. compressus> C. odorata> A. africana.* Also observed in the leaf of test plants grown in higher concentrations of spent engine oil are defoliation, yellowing of leaf, chlorosis and necrosis.

Effects on root number fresh and dry weight of *C. odorata, A. africana* and *A. compassus* exposed to different concentrations of spent engine oil: There was a progressive decrease in root number with an increase in the level of contaminant as shown in Table 3. The highest root number, fresh and dry weights were observed in control in comparison with other treatments. There was a gross reduction in root number in *A. compressus, C. odorata* and *A. africana* compared with the control. *Aspilia africana* produced the highest root number followed by *A. compressus* and then *C. odorata*. There was a significant difference (p<0.05) among the root number in all the test plants. The responses of the plants about fresh and dry weights varied consistently with a corresponding increase in treatment than control.

Table 1: Mean height of *C. odorata, A. africana* and *A. compressus* exposed to different concentrations of spent engine oil polluted and unpolluted soil after 12 weeks

| Treatment (%) | Varieties | 2 WAP | 4 WAP | 6 WAP | 8 WAP | 10 WAP | 12 WAP |
|---------------|---------------|-----------------------|------------------------|------------------------|------------------------|------------------------|------------------------------|
| 0 | | 9.0±1.60 ^a | 10.1±1.64 ^b | 12.8±1.77ª | 14.8±1.82° | 16.8±1.87° | 20.3±2.01° |
| 4 | C. odorata | 6.8±1.23 ^b | 7.8±1.15° | 9.5±1.20° | 12.3 ± 1.23^{d} | 14.6±1.59d | 18.3 ± 1.87 ^d |
| 8 | | 6.7±1.22 ^b | 7.1±1.29 ^c | 8.6±1.13° | 11.9±1.16 ^d | 13.0±1.46 ^d | 18.0 ± 1.82^{d} |
| 12 | | 5.9±1.17 ^b | 6.9±1.05 ^c | 8.0±1.07 ^c | 10.8±1.10 ^e | 12.8±1.34 ^d | 16.8±1.77e |
| 16 | | 4.8±1.11° | 5.3±1.01 ^d | 7.2±1.05° | 10.1±1.08 ^e | 12.9±1.25d | 14.2±1.73e |
| 4 | A. africana | 5.3±1.24° | 8.3±1.09° | 11.2±1.11 ^b | 13.2±1.58° | 17.2±1.74° | 20.8±2.06 ^c |
| 8 | | 5.0±1.15° | 7.2±1.06 ^c | 10.9±1.09 ^b | 12.9±1.35 ^d | 16.3±1.68 ^c | 19.3±1.90° |
| 12 | | 4.2±1.00° | 7.0±1.04 ^c | 9.9±1.24° | 12.0±1.29 ^b | 16.2±1.65° | 17.3±1.79 ^d |
| 16 | | 3.0 ± 0.93^{d} | 5.2±1.26 ^d | 9.0±1.27 ^c | 11.2±1.37° | 13.2±1.53 ^d | 15.0±1.76 ^e |
| 4 | A. compressus | 11.1±1.76ª | 13.2 ± 1.79^a | 15.2±1.83° | 19.3 ± 1.98^a | 24.4±2.27 ^a | 28.8 ± 2.34^{a} |
| 8 | | 10.3±1.67° | 12.8 ± 1.79^a | 13.2±1.81 ^b | 17.3±1.94 ^b | 21.9±2.19 ^b | 25.3±2.23 ^b |
| 12 | | 9.9±1.71ª | 11.3±1.73 ^b | 11.9±1.77 ^b | 16.9±1.84 ^b | 20.4±2.17 ^b | 24.1±2.21 ^b |
| 16 | | 7.7±1.31 ^b | 10.3 ± 1.43^{b} | 10.8±1.45 ^b | 14.8±1.61° | 18.1±1.86 ^c | 20.3±2.01° |

Values are Means \pm SE, Mean along the column having different superscript of letters different significantly at p \leq 0.05 levels according to Duncan's Multiple Range Test (DMRT) and WAP: Weeks after planting

Table 2: Number of leaves of *C. odorata, A. africana* and *A. compressus* exposed to different concentrations of spent engine oil polluted and unpolluted soil after

| 12 WCC | 12 WCG | | | | | | |
|---------------|---------------|---------------------|------------------------------|-----------------------|------------------------|----------------------|-----------------------|
| Treatment (%) | Varieties | 2 WAP | 4 WAP | 6 WAP | 8 WAP | 10 WAP | 12 WAP |
| 0 | | 11±1.32° | 12±1.35° | 13±1.361 ^b | 15.8±1.47° | 19±1.54ab | 23±2.22 ^d |
| 4 | C. odorata | 5±0.22 ^b | 5±0.22° | 6±1.24 ^e | 10±1.29 ^{de} | 14±1.50° | 16±1.58 ^{ef} |
| 8 | | 6±1.24bc | 7.1 ± 1.26 ^{bc} | 8.6 ± 1.30^{d} | 11.9±1.34 ^d | 13±1.41° | 18±1.52 ^e |
| 12 | | 4±0.21° | 4±0.21° | 4±0.21 ^f | 9±1.32 ^e | 13±1.36° | 14 ± 1.50^{f} |
| 16 | | 8±1.27 ^b | 1±0.01 ^d | 3±0.04 ^f | 7±1.25 ^e | 11±1.34 ^d | 11±1.34 ⁹ |
| 4 | A. africana | 11 ± 1.3^{a} | 13±1.36° | 15±1.55° | 19±1.58° | 20 ± 1.74^{a} | 33 ± 2.06^a |
| 8 | | 10±1.29° | 10±1.29 ^b | 14±1.50ab | 17±1.35 ^b | 18±1.68 ^b | 30 ± 1.90^{b} |
| 12 | | 8±1.27 ^b | 9±1.32 ^b | 13±1.36 ^b | 14±1.29 ^c | 15±1.65° | 28±1.79° |
| 16 | | 7±1.26 ^b | 8±1.27 ^b | 11±1.27° | 12±1.35d | 13±1.36° | 24 ± 1.76^{d} |
| 2 | A. compressus | 3 ± 0.20^{d} | 5±0.22e | 6±1.24 ^e | 8±1.27 ^e | 9±1.54 ^d | 11 ± 1.32^{9} |
| 8 | | 1±0.01 ^d | 2 ± 0.18^{d} | 3±0.20 ^f | 4±0.21 ^f | 4±0.21e | 5±0.22 ^h |
| 12 | | 2±1.18 ^d | 2 ± 0.18^{d} | 3±0.20 ^f | 3±0.20 ^f | 4±0.21e | 4±0.21h |
| 16 | | 1±1.01 ^d | 2 ± 0.18^{d} | 3±0.20 ^f | 3±0.20 ^f | 4±0.21e | 4±0.21 ^h |

Values are Means \pm S.E, Mean alone the column having different superscript of letters different significantly at p \leq 0.05 level according to Duncan's Multiple Range Test (DMRT) and WAP: Weeks After planting

Table 3: Mean Number of the root, fresh and dry weight of C. odorata, A. africana and A. compressus exposed to different concentrations of spent engine oil

| Concentration (%) | Varieties | Number of roots | Fresh weight (g) | Dry weight (g) |
|-------------------|---------------|-----------------------|-------------------------|-------------------------|
| 0 | | 22±1.43 ^{ab} | 20.8±1.26 ^b | 17.7±1.19 ^b |
| 4 | C. odorata | 20±1.33° | 19.8±1.21 ^{bc} | 16.9±1.18 ^{bc} |
| 8 | | 19±1.22° | 16.6±1.15 ^d | 16.3±1.14bc |
| 12 | | 17±1.17 ^d | 13.8±1.11 ^e | 14.8±1.12° |
| 16 | | 15±1.11e | 27.3±1.31 ^a | 20.3 ± 1.24^{a} |
| 4 | A. africana | 23±1.24° | 19.9±1.20 ^{bc} | 16.8±1.14bc |
| 8 | | 21±1.15 ^b | 19.0±1.19 ^{bc} | 15.9±1.13° |
| 12 | | 19±1.00 ^b | 17.9±1.17 ^d | 14.5±1.12° |
| 16 | | 15±0.93 ^e | 12.2±1.80 ^e | 11.3±1.05 ^d |
| 4 | A. compressus | 16±1.76° | 21.8±1.28 ^b | 18.6±1.23b |
| 8 | | 20±1.67° | 21.0±1.27 ^b | 16.3±1.13 ^{bc} |
| 12 | | 18±1.71° | 18.2±1.18 ^{bc} | 13.3±1.10 ^c |
| 16 | | 21±1.31 ^b | 15.3±1.13 ^d | 11.2±1.04 ^d |

Values are Means ± S.E, Mean alone the column having different superscript of letters differ significantly at p≤0.05 level according to Duncan's Multiple Range Test (DMRT)

DISCUSSION

Growth parameters assessed in this study were plant height, number of leaves, number of roots and fresh and dry weight, respectively. Spent engine oil affected plant height at 4, 8, 12 and 16% treatment levels. Although there was a marked increase in plant height as the weeks passed with a marked reduction as the concentration increased. The highest plant height performance was observed in control soil (0%) compared with other treatments. The spent engine oil in soil may have interfered with aeration, mineral availability, plant water relation and suitable warmth that are required for plant growth and development. This finding was in line with the findings of Azorji *et al.*¹⁷, who reported that treatment of soil with spent engine oil consistently inhibited plant growth.

As opined by authors^{18,19}, plant growth stimulatory effect at 1% spent engine oil contamination was observed when compared to the control for various plant species. Generally, the behavior of the 4% spent engine oil in this study may be related to the findings of Emoyan *et al.*²⁰, who reported that

contamination of soil with 1-5% petroleum hydrocarbon normally acts as a boost to soil organic matter. However, maximum growth inhibition was observed at the highest treatment level (16%) in all the plants. Increased contamination by 12 and 16% had observable effects on the mean plant height at 12 weeks when compared to other treatments studied. The order of effects on plant height was as follows: *A. compressus> A. africana> C. odorata.* The result showed that spent engine oil retarded the height of the plants under study as evidenced by the reduction in growth of the plants. This corroborated the work of Saha²¹.

The exposure of the test plants resulted in a decrease in leaf number with an increase in concentration and the yellowing of the leaves. Photosynthesis might have been affected. According to study by researchers²²⁻²⁴, leaf number was reduced by the hydrocarbon content of the spent engine oil in *Khaya senegalensis* and *Terminalia superba*. The reduction in the mean number of leaves recorded in this study compares well with the report of reporters²⁵⁻²⁸. This implied that spent engine oil affected the leaf and this could be

attributed to the large amount of hydrocarbons contained in the oil which includes the highly toxic PAHs. This is in line with the report of Azorji et al.17. Similarly, Ahmad et al.12 reported a reduction in the fresh and dry weight of soybean planted in crude oil-polluted soil. The higher performance of control over other treatments (4, 8, 12 and 16%) is an indication that the control was in a higher supply of nutrients over time with a resultant decrease in fresh and dry yield. This is congruent with the findings of researchers^{3,29-33}. According to Essien et al.23 reductions in the number of roots and low biomass production could also be interpreted as being due to the gross effects of the spent engine oil in the soil. Multiple comparisons of the growth properties measured indicated that the 16% concentration had the highest inhibitory effect on the plants under study. This was similar to the report by Enyoh and Isiuku²². The negative effects of spent engine oil disposal may distort the proper functioning of the soil which could result in biodiversity loss with man at the receiving end of the food chain. The ability of C. odorata, A. africana and A. compressus to tolerate suggested that these plants could be used to remediate spent engine oil contaminated sites. Thus, the three plant species have demonstrated the ability to tolerate heavy metal stress, grow and accumulate biomass in spent engine oil-polluted soil. This underscores the need to further explore the adaptability of these indigenous plant species to heavy metals for their selective exploitation in phytoremediation of spent engine oil polluted sites.

CONCLUSION

The crux of this study was to evaluate and appraise the effects of indiscriminate disposal of spent engine oil on the environment using *C. odorata, A. africana* and *A. compressus* as test plants. The observations made in this study showed that although spent engine oil affected the growth parameters measured, the plant species were able to tolerate and grow in various concentrations. This underscores the need to enact laws prohibiting indiscriminate disposal of spent engine oil on the environment. These findings validate the need for recycling and reuse of spent engine oil to forestall further pollution of the environment. It is recommended that the three plant species be tried in decontamination of spent engine oil-polluted soils in the tropics.

SIGNIFICANCE STATEMENT

This study seeks to bring to the fore potential ecological risks associated with indiscriminate disposal of spent engine oil agricultural soils in Imo State and the Nigerian environment

at large. This study has given us insight into the possibility of applying the test plants in the phytoremediation of used oil-polluted agricultural soils.

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