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THE EFFECT OF SPENT ENGINE OIL ON SOME VEGETATIVE PARAMETERS OF *EICHHORNIA CRASSIPES*

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ABSTRACT

The effect of spent engine oil pollution on some vegetative parameters of *Eichhornia crassipes* was studied in a greenhouse for eight (8) weeks to evaluate the level of tolerance of *E. crassipes* to spent engine oil pollution, and to make recommendations on the use of *E. crassipes* for phytoremediation of spilled engine oil. Three levels of spent engine oil: 5 ml, 15 ml and 45 ml were applied to pollute the water culture container. A plant grown in an unpolluted medium served as the control. Results showed that there were significant ($p<0.05$) reductions in plant height, width of lamina, leaf area and petiole length of all the plants in polluted media compared with the control. Among the treatments, 15 ml treatment had minimal pollution effect on plant growth and produced the highest percentage flowering whereas 45 ml treatment gave the highest pollution effect on the plant, resulting in low percentage flowering. The growth of the vegetative parameters of plants cultured in both polluted and unpolluted water was enhanced by the application of fertilizer. It is recommended that *E. crassipes* be employed in phytoremediation trial experiments since the vegetative parameters studied were not adversely affected by spent engine oil when supplied with nutrients like NPK fertilizer.

Keywords: *Eichhornia crassipes;* spent engine oil; pollution; phytoremediation

INTRODUCTION

In urban areas, various types of activities like agriculture, industrial production and transportation produce large amount of wastes, which are classified as agricultural, industrial, municipal or nuclear wastes (Onwuka *et al*., 2012). These wastes from various sources are deposited on the soil surfaces either deliberately applied as fertilizer, sprays or pesticides (Lauhanen *et al*., 2004) or inadvertently through small or large leaks (Adesodun, 2014) as solids, plastics, crude oil or spent engine oil. Some of these wastes are recycled into some important products that are used to meet the challenges of the increasing population of Nigeria. Some of the wastes are recycled into manures and fertilizers for crop and animal production (Onwuka *et al*., 2012). There are some wastes that cannot be converted into any beneficial secondary uses and, therefore, pose a serious threat to the environment, one of such wastes being spent engine oil (Onwuka *et al*., 2012).

Spent engine oil refers to expended or used motor oils collected from mechanical or automobile workshops, garages, and industrial sources like hydraulic oil, turbine oils, engine oils, process oil and metal working fluids (Olugboji and Ogunwole, 2008). Spent engine oil is a mixture of several different chemicals including low and high molecular weight (C_{15}-C_{20}) aliphatic hydrocarbons, aromatic hydrocarbons, polychlorinated biphenyls, lubricant additives, chlorodibenzofurans, decomposition products, heavy metal contaminants such as aluminum, chromium, tin, lead, manganese, nickel and silicon that come from engine parts as they wear and tear down (Wang *et al*., 2000).

The disposal of spent oil into open vacant plots and farms, gutters and water drains is an environmental risk (Odjegba and Sadiq, 2002). Since it is liquid, it easily percolates into the soil and leaches into the environment from where it eventually pollutes either water or soil (Olugboji and Ogunwole, 2008). Pollution from waste engine oil has been reported to be more widespread than that of crude oil (Odjegba and Sadiq, 2002). This unregulated discharging of

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spent engine oil undesirably affects the flora, 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).

The plant, commonly known as water hyacinth (*Eichhornia crassipes* (Mart.) Solms), is a free-floating perennial herb of fresh water ecosystems. It is found on the surface of rivers, lakes, canals and ponds and may root in the mud of shallow waters. It is generally 10-20 cm high but can reach a height of 1m (Pieterse, 1997). It possesses a dense highly branched, fibrous root system and it floats on water (Ukiwe and Ogukwe, 2007). The plant has considerable buoyancy and the leaves act as sails in the wind (Hasan and Chakrabarti, 2009). Water hyacinth can be useful for the remediation of water contaminated with heavy metals by having the roots trap and remove large amounts of the oils, minerals, including heavy metals and radioactive elements from the water (Hasan and Chakrabarti**,** 2009). It has been effective in removing cadmium and zinc from moderately contaminated water (Lu *et al.,* 2004).

After studying the degradation of polycyclic aromatic hydrocarbons (PAH) from soil leachates, Ukiwe *et al*. (2012) concluded that *E. crassipes* is a good phytoremediator in the removal of PAHs from polluted water bodies.
Water hyacinth may be used in remediating soils contaminated with 3% and above crude oil but will not be needed for soils where contamination is 1- 3%. Time is also an essential factor in the remediation (Udeh *et al*., 2013). Investigations on the soil properties and growth of *Zea mays* have shown that spent engine oil had no effect on both the texture and pH of the soil. However, organic C, N and Mg as well as heavy metals (Fe, Cu, Zn, Cd, Mn, Ni, Cr and Pb) increased while P and yield of the plants grown on contaminated soil decreased. Also affected were the plant height, root number and root length (Okonokhua *et al*., 2007). Uhegbu *et al*. (2012) also reported a decrease in the moisture content of spent engine oil-polluted soil as well as delay in seed germination, leaf spread and rate of growth of *Zea mays* (Nwite and Alu, 2015).

There was also a significant reduction in plant height, percentage germination, biomass, number of leaves and leaf area thereby reducing yield of *Arachis hypogaea, Telfairia occidentalis, Vigna unguiculata* and *Jatropha curcas* where it also increases trace elements. Yellowish colouration was also observed in the leaves of *Telfairia occidentalis* (Kayode *et al*., 2009; Ogbuehi *et al.,* 2011; Agbogidi and Eruotor, 2012; Onwusiri *et al*., 2017). Results showed that there were significant reductions in yield, plant height, velocity of germination and leaf area index of *Zea mays* and *Arachis hypogaea* grown on spent engine oil-polluted soil. However, the use of *Bacillus cereus*, *Pseudomonas aeruginosa* and fertilizer significantly improved the coefficient of variation of germination, leaf area and fresh weight of *Zea mays* (Abdulhadi and Kawo, 2006) while *Arachis hypogaea* was shown to have the capacity to reduce heavy metal concentration in soils polluted with spent engine oil (Ogbuehi *et al.,* 2011). In *Abelmoschus esculentus* (L.), spent engine oil was found to adversely decrease the plant growth parameters, dry weight and number of fruits. There was also increase in the number of stomata, K, Cd, Na, Cr and Fe. However, the addition of organic nutrient supplements such as poultry waste was found to remedy the adverse effects of the spent engine oil-polluted soil (Okon and Mbong, 2013; Oluwanisola and Abdulrahaman, 2018). Further studies show that spent engine oil-pollution could cause inhibitions in radicle, plumule and early seedling growth of *Telfairia occidentalis, Vigna unguiculata* and poor performance in *Zea mays* (Kayode *et al*., 2009; Otunne and Kinako, 2010; Njoku *et al*., 2012). Obazuaye *et al*. (2016, 2017) reported reductions in germination rate, mean leaf area, root elongation and growth inhibition in *Capsicum chinesse*, *C. frutescens* and *C. annum* grown on spent engine oil-polluted soil.

Further studies on effects of spent engine oil on soil and *Zea mays* showed an increase in nitrogen, bulk density and organic carbon of the soil as well as reduced porosity, moisture content, hydraulic conductivity, Na, P, Mg, Ca and K (Udonne and Onwuma, 2014; Nwite and Alu, 2015).

Akingunsola *et al*. (2018) reported a decrease in plant growth parameters of *Lycopersicum esculentum* and *Amaranthus hybridus.* The number of leaves, stem diameter and percentage survival of *L. esculentum* was greater than that of *A. hybridus*, whereas the height of *A. hybridus* was higher than *L. esculentum.* It has been observed in most instances that spent engine oil tends to affect the vegetative parameters of plants negatively. However, it increases the ion and heavy metal concentration in soils and water bodies.

MATERIALS AND METHODS

The experiment was performed at the Botanical Garden of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. Using a 1000 ml measuring cylinder, 2,500 ml of water was measured and added into each of the 36 pieces of 5-litre capacity buckets. *E. crassipes* plants were uprooted from a shallow small pond located inside the Botanical Garden where they grew in clusters in the open. The shoots were separated and placed into the labelled buckets containing water. The set-up was left to stabilize for 28 days. Nine plants were exposed to pollution with 5 ml of spent engine oil in each bucket. This was repeated with 15 ml and 45 ml of spent engine oil, respectively. Nine plants used as the control were not exposed to pollution. The experiment was carried out in three replicates and set up in a greenhouse using the completely randomized design. In order to supply nutrients to the plants, 1.91 gm of NPK fertilizer was added to each plant in the medium.

The following vegetative parameters were measured: height of plant which was measured with a metre rule and number of leaves. The leaf lamina area was determined by planimetric method. The leaf was placed on a standard calibrated graph paper ($1cm^2$), traced out and the areas (squares) covered by the leaf outline were counted. Areas that were more than $^3/_4$ of the entire square were counted as an individual unit. The results, expressed as mean leaf area, were determined. Circumference of stem was taken with a measuring tape. The length of petiole was measured with a ruler, from the base of the leaf lamina to the point where the petiole attaches to the stem. Circumference of inflated portion of petiole was taken with a measuring tape. The final vegetative parameters were taken 61 days after pollution. During the experiment, the plants were watered with tap water in the range of 6 to 9 litres, depending on the need. The results were subjected to Analysis of Variance (ANOVA) and means were separated using the Duncan's Multiple Range Test.

RESULTS

The height of *E. crassipes* polluted with different concentrations of spent engine oil showed that the control had significantly ($p<0.05$) the highest value (34.83 ± 2.33 cm), followed by treatments with 15 ml and 5 ml (Table 1). The lowest value was that of 45 ml treatment (20.00 ± 4.93 cm). Analysis of variance (ANOVA) showed that the effect of spent engine oil on the height of the plant was significant at $p<0.05$. The control was not significantly different from 15 ml treatment but was significantly ($p<0.05$) different from 5ml and 45ml treatments. However, the latter two treatments were the same. The overall height was retarded by the spent engine oil treatment. Two out of 36 plants died. Therefore, percentage survival was 94.4%. Due to lack of nutrients, some shoots started wilting. 1.91g of NPK fertilizer was added to each bucket to boost the performance of the plants. Thereafter, many shoots started regenerating.

The control produced the highest number of generated shoots (3 ± 0.62) followed by 15 ml and 45 ml treatments. The least was that of 5 ml treatment. There was no significant difference among the treatments.

For stem circumference, the 5 ml treatment had the highest value (10.67 ± .82 cm). This was followed by the control and 15 ml treatment. The 45 ml treatment had the lowest stem circumference (6.89 ± 1.40 cm). The effect of spent engine oil on the stem circumference of the plant did not differ significantly. The control, 5 ml and 15 ml treatments were not significantly different from one another.

In addition, the control, 15 ml and 45 ml treatments were not significantly different from each other. However, 5 ml and 45 ml treatments differed significantly ($p<0.05$) from each other.

Results of the inflated petiole circumference of *E. crassipes* showed that the control had the highest value (4.33 ± .67 cm) followed by 5 ml and 15 ml treatments. The 45 ml treatment had the lowest value (2.11 ± .70 cm). The effect of spent engine oil on the inflated petiole circumference of the plants was not significant. The control, 5 ml and 15 ml treatments were similar but the three were significantly ($p<0.05$) different from 45 ml treatment. The overall inflated petiole circumference decreased with increasing spent engine oil concentration.

TABLE 1: Effects of different concentrations of spent engine oil on growth and vegetative parameters of *Eichhornia crassipes*

Treatment	Height (cm)	No. of regene-rating shoots (cm)	Stem circumference (cm)	Inflated petiole circumference
Control	34.83 ± 2.33^{b}	$3.22 \pm .62^{a}$	$10.00 \pm 1.01^{a,b}$	$4.33 \pm .67^{b}$
5 ml	22.52 ± 1.69^{a}	$1.44 \pm .63^{a}$	$10.67 \pm .82^{b}$	$3.89 \pm .45^{b}$
15 ml	$25.83 \pm 3.44^{a,b}$	$1.78 \pm .55^{a}$	$9.61 \pm 1.11^{a,b}$	$3.83 \pm .44^{b}$
45 ml	20.00 ± 4.93^{a}	$1.67 \pm .65^{a}$	6.89 ± 1.40^{a}	$2.11 \pm .70^{a}$

Values represent means ± standard error.

Mean values with the same letter(s) in the same column are not significantly different at $p<0.05$.

The number of leaves of *E. crassipes* in the medium polluted (15 ml) plant was observed to be highest (6.22 ± 1.18) (Table 2). This was followed by 5 ml and the control. The least number of leaves was that of high pollution (45 ml) treatment. There was no significant difference among the treatments (Table 4).

Results showed that the control treatment significantly ($p<0.05$) had the longest petiole (19.00 ± 1.26 cm).The 15 ml treatment had a length of 12.52 ± 1.77 cm and the 5 ml treatment had 9.61 ± .83 cm. The 45 ml treatment had the shortest petiole (8.83 ± 2.11 cm). The spent engine oil had a highly significant ($p<0.01$) effect on the length of the petiole of the plants. The 5 ml, 15 ml and 45 ml treatments were similar but the three were significantly ($p<0.05$) different from the control.

The control was observed to have the highest mean leaf area (27.50 ± 7.76 cm^2), which was followed by 15 ml treatment, with a mean leaf area of 16.00 ± 3.76 cm^2. The 45 ml treatment had a mean leaf area of 13.50 ± 5.59 cm^2 while the least mean leaf area was that of 5 ml treatment (8.50 ± 1.26 cm^2). The spent engine oil had a significant effect on the area of the leaf. The control, 15 ml and 45 ml treatments were not significantly different from each other. In addition, 5 ml, 15 ml and 45 ml treatments were not significantly different from each other. However, the control and 5 ml treatments differed significantly ($p<0.05$) from each other.

TABLE 2: Effects of different concentrations of spent engine oil on leaf and parameters of *Eichhornia crassipes*

Treatment	No. of leaves (cm^2)	Area of leaf (cm)	Length of petiole
Control	5.11 ± .63[a]	27.50 ± 7.76[b]	19.00 ± 1.26[b]
5 ml	6.11 ± 1.69[a]	8.50 ± 1.26[a]	9.61 ± .83[a]
15 ml	6.22 ± 1.18[a]	16.00 ± 3.76[a, b]	12.52 ± 1.77[a]
45 ml	3.67 ± .96[a]	13.50 ± 5.59[a,b]	8.83 ± 2.11[a]

Values represent means ± standard error.
Mean values with the same letter(s) in the same column are not significantly different at $p<0.05$.

The percentage of flowered plants before pollution was 25%, 80.55% of the plants flowered after pollution. The 15 ml treatment produced the highest average number of flowers, followed by the control (Table 3). The least number of flowers was produced by 45 ml treatment.

TABLE 3: Effects of different concentrations of spent engine oil on number and percentage flowering in *Eichhornia crassipes*

Treatment	Average no. of flowers	Percentage flowering
Control	8.67	24.08%
5ml	6.67	18.53%
15ml	10.33	28.69%
45ml	3.33	9.25

DISCUSSION

The results of this study showed that spent engine oil significantly reduced the plant height, leaf area and petiole length of *E. crassipes*. This is similar to the report of Ogbuehi *et al*. (2011), who observed that spent engine oil caused a significant reduction in plant height, number of leaves, percentage germination and leaf area of *Arachis hypogaea*. Agbogidi and Eruotor (2012) also reported reductions in plant height, leaf area and number of leaves of *Jatropha curcas* polluted with spent engine oil. Otunne and Kinako (2010) reported reduction in the number of leaves and inhibition of radicle and plumule growth during early seedling growth of *Telfaria occidentalis* and *Zea mays* grown on spent engine oil-polluted soil. Even though the present results showed reductions in plant height and leaf area of *E. crassipes*, the vegetative parameters were enhanced by the application of fertilizer. This is similar to the work of Abdulhadi and Kawo (2006), who reported that the use of microorganisms and fertilizer application significantly improved the leaf area, coefficient of velocity of germination and fresh weight of *Zea mays*.

There was a reduction in the height and the petiole length which decreased with increase in the concentration of spent engine oil applied. This result is similar to Otunne and Kinako (2010), who reported that inhibition of growth of seedlings of *Telfaria occidentals* and *Zea mays* increased with increase in concentration of spent engine oil. Also, Okon and Mbong (2013) reported a decrease in height, petiole length and leaf area with increasing concentration of spent engine oil pollution in *Abelmoschus esculentus* (L.) Monech. in South-South Nigeria. They also reported reductions in internode length, root length, number of fruits, moisture content and percentage germination. Nutrient amendment was used to remedy the effects of the spent engine oil.

The high number of flowers observed 28 days after pollution showed that spent engine oil, in addition to extended maturity time, enhanced flowering after pollution. Nwadinigwe and Olawole (2010) reported that crude oil pollution did not hinder or delay flowering in *Sorghum vulgare* polluted with crude oil. This result is in contrast with the work of Nwadinigwe and Onyeidu (2012) and Nwadinigwe and Onwumere (2003), who reported that flowering was delayed in soybean planted in crude oil-contaminated soil. Pezeshki and Delaune (1993) reported delay in flower production of *Spartina alterniflora* and *Juncus roemeranus* due to crude oil pollution.

CONCLUSION

This study showed that *Eichhornia crassipes* can be used in phytoremediation trial experiments since the vegetative parameters studied were not adversely affected by spent engine oil pollution, especially when supplied with nutrients like NPK fertilizer. These trial experiments may be more appropriate when water bodies are polluted by the flushing of spent engine oil into them. The study shows that the plant can tolerate 15 ml concentration of spent engine oil pollution.

TABLE 4: Analysis of variance (ANOVA) Table

		Sum of Squares	df	Mean Square	F	Sig.
Height	Between Groups	1133.874	3	377.958	3.776	.020
	Within Groups	3203.316	32	100.104		
	Total	4337.190	35			
Number of Leaves	Between Groups	37.889	3	12.630	1.012	.400
	Within Groups	399.333	32	12.479		
	Total	437.222	35			
Circumference of Stem	Between Groups	74.410	3	24.803	2.256	.101
	Within Groups	351.778	32	10.993		
	Total	426.187	35			
Length of Petiole	Between Groups	576.363	3	192.121	8.670	.000
	Within Groups	709.124	32	22.160		
	Total	1285.488	35			
Inflated petiole circumference	Between Groups	25.910	3	8.637	2.886	.051
	Within Groups	95.778	32	2.993		
	Total	121.687	35			
Number of Regenerating Shoot	Between Groups	17.639	3	5.880	1.753	.176
	Within Groups	107.333	32	3.354		
	Total	124.972	35			
Leaf Area	Between Groups	1165.125	3	388.375	2.416	.096
	Within Groups	3214.500	20	160.725		
	Total	4379.625	23			

Figure 1: Experimental set up of *Eichhornia crassipes* polluted with different concentrations of spent engine oil

C_1R_1 in flower

C_2R_1 in flower

C_3R_1 in flower

Withered flower of C_3R_1

C_8R_3 in flower

Withered flower of C_8R_3

Plate 1: Withered and open flowers of *Eichhornia crassipes* not polluted with spent engine oil

Legend:

C_1R_1 = Control plant 1 replicate 1

C_2R_1 = Control plant 2 replicate 1

C_3R_1 = Control plant 3 replicate 1

C_8R_3 = Control plant 8 replicate 3

Plate 2: Flowers of *Eichhornia crassipes* polluted with 15 ml and 45 ml concentrations of spent engine oil

Legend:

45_4R_2 = 45 ml plant 4 replicate 2

45_1R_1 = 45 ml plant 1 replicate 1

15_9R_3 = 15 ml plant 9 replicate 3

15_8R_3 = 15 ml plant 8 replicate 3

15_1R_1 = 15 ml plant 1 replicate 1

15_1R_1 flowering a second time

Withered flower of 5_3R_1

Withering flower of 15_1R_1

15_1R_1 flowering a third time

Withered first flower of 15_1R_1

Plate 3: Withered and open flowers of *Eichhornia crassipes* polluted with 5 ml and 15 ml concentrations of spent engine oil

Legend:

5_3R_1 = 5 ml plant 3 replicate 1

15_1R_1 = 15 ml plant 1 replicate 1

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