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Impact of Crude Oil Treatments on the Growth, Survival, Morphology and Plant Based Component of *Eichhornia crassipes*

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ABSTRACT: This experimental study was carried out to investigate the growth response, survival and morphology of *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae) in crude oil polluted freshwater. The parameters measured include leaf diameter, stem girth, root length, chlorophyll content index and biomass production. The experimental treatment concentrations used were 0% (control), 1%, 2%, 3% and 4% (v/v). Using three replicates per treatment, the plants were exposed to all concentrations for eighteen days. The result showed that leaf diameter, stem girth, root length and chlorophyll content index were significantly reduced ($P<0.05$) in *E. crassipes* when exposed to all concentrations of crude oil for eighteen days when compared with samples grown without treatment (0%). The effects were concentration dependent. The leaves of the test plant also showed signs of wilting and chlorosis at the end of the experiment. The study has shown that *E. crassipes* can play a role in phytoremediation at low level of oil pollution and can also be used as a biomonitor of high concentration of crude oil pollution in fresh water habitats.

Keywords: Crude oil, Parameters, *Eichhornia crassipes*, Morphology, Biomonitor

Introduction

Water bodies undergo eutrophication generally with time and this eutrophication can occur either naturally or culturally. Cultural eutrophication results from both point and non-point source addition of nutrients such as municipal sewage, septic tank effluents, agricultural and urban run offs, industrial effluents and oil spillage. Such discharges into the water bodies confer changes on the physical and chemical as well as biological (flora and fauna) characteristics of such water bodies (Sankaranarayanan *et al.,* 1986; Joy *et al.,* 1990; Teltsch *et al.,* 1989, 1992). The effect in the long run includes water quality degradation, death of aquatic organisms, oxygen deficiency and sedimentation. The effects of industrial effluents on aquatic organisms range from growth stimulation through growth inhibition, to stimulation at low concentration and inhibition at high concentration (Walsh *et al.,* 1980, Walsh and Merrit, 1984).

Crude oil as a source of energy was first discovered in commercial quantity in Bayelsa State, Nigeria in the year 1958. It was discovered by Shell Petroleum Development Company (SPDC). Crude oil is a naturally occurring, unrefined petroleum product composed of hydrocarbon deposits and other organic materials. The exploitation of this natural resource has immensely contributed to the growth and development of Nigeria. The discovery and

exportation of crude oil resulted to the oil boom period of the 1970s with oil contributing about 90% of export earnings and 80% of the nation's gross domestic product (GDP).

Crude oil is a naturally occurring flammable liquid consisting of complex various molecular weight and other liquid organic compounds that occur in geologic formation beneath the earth surface. The hydrocarbon in crude oil are mostly alkanes, cycloalkanes and various aromatic hydrocarbons while the other organic compounds contain nitrogen oxygen, sulphur and trace amount of metals such as iron, nickel, copper and vanadium. Paraffin, naphthenic, aromatics and asphaltics are the four different types of hydrocarbon molecules found in crude oil and their relative percentages vary from one oil type to the other.

Oil pollution is the unintentional or intentional release of liquid petroleum hydrocarbon into the environment as a result of human activity. It is the introduction by man directly or indirectly any hydrocarbon material especially crude oil and its refined products into the environment (Adeyanju, 2004). The Nigerian physical environment has been impacted negatively by the activities of oil companies. Oil pollution which arises mainly from oil spills has serious implication for biodiversity as most biotic habitats are either destroyed or altered making them unsuitable for habitation. Example is seen when floating oil slick in a water body forms a layer that prevents oxygen from dissolving in water. Crude oil contains toxic components which causes outright mortality of plants and animals as well as other sub lethal impacts. Oil does not dissolve in water hence it undergoes biological, physical and chemical process called weathering (Farrington and Mac-Dowell. 2004). Crude oil in the soil reduce sediment porosity and gaseous exchange that result in negative effect on the physiological functions of plants (Amadi *et al.,* 1997). This study thus aims to assess the effects of unrefined crude oil on the growth, morphology and chlorophyll content of *E. crassipes* and determine the extent of tolerance of *E. crassipes* upon exposure to crude oil pollution.

Materials and Methods

Study Site: This study was carried out in the screen house of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State.

Plant Collection: Fresh samples of *E. crassipes* were collected from Ologbo Pond, Edo State, Nigeria. The plants were carefully collected from the water to avoid root damage. The plants were placed in a plastic bowl and the roots were covered with pond water, to prevent dehydration before getting to the screen house. Identification of the test plants was done at the department of Plant Biology and Biotechnology, University of Benin.

Experimental Set Up: Different treatments of 0%, 1%, 2%, 3% and 4% (v/v) were used for the study. The test *E. crassipes* was thoroughly rinsed with tap water to wash off any particles attached to the leaf surfaces and roots. It was there after transferred to the 15 bowls. The bowls were categorized into five places as follows:

- 0% $^{v}/_{v}$ containing 1000ml deionized water as control.
- 1% $^{v}/_{v}$ containing 990ml deionized water and 10ml crude oil.
- 2% $^{v}/_{v}$ containing 980ml deionized water and 20ml crude oil.
- 3% $^{v}/_{v}$ containing 970ml deionized water and 30ml crude oil.
- 4% $^{v}/_{v}$ containing 960ml deionized water and 40ml crude oil.

There were three replicates of each which was accordingly. The experimental setup was left for 18 days and readings were taken at 3 days interval. All the experimental materials were placed under the same environmental conditions, to ensure completeness and accuracy of data.

Data Recording: Morphological observations of the plants were made to ascertain change in leaf diameter, leaf colour, texture, bulb colour, stem girth, chlorophyll content index and root length. The following data were collected during the experiment.

Leaf diameter measurement: The diameter of the sample plant leaves were measured using a metre rule.

Stem Girth Measurement: The stem girth of the plants was measured using a digital vernier calliper.

Chlorophyll content Index Determination: Chlorophyll contents index of the leaves were measured using the Apogee^{TM} chlorophyll content meter. Measurement was done by holding the arm of the chlorophyll content meter in direct contact with the leaf until it made a beep. The chlorophyll content index was displayed on the screen of the device and was recorded before treatment (day 0) and after treatment (day 18) accordingly.

Root length measurement: The length of the root was taken by the use of a measuring tape. The root of the test plant was measured before introducing it to the treatment medium and also measured at the end of the experiment

Fresh and dry weight determination: The fresh and dry weights were determined after eighteen (18) days of treatment. After recording all observations on day 18, the plants were separated into leaves, stem and root. The fresh weight was obtained after weighing using an electronic sensitive balance. Newspapers were used to package the various plant portions and thereafter labeled accordingly. The dry weight was also obtained by

drying the plant parts packaged in the newspaper in a ventilated oven at 70 ºC for 24 hours, after which dry weight was determined using an electronic sensitive balance.

Statistical Analysis: The results are the means ± S.E. of three independent replicates. All obtained data were subjected to statistical analysis using statistical package for social science (SPSS) version 16.0. Analysis of variance (ANOVA) was performed appropriate to the experimental design used. The post-hoc procedure employed was Duncan Multiple Range Test.

Results

Table 1 shows the effect of crude oil treatment on the leaf diameter of *E. crassipes*. At day 18, *E. crassipes* leaves with control treatment 0% had the highest leaf diameter as 8.83±0.57 cm while the 4% (v/v) treatment with conspicuous shrinkage and brown leaves had the least leaf diameter as 2.25±0.05 cm. All the concentrations had unequal number of leaves at day 18.Water hyacinth played in control treatment grew normally with no discolouration of the leaves. At 6 days after treatment (6 DAT), all test plants subjected to crude oil treatments (1,2,3 and 4% v/v) had gradual discolouration while at 12 DAT, wilting and mortality was observed although this was more in *E.crassipes* treated with 4% (v/v) of crude oil.

Table 1: Effect of crude oil on the leaf diameter of *E. crassipes*

Treatment % (v/v)	**Days after treatment (cm)**						
	0	3	6	9	12	15	18
0	7.17±0.35^{b}	7.07±0.32^{bc}	7.10±0.35^{b}	7.10±0.35^{b}	8.13±0.90^{b}	8.30±0.85^{b}	8.83±0.57^{b}
1	5.60±0.15^{a}	5.43±0.20^{a}	5.47±0.18^{a}	5.40±0.23^{a}	4.97±0.20^{ab}	2.90±0.00^{a}	2.83±0.02^{a}
2	6.47±0.66^{ab}	6.33±0.65^{abc}	6.03±0.61^{ab}	5.80±0.40^{a}	3.93±1.97^{a}	2.77±0.47^{a}	2.64±0.04^{a}
3	6.13±0.03^{ab}	5.93±0.09^{ab}	5.97±0.17^{ab}	5.97±0.09^{ab}	5.87±0.07^{ab}	2.52±0.02^{a}	2.46±0.02^{a}
4	7.50±0.60^{b}	7.63±0.62^{c}	5.33±0.39^{b}	7.07±0.57^{b}	5.40±0.58^{ab}	2.27±0.02^{a}	2.25±0.05^{a}
	*	*	*	*	*	*	*

Key. * = Significant (P > 0.05).

Values are means and standard errors of 3 replicates. Means in the column sharing the same superscript alphabet are not significantly different at P > 0.05.

Table 2 shows the effects of crude oil on the stem girth of *E. crassipes*. At day 0, 3, 6 and 9, *E. crassipes* showed no significant differences in stem girth of the test plant. At 9 DAT, *E. crassipes* control had the highest stem girth as 19.99±1.91 and *E. crassipes* treated with 4% (v/v) crude oil had the least stem girth as 16.32±1.81. At day 18, *E. crassipes* control treatment had the highest stem girth as 23.12±2.94 and the 4% (v/v) treatment recorded as 6.68±0.86 had the least stem girth.

Table 2: Effect of crude oil on the stem girth of *E. crassipes*

Treatment % (v/v)	**Days after treatment (cm)**						
	0	3	6	9	12	15	18
0	21.26±3.24^{a}	21.05±2.56^{a}	21.17±2.82^{a}	19.99±1.91^{a}	21.00±2.63^{b}	20.59±1.26^{c}	23.12±2.94^{c}
1	18.80±2.65^{a}	18.19±2.02^{a}	18.10±1.93^{a}	18.72±2.40^{a}	18.22±2.10^{b}	16.68±1.67^{bc}	16.72±1.72^{b}
2	16.52±1.79^{a}	16.91±1.72^{a}	16.86±1.99^{a}	16.99±1.74^{a}	17.12±1.80^{ab}	16.80±1.56^{bc}	16.67±1.64^{b}
3	17.43±1.94^{a}	17.87±1.90^{a}	19.97±2.99^{a}	18.86±2.20^{a}	17.41±1.83^{ab}	13.66±3.12^{ab}	11.33±0.88^{ab}
4	20.70±0.68^{a}	20.31±0.35^{a}	19.64±0.38^{a}	16.32±1.81^{a}	11.33±0.29^{a}	10.30±0.36^{a}	6.68±0.86^{a}
	N.S	**N.S**	**N.S**	**N.S**	*	*	*

Key. N.S = Non significant (P < 0.05) * = Significant (P > 0.05)

Values are means and standard errors of 3 replicates. Means in the column sharing the same superscript alphabet are not significantly different at P < 0.05.

Table 3 shows the effect of crude oil on the root length of *E. crassipes*. There was no significant difference on the root length before the treatment but significant differences occurred after the treatment. There was a decline in the length of the root generally with the control having the longest root length as 48.56±0.92 cm and the 4% (v/v) treatment had the least root length as 33.36±2.84 cm.

Table 3: Effect of crude oil on the root length of *E. crassipes*

Treatment % (v/v)	Before Treatment (cm)	After Treatment (cm)
0	49.10±1.34[a]	48.56±0.92[c]
1	44.40±1.51[a]	42.88±2.18[bc]
2	44.87±0.52[a]	40.89±1.05[b]
3	44.67±1.20 [a]	38.00±2.51[ab]
4	44.67±1.90[a]	33.36±2.84 [a]
	N.S	*

Key. N.S = Non significant (P < 0.05) * = Significant (P > 0.05). Values are means and standard errors of 3 replicates. Means in the column sharing the same superscript alphabet are not significantly different at P < 0.05.

Table 4 shows the fresh and dry weight of *E. crassipes* leaves after crude oil treatment. The highest fresh weight was in the control treatment as 8.86±1.67 g and the 1% (v/v) treatment was recorded as the least with 2.45±0.41 g. For the dry weight, the least remained the 1% (v/v) treatment recorded as 0.77±0.17 g and the highest was seen in the 4% (v/v) treatment as 2.00±0.77g.

Table 4: Fresh and dry weight of *E.crassipes* leaves after crude oil treatment

Treatment %(v/v)	Fresh weight (g)	Dry weight(g)
0	8.86±1.67[b]	1.12±0.17[a]
1	2.45±0.41[a]	0.77±0.17[a]
2	2.92±1.12[a]	1.09±0.31[a]
3	4.65±0.32[a]	1.31±0.57[a]
4	3.87±0.69[a]	2.00±0.77[b]
	*	*

Key. * = significant (P > 0.05). Values are means and standard errors of 3 replicates. Means in the column sharing different superscript alphabets are significantly different at P > 0.05

Table 5 shows the fresh and dry weights of *E. crassipes* stem after crude oil treatment. The highest fresh weight was 48.24±4.12g recorded for the control while the lowest fresh weight of 19.38±3.55g was recorded for 1% (v/v) treatment. For dry weight, a maximum value (4.80±0.38g) was recorded for 3% treatment while the minimum value of 1.83±0.50g was recorded for 1% (v/v) treatment.

Table 5: Fresh and dry weight of *E. crassipes* stem after crude oil treatment

Treatment % (v/v)	Fresh weight (g)	Dry weight (g)
0	48.24±4.12[c]	3.07±0.56[ab]
1	19.38±3.55[a]	1.83±0.50[a]
2	25.95±5.36[ab]	2.86±0.93[ab]
3	41.18±4.04[c]	4.80±0.38[b]
4	36.55±3.82[bc]	4.12±0.54[b]
	*	*

Key. * = significant (P > 0.05)
Values are means and standard errors of 3 replicates. Means in the column sharing different superscript alphabets are significantly different at P > 0.05.

Table 6 shows the fresh and dry weight of *E.crassipes* root after crude oil treatment. The highest fresh and dry weight was in the 4% (v/v) treatment recorded as 42.20±17.94 g and 7.56±4.32 g respectively, while the lowest fresh and dry weight of the root was recorded in the 1% (v/v) treatment as 21.83±4.32 g and 2.05±0.53 g respectively.

Table 6: Fresh and dry weight of *E. crassipes* root after crude oil treatment

Treatment % (v/v)	Fresh weight(g)	Dry weight(g)
0	35.42±4.41[a]	2.83±0.48[a]
1	21.83±4.32[a]	2.05±0.53[a]
2	25.51±12.53[a]	3.23±1.70[a]
3	34.70±4.69[a]	4.56±1.27[a]
4	42.20±17.94[a]	7.56±4.23[a]
	N.S	**N.S**

Key: N.S = Non significant ($P < 0.05$)
Values are means and standard errors of 3 replicates. Means in the column sharing the same superscript alphabets are not significantly different at $P < 0.05$.

Figure 1 shows the chlorophyll content index of *E. crassipes* leaves before and after crude oil treatment. Before treatment, *E. crassipes* with 3% (v/v) treatment had the highest chlorophyll content index as 28.07 ±0.78 and the control had the least chlorophyll content index as 26.30±0.75. After the treatment, the highest chlorophyll content index was recorded as 29.87±0.69 with the control treatment, whereas the 4% (v/v) treatment had the least which is 15.37±3.36.

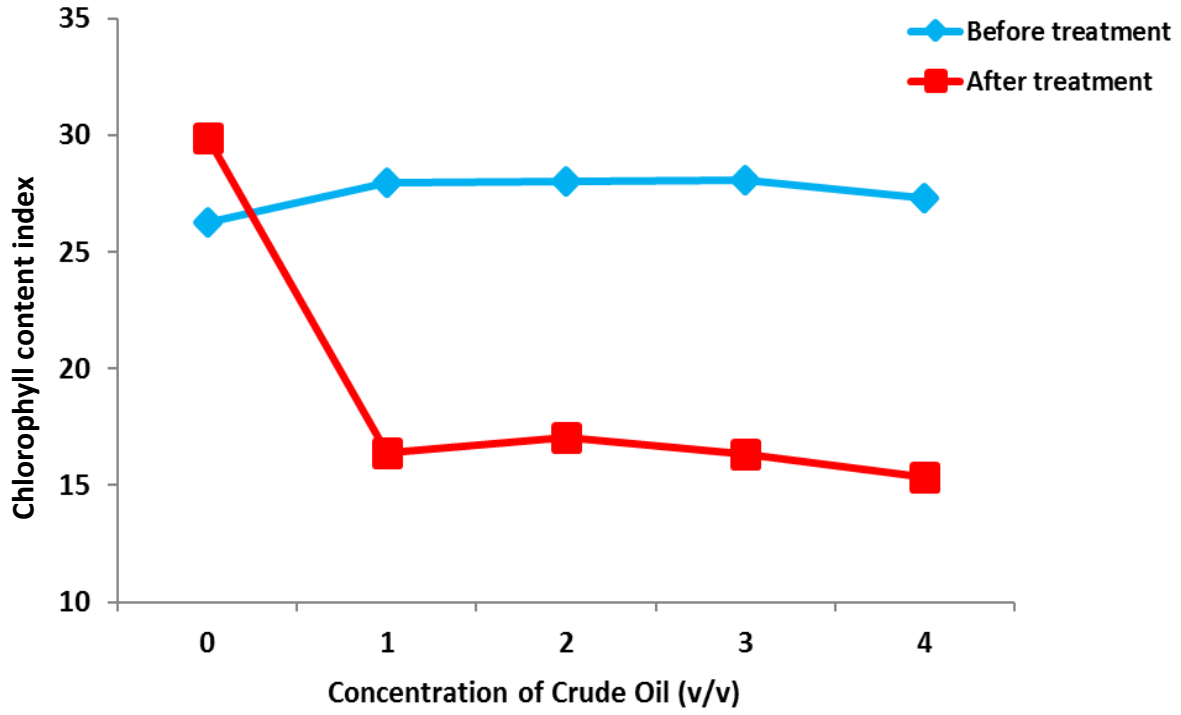

Figure 1: Effect of crude oil on the chlorophyll content of *Eichhornia crassipes* leaf

Discussion

The introduction of petroleum products into a wetland habitat can alter the physical and chemical features of such habitat. Macrophytes take up elements in their ionic form (Ali and Mai, 2007). Adedokun and Ataga (2007) stated that crude oil, automotive gasoline oil and spent engine oil have a differential effect on germination and growth of cowpea (*Vigna unguiculata*). Plants exposed to oil polluted soil results in inhibition of growth and biomass production in grasses and legumes. The reduction in plant biomass results indirectly from the difficulty of the plant to adapt to the obstruction of soil pores that causes poor gas exchange, and to lower water flow, which reduces nutrients supply and availability of the plant (Olorunfemi *et al*., 2008).

The effect of crude oil on the growth of the leaf diameter *E. crassipes* was investigated. From Table 1, the result showed that at day 0, the leaf diameter of *E. crassipes* for the control (0%) treatment was 7.17±0.35 cm for crude oil while 6.13±0.03 cm was leaf diameter for the crude oil 3% (v/v) treatment This varies significantly with the resultant leaf diameter of 8.30±0.85 cm for crude oil control treatment, and 2.52±0.02 cm for the 3% (v/v) crude oil treatment, This indicates that 15.8% increase for control, 58.9% decrease for crude oil 3% (v/v) treatment at 15 days after treatment (15DAT). There was significant difference observed between the control and other treatment from day 0 to day 18 in the crude oil treatment. High concentration (4% v/v) of crude oil caused a decrease in the leaf diameter of *E. crassipes* indicating their degree of sensitivity to oil pollution, however, at 0%, there was appreciable increase in the leaf diameter of *E. crassipes*, suggesting that control condition enhanced the growth of the plant.

The reduction in the leaf diameter of *E. crassipes* as recorded in both crude oil and kerosene treatments after day 0 corresponds with the findings of Lopes *et al.*, (2009) who observed that exposure of this species to Urucu crude oil concentrations between 0.08 and 15.89L.m^{-2} reduced the number and size of leaves. This observation is also in line with the report of Bamidele *et al*., (2007) who opined that the exposure of *I. rugosum* to gasoline caused the observed reduction of the plant´s growth characters, and this could be attributable to a reduction in carbon fixation consequent upon oxygen tension.

Chlorosis and wilting of leaves were also one of the visible effects of crude oil exposure on *E. crassipes* at higher concentrations. There was significant reduction in chlorophyll content as shown in Figure 1. For the control plant, the chlorophyll content index was 29.87±0.69 at 18DAT. A 13.6% increase when compared to the initial chlorophyll content index before treatment, while the 1,2,3 and 4% (v/v) crude oil treatment registered a significant reduction in chlorophyll content index (16.37±2.82, 17.03±1.92, 16.33±1.62, 15.37±3.36). This reduced chlorophyll content index might be as a result of lipid peroxidation or due to increased cell or tissue damage as supported by studies on tomato by Cho and Park (1999). The apparent chlorosis, a result of the reduced chlorophyll content index may be an implication of heavy metals absorbed by the plant (Ochekwu and Madagwa, 2013). This observation is also in line with the scientific findings of Turkoglu *et al*. (2011) who noted that salt exposure had negative effects on chlorophylls a and b as well as the carotenoid contents of *Hyacinthus orientalis*, hence, a deceleration of the plants metabolism. This is also in line with the work carried out by Eguagie and Orji, (2015) and Bamidele *et al*. (2015) who both noticed a decrease in the chlorophyll content of plants exposed to various refined products.

The fresh and dry weight of leaves of *E. crassipes* at control was higher than those exposed to high concentration of crude oil treatments, (Table 4). It was observed that at high concentrations (3% and 4%) of crude oil, a decrease in the values of the fresh weight and dry weight of *E. crassipes* was recorded. This observed reduction in biomass of *E. crassipes* with increasing concentration of crude oil maybe as a result of disruption in the plants photosynthetic rate and hence its rate of dry matter accumulation as reported by Bamidele *et al.* (2007). The reduction in fresh weight and dry weight as observed in this study is also in accordance with Emakpor, who reported a decrease in dry weight and fresh weight of *Pistia stratoites, Ludwigia abysinnica* and *Ipomoea aquatic* with increase in brewing effluents. This result is also consistent with the observation of Qianxin and Mendelssohn (1996) that the biomass of *spartina patens* was significantly reduced with increasing oil in soil.

At day 18, *E. crassipes* present in 4% (v/v) treatment of crude oil was unable to survive. The high mortality of water hyacinth grown in crude oil treatment could be attributed in part to the toxicity of crude oil as well as the buildup of metals as opined by Bamidele *et al.* (2007) who observed similar mortality of *Ischaemum rugosum* plants sown in gasoline treated soils. This result is also not contradictory to the result of Silva and Camargo (2007) that revealed *Pistia stratoites* is extremely sensitive to Urucu petroleum at low doses (0.1, 0.2 and 0.3 Lm^{-2} concentration) caused death in plants.

Conclusion

Fresh water plants are very important because of their ecosystem functions and they play a major role in phytoremediation at low levels of oil pollution. The results of the present study show that crude oil had concentration dependent effects on *E. crassipes*. A negative interaction was observed between the plant parameters measured and the level of crude oil treatment. *E. crassipes* plants grown in the uncontaminated water (control) had the highest values in all growth variables considered and they were significantly ($P \geq 0.05$) greater than those exposed to the other treatments. Proper measures should be put in place to prevent release of these products into the environment where it can adversely affect plants.

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