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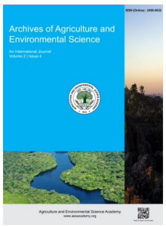

ORIGINAL RESEARCH ARTICLE

CrossMark

Effects of crude oil treatment on the morphology and performance of water hyacinth (*Eichhornia crassipes* (Mart) Solms) in Niger-Delta region of Nigeria

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ABSTRACT

Many aquatic plants existing in the Niger Delta area of Nigeria are at the risk of extinction due to oil spill. The present study was aimed at investigating the effect of different concentrations of crude oil on the morphology and growth performance of the hydrophyte, *Eichhornia crassipes* present in coastal waters of Nigeria's Niger Delta region, where crude oil exploitation as well as oil spill is high. The plant, *E. crassipes* was subjected to varying concentrations of crude oil treatments (1.25%, 2.5%, 5%, 7.5% and 10%). Each treatment was replicated three times. The performance was measured using various growth parameters which include height, number of leaves, leaf area, fresh weight and dry weight. The results showed that the control plant performed better than the treated plants with respect to all the morphological characters considered. The control plant of *E. crassipes* produced a height of 7.56±0.028 from an initial height of 6.02±0.028. Among the treated plants, 1.25% and 2.5% promoted height, leaf area, number of leaves, fresh and dry weight up to the 6th week of growth while 7.5% and 10% decreased plant height up to the 4th week. The *E. crassipes* treated with 7.5% and 10% crude oil concentrations died 6 weeks following the treatments. The ability of *E. crassipes* to tolerate the different levels of crude oil was authenticated in this study. However, the study showed that there is a limit of sustenance of petroleum hydrocarbons pollution for *E. crassipes* above which toxicity will apply.

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INTRODUCTION

Coastal marshes are important ecosystems because of their high biological productivity and role as nurseries for coastal fishes, habitat for wildlife, flood mitigation, shoreline protection from erosion, and water quality enhancement (Mitsch and Gosselink, 2007). However, these important functions are at risk in areas such as the oil-rich Niger Delta region of Nigeria, where crude oil exploration, production, transportation and refining are extensive, and the potential for oil spill is consequently high (Njoku *et al*., 2009). According to U.S Environmental Protection Agency (USEPA, 2007) "Oil released threaten public health and safety by contaminating drinking water, causing fire and explosion hazards, diminishing air and water quality, compromising agriculture, destroying recreational areas, and wasting non-renewable resources. Oil spills also have a severe environmental impact on ecosystems by harming or killing wildlife and plants, and destroying habitat and foods (Lijuan, 2012). They can influence an ecosystem directly or indirectly (Lijuan, 2012). The social and economic lives of people living in such communities are also affected because their rivers and other water bodies can no longer sustain aquatic life and so their primary source of livelihood is affected (Ochekwu and Madagwa, 2013). Ochekwu and Madagwa (2013) also posited that they (people in oil polluted areas) can no longer drink or swim in their river as they used to and this affect their social life.

In Nigeria, the major cause of crude oil pollution is as a result of pipeline vandalization by saboteurs (individuals and group) seeking government attention to correct economic marginalization and ecological disaster occasioned by many years of unregulated crude oil exploration and exploitation by foreign companies (Nwilo and Badejo, 2006). This has led to loss of species diversity, loss of habitat, destruction of breeding grounds of aquatic organisms and sometimes death of organisms including man (Ndimele, 2008). Many persons/group have however faulted the claims by oil companies that most oil spill is due to sabotage (Anonymous 2014a). They rather posit that poor maintenance of oil infrastructure, equipment failure, sabotage of oil infrastructure, theft of oil and illegal refining all contributes to oil pollution in the region (Amnesty 2009; Anonymous 2014a). The basis for the claim is the outcome of oil spill investigation in the Niger Delta region (Anonymous 2014a). The investigation process has been the subject of community complaints over many years, with allegations that the process lacks transparency, does not always comply with national law and standards and the data recorded in oil spill investigations forms are inaccurate.

Conventional oil spill counter measure of physical, chemical and biological methods have been used over time (Ochekwu and Madagwa, 2013). Commonly used physical methods include booming and skimming, manual removal (wiping), mechanical removal, water flushing, sediment relocation and tilling (Ochekwu and Madagwa, 2013). Chemical methods involve the use of dispersants and this has done more damage to the aquatic ecosystem than the crude oil itself (Lin and Mendelessohn, 1998; Anukwuorji *et al.*, 2012; 2013; 2016). Also, biological method (Bioremediation) uses naturally occurring organisms to break down hazardous substances into less toxic or non-toxic substance (Anonymous, 2014b; Kumar *et al.*, 2018). Some examples of bioremediation related technologies are phytoremediation, bionventing, bioleachimg, landfarming, bioreactor, composting, bioaugmentation, rhizofiltration, and biostimulation. Several aquatic plants have been shown to have the ability to filter contaminants on polluted water (Brooks and Robinson, 1998; Kumar *et al.*, 2016). Some aquatic plants accumulate metals and many species suffer phytoxicity while others grow easily in the presence of metals (Ochekwu and Madagwa, 2013). In the majority of studies, grasses and legumes have been singled out for their potential in this regard (Qui *et al.*, 1997; Gunther *et al.*, 1996; Reilley *et al.*,1996). However, studies have shown that *Eichhornia crassipes* in crude oil contaminated water affects the physico-chemistry of the water thereby enhancing degradation of crude oil (Ochekwu and Madagwa, 2013).

Eichhornia crassipes (Mart) Solms-Lamb (Family Pontederiacea) a monocot, commonly known as water hyacinth, is a floating aquatic plant with inflated petioles native to the Amazon basin, and it's often considered a highly problematic invasive species outside its native range (Anonymous, 2015a; Hutchinson and Dalziel, 1968). Water hyacinth (*Eichhornia crassipes*) is an invasive species that has changed the functioning of the ecosystem (Tobias *et al.*, 2019). Reports by so many scientists indicated that water hyacinth alters water quality. In tidal systems, such as the Delta, water moves back and forth through the water hyacinth patch so water quality directly outside the patch in either direction is likely to be impacted. The intricate and distinctive characteristics of water hyacinth make it one of the most ecologically resilient aquatic plants enabling it to invade major water systems (Chapungu *et al.*, 2018). The *E. crassipes* was introduced into the Nigerian coastal waters in September 1984 from Port Novo creek (Benin Republic) and has continued to flourish (Inyang *et al.*, 2015). The plant has subsequently invaded and established itself on the waterways of Niger Delta oil rich region of Nigeria (Anonymous, 2015a; Akinyemiju, 1987). Some of the fastest growing plants known, water hyacinth reproduces primarily by way of runners or stolons, which eventually form daughter plants (Anonymous, 2015a). The roots of *E. crassipes* naturally absorb pollutants, including lead, mercury and strontium-90, as well as some organic compounds believe to be carcinogenic, in concentrations 10,000 times that in the surrounding water (Anonymous, 2015a). Water hyacinth is sometimes cultivated for waste water treatment (Anonymous, 2015a) and has been used for environmentally sustainable phytoremediation of water, though its use has been geographically restricted (Jones *et al.*, 2018) hence the need for this study. Also, several successful researches have been carried out to determine the potentials of *E. crassipes* to clean-up crude oil contaminated sites (Ochekwu and Madagwa, 2013; Udeh *et al.*, 2013). Its phytoremediation potential therefore cannot be overemphasized.

Therefore, *Eichhornia crassipes* is among the plants that maintain the biological diversity of coastal areas and is useful in filtering the environment. But oil pollution seems to be negatively affecting it adversely. The aim and objective of the present study is to investigate the changes in growth and performance of *E. crassipes* grown in crude oil contaminated environment and to determine the extent of *E. crassipes* tolerance to toxicity with a view to inferring their possible use in phytoremediation.

MATERIALS AND METHODS

Sampling area

Crude oil sample was collected from Shell Petroleum Development Company, Uzere flow station. The experimental plant was collected from Ase River in Ndokwa-East local Government area of Delta state, a boundary town with Uzere.

Plant collection

The plant was collected by hand. The study was carried out in a screen house. They were transferred into buckets filled with measured amount of water. The plant, *Eichhornia crassipes* (Mart) Solms-Lamb (Water hyacinth) was authenticated by a taxonomist, Prof C.U Okeke of Botany Department, Nnamdi Azikiwe University, Awka.

Preparation of samples

Ten litres of water from Ase River were poured into thirty different 15 litres containers. Various concentrations of crude oil were administered into the bowls. The treatment used include: 1.25%, 2.5%, 5%, 7.5% and 10%, respectively. The control, 0ml had no crude oil on it. The treatments were replicated 3 times.

The test plant, *E. crassipes* was introduced into each container a week after pollution.

Plant performance

The performance of the *E. crassipes* plants was measured using height, number of leaves, leaf area, fresh weight and dry weight.

Determination of height of plants

This was done on weekly bases. First a base marked and a string attached. Each week a metre rule was placed at the base and measurement of the plant height was taken and recorded. The height of *E. crassipes* was determined by measuring from the base level to the tip of terminal leaf (Omosun *et al*., 2008). This was done for 8 weeks.

Determination of leaf area

Leaf area of *E. crassipes* plants was got by applying the traditional short cut field method by first getting the actual leaf area through taking the entire leaf perimeter and plotting this against leaf length × leaf breadth readings. The slope was used as the multiplying factor for subsequent leaf breadth × leaf length readings. This gave the leaf area for all leaves that sprouted (Pearce *et al*., 1975).

Determination of number of leaves

On weekly bases, the numbers of leaves were counted. Careful notes were taken of new sprout and flowering.

Determination of weight of plants

In order to determine the fresh weight of the *E. crassipes* plant, samples were weighted on a scale with 0.0001g readability and after drying at 100^{oc} for 24hr (till constant weight), dry weight were determined

Data analysis

The data collected in this research was subjected to independent sample effect and one-way analysis of variance (ANOVA). The Duncan multiple range tests were used for means separation.

RESULTS AND DISCUSSION

General observation

The health of *E. crassipes* was adversely affected at exposure to increasing crude oil treatments. Chlorosis of leaves, plant dehydration, stunted growth and death of the growing point was the effect of exposing *E. crassipes* to increasing concentration of crude oil. Another striking observation is the flowering of *E. crassipes* in treatment 5% at 6 weeks of planting.

Effect of crude oil treatment on the growth of *E. crassipes*

Results of the effect of different concentrations of crude oil on the stem height of *E. crassipes* revealed an increase in the length of control plants as well as those treated with 1.25%, 2.5% and 5% crude oil. Nevertheless, the growth rate of the control was highest (6.02+0.028 to7.56+0.028). There was death of *E. crassipes* in 7.5% and 10.0% concentration of crude oil from the 6th week (Table 1), this agrees with the documentation of Ochekwu and Madagwa, (2013) who reported that a significant increase in the morphological parameters (plant height, number of leaves and leaf area) of *E. crassipes* was observed after 2 weeks and further increase were observed at 4 weeks. At 8weeks and 12 weeks which the experiment lasted, the growth rate reduced greatly. Analysis of variance showed a significant ($P<0.05$) difference in the weekly stem height of *E. crassipes* between concentrations of crude oil (Table 1).

Results of the effect of different concentrations of crude oil on the leaf area of *E. crassipes* showed that the control and 1.25% concentrations induced a weekly increase in leaf area while concentrations of 2.5%, 5.0%, 7.5% and 10.0% showed a weekly decrease in leaf area. The control plants showed changes in leaf area from 30.10±0.007 cm^2 to 33.70±0.001cm^2 while 2.5% concentration showed decrease in leaf area from 32.35±0.015 cm^2 to 12.16±0.002cm^2. The *E. crassipes* treated with 7.5% and 10.0% crude oil concentrations died 6 weeks following treatment. Analysis of variance showed a significant ($P<0.05$) difference in the weekly leaf area of *E. crassipes* between concentrations of crude oil (Table 2).

Table 1. Changes in plant height (cm) of *E. crassipes* as influenced by concentrations of crude oil.

Crude oil concentration %	Plant height (cm) per age (weeks)				
	0	2	4	6	8
0	6.02±0.028	6.20±0.001	6.90±0.001	7.23±0.001	7.56±0.028
1.25	4.32±0.021	4.50±0.002	4.86±0.000	5.11±0.007	5.22±0.007
2.5	8.61±0.014	8.71±0.037	8.90±0.002	9.03±0.035	9.10±0.001
5.0	10.01±0.007	10.20±0.004	10.25±0.057	10.30±0.001	10.40±0.006
7.5	7.01±0.015	7.00±0.002	5.60±0.004	-	-
10.0	8.52±0.021	6.70±0.006	6.50±0.000		
	**	**	**	**	**

Results are in Mean ± SD; **Significantly different at $P<0.05$ significance level.

Table 2. Changes in leaf area (cm^2) of *E. crassipes* as influenced by varying concentrations of crude oil.

Crude oil concentration %	Leaf area (cm^2) per age (Weeks)				
	0	2	4	6	8
0	30.10±0.007	32.53±0.009	33.56±0.001	33.60±0.006	33.70±0.001
1.25	12.21±0.008	12.24±0.005	12.28±0.008	12.29±0.001	12.38±0.007
2.5	32.35±0.015	12.81±0.013	12.16±0.013	12.16±0.012	12.16±0.002
5.0	59.82±0.003	59.80±0.011	50.47±0.003	33.72±0.004	32.48±0.001
7.5	25.43±0.014	22.33±0.008	20.33±0.012	-	-
10.0	24.77±0.005	21.67±0.012	18.06±0.001	-	-
	**	**	**	**	**

Results are in Mean ± SD; **Significantly different at *P<0.05* significance level.

Table 4. Changes in fresh weight (g) of *E. crassipes* as influenced by concentrations of crude oil treatment.

Crude oil concentration %	Fresh weight (g) per age (Weeks)				
	0	2	4	6	8
0	14.22±0.003	33.05±0.004	48.09±0.001	60.37±0.008	62.43±0.009
1.25	17.53±0.012	28.99±0.006	40.06±0.002	52.38±0.012	54.25±0.005
2.5	19.34±0.001	42.66±0.000	45.03±0.009	47.07±0.001	48.88±0.013
5.0	15.51±0.006	37.00±0.001	38.59±0.005	39.95±0.002	40.66±0.011
7.5	18.22±0.001	18.11±0.007	18.10±0.013	-	-
10.0	17.19±0.002	19.13±0.002	16.28±0.011	-	-
	**	**	**	**	**

Results are in Mean ± SD; **Significantly different at *P<0.05* significance level.

Table 5. Changes in dry weight (g) of *Eichhornia crassipes* as influenced by crude oil concentrations during growth.

Crude oil concentration %	Dry weight (g) per age (Weeks)				
	0	2	4	6	8
0	2.84±0.001	6.61±0.006	9.62±0.002	12.07±0.002	12.49±0.006
1.25	3.51±0.006	5.80±0.002	6.02±0.006	6.48±0.003	6.85±0.003
2.5	3.87±0.004	8.53±0.000	11.00±0.001	11.41±0.003	11.77±0.000
5.0	3.10±0.002	7.40±0.004	7.72±0.002	7.99±0.001	8.14±0.012
7.5	3.65±0.009	6.60±0.006	5.43±0.000	-	-
10.0	3.43±0.003	3.43±0.003	3.46±0.006	-	-
	**	**	**	**	**

Results are in Mean ± SD; ** Significantly different at *P<0.05* significance level.

Results of the effect of different concentrations of crude oil on the number of leaf of *E. crassipes* showed that the control plant gave the highest number of leaves as 17.00±1.414 after eight (8) weeks of growth while plants treated with 10.0% of crude oil gave the least number of leaves as 2.00±0.002 after four (4) weeks of growth. Thereafter, plants treated with 7.5% and 10.0% crude oil died so that by the 6th week they lost all their leaves. Analysis of variance showed a significant (*P<0.05*) difference in the weekly number of leaf of *E. crassipes* between concentrations of crude oil (Table 3).

Results of the effect of different concentrations of crude oil on the fresh weight of *E. crassipes* showed that the control plants gave the highest fresh weight from initial weight of 14.22±0.003g to 62.43±0.009g in week 8 while 10.0% concentration gave the least fresh weight increase from 17.19±0.002g initial weight to 16.28±0.011g in week 4. There was death of *E. crassipes* in 7.5% and 10.0% concentration of crude oil by the 6th week of growth. Analysis of variance showed a significant difference (*P<0.05*) in the weekly fresh weight of *E. crassipes* between concentrations of crude oil (Table 4).

Again the results revealed that the dry weight of *E. crassipes* of the control plant was the highest with a value 9.62±0.002g after 4 weeks of growth while crude oil treatment, 10%, gave 3.46±0.006g dry weight after 4 weeks of growth. Thereafter, plants treated with 7.5% and 10% died. The control plant increased its dry weight to 12.49±0.006g by the 8th week of growth. Analysis of variance showed a significant difference (*P<0.05*) in the weekly dry weight of *E. crassipes* between concentrations of crude oil (Table 5).

The response of *E. crassipes* to crude oil contamination showed a reduced plant height, leaf area, number of leaves, fresh weight and dry weight as the concentration (treatment) increased. These results are in line with the report of Bailey and McGill (1999) who stated that plants tolerate increased exposure to crude oil and creosote-contaminated soil with minimal growth rate. Crude oil spills affect plants adversely by creating conditions that make essential nutrients like nitrogen and oxygen needed for plant growth unavailable to them (Wright *et al.*, 1997).

According to Erute *et al.* (2009), oil contamination causes slow rate of germination in plants. Adam and Duncan (1999) reported that this effect could be due to the oil which acts as a physical barrier (hydrophobic layer), preventing or reducing access of the seeds to water and oxygen.

A significant increase in the morphological parameters (plant height, number of leafs, leaf area, fresh weight and dry weight) as observed after two weeks of planting in plants treated with 1.25%, 2.5%, and 5% agrees with the work done by Ochekwu and Madagwa (2013) on phytoremediation potentials of water hyacinth in crude oil polluted water. Also, the findings that after 6 weeks of growth, *E. crassipes* treated with 7% and 10% crude oil died is in consonance with Ochekwu and Madagwa (2013) report that in their experiment to determine the phytoremediation potentials of *E. crassipes*; at 8 and 12 weeks, the growth rate reduced greatly indicating that *E. crassipes* can hardly tolerate crude oil with time. Frick *et al.* (1999) had stated that phytoremediation of petroleum hydrocarbons may be ineffective, if concentrations of the contaminants are either too high (causing toxicity) or too low (resulting in poor bioavailability). This statement therefore implies that there is a limit of sustenance of petroleum hydrocarbon pollution for every plant above which toxicity will apply.

The findings that the morphological nature of these plants were tortured especially by loss of chlorophyll and clear chlorosis immediately following crude oil pollution is in line with the general physiology of plants since nutrients absorption such as nitrogen uptake cannot be achieved smoothly. Such minerals are needed for chlorophyll synthesis. This corresponds with Brooks and Robinson (1998) work on aquatic phytoremediation by accumulator plants. Ochekwu and Madagwa (2013) had posited that chlorosis of the leaves may be an implication of the persistent organic compound and heavy metals absorbed by the plant. Pezeshki and Delaune (1992), however, stated that the effect of crude oil on plants could be short term under field conditions, since plants would likely recover once residual oil is removed by rainfall. The observation that *E. crassipes* plants started flowering 6 weeks at treatment 5% could have been exploited as normal point reaction to stress. According to Kaede and Kiyotoshi (2010), many plant species can be induced to flower by responding to stress factors.

Conclusion

It is evident from the study that *E. crassipes* have demonstrated promising potential to phytoremediate petroleum hydrocarbons. At a very low concentrations *E. crassipes* survived for a long period of time, however, high concentration decreases the morphology of the test plant. The ability of the plant to tolerate different levels of petroleum hydrocarbons was also proved in the study. The study however advocates the use of alternative clean–up method in cases of excessively high concentrations of crude oil concentrations before reclaiming with the plant. This also suggests that the test plant can be used in cases with low to moderate crude oil contamination. It also proposes the use of the plant as possible bio-indicator for the detection of crude oil using the plant growth at lower doses as markers. Further assessment of the plant in field situation would be useful.

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