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Growth and tolerance evaluation of selected plants to crude oil contamination in the Niger Delta

Monday Ubogu[1,*], Lucky O. Odokuma[2]

[1]*Dept. of Microbiology, Federal University of Agriculture Makurdi, Nigeria.*

[2]*Dept. of Microbiology, University of Port Harcourt, Nigeria.*

[*]*Corresponding author: ubomon@yahoo.co.uk*

Abstract

Little is known about Niger Delta plant species that can adapt to survive in oil-contaminated soil. To investigate this, the following plant species *Zea mays, Telfairaoccidentalis, Saccharum officinarum, Kalanchoe pinnata, Phaseolus vulgaris, Arachis hypogaea, Phragmitis australis, Azolla pinnata* and *Eichornia crasssipes* were screened for growth and tolerance to 0, 1, 3 and 6% w/w crude oil contamination for a 120-day period to determine the influence of oil on plant germination, height, root length, leaf area growth and survival/death time. With the exception of *P. vulgaris* and *A. hypogaea*, plant germination was delayed with increased concentration of oil. The effect of oil concentration on height, root length and leaf area growth varied with plant species ($P < 0.05$). Among the nine plants tested only *E. crassipes, P. australis* and *S. officinarum* survived for the 120-day period of the study at 6% w/w contamination. The survival of these plants in oil-contaminated soils indicates that they could be used for rhizoremediation in the Niger Delta.

Keywords: Contamination; crude oil; Niger Delta; plant species; rhizoremediation.

1. Introduction

The Niger Delta region is a tropical rainforest harboring diverse plant and animal species both in the terrestrial and aquatic ecosystem (Ayuba, 2012). It is ranked among the world largest wetlands (SPDC, 1999).

Crude oil amounting to approximately 13 million barrels have been spilled into the Niger Delta environment in the past 50 years (FME2006), making it one of the world's five most negatively impacted environment by petroleum (Ayuba, 2012).

The high cost and low efficacy of the usual physicochemical remediation techniques have necessitated the creation of alternative options for *in situ* applications, particularly remediation techniques, that are dependent on the activities of microorganisms and plants (Snigh and Chaudhry *et al*., 2005). This is known as bioremediation, and one of such techniques is rhizoremediation. Rhizoremediation involves the removal of pollutants from a contaminated environment via mutual interaction of plant roots and the associated microorganisms (Shukla *et al*., 2010).

Intensive selection studies are required to choose the most appropriate plants that can survive oil contaminated soils (Pivetz, 2001). Little is known about plant species from the tropics that can be deployed for this purpose (Ogbo *et al*., 2009). Despite the overwhelming numbers of potential plants, a comparatively small numbers of plants have been studied (Pivetz, 2001).The aim of this study is to identify potential plants capable of growing in crude oil contaminated soil. Those plants could be used for rhizoremediation in the Niger Delta.

2. Materials and methods

2.1 Evaluation of baseline physicochemical properties of soil

Prior to plant propagation, soils were analyzed for physicochemical characteristics. Total petroleum hydrocarbon (TPH) in soil was determined using US EPA-Method 8015C (2007). A hydrometer method was used for soil textural component determination (Oyeyiola, 2011). Nitrogen content was analyzed by micro-Kjeldahl (Hesse, 1979). Total organic carbon (TOC), phosphorus, pH and soil porosity were determined by the methods of Black (1965), Bray and Kurtz (1971), Black (1965) and Ezzati *et al*. (2012), respectively.

2.2 Soil preparation and treatments for plant propagation

Rainforest and mangrove soil for plant propagation were collected from the Niger Delta (Warri axis) from the top layer (0-15 cm) of soil in vertical profiles. Different amounts of crude oil (specific gravity of 0.818 g/cm^3) (0.0 g (0.0 ml), 40.0 g (48.9 ml), 120.0 g (146.7 ml) and 240.0 g (293.4 ml) were mixed with 4000.0 g soil in plastic pots to attain contamination levels of 0.0 (control), 1.0, 3.0 and 6.0% w/w concentrations of oil in soil, respectively.

2.3 Selection of plants for propagation

Zea mays, *Telfaira occcidentalis*, *Saccharum officinarum*, *Kalanchoe pinnata*, *Phaseolus vulgaris* (white bean), and *Arachis hypogaea* L. were selected from the rainforest. *Phragmitis australis*, *Azolla pinnata*, *Eichornia crasssipes* were selected from the mangrove swamp. Plant selection, propagation and evaluation hinged on the ease of cultivation, common occurrence and adaptability to the Niger Delta. Selection was also based on rhizosphere effect, total population, and percentage population of hydrocarbon utilizers in the rhizosphere of these plants (Odokuma & Ubogu, 2014).

2.4 Healthy seed testing and selection

Healthy seeds of *T. occidentalis, P. vulgaris, Z. mays* and *A. hypogaea* obtained from the local market were selected using the floatation method. Floating seeds were then discarded, while submerged viable seeds were selected for propagation.

2.5 Plant propagation

Rainforest and mangrove swamp plants were all propagated in a greenhouse. Viable seeds of *T. occidentalis, P. vulgaris, Z. mays, and A. hypogaea* were planted in rainforest soil. Two seeds were soaked per pot in crude oil concentrations of 0.0, 1.0, 3.0 and 6.0% w/w. Three pots were used in each treatment.

Stem cuts from *S. Officinarum* measuring 22.0 cm in length, 3.5 ± 0.1 cm in diameter were propagated at a ratio of one/pot in triplicate in rainforest and mangrove swamp soil at crude oil concentrations of 0.0, 1.0, 3.0 and 6.0%, respectively. However, *K. pinnata* stem cuts of 15.0 cm in length and 1.0 ± 0.1 cm width were propagated in triplicate in rainforest soil at crude oil concentrations of 0.0, 1.0, 3.0 and 6.0% w/w. Similarly, *P. australis* stem cuts of 30.0 cm in length and diameter of 0.8 ± 0.1 cm were propagated at a ratio of two/pot in triplicate in mangrove swamp soil at crude oil concentrations of 0.0, 1.0, 3.0 and 6.0% w/w.

Young and tender *E. crasssipes* and *A. pinnata* plants were collected from the wild and transplanted into respective pots. For *E. crasssipes,* whole plants (shoots consisting of five bulbed-leaves, measuring 12.5 ± 1.5cm in height, together with roots of approximate equal lengths), were planted at a ratio of one set per pot. *A .pinnata* rhizomes measuring 15 cm in length and 0.5 ± 0.1cm in diameter together with fronds measuring 12.0 ± 1.0 cm in height (one per rhizome) were planted, one per pot in triplicates in mangrove swamp soil at crude oil concentrations of 0.0, 1.0, 3.0 and 6.0% w/w, respectively.Plants in rainforest soil were regularly watered, while mangrove swamp soil was kept perpetually flooded with tap water. Finally, all seeds and stems of each plant species were planted at approximately the same soil depth to ensure consistency of propagation.

2.6 Calculation of emergence time and percentage

Time (days) taken for plant shoots to emerge from the soil surface was regarded as the emergence time. However, sprouting time in plants propagated from stem cuts was taken as emergence time of leaves on aerial nodes or tips of cut stems. The emergence percentage was calculated as the number of successful emergences out of the total number of used cuts.

2.7 Growth measurements

Plant height and root length were determined using the method by Omosun *et al*. (2008), while and leaf area was determined by the method from Pearce *et al*. (1975). These measurements were made by the end of the experiment (after 120 days). However, for *E. crasssipes* and *A. pinnata,* measurements were taken at day-0 of transplantation and after 120 days. The growth was measured as the net increment between the two time intervals.

$$\text{Percentage germination} = \frac{Number\ of\ sprouted\ stem\ or\ emerged\ seedlings}{Total\ number\ of\ propagated\ stems\ or\ seeds}\ x\ 100$$

$$Increment\ in\ height, root\ and\ leaf\ area = Measurement\ at\ day\ 120 - Measurement\ at\ day\ 0$$

2.8 Calculation of plant death time

Death time were determined by observing the time taken for plants to irreversibly wither (leaf became senescence) and die in the various crude oil concentrations in mangrove and rainforest soils within the 120 days of the experiment. This process was evaluated by adapting the method from Hensel, *et al*. (1993) as described by Nooden (2003). A plant was regarded as dead when more than half of the surface of its apex leaves had become flaccid or dried.

T. occidentalis, and *K. pinnata* showed 50.0 and 66.7% emergence, respectively, at 3.0% contamination (the highest concentration of oil that produced germination) (Table 2). In other studies, *Paspalum scrobiculatum* and alfalfa germinated in 15.0 and 5.0% crude oil contaminated soil, respectively (Ogbo *et al*., 2009; Wiltse *et al*., 1998). It has been reported that the germination test is a key measurement for the determination of plant species tolerance for rhizoremediation purposes (Gaskin, 2008).

Table 1. Physicochemical properties of soils used for plant propagation (Mean ± SD)

Characteristics	Environment Type	
	Rainforest	**Mangrove swamp**
TPH (mg/kg)	61.59± 1.2	379.50± 1.5
TOC (%)	0.01± 0.00	0.06± 0.01
Nitrogen (%)	0.15± 0.01	0.15± 0.01
Phosphorus (mg/kg)	43.70± 2.0	44.04± 1.5
Porosity (%)	60.0± 1.5	32.00± 1.0
pH	5.60± 0.5	5.05± 0.5
Sand	90.0± 2.0	47.50± 2.0
Silt	4.20± 1.0	23.80± 1.0
Clay	5.80± 1.0	28.70± 2.0

2.9 Data analysis

Accumulated data were analyzed using the measure of central tendency and dispersion, Student's *t*-test and analysis of variance ($P < 0.05$).

3. Results and discussion

The baseline physicochemical properties of the soils that were used in this study showed a relatively low TPH and TOC (Table 1), indicating that crude oil had not had any significant impact.

In this study, all the crude oil concentrations attained 100.0% emergence for propagated stems of *P. australis* and *S. officinarum*. However, seedling emergence in *P. vulgaris, Z. Mays,* and *A. hypogeae was* recorded at 66.7, 66.7, and 50.0%, respectively, at 6.0% contamination. Oil slows germination as it impede access to oxygen and water (Ogbo *et al*., 2009). In this study, with the exception of *P. vulgaris* and *A. hypogaea* all the other tested plants (*T. occidentalis, Z. mays, K. pinnata, S. officinarum* and *P. australis*) showed delayed emergence with an increase in crude oil concentrations (Table 3). This effect was more severe with *T. occidentalis* and *K. pinnata,* as these plants did not only showed delayed germination but also failed to germinate at 6.0% crude oil contamination. Although there was delayed germination of *S. officinarum* and *P. australis* by a period of five and two days, respectively at 6.0% when compared to the control, the 100% germination recorded in these plants indicates some level of tolerance at lower levels of contamination.

Table 2. Influence of crude oil concentrations on plant percentage (%) sprouting/germination

	Crude Oil Concentration (% w/w)			
Plant Species	**0**	**1**	**3**	**6**
P. vulgaris	100.0	66.7	66.7	66.7
A. hypogaea	83.3	66.7	66.7	50.0
T. occidentalis	100.0	100.0	50.0	0
Z. mays	100.0	100.0	100.0	66.7
K. pinnata	100.0	100.0	66.7	0
S. officinarum	100.0	100.0	100.0	100.0
P. australis	100.0	100.0	100.0	100.0
E. crassipes	ND	ND	ND	ND
A. pinnata	ND	ND	ND	ND

ND = Not determined

Table 3. Influence of crude oil concentrations on plant germination/sprouting time (mean number in days ± SD)

	Crude Oil Concentration (% w/w)			
Plant Species	**0**	**1**	**3**	**6**
P. vulgaris	3.3 ± 0.6^{a}	4.0 ± 1.0^{a}	4.3 ± 0.6^{a}	5.3 ± 1.5^{a}
A. hypogaea	4.7 ± 0.6^{a}	6.0 ± 1.0^{a}	6.3 ± 1.5^{a}	6.7 ± 1.5^{a}
T. occidentalis	10.7 ± 0.6^{a}	14.0 ± 1.0^{b}	27.3 ± 0.6^{c}	0
Z. mays	3.0 ± 0.0^{a}	3.0 ± 0.0^{a}	$3.7 \pm 0.^{ab}$	4.7 ± 0.6^{b}
K. pinnata	9.0 ± 1.0^{a}	11.7 ± 1.5^{b}	18.6 ± 0.6^{c}	0
S. officinarum	9.0 ± 1.0^{a}	9.3 ± 1.2^{a}	10.3 ± 0.6^{a}	14.3 ± 0.6^{b}
P. australis	5.0 ± 0.4^{a}	5.3 ± 0.5^{a}	5.3 ± 0.5^{a}	7.0 ± 1.0^{b}
E. crassipes	ND	ND	ND	ND
A. pinnata	ND	ND	ND	ND

*Values with the same letter within a row did not differ significantly ($P < 0.05$).
ND = Not determined

With the exception of *S. officinarum* and *E. crassipes*, for which increased concentrations of crude oil did not significantly affect plant height, all other tested plants (*A. pinnata, P. ausralis, P. vulgaris, A. hypogeae, T. occidentalis, Z. mays* and *K. pinnata*) showed a reduction in height with increased crude oil contamination (Table 4).A reduction in plant height under the influence of increased concentrations of crude oil in soil has been widely reported (Anoliefo & Vwioko, 1995; Omosun *et al*.,2008; Ogbo *et al*., 2009). The presence of oil in soil impedes plant growth due to aeration and water infiltration impairments (Samira *et al*., 2016). A reduction in plant height with concomitant increase in crude oil contamination in soil may be ascribed to a higher stress imposition on these plants. Microorganisms involved in the breakdown of hydrocarbons strive to outperform plants in their contest for nutrients and oxygen, which are usually limited in soil. The creation of an anaerobic environment resulting from oxygen depletion may trigger microbial formation of hydrogen sulfide, which

is phytotoxic to plants (Dejong 1980). The ability of *S. officinarum* and *E. crassipes* to withstand increased crude oil contamination without any significant effect on their growth is noteworthy as it indicates that these plants show some degree of tolerance to crude oil contamination.

Previous reports indicate that increased crude oil concentrations in soil decreased growth in leaf area (Vwioko & Fashemi, 2005; Omosun *et al*., 2008; Ogbo *et al*., 2009).The plant specimens in this study exhibited differential responses to increased crude oil contamination. While the leaf area growth of *P. vulgaris, A. hypogaea, T. occidentalis, Z. mays,* and *K. pinnata* were affected by increased contamination, the effect was only significant on the leaf area of *S. officinarum* and *A. pinnata.* Increased crude oil contamination did not significantly affect the leaf area growth of *E. crassipes* and *P. australis* (Table 5).

Table 4. Influence of crude oil concentrations on plant height (cm) (mean plant height ± SD)

	Crude Oil Concentration (% w/w)			
Plant Species	**0**	**1**	**3**	**6**
P. vulgaris	364.0 ± 9.8[a]	19.3 ± 1.1[b]	13.0 ± 1.4[c]	-
A. hypogaea	92.3 ± 8.8[a]	46.1 ± 1.0[b]	-	-
T. occidentalis	140.0 ± 9.8	-	-	-
Z. mays	118.2 ± 1.0	-	-	-
K. pinnata	37.0 ± 5.5[a]	13.0 ± 0.0[b]	6.3 ± 0.4[c]	-
S. officinarum	141.7 ± 10.5[a]	145.7 ± 6.7[a]	138.8 ± 1.6[a]	108.2 ± 6.9[a]
P. australis	207.3 ± 9.2[a]	208.0 ± 8.0[a]	199.0 ± 9.0[b]	187.3 ± 9.5[c]
E. crassipes	10.4 ± 2.5[a]	16.2 ± 1.4[b]	18.9 ± 3.4[c]	19.3 ± 0.5[c]
A. pinnata	45.5 ± 0.5[a]	45.3 ± 0.8[a]	38.8 ± 4.8[a]	-

*Values with the same letter within a row did not differ significantly ($P < 0.05$).

Table 5. Influence of crude oil concentrations on plant leaf area (cm^2) (mean leaf area ± SD)

	Crude Oil Concentration (% w/w)			
Plant Species	**0**	**1**	**3**	**6**
P. vulgaris	17.7 ± 1.1[a]	5.0 ± 2.3[b]	2.2 ± 0.0[c]	-
A. hypogaea	13.1 ± 0.2[a]	7.2 ± 0.7[b]	-	-
T. occidentalis	19.1 ± 1.2	-	-	-
Z. mays	82.6 ± 10.6	-	-	-
K. pinnata	25.9 ± 5.2[a]	6.8 ± 0.1[b]	0.8 ± 0.0[c]	-
S. officinarum	89.4 ± 9.5[a]	80.9 ± 1.9[a]	70.1 ± 5.8[b]	48.5 ± 3.0[c]
P. australis	84.5 ± 9.6[a]	64.4 ± 9.8[a]	62.1 ± 7.7[a]	62.2 ± 5.1[a]
E. crassipes	1.0 ± 0.5[a]	1.0 ± 0.8[a]	0.5 ± 0.0[a]	0.5 ± 0.4[a]
A. pinnata	6.6 ± 0.5[a]	6.1 ± 0.8[a]	6.1 ± 0.6[a]	-

*Values with the same letter within a row did not differ significantly ($P < 0.05$).

Measurements taken at day-120 showed that the root lengths of *P. vulgaris, A. hypogeae, K. pinnata, A. pinnata* and *S. officinarum* decreased with increased crude oil contamination except for *K. pinnata*, where 1.0% contamination showed a greater root growth compared to that of the control. This suggests that 1.0% crude oil in soil had a stimulating effect on root growth of *K. pinnata, T. occidentalis* and *Z. mays*, despite the fact that these plants did not survive up to day-120 in any of the tested contaminated soil. However, growth in the roots of *P. australis* and *E. crassipes* were not significantly affected by the increase in concentration levels (Table 6 and Figure 1(a), 2(a), 3(a)).

Table 6. Influence of crude oil concentrations on plant root length (cm) (mean root length ± SD)

	Crude Oil Concentration (% w/w)			
Plant Species	**0**	**1**	**3**	**6**
P. vulgaris	41.0 ± 8.0^{a}	6.5 ± 0.5^{b}	5.1 ± 0.2^{c}	-
A. hypogaea	31.0 ± 6.0^{a}	22.7 ± 5.5^{b}	-	-
T. occidentalis	59.8 ± 3.0	-	-	-
Z. mays	62.7 ± 0.6	-	-	-
K. pinnata	20.5 ± 3.5^{a}	28.8 ± 1.5^{b}	3.4 ± 0.1^{c}	-
S. officinarum	73.2 ± 3.3^{a}	64.7 ± 3.0^{b}	54.1 ± 1.9^{c}	40.2 ± 0.3^{d}
P. australis	46.3 ± 0.4^{a}	46.8 ± 0.4^{a}	43.5 ± 2.1^{a}	42.0 ± 1.4^{a}
E. crassipes	18.4 ± 1.2^{a}	18.5 ± 1.5^{a}	18.8 ± 0.3^{a}	19.9 ± 1.4^{a}
A. pinnata	34.3 ± 0.8^{a}	27.7 ± 0.5^{b}	14.8 ± 0.8^{c}	-

*Values with the same letter within a row did not differ significantly ($P < 0.05$).

Fig. 1(a). Influence of crude oil concentrations 0, 1, 3 and 6% w/w on the root length of *S. officinarum* (day 120 from left to right)

Fig. 2(a). Influence of crude oil concentrations 0, 1, 3 and 6% w/w on the root length of *P. australis* (day 120 from left to right, respectively)

Fig. 3(a). Influence of crude oil concentrations 1, 3 and 6% w/w on the root length of *P. australis* (day120fromleft to right, respectively)

In a similar study, Gaskin (2008) reported that while the root lengths of *Cymbopogon ambiguous* increased in oil contaminated soil, roots of *Brachiaria decumbens* did not show similar results at 0.5 and 1.0% w/w. These findings indicate that growth response of roots in the presence of oil is largely dependent on species and oil concentrations. The remarkable absence of a perceivable negative growth effect on the root lengths of *E. crassipes* and *P. australis* at higher concentrations of crude oil clearly indicates the degree of tolerance. One major limitation to effective rhizoremediation is the restriction of the process to the area of plant root depth; many plants have relatively shallow roots (Pivetz, 2001).

Results from this study showed that *P. australis* and *S. officinarum* root lengths were in the range of 42.0 - 46.8 and 40.2 - 64.7 cm, respectively in the contaminated soil. This strongly suggests that both plants are adequately suitable for rhizoremediation purposes. Regardless of the fact that oxygen and nitrogen, which are essential nutrients for plant growth, are rendered unavailable in soil in the event of oil spills, adversely affecting plant growth (Ogbo *et al*., 2009), nutrient additives can be incorporated into the soil by the tilling action of plant roots, which improve aeration (April & Sims, 1990). Some plants such as willow, popular, *S. officinarum* and *P. australies* that possess root system that are well developed together with the possession of aerenchyma (specialized root vessels) that facilitate oxygen release in the rhizosphere into greater soil depth (Zalesny, 2005) makes the preferential selection of these class of plant easy for the purpose of rhizoremediation.

For specimens investigated for growth and tolerance to various concentrations of crude oil contamination, only *S. officinarum, P. australis* and *E. crassipes* tolerated and survived in all the concentrations for the 120-day experiment period (Figure 1b, 2b and 3c). *T. occidentalis* only survived 1% oil, while *P. vulgaris, A. hypogeae, Z. mays, K. pinnata* and *A. pinnata* survived 3% for the entire period.

Fig. 1(b). *S. officinarum* growing in 0, 1, 3 and 6%w/w concentrations of crude oil (day120)

Fig. 2(b). *P. australis* growing in 0, 1, 3 and 6% w/w concentrations of crude oil (day120)

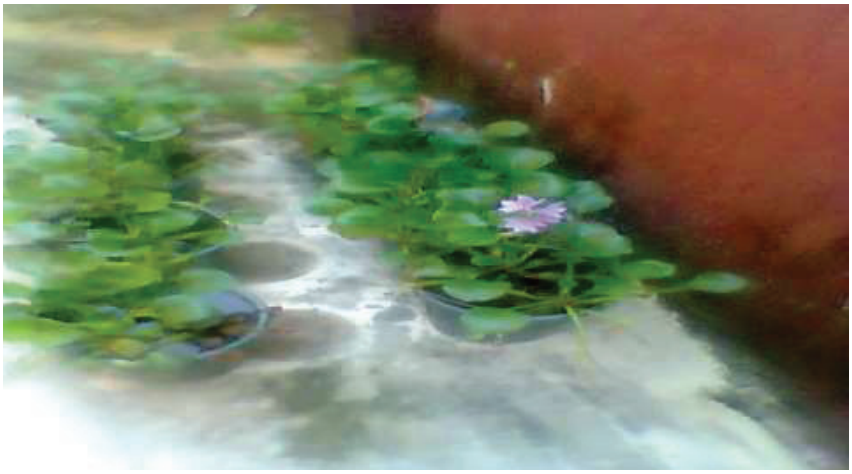

Fig. 3(b). *E. crassipes* growing in 0, 1, 3 and 6%w/w concentrations of crude oil (day120)

4. Conclusions

The findings evidently suggest that *S. officinarum*, *P. australis* and *E. crassipes* are the most tolerant of crude oil contamination, surviving the 120-daystudy period. Therefore, these plants hold great rhizoremediation potential, meaning they are best for the cleanup of crude oil impacted soils in the Niger Delta.

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تقييم النمو في نباتات مختارة وقدرتها على تحمل التلوث بالنفط الخام في دلتا النيجر

[1]موندای أوبوجو، [2]لاكي أودوكوما

[1]قسم الأحياء الدقيقة، الجامعة الفيدرالية للزراعة بماكوردي، نيجيريا.

[2]قسم الأحياء الدقيقة، جامعة بورت هاركورت، نيجيريا

الملخص

لا يُعرف سوى القليل عن الأنواع النباتية التي يمكنها أن تتكيف مع بقايا التربة الملوثة بالزيت في دلتا النيجر. وللتأكد من ذلك، فإنه تم فحص النمو والقدرة على تحمل التلوث بالزيت الخام بنسبة 0 ، 1 ، 3 و 6% من الوزن الرطب لمدة 120 يوماً لتحديد تأثير الزيت على نمو النبات، وارتفاعه، وطول الجذور، ومساحة الأوراق ومدة البقاء على قيد الحياة أو الموت في أنواع النباتات التالية: *Zea mays* و *Telfaira occidentalis* و *Saccharum officinarum* و *Kalanchoe pinnata* و *Phaseolus vulgaris* و *Arachis hypogaea* و *Phragmitis australis* و *Azolla pinnata* و *Eichornia crasssipes*. وباستثناء الفاصوليا (*P. vulgaris*) والفول السوداني (*A. hypogaea*)، فقد تأخرت عملية الإنبات مع زيادة تركيز الزيت. وقد تنوع تأثير تركيز الزيت على ارتفاع النبات وطول الجذور ومساحة الأوراق باختلاف الأنواع النباتية ($P < 0.05$). من بين النباتات التسع التي تم اختبارها، بقي على قيد الحياة كل من *P. australis* ، *E. crassipes* و *S. officinarum* فقط لمدة 120 يوماً من الدراسة بتلوث بنسبة 6% من الوزن الرطب. إن بقاء هذه النباتات على قيد الحياة في التربة الملوثة بالنفط أشار إلى أنه يمكن استخدامها لعلاج الجذور في دلتا النيجر.