International Scholars Journals

Advanced Journal of Environmental Science and Technology ISSN 7675-1686 Vol. 10 (2), pp. 001-005, February, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

The effect of crude oil on growth of the weed (*Paspalum scrobiculatum L.*) –phytoremediation potential of the plant

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Accepted 13 November, 2018

The use of grasses for phytoremediation of crude oil polluted soils especially in the tropics is a new area of study with a lot of potentials. The effect of different levels (0.00, 2.50, 5.00, 7.50, 10.00, 12.50 and 15.00%) of crude oil contamination on the growth of *Paspalum scrobiculatum*, a common weed in Nigeria was investigated. The weed is found growing luxuriantly in crude oil contaminated sites in Delta State, Nigeria. 3, 6 and 9 plants per pot of standing crops of the test plant were used for the study. The results show that the plant has potential for phytoremediation as it grew successfully in the different levels or concentrations of crude oil contamination. The different levels of crude oil contamination caused significant reduction in the growth of the plant using plant height, fresh weight and leaf area. The effect increased with increasing levels of contamination (e.g. there was reduction in the leaf area from 68.47 cm^2 in control to 34.07 cm^2 in 15.00% level of contamination). The contamination did not cause significant reduction in the dry weights of the plant in 3 plants per pot and 6 plants per pot treatments but only in the 9 plants per pot treatment where the control recorded significantly higher dry weight than all other treatment. The weed can be used for the restoration of crude oil contaminated soils as it showed great potentials by being able to withstand the high levels of crude oil contamination. It was observed that crude oil contamination did not affect biomass production of the test plant using dry weight basis and plant density improved the performance of the plant in the contaminated soils.

Key words: Phytoremediation, *Paspalum scrobiculatum*, crude oil contamination, phytotoxicity, biomass.

INTRODUCTION

Contamination of soil by crude oil spills is a wide spread environmental problem that often requires cleaning up of the contaminated sites (Bundy et al., 2002). Disposal of oil based wastes, oil spills from well blow outs and pipeline ruptures are the most common sources of petroleum contamination (Reis, 1996). Crude oil spills affect plants adversely by creating conditions which make essential nutrients like nitrogen and oxygen needed for plant growth unavailable to them. It has been recorded that oil contamination causes slow rate of germination in plants. Adam and Duncan (2002) reported that this effect could be due to the oil which acts as a physical barrier preventing or reducing access of the seeds to water and oxygen.

Some plants can render harmless, extract or stabilize a contaminant in soil, thus making it unavailable for other organisms and reducing environmental hazards in a process termed phytoremediation (Cunningham et al., 1996). Current phytoremediation techniques require that plants live in the zone of contamination. Consequently plants viability is a critical issue in the successful application of phytoremediation. If the contaminant in its present concentration is not phytotoxic, cultivation of plants can be a valuable tool in soil remediation (Merkl et al., 2004). The mechanisms and efficiency of this technology called phytoremediation depend on the type of

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contaminant, bioavailability and soil properties (Cunningham and Ow, 1996). The mechanism believed to be responsible for most of the degradation of petroleum hydrocarbons in vegetated soil is the stimulation of growth and activity of degrading micro-organisms in the rhizosphere (Frick et al., 1999). There are several approaches to selecting candidate plants for phytoremediation of soils contaminated with organic pollutants. These approaches have been based on the occurrence of plants under specific climatic conditions (Gudin and Syratt, 1975; Banks et al., 2003) their resistance to pollutant phytotoxicity (Kirk et al., 2002), the presence of phenolic compounds in the plant root exudates (Hegde and Fletcher, 1996; Liste and Alexander, 1999) or their capability to reduce the pollutant concentration in soil. Most studies on the phytoremediation of petroleum hydrocarbon contaminated soils have employed grasses (poacae) and legumes (leguminosae) (Aprill and Simms, 1990; Gunther et al., 1996; Merkl et al., 2005; Kirkpatrick et al., 2006; Qui et al., 1997; Schwab et al., 2006; Kaimi et al., 2006).

Grasses are considered to be particularly suitable for phytoremediation since they offer an increased rhizosphere zone because of their multiple ramified root systems. This gives room for more microbial activity and growth around the root zone (Aprill and Sims, 1990). Researchers like, Adam and Duncan (1999), Merkl et al. (2004, 2005) have concluded that grasses and legumes are the best candidates for the process of phytoremediation or rhizoremediation because of their root systems. Bioremediation is generally considered a promising technology for the tropics because climatic conditions favour microbial growth and activity (Merkl et al., 2004). Grasses like *Panicum maximum* and *Brachiara brizantha* were able to degrade 55 and 63% of oil and grease present in the contaminated soils in the tropics. The screening of plant species for their ability to grow and establish in contaminated soil is one of the first steps in the selection of species for phytoremediation in the tropics, followed by the evaluation of their influence on the degradation of petroleum hydrocarbons in soil (Merkl et al., 2004). However little is known about tropical species that could serve for the cleanup of oil contamination. In crude oil contaminated sites visited in Delta State, the most prominent weed found growing luxuriantly was *Paspalum scrobiculatum* hence the interest in investtigation of this plant for the purpose of oil clean-ups. *P. scrobiculatum* is commonly known as Kodo-millet. It is of ancient cultivation in Africa and Asia and also well known in India. *Paspalum* is a glabrous, tufted perennial grass of about 60 - 100 cm high. *Paspalum* is a common weed of flood plains and valley bottoms both in savannah and forest zones frequently found in rice fields. They can be grown in poor thin soils, swampy ground, wet open cultivated places, pasture and waste land (Akobundu and Agyakwa, 1998). The objective of this study is thus to find out if the weed can be used to remediate crude oil contaminated sites by investigating its growth habit in the presence of crude oil.

MATERIALS AND METHOD

P. scrobiculatum was collected from the wild from fallow farmlands in Abraka Delta State, Nigeria. The roots and stems were cropped leaving behind 5 cm of stems. Soil samples were collected from a fallow plot of land in Abraka, Nigeria. The soils were air dried and filtered using 2 mm mesh gauze to remove debris. A composite mixture was made and mixed homogenously. Crude oil was got from SPDC (Forcados blend). The control (0%) had no crude oil while the 5 different levels of contamination was obtained by adding 30.68, 62.66, 96.53, 132.28, 170.08 and 210.08 ml to 1000 g of soil get 2.50, 5.00, 7.50, 10.00, 12.50 and 15.00% levels or concentration of contamination respectively.

The effect of stumping on the performance of the plant in crude oil contaminated soil was also tested. 3, 6 and 9 plants per pot of the test plant were used as treatment options for the various levels of contamination (Agha et al., 2009) to check the effect of plant density on the performance of the plant in contaminated environment. Plant height was determined by using a meter rule from the soil level to the tip of the youngest leaf (Omosun et al., 2008). Leaf area 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 x leaf breath readings. The slope was used as the multiplying factor for subsequent leaf breath x leaf length readings. This gave the leaf area for all leaves that sprouted (Pearce et al., 1975). The fresh weight was got by weighing with Metler's weighing balance and the dry weight was determined after drying to constant weight at 40 °C.

5 replicates were made for each treatment. Differences (≤ 0.05) among treatments were tested using Analysis Of Variance followed by Duncan's multiple range test (SPSS for windows version 13.00).

RESULTS

The growth of the *P. scrobiculatum* in the different levels of contamination varied. The test plant however grew successfully in all the levels of contamination. The shoot length was significantly reduced by the presence of crude oil. Increasing oil concentration from 2.50 to 15.00% however did not cause additional significant decrease of shoot growth using 9 stump values (Table 1). The shoot growth of the plant in the 5.00% level of contamination using the 6 stumps records was comparable with the control as there was no significant difference between them (Table 1). The number of plants per pot affected the plant height as the level of contamination increased. The pots with 6 and 9 plants per pot performed better than those with 3 plants per pot at 12.50 and 15.00% (Table 1).

Biomass or yield of the test plant showed that crude oil contamination affected the plant negatively reducing the yield using fresh weight values. In the 6 stump treatment however there was no significant difference between the control and the 2.50, 5.0, 7.50 and 10.00% levels of contamination. Inhibition of yield was only noticed in the 12.50 and 15.00% levels of contamination which were significantly lower than the control (Table 2). The 3 plants per pot and 9 plants per pot treatments showed signifycant reduction of yield in the 2.50% level of contamination. In the 9 plants per pot treatments however, crude

Table 1. Plant height (cm) of *Paspalum scrobiculatum* grown in crude oil contaminated soils.

	Number of plants per pot		
Level of contamination (%)	**3**	**6**	**9**
Control (0.00)	84.93 ± 11.30^{a}	76.83 ± 7.47^{a}	83.07 ± 6.82^{a}
.50	50.93 ± 4.83^{b}	57.80 ± 8.40^{b}	43.97 ± 2.93^{b}
.00	53.70 ± 9.98^{b}	73.70 ± 4.75^{a}	41.13 ± 3.35^{b}
7.50	32.73 ± 6.46^{c}	52.57 ± 5.42^{b}	45.87 ± 2.27^{b}
10.00	30.83 ± 2.77^{c}	44.50 ± 3.80^{b}	32.60 ± 2.26^{b}
12.50	26.60 ± 7.47^{c}	15.57 ± 4.48^{c}	43.87 ± 2.37^{b}
15.00	21.73 ± 4.17^{c}	44.13 ± 7.03^{b}	45.70 ± 4.68^{b}

Letters with same superscript are not significantly different within the same column at $P < 0.05$.

Table 2. Fresh weight (g) of *Paspalum scrobiculatum* grown in different levels of crude oil contaminated soils.

	Number of plants per pot		
Level of contamination (%)	**3**	**6**	**9**
Control (0.00)	29.88±2.43^{a}	25.48±3.37^{a}	47.79±7.09^{a}
2.50	17.19±2.62^{b}	25.40±1.42^{a}	21.78±1.63^{b}
5.00	13.22±1.94^{b}	26.69±4.96^{a}	19.62±4.27^{b}
7.50	7.05±0.95^{c}	27.33±4.51^{a}	20.64±0.87^{b}
10.00	6.45±0.26^{c}	19.98±3.26^{a}	19.12±2.45^{b}
12.50	5.14±0.49^{c}	8.30±0.02^{b}	25.99±3.19^{b}
15.00	9.15±0.85^{bc}	11.88±2.64^{b}	25.55±2.98^{b}

Letters with same superscript are not significantly different within the same column at $P < 0.05$.

oil contamination caused reduction in yield but increasing levels of contamination did not show further reduction in the yield using fresh weight indices (Table 2).

There was no significant reduction in the dry weight of the 3 plants per pot and the 6 plants per pot treatments but only in the 9 plants per pot treatment, where the contamination caused significant reduction in yield between the control and all other treatments of *P*. *scrobi-culatum* (Table 3). The 15.00% level of contamination recorded higher yields than the 10.00 and 12.50% levels of contamination in the 3 plants per pot treatment with regards to their dry weights. In the 9 plants per pot treatments, the 15.00% fared better than the 5.00% to the 7.50% indicating that the plants perform optimally at high levels of crude oil contamination with higher plant density (Table 3).

The crude oil contamination affected the leaf area of the test plant reducing it significantly. The leaf area in the 3 plants per pot treatments was higher than that in the 6 plants per pot and 9 plants per pot in some levels of contamination because while all the all plants grew in the 3 plants per pot treatments, some died off in the 6 plants per pot and the 9 plants per pot treatments (Table 4). The leaf area recorded in 12.50% level of contamination was higher than that for the 2.50 - 10.00% level of contami-nation using the 3 plants per pot treatment. The 12.50% level of contamination in the 9 plants per pot treatments was significantly higher than the 2.50 and 7.50% levels of contamination (Table 4).

DISCUSSION

Crude oil contamination affected the growth indices of the plant negatively shown by reduced biomass, plant height and leaf area. This was expected as it is already docu-mented in literature. Researchers like Chaineau et al. (1997) recorded a growth rate reduction of beans and wheat by as much of as 80% caused by the effect of petroleum hydrocarbons. Similarly, Gallegos Martinez et al. (2000) reported a reduction in biomass as is seen here in this study, of 3 non-crop plants. On the other hand Merkl et al. (2004) working on weeds like *Centro-sema brasillianum* and *P. maximum* also recorded re-

Table 3. Dry Weight (g) of *Paspalum scrobiculatum* grown in different levels of crude oil contamination.

	Number of plants per pot		
Level of contamination (%)	**3**	**6**	**9**
Control	5.22±0.82[a]	6.92±1.20[a]	16.18±3.77[a]
2.50	4.68±0.67[a]	6.94±0.41[a]	5.95±0.95[b]
5.00	4.28±0.76[a]	5.53±0.81[a]	5.63±1.50[b]
7.50	2.05±0.47[a]	4.50±1.00[a]	6.23±0.22[b]
10.00	1.60±0.16[a]	7.58±1.30[a]	9.90±0.49[b]
12.50	1.60±0.11[a]	7.58±1.02[a]	9.90±1.29[b]
15.00	3.33±0.63[a]	5.82±1.04[a]	7.64±0.14[b]

Letters with same superscript are not significantly different within the same column at $P < 0.05$.

Table 4. Leaf area (cm^2) of *P. scrobiculatum* grown in crude oil contaminated soils.

	Number of plants per pot		
Level of contamination (%)	**3**	**6**	**9**
Control (0.00)	68.47±1.09[a]	64.00±0.35[a]	54.73±1.71[a]
2.50	45.10±7.41[b]	57.20±5.30[b]	47.80±6.57[b]
5.00	50.33±4.07[b]	61.37±7.22[a]	61.53±7.92[a]
7.50	40.33±6.54[c]	55.47±8.39[b]	48.73±5.81[b]
10.00	44.23±6.81[bc]	38.40±2.40[c]	57.67±0.33[a]
12.50	55.43±2.80[c]	48.33±3.06[c]	56.47±1.58[a]
15.00	34.07±1.00[c]	44.83±1.01[c]	41.77±0.82[b]

Letters with same superscript are not significantly different within the same column at $P < 0.05$.

duced biomass. This study also recorded reduced plant height which agrees with the work of Molina-Baharahona et al. (2005). The authors recorded similar results caused by petroleum hydrocarbons in diesel fuel and inferred thatthe negative effect could be due to the impermeability effect of petroleum hydrocarbons or immobilization of nutrients mainly nitrogen or inhibitory effect of some poly-cyclic aromatic compounds. Baud-Grasset et al. (1993) on the other hand says the effect is a plant sensitive response to chemical substances. Research also show that the plant that are able to grow in contaminated sites take up long chain (heavy) alkanes into their roots rapidly and slowly translocate them stems and leaves as result of their low solubility in water (Trapp et al., 1994; Palmouth et al., 2002). Other explanations proffered for this reduced growth is the effect of small aliphatic, aroma-tic, naphthalic and phenolic like compounds in crude oil that may reduce respiration, transpiration and photosyn-thesis II and hormonal stress response (Baker, 1970; Vouillamoz and Milke, 2001; Trapp et al., 2005). These effects however vary with individual plant species and their physiological responses to contaminants (Vega-Jarquin et al., 2001). Phytotoxicity assays help in selecting plant species that are able to withstand high levels of contaminants and screening out those that are not able to establish themselves in such conditions as present in contaminated sites (Kirk et al., 2002). The continued growth of the plant in the presence of the contaminant and other growth indices particularly biomass will determine if the plant is a potential phytoremediant. In this study, *P. scrobiculatum* was able to withstand high levels of crude oil contamination and the dry weight records show that there was no significant difference between the plants that received high doses of the contaminant and the control that had no contaminant. The plant is therefore a good candidate for remediation of crude oil contaminated sites. This agrees with Kirk et al. (2002) which selected alfalfa as the best candidate for petroleum contaminated sites on the basis its high resistance to phytotoxic compounds in petroleum contaminated sites.

The number of plants used per pot in this study affected its performance in the presence of the contaminants. Higher plant density improved performance of the plant against contaminant stress. Dry weights were higher in 8 plants per pot treatment than in 2 plants per pot treatment in the study by Agha et al. (2009) as recorded here in this study where dry weight was higher in the 9 plants per pot treatment than in the 3 plants per pot treatment. This study also agrees with that of Agha et al. (2009) which concluded that higher plant density improves performance of the plant against environmental stress of contamination. The study also agrees with that of Onwugbuta-Enyi and Onuegbu (2008) which concludeed that higher standing crop density improved the performance of plant like *Paspalum vaginata* in the remediation of dredge spoils in the Niger Delta of Nigeria.

In conclusion, this species is worthy of further study with respect to its use in phytoremediation of crude oil polluted sites which abound in oil producing countries like Nigeria, Venezuela, Ecuador and Indonesia that are found in the tropics.

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