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Phytoremediation of xylene polluted environment, using a macrophyte *Commelina benghalensis L.*

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ABSTRACT

Phytoremediation of xylene polluted environment was carried out in the laboratory using Commelina benghalensis. The plant was grown in 0%, 2%, 4%, 6% and 8% concentration of water saturated fractions of xylene. Growth parameters such as numbers of leaves, formation of aerial root along with the uptake of petroleum hydrocarbon by Commelina benghalensis was used to determined its hyperaccumulative capacity. A range of (0.018- 0.027) mg/L was recorded in 0%-8% concentration respectively. Maximum uptake of (0.034mg/L) was recorded for 8% while the minimum uptake of (0.002 mg/L) was recorded for 2%. Death rate of plants in lower concentration (2%) was observed to be less pronounced than other concentration (4%-8%). Generally, formation of aerial root was observed in all concentrations of treatment culture, this was observed to be higher also in lower concentrations(2% and 4%) than higher concentrations(6% and 8%). The result of this study shows the ability of the macropphyte Commelina benghalensis in uptake and hyperaccumulation of xylene and could be useful in environmental management of hydrocarbon pollution with special emphasis on xylene.

Keywords: *Commelina benghalensis,* phytoremediation, hyperaccumulation, Aquatic macrophtye, environmental management and hydrocarbons pollution.

INTRODUCTION

Commelina benghalensis” Linn; belongs to the family Commelinaceae (Wunderlin and Hansen, 2008). The plant is commonly known as the Benghal day flower or tropical spiderwort. *Commelina benghalensis* is a wide-ranging plant, being native to tropical and sub-tropical Asia and Africa, an area otherwise known as the paleotropics. The plant is a widely distributed herbaceous weed that commonly invades agricultural site and disturbed areas (USDA, ARS, 2008). Though not commonly reported to invade natural areas, this rapidly reproducing plant is considered as one of the most troublesome weeds for 25 crops in 29 different countries (Webster *et al*., 2003). In its native range, it is a rainy season weed which requires moist soil condition for establishment. Once established, it has a high drought tolerance. It grows well on all soil types of variable pH and moisture levels (Webster *et al*., 2003). It flourishes well in flooded plains. It is a common inhabitant of flooded ponds.

Phytoremediation is the use of plants and their associated microbes for environmental cleanup. It can be defined as the enhancing of rehabilitation of an impacted ecosystem by plants (Rupassara, 2002).This technology is increasingly becoming an important application in environmental and ecological research owing to its cost-effective and environmentally-friendly nature. The efficiency of phytoremediation depends mostly on the establishment of robust plant-microbe interactions. Plants, through their ‘rhizosphere effects’, support hydrocarbon-degrading microbes that assist in phytoremediation in the root zone. In turn, healthy microbial communities enhance soil nutrient availability to the plants. However, petroleum hydrocarbons are known to be harmful not only to plant growth and development, but also to microbial processes. This is because petroleum hydrocarbons negatively affect photosynthesis and therefore reduce nutrient assimilation and biomass accumulation. In addition, petroleum pollution often results in altered microbial community structure and negatively influence consumption rate of soil resources, soil structure and water stress in petroleum-polluted soil. Petroleum hydrocarbon pollution also intensifies competition between plants and microbes for nutrients during phytoremediation (Nie*et al.*, 2011).However, one major disadvantage of phytoremediation is that it requires a long-term commitment, as the process is dependent on plant growth, tolerance to toxicity and bioaccumulation capacity (Fosket, 1994 andBurken, 2004).

Research and application of phytoremediation for treatment of petroleum hydrocarbon contamination over the past decades has provided much useful information that can be used to design effective remediation systems and drive further improvement and innovation (Kamath, *et al.,* 2007). Plant species are selected for phytoremediation based on their potential to evapotranspirate groundwater, the degradative enzymes they produce, their growth rates and yield, the depth of their root zone, and their ability to bioaccumulate contaminants (Chappell, 1997). This study aims to investigate the use of *Commelinabenghalensis* in phytoremediation of xylene polluted environment.

MATERIALS AND METHODS

Site Description and Samples Collection

Commelina benghalensis L. was collected at Afaha Oku stream in Uyo Local Government Area and the chemical (xylene) was collected at the Department of Botany and Ecological Studies, University of Uyo. The experiment was conducted in research lab II of Botany and Ecological Studies, University of Uyo between August to December, 2012.

Material used

Xylene, distilled water, round bottom transparent bottle, masking tape, marker, cotton wool, hand towel, tissue paper, culture medium, pen, notebook.

Experimental Vessels

Two hundred and fifty mililitres conical flasks were used for both sub culturing and in the main experiment. They were washed thoroughly with detergent and further rinsed with a 40% solution of nitic acid and sulphuric acid separately to remove any trace of algal spore present. The flasks were then oven-dried at 35^{o}c for 72 hrs.

Preparation of Water Saturated Fraction(WSF)

The WSF was prepared according to the method of Anderson *et al.*, (1974). A sample of xylene (75ml) was slowly mixed in an equal volume of distil water in ratio 1: 9 in a 2 litre screw-cap conical flask. This was then placed on Gallen-kamp table top magnetic stirrer and stirred with 7/cm magnetic rod for 24 hrs at room temperature (27^{o}c±2^{o}c). After mixing, the oil-water mixture was allowed to stand overnight in a separating funnel. The filtrate which is the water saturated fraction was separated from the supernatant and referred to as stock or 100% WSF. The stock WSF was diluted with the culture medium serially to give 2%, 4%, 6%, 8% and 0% (control).

Experimental design

Three replicates were made of each treatment concentration in a randomized design.

Experimental Set Up

Three different plants of *Commelina benghalensis L* were grown in each of the replicates of the different concentration of xylene2%, 4%, 6%, 8% and 0% concentration giving a total of nine plants per concentration. The experiment was setup at a south window for sunlight at the research laboratory II of the Department of Botany and Ecological Studies University of Uyo. Daily observation of experimental plant was carried out and when there were observations, such were recorded in our experimental note book. Emphasis was placed on the formation of aerial root as well as root degradation and the nature of leaf.

RESULTS

Table 1 shows morphological responses of *C. benghalensis* to varying concentrations of WSF of xylene. There was increase in death rate of *C. benghalensis* grown in different concentrations. This decrease was observed to be more pronounced in higher concentrations (6% &8%) than lower concentrations (2% &4%). Statistical analysis shows a correlation between concentration and the death rate of *C. benghalensis*. Scorching of leaves was also observed in all experimental concentrations. This was higher in lower concentrations than in higher concentrations then lower concentrations.

Effect of WSF of xylene on root formation of *C. benghalensis* is illustrated in table 2, there was an increase in the root formation in all concentrations of WSF of xylene. There was high evidence of promotion of roots formation by the WSF of xylene at lowers concentration and retards its formation at higher concentrations.

Table 3 illustrates the mean concentration of xylene in *C. benghalensis* exposed to different concentrations of WSF xylene. There was generally uptake in all experimental plant grown in all concentration except control experiment and there was increasing uptake of xylene with respect to increasing concentrations. The pattern of uptake shows that lower uptake was recorded in lower concentration while maximum uptake was recorded in higher concentrations. Significant difference ($p<0.05$) in uptake by the plant was observed in all investigated concentrations.

Table 1: Response of Leaves and Roots of *C. benghalensis* to Different Concentrations of WSF of xylene

	Day 0		Day 2		Day 4		Day 8		Day 12	
Conc	**NHL**	**NHR**	**NHL**	**NHR**	**NHL**	**NHR**	**NHL**	**NHR**	**NHL**	**NHR**
Control	10	3	8± 0.000	3± 0.000	8± 0.000	6± 0.000	10± 0.000	5± 0.000	11± 0.000	4± 0.000
2%	8	5	8.3± 1.155	4.3± 0.577	1.5± 0.000	0± 0.00	1.2± 1.142	0± 0.00	6.3± 0.578	0± 0.00
4%	8	6	6.6± 2.082	3± 1.00	5.6± 1.155	0± 0.00	4.5± 2.122	0.3± 0.83	5.3± 3.05	0± 0.00
6%	11	4	9± 3.615	2.6± 1.155	3.3± 0.577	1.0± 0.00	5.3± 1.523	0± 0.00	7.3± 3.22	0.3± 0.33
8%	7	5	7.3± 0.577	4.3± 0.155	1.6± 0.80	0± 0.00	5.3± 3.22	0± 0.05	8.3± 4.60	0± 0.00

Mean value of replicate
NHL=Number of Healthy Leaf
NHR=Number of Healthy Root

Table 2: Effect of WSF of Xylene on Root Formation of *Commelina benghalensis*

	Day 0		Day 2		Day 4		Day 8		Day 12	
Conc	**NAR**	**NNR**	**NAR**	**NNR**	**NAR**	**NNR**	**NAR**	**NNR**	**NAR**	**NNR**
Control	9	0	8	2	7	2	7	3	7	5
2%	13	0	12	6	11	7	9	8	11	8
4%	18	0	16	6	15	15	14	13	15	13
6%	11	0	10	6	7	11	8	8	10	7
8%	11	0	10	4	5	7	5	8	9	8

NAR = No of aerial roots
NNR = No of normal roots

Table 3: Mean Concentration of Xylene mg/L in *Commelina benghalensis*

Treatments	**0%**	**2%**	**4%**	**6%**	**8%**
Conc mg/L	0.005+ .01	0.007+ 012	0.018+.12	0.027+0.034	0.034+0.023

DISCUSSION

The ability of plants to bioaccumulate, degrade or render harmless contaminants in soil, water or in air varies from plant to plant also the prevailing factors in such habitat. Contaminants such as metals, pesticides, solvents, explosives, crude oil and its derivations have been mitigated by phytoremediation. Many plants such as mustard plants, alpine pennycreas, hemp, and pigweed have proven to be successful at hyperaccumulating contaminants at toxic waste sites (Salt *et al.*, 1998).

In this study, *C. benghalensis* was found to hyperaccumulate xylene to varying degree when exposed to different doses of the WSF. Various responses were shown by the plant which suggestion its ability to bioaccumulate the investigated hydrocarbon.

The following findings were obvious from this study:
- The experimental plant (*C. benghalensis*) grew luxuriantly in both control and treatment culture
- Degenerating rate of roots in higher concentration (8%) was more pronounced than other concentrations (2%, 4% and 6%) concentration respectively (Table 1).
- There was increase in number of leaves in control as expected from the beginning of the experiment to the end of the experiment (Table 1)
- Root formation was enhanced by WSF of xylene at lower concentration (Table 2).

The growth pattern of *C. benghalensis* in control in this study was expected, various authors have reported the growth of plants in environment which are conducive for their growth, such as presence of growth factors (nutrients), water etc. Since the control was made up of distilled water and free from toxic materials, it is expected that the plant will follow the normal growth pattern or growth curve as described by sigmoid. The growth in treatment cultures shows that the plant was utilizing the hydrocarbon content. This finding is supported by the finding of De - yong (1986).

The enhancement of root formation by WSF of xylene (Table 2) in*C. benghalensis* could be due to physiological reasons such as increase in uptake of substance and increase in proliferation of meristematic cells giving rise to formation of new roots. It could also be a survival measure. If the older roots are affected physiologically due to uptake of hydrocarbons and could no longer carry out their functions, then, the formation of new roots within the shortest period of exposure could be a survival mechanism to overcome the effect of the hydrocarbon.

CONCLUSION

The macrophyte*C. benghalensis* show excellent ability in bioaccumulation of xylene and thus could be useful in bioremediation xylene.

This work only accessed the effect of WSF of xylene. I hereby recommend that other common persistent hydrocarbons should be used to give a firm conclusion on its ability as a hyperaccumulator.

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