IMPACT OF PETROLEUM WASTE WATER ON THE MORPHOLOGICAL ATTRIBUTES OF *Heteranthera callifolia* RCHB. EX KUNTH

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ABSTRACT

Morphological responses of *Heteranthera callifolia* to petroleum waste water was investigated in the Laboratory. A 96 hour toxicity test was carried out by exposing stabilized suckers of *H. callifolia* to 0%, 2%, 4%, 6% and 8% concentrations of Water Saturated Fraction (WSF) of hexane. Distinctive marginal leaf burn was observed in treatments with higher concentrations (6% and 8%) of WSF. Other adverse impact include serious damage to terminal buds, suppression and loss of apical dominance, increased deterioration of lower and mid petioles of leaves with increasing exposure and concentrations. Morphological stress was persistent and continued with decline in quality of growth. This eventually led to complete death of plant within eight days of exposure at all concentrations except the control. Scorching of leaves was also observed in mid and higher concentrations (4%, 6% and 8%). There was complete smothering in higher concentration while in lower concentrations there was evidence of regeneration after twenty eight day of exposure. Regrowth was completely absent in plants exposed to 8% level of pollution. Root formation was also seriously impacted by 8% concentration level. The result shows that *H. callifolia* is sensitive to WSF of petroleum hydrocarbon (hexane) and as such could be an addition to the list of existing aquatic macrophyte that could be used as bio-indicator of hydrocarbon pollution in aquatic ecosystem.

INTRODUCTION

Remediation is a programme of activities designed to rehabilitate an impacted ecosystem. Phytoremediation is a form of bioremediation which applies the use of biological processes to detoxify polluted sites. It can be defined as the enhancing of rehabilitation of an impacted ecosystem by plants (Rupassara, 2002). It may be applied wherever the soil or static water environment has become polluted or is suffering ongoing chronic pollution. It has been used successfully in the restoration of abandoned metal-mine, reducing the impact of sites impacted with polychlorinated biphenyls, mitigation of on-going coal mine discharges. Phytoremediation focuses on the natural ability of certain plants able to bio-accumulate, degrade or render harmless contaminants in soils, water or air (Gresshoff, 1993). Contaminants such as metals, pesticides, solvents, explosives and crude oil and its derivatives have been mitigated in phytoremediation project worldwide.

In modern day technology phytoremediation is considered a clean, cost-effective and non-environmentally disruptive technology. This technology has become increasingly popular over the past 20 years and has been employed at sites with soil contaminated with lead, uranium and arsenic. 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 and Burken *et al*., 2011).

This study is aimed at assessing the effect of water saturated fraction of hexane on morphological attributes of *Heteranthera callifolia*.

MATERIALS AND METHODS

Climate of the Study Area:

Uyo Local Government Area is located north of the equator, within the humid tropics and its proximity to the sea makes it generally humid. It is characterized by two season, dry and wet or rainy seasons. Generally, the wet season is characterized by relatively heavy rainfall and high humidity with heavy clouds covering the sun. Less rainfall, low cloud cover and air increase in solar radiation reaching the earth surface due to less cloud cover in contrast characterizes the dry season.

Uyo Local Government Area lies between latitude 5.05$^{\mathbf{0}}$N and longitude 80$^{\mathbf{0}}$E. This is within the equatorial rainforest belt, which is a tropical zone that house vegetation of green foliage of trees, shrubs and oil palm trees.

Experimental plant

The experimental plant (*H. callifolia)* was collected from a drainage system emptying into a stream at Ikpa road behind University of Uyo, Uyo, Akwa Ibom State. The plant species was ascertained by a taxonomist before used in the study.

Preparation of Water Saturated Fraction of Hexane

Water saturated fraction (WSF) of hexane was prepared according to the method of Anderson *et al*. (1974). A sample of hexane was slowly mixed in an equal volume of distil water in 1:1 ratio in a 2 litre screw – cap conical flask. This was placed on Gallen-kamp table top magnetic stirrer and stirred with 7cm magnetic rod for 24hrs at room temperature ($28^0 \pm 2^0$C)**.** After mixing the water and oil, the 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 was diluted with distilled water serially to give 0%, 2%, 4%, 6% and 8% WSF respectively.

Stabilization of Test Plant

The experimental vessels used were 950ml round bottom transparent bottle. They were washed thoroughly with detergent and further rinsed with 70% sulphuric acid solution to remove organic contaminants. Prior to the introduction of *H. callifolia* into the laboratory macrocosm, the roots were rinsed with running tap water to remove any contaminant from the field from where it was harvested. It was then transferred to a 950ml transparent round bottom culture bottle filled with tap water and left for four days to stabilize and adjust to culture life. After four days of stabilization the plants were then transferred to experimental set up containing various concentrations (0%, 2%, 4%, 6% and 8%) of water saturated fraction of hexane.

Statistical analysis

The results were subjected to a two-way analysis of variance (ANOVA) to determine the level of significance (Ubom, 2004).

RESULTS

The effects of WSF of hexane on the morphological attributes of H. Callifolia are presented in Tables 1- 4. Table 1 shows a 96hours comparative marginal leaf burn pattern of leaves of *H. callifolia* in WSF of hexane at different concentrations. The results revealed a range of 0% - 25% PNLWAE (Percentage numbers of leaves with adverse effects) for plants grown in control (0%) and those in lower concentration (2%). Plants grown in mid concentration (4%) recorded a range of 0%-46% of PNLWAE while those grown in higher concentrations (6% and 8%) have a range of 0%- 100%.

Table 1: Comparative marginal leaf burn pattern of leaves of *H. callifolia* exposed to WSF of hexane for 96 hours

Concentrations	24hrs (PNLWAE)	48hrs (PNLWAE)	72hrs (PNLWAE)	96hrs (PNLWAE)
0%	0	0	0	0
2%	0	10	15	25
4%	0	28	39	46
6%	0	55	100	100
8%	0	70	100	100

PNLWAE: Percentage numbers of leaves with adverse effects, n= 12

Table 2: Comparative terminal buds of leaves of *H. callifolia* exposed to WSF of hexane for 96 hours

Concentrations	24hrs PTBWAE	48hrs PTBWAE	72hrs PTBWAE	96hrs PTBWAE
0%	0	0	0	0
2%	0	7	18	22
4%	0	12	28	33
6%	0	17	35	77
8%	0	23	56	92

PTBWAE: Percentage terminal buds with adverse effects, n = 24

Table 3: Percentage deterioration of petioles of *H .callifolia* exposed to WSF of Hexane for 28 days.

Concentrations	Day 7 PDPWAE	Day14 PDPWAE	Day 21 PDPWAE	Day 28 PDPWAE
0%	0	0	0	0
2%	0	0	0	2
4%	0	0	0	2
6%	0	0	2	5
8%	0	0	5	8

PDPWAE: Percentage deteriorated petioles with adverse effects, n = 12

The results also revealed a gradual increase in adverse effect with increasing concentration and age of the culture. Apart from the control (0% WSF) which had normal plants growth, treatment with 2% WSF led to 10% marginal leaf burn at 48hrs, 4% WSF led to 28% marginal leaf burn, 6% and 8% WSF concentrations led to 55% and 100% marginal leaf burn respectively after 48hrs exposure. However, 100% marginal leaf burn were recorded after treatment with higher levels of WSF for 96hours. The effect of WSF of hexane on terminal bud of *H. callifolia* varied with the WSF concentration and duration of exposure. At 48hrs of exposure, percentage terminal buds with adverse effects (PTBWAE) was 7% in plants exposed to 2% WSF, 12% in plants exposed to 4% WSF, 17% in plants exposed to 6% WSF and 28% in plants exposed to 8% WSF. At 96hrs, PNLWAE for plants cultured in 2% WSF was 22%, 33% for those in 4% and, 77% and 92% for those grown in 6% and 8% WSF concentration respectively. No deterioration of plant petioles was observed in the first 7 days of exposure to all concentrations of WSF. However, 2% and 5% of experimental plant petioles exposed to 6% and 8% WSF showed signs of petiole deterioration after 14 days of exposure and significant deterioration of plant petioles was noticed after 28 days exposure to 6% and 8% WSF (Table 3). On the other hand, high numbers of root were formation was observed within 2 weeks of exposure to all WSF concentrations. The highest root development was observed in control culture and those exposed to 2 – 4% WSF. However, there was a gradual decrease in roots formation rate overtime (Table 4).

Table 4: level of root formation of *H. callifolia* exposed to WSF of Hexane for 28 days.

Concentrations	Day 7 NRF	Day 14 NRF	Day 21 NRF	Day 28 NRF
0%	11	17	5	3
2%	10	6	2	2
4%	8	7	2	1
6%	6	3	0	0
8%	3	2	0	0

NRF = numbers of roots formed

DISCUSSION

Environmental pollutants and contamination has its own damaging effects to plants, animals and ultimately to human health. It has been noted that different organisms reacts differently to the same dose of pollutants. While some show sign of toxicity to a particular pollutants, others may not and some may even have added advantage of inherent capacity to mineralize such pollutant. While some pollutants are persistent and thus add greatly to the total load of such environments others are not as they are constantly be removed by the activities of plants and microorganisms. Carcinogenic ones may thus pose serious health challenge to humans. Of recent, great efforts have been made to reduce pollution sources and its effects on environment. Contaminated soil, air and water resources have recently been remedied using phytoremediation approach. More than 400 plant species have been identified to have potential for soil and water remediation (McGrath *et al.*, 2001, Nriagu and Pacyna, 1988).

Phytoremediation approaches have been widely used for remedying polluted soil and water, especially at small scale and the use of plants species for cleaning polluted soils and waters has gained increasing attention. In this study, it was observed that *H. callifolia* responded differently to increasing concentrations of WSF of hexane. Morphological responses to hydrocarbon pollution shown include serious damage to leaves and petioles. Distinctive marginal leaf burn was observed in treatments with higher concentrations of WSF (Table1). Other additional responses include serious damage to terminal buds, suppression and loss of apical dominance, increased deterioration of lower and mid petiole of leaves with increase in concentrations of WSF and exposure time (Table 3). Responses persist and were characterized by a decline in quality of growth which eventually led to complete death of experimental plants in all concentration except control after twenty eight days of exposure. Scorching of leaves was also observed in mid and higher concentrations. There was complete death of all foliage leaves (smothering) in high concentrations while in lower concentrations there was evidence of regrowth of new leaves after twenty eight day of exposure. Root formation was highly affected by exposure to high WSF concentrations (Table 4).

The results of this study show that *H. callifolia* is very sensitive to WSF of petroleum hydrocarbon (hexane), the effect however vary with concentrations of petroleum pollutant and duration of exposure. However, the finding is different from results of other authors on hydrocarbon uptake by different plants. Durmishide (1977) reported that benzene, toluene and xylene were metabolized by cereal grasses in only two to three days; within the green mass of corn in four to five days and by root crops in five to six days. Edwards *et al.,* (1982) also reported that soybean was capable of degrading C-anthracene. Evidence of the degradation was given by measuring the $^{14}Co_2$ given off from the plants placed in ^{14}C- anthracene – contaminated soil. Yateem *et al.,* (2000) investigated the degradation of Total Petroleum Hydrocarbons (TPH) in the rhizosphere and non-rhizosphere soil of three domestic plants namely *Medicago Sativa, Vicia faba* and *Lolium perenne*. Although the three domestic plants exhibited normal growth in the presence of 1% TPH, the degradation was more profound in the case of leguminous plants. They found that the degradation rates in soil cultivated with (broad bean) *Medicago sativa, Vicia faba* (alfalfa) were 36.6 and 35.8% respectively, as compared with 2% degradation in case of *Lolium perenne* ryegrass. Rosada and Pichtel (2004) studied the decomposition of used motor oil in soil as influence by plant treatment. Soil contaminated with used oil (1.5% w/w) was seeded with soybean (*Glycine max*), green bean (*Phaseolus vulgaris),* sunflower (*Helianthus annus*) and Indian mustard (*Brassica juncea)* mixed with *Zea mays* and *Trifoleum repense*. After 150 days in the clover treatment the added oil was no longer detected.

CONCLUSION

This study shows that *H. callifolia* is sensitive to WSF of petroleum hydrocarbon (hexane) and may not be a useful phytoremediation agent but could be an addition to the list of existing aquatic plants (macrophyte) that could be used as bio-indicator of hexane pollution in aquatic ecosystem. However we recommend the trials of other aquatic macrophytes to compare their responses to water saturated fraction of hexane and other petroleum hydrocarbons

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