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Phytoextraction of Total Petroluem Hydrocarbon In Polluted Environment Using An Aquatic Macrophyte Heteranthera callifolia Rchb. Ex Kunth

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ABSTRACT
Laboratory study on phytoextraction of total petroleum hydrocarbons (TPH) in polluted Environment using Heteranthera callifolia was carried out. The sea weed was grown in the laboratory in 0%, 2%, 4%, 6% and 8% concentrations of water saturated fraction (WSF) of Hexane for 4 weeks. The various concentrations of TPH bioaccumulated in roots, petioles and leaves were estimated using standard laboratory procedures. The leaves had the highest concentration of TPH (0.434 ± 0.170) mg/L followed by the petioles (0.202l ± 0.116) mg/L while the roots had the least uptake of TPH (0.096 ± 0.080) mg/L. The result of this study shows that the experimental plant exhibit high level of uptake of TPH. This could be useful in setting up a list of aquatic macrophyte that could be used as bio-indicator of TPH pollution in aquatic ecosystem and could be added to the list of aquatic plants with TPH uptake potential and can also be implicated in bioremediation protocol.

KEYWORDS: *Sea weeds, Bioenergy, Phytoextraction, uptake potential, bioremediation protocol, Petroleum hydrocarbons, pollution, bioindicator*.

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I. INTRODUCTION

Heteranthera callifolia is a glabrous, aquatic herb about 2.5cm high with submerged stems rooting in the substrate that reproduces from seed. The leaves have long hollow petioles up to 25cm long that raise the leave blades above the water. The blade are ovate to lanceolate, about 5-7cm long and 1.5cm wide, heart shaped or rounded at the base rather thin, smooth and with numerous ascending nerves at the undersurface. The inflorescence is a few-flowered spike; the flowers are small and white and have tubes about 5mm long and lobes that are about 4mm long. It is found in shallow water or immersed at edges. It is also a weed of lowland rice and water logged soils, it is widely distributed throughout West Africa.

The plant can tolerate extremes water level fluctuation and seasonal variations in flow velocity and variation of nutrient availability, pH, temperature and toxic substances [5]. Due to its extremely high rate of development is an excellent source of biomass and thus Play unique role in bioenergy production.

Various plants have been identified for their potential to facilitate the phytoremediation of sites contaminated with petroleum hydrocarbon. In the majority of studies, grasses and legumes have been singled out for their potential [4,10,7]. However, [11] reported that water hyacinth *(Eichhornia Crassipes*) significantly accumulated petroleum hydrocarbon. Prairie grasses are thought to make superior vehicles for Phytoremediation because they have extensive, fibrous root system [11]. They also exhibit an inherent genetic diversity, which may give them competitive advantage in becoming established under unfavourable soil condition [4]. Legumes are thought to have an advantage over non-leguminous plants in phytoremediation because of their ability to fix nitrogen. They do not have to compete with micro-organisms and other plants for limited supplies of available soil nitrogen at oil contaminated sites [6].

Heteranthera califolia belongs to the family Pontederiaceae, and this family possesses some species of weeds described as the most troublesome in the world, they have been linked to several problems like obstruction to water transportation, micro-habitat for disease vectors, obstruction to fishing and reduction in biodiversity. Recent studies have shown that this macrophyte can be used for the production of paper, biogas, fertilizer and fish feed.

This paper is aimed at evaluating the ability of *H. califfolia* to grow in polluted environment with petroleum hydrocarbon and also to ascertain its uptake potential and finally to assess its bioremediation capacity in cleanup of the environment.

II. MATERIALS AND METHODS

Experimental Plant:

The plant used in this study is *Heteranthera callifolia*. It was collected from a drainage system emptying into a stream at Ikpa road in Afaha Oku village, behind University of Uyo, Uyo Local Government Area of Akwa Ibom State. This was identified using West African weeds by [2].

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.

Geography of the area:

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

Stabilization of Test Plant:

On introduction of *Heteranthera callifolia* into the laboratory, 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. These were then Transfer to experimental set up containing the various concentrations of the petroleum hydrocarbons after four days of stabilization.

Experimental Vessels:

The experimental vessels used were 950ml round bottom transparent bottle. They were washed thoroughly with detergent and rinsed with 70% sulphuric acid and nitric acid solution to remove any trace of algal spore present.

Preparation of water saturated fraction of hexane:

Water saturated fraction (WSF) was prepared according to the method of [3]. A sample of hexane was slowly mixed in an equal volume of distil water in ration 1: 9 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 ($27^{0}C+2^{0}C$)**.** After mixing, the water and oil 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 the various experimental concentrations (0%, 2%, 4%, 6% and 8%) respectively.

Statistical Analysis:

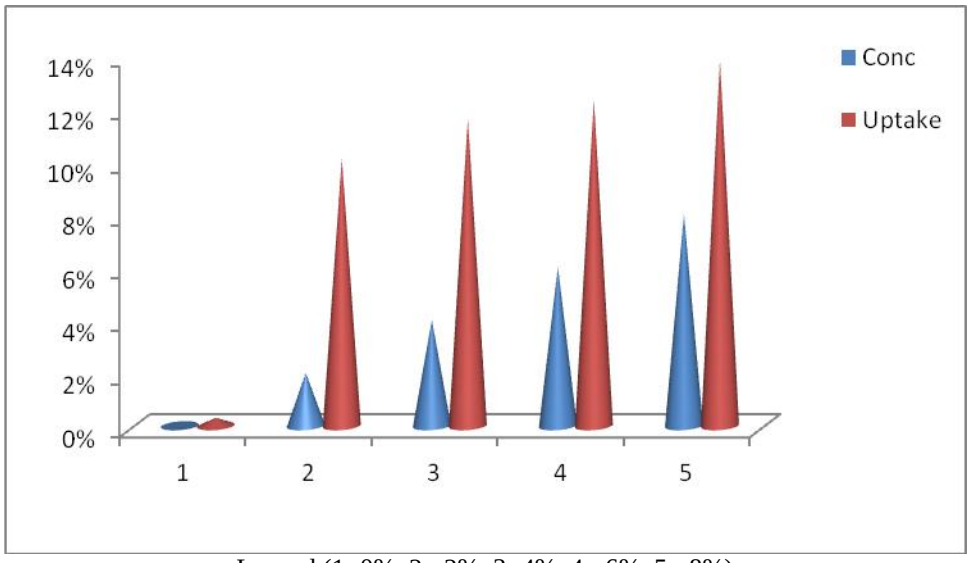
The results were subjected to a two-way analysis of variance (ANOVA) to determine the level of significance [14].

III. RESULTS

Percentage uptake and extraction potential of *H. callifolia* in different concentrations of WSF of hexane is shown in fig 1-3. There was substantial uptake in the three different parts of the experimental plant namely roots, petioles and leaves. Uptake followed expected pattern. The highest uptake (0.434 ± 0.170) mg/L was recorded in the leaves of the experimental plant.

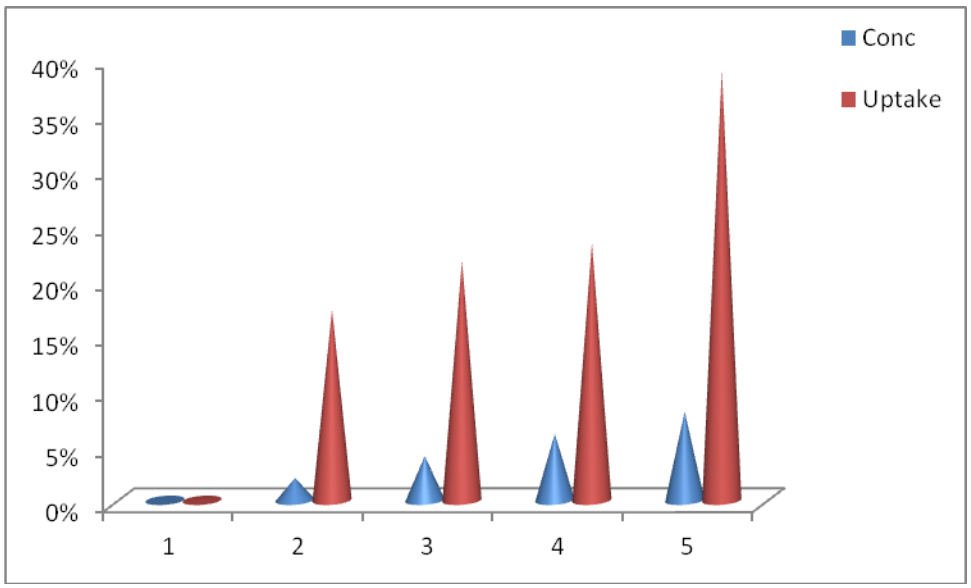
Fig 1: shows percentage uptake of petroleum hydrocarbon (hexane) in roots of *H. callifolia* at different concentrations. The value (0.101 ± 0.044) mg/L, (0.116 ± 0.011) mg/L, (0.123 ± 0.014) mg/L, and (0.0138 ± 0.033) mg/L were taken up by the root of *H. callifolia* in 2%, 4%, 6% and 8% concentrations respectively. These values were highly significant ($p<0.05$) when compared to (0.003 ± 0.001) mg/L in the root of the control plant. Percentage uptake of petroleum hydrocarbon in petioles of *H. callifolia* in different concentrations is shown in fig 2. A mean of (0.172 ± 0.008) mg/L, (0.216 ± 0.005) mg/L, (0.232 ± 0.013) mg/l and (0.388 ± 0.012) mg/l were recorded for petioles of *H. callifolia* grown in 2%, 4%, 6% and 8% concentrations. These values were also significantly high compare to (0.001 ± 0.005) mg/l obtained in control plant.

Fig 3 shows the percentage uptake of petroleum hydrocarbon in leaf of *H. callifolia* in different concentrations of water saturated fractions of hexane. A total mean of (0.431 ± 0.016) mg/L, (0.518 ± 0.082) mg/L, (0.542 ± 0.008) mg/L, (0.684 ± 0.037) mg/L were taken up by the leaves of *H. callifolia* in the different concentrations. In general, the amount of petroleum hydrocarbons taken up by *H. callifolia* increased gradually with increase in concentration. The highest uptake was recorded in 8% concentration in all three parts of the plant investigated.



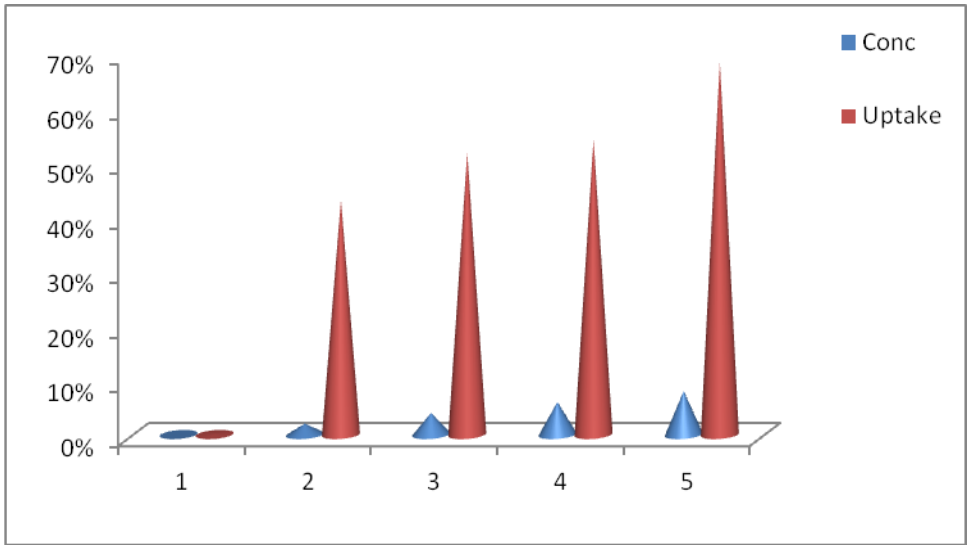
Legend (1=0%, 2= 2%, 3=4%, 4= 6%, 5= 8%)

Fig 1: Percentage uptake of petroleum hydrocarbon in root of *H. callifolia* in different experimental concentrations



Legend (1=0%, 2= 2%, 3=4%, 4= 6%, 5= 8%)

Fig 2: Percentage uptake of petroleum hydrocarbon in petioles of *H. callifolia* in different experimental concentrations



Legend (1=0%, 2= 2%, 3=4%, 4= 6%, 5= 8%)

Fig 3: Percentage uptake of petroleum hydrocarbon in leaves of *H. callifolia* in different experimental concentrations

IV. DISCUSSION

Land and water are precious natural resources on which rely the sustainability of agriculture and the civilization of mankind. Unfortunately, they have been subjected to maximum exploitation and severe degraded and pollution due to anthropogenic activities. The pollution includes point sources such as emission, effluents and solid discharge from industries, vehicle, exhausts and nonpoint sources such as soluble salts, insecticides, pesticides, disposal of industrial and municipal wastes and excessive use of fertilizers [8,9,13]. One of the most common pollutants in the Niger delta coast is crude oil pollution. This has released tremendous amount of hydrocarbons into the coast and its surrounding water sheds and wetlands.

This study however seeks to evaluate the efficiency of using *H. callifolia* in cleanup of petroleum hydrocarbon (Hexane). The following findings were observed in this study.

- *Heteranthera callifolia* was able to absorb various amounts of water saturated fractions of Hexane.
- The highest uptake was observed in plants grown in higher concentrations 6% and 8%
- The leaves had the highest concentration of the TPH (0.434 ± 0.170) mg/L followed by the petioles (0.202l ± 0.116) mg/L, the roots had the least uptake of TPH (0.096 ± 0.080) mg/L.
- The result of this study shows that the experimental plant exhibit high level of uptake of TPH.

This finding corroborates the findings of [10] who observed that in a three year field plot that prairie buffalo grass accelerated the reduction of naphthalene in a clay soil compared to unpolluted clay soil. The authors conducted a parallel experiment to assess the performance of 12 warm seasons grass species to remove various PAHs from contaminated soil. Results indicated that prairie buffalo grass, common buffalo grass, Meyer Zoysia grass and Verde Klein grass accelerated the loss of the low molecular weight PAHs naphthalene, fluorine and phenanthrene compared to the control. [15] investigated the degradation of total petroleum hydrocarbons (TPH) in the rhizosphere and non-rhizosphere soil of three domestic plants namely alfalfa (*Medicage sativa*), broad bean (*Vicia faba*) and ryegrass *(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. [1] found that the legume plant (*Vicia sativa*) was able to grow in soil contaminated with diesel fuel and the total numbers of nodules were significantly reduced in contaminated plants as compared to control plants, but nodules on contaminated plants were more developed than corresponding nodules on control plants. These authors found that the amount of diesel fuel remaining after 4 months in the legume plant *Vicia sativa* was slightly less than in the ryegrass planted soil. [12] studied the decomposition of used motor oil in soil as influenced by plant treatment. Soil contaminated with used motor oil (1.5% w/w) was seeded with soybean (*Glycine max*) green bean *(Phaseolus Vulgaris)*, sunflower *(Helianthus annus)*, Indian mustard *(Bressica juncea)*, mixed grasses/maize *(zea mays)* and mixed clover *(Trifoleum partense*, L. *Trifoleum repense)*. After 150 days in the clover treatment, the added oil was no longer detected. A total of 67% of the oil was removed in sunflower/mustard and with addition of NPK fertilizer; treatment resulted in a 38% oil reduction, which increased to 67% with fertilizer application.

The experimental plant exhibits high level of uptake of TPH. The leaves had the highest concentration of the TPH (0.434 ± 0.170) mg/L followed by the petioles (0.202l ± 0.116) mg/L, the roots had the least uptake of TPH (0.096 ± 0.080) mg/L. This pattern of uptake could have been influenced by physiological process involves in translocation and transpiration in plants. Manufactured products of photosynthesis are reported to be transported down from the regions of production. Water and mineral salts are transported in opposite direction to products of photosynthesis. The various concentrations in leaves and petioles could have been transported to this region for storage and utilization in photosynthesis.

V. CONCLUSION

The result of this study shows that *H. callifolia* exhibits excellent capacity to biaccumulate petroleum hydrocarbon (hexane) in the roots, petioles and leaves and thus could be assayed for bioremediation potential for petroluem hydrocarbons in respect to hexane pollution.

VI. RECOMMENDATION

Based on these findings, and the level of work done, it is recommend that further work should be carried out using other petroleum hydrocarbons to compare the uptake ability of these hydrocarbons by this aquatic macrophyte.

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