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Bioremediation Study of Crude Oil Polluted Soil with Water Hyacinth (*Eichhornia Crassipes*) and Spent Mushroom Compost

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ABSTRACT

A lot of hazard from polluted environment in the Niger Delta have obstructed Ecosystem balance, comparing bioremediation study with water hyacinth (Eichhornia crassipes) have done good to the environment analysis to ascertain the Stoichiometric loss in TPH which ranging from, Changes in soil nitrate and phosphate levels, Levels of heavy/trace metals, Changes in soil pH composted soil was tested for bioremediation of aged crude oil-polluted soil. spent mushroom compost, water hyacinth compost, water hyacinth compost + spent mushroom compost, and unamended soil were all applied at 10% each to 1000g of polluted soil. TPH degradation occurred in the following order: control< SMC < WHC< SMC+WHC. All treatments increased TPH with WHC having the highest bio-stimulatory effect followed by SMC+WHC. This study will help in restoration of agricultural land and bio-diversity and large scale control of water hyacinth in Nigerian waters, the research is necessitated by the need to proffer an appropriate solution to the menace of Water Hyacinth (Eichhornia crassipes) in Nigerian fresh waters. In addition, this research will address the insufficient supply of biofertilizer to improve crude oil impacted sites in the Niger Delta. As a supply of limiting nutrients required for the bioremediation of crude oil-impacted media, organic nutrient sources like Eichhornia crassipes nutrient powder are beneficial.

Keyword: *Ecosystem, bioremediation, water hyacinth, nutrient, restoration.*

INTRODUCTION

The use of organic amendment would enhance a more eco-friendly remediation approach with consequent improvement of the soil after the remediation exercise. Food security, agricultural sustainability and environmental pollution have been critical issues of global concern. Environmental and anthropogenic activities have resulted in depletion of soil nutrients (nitrogen (N) and phosphorus (P) which are the limiting nutrients based on Liebig's Law of the Minimum). This has led to a drastic drop in agricultural yield in the Niger Delta. Development in the Niger Delta has inevitable impacts arising from crude oil activities, pipeline sabotage and artisanal refining activities (kpo-fire) with resultant leaks of hydrocarbon into the environment have caused loss of coastal and agricultural lands among others.(Bian et al 2019), Water Hyacinth (*Eichhornia crassipes*) is a renewable aquatic resource that is nuisance (waste) in Nigeria but can be turned to product (wealth) by its application as either a nutrient source for oil eating microbes or soil conditioner, (Chikere et al., 2019) if composted Compost is a green chemistry product, that is cheap, safe and ecofriendly. (Shen et al 2019) it is an excellent substitute to inorganic fertilizer. Water Hyacinth has spread in Niger Delta Fresh Waters and also infested 20 of the 36 States as well as Abuja the Federal Capital

Territory (NIFF, 2000). The high financial cost and health issues associated with the use of chemical fertilizer have made this research important in order to proffer a more economic and eco-friendlier alternative. Having negative impact on the socio-economic activities on rivers and communities thus threatening fishing jobs and a vital food source. The use of water hyacinth grass (*Eichornia crassipes*) as an organic amendment, (bio-fertilizer), have significantly aided in the implementation of bioremediation procedures, which provide a range of measures that are not only economical and successful in restoring the site, but also have improved the overall cleanup efforts of contaminated areas (Rothe et al 2019).

The potential use of water hyacinth as a nitrogen supply in the bioremediation process of soils contaminated by crude oil was examined in this study.

Fig. 1:Water Hyacinth (*Eichhornia crassipes*) Plant Studied.

METHODLOGY

Collection and Preparation of Materials

Water hyacinth plant was hunt and gotten from a new calabar river in River state. Both a manual and an industrial grinder were used to shred it after it had been sun-dried. The amount of carbon, nitrogen, and phosphorus in the groundwater hyacinth was measured in order to validate the water hyacinth grass's remediation capabilities. From the Ahoda community River states, crude oil samples were gathered, and their physicochemical characteristics were examined. Samples of agricultural soil (subsoil) were gathered from a farm in the Choba village. The following physicochemical characteristics of the soil samples were examined: pH, phosphate, potassium, nitrate, sodium, calcium, magnesium, organic matter, total organic carbon (TOC), volatile matter, and ash content. (Orji and others, 2013). In compliance with Standard Test Methods (ASTMD), the solvent extraction method was used to perform the physicochemical evaluations of each test sample.

Treatment and Analysis

Aside et al. (2019) state that heavy crude oil and appropriate agricultural soil were used to imitate crude oil-contaminated soil samples in a lab setting. Remediation was only examined at 5, 10, and 20% crude oil contaminated soil, respectively, because soil containing more than 5% TPH by weight of crude oil does not decompose well (Deuel & Holliday, 2016). Additionally, the 140–160 mm container used for bioremediation held soil tainted by crude oil.

Remediation Method:

Different concentrations of heavy crude oil (1.0, 2.0, 3.0, and 4.0%) were combined with equal amounts (1 kg) of soil (Sherem et al 2019). Different amounts of water hyacinth (10, 20, 30, and 40g) were combined with various concentrations of the contaminated soil samples. A 1.5-liter jar held the different combinations (Brown et al., 2008). Each setup's contents were well combined to guarantee that the water hyacinth and crude oil were distributed evenly. In order to give the containers enough oxygen and a favorable environment for the growth of bacteria, the experiment was permitted to begin and they were watered and stirred twice a week. Measurements were made of the total hydrocarbon content (THC) and pH. Throughout the experiment, an ambient temperature range of 25 to 35 degrees Celsius was maintained. The chemical makeup was consistent with Chang (2000). Using a 10 m soil auger, 50 cm of loamy soil samples were taken straight from the botanical garden for physicochemical examination and soil preparation. Before being used for amendment, the physicochemical parameters of the control and crude oil-contaminated soils were assessed in accordance with the Association of Official Analytical Chemists' (AOAC) standard methods.

Statistical analysis

Using SPSS (8th edition), data were evaluated as mean and standard error after a two-way analysis of variance (ANOVA). A p-value of (0.05) was used to determine the statistical significance of the values acquired.

RESULTS AND DISCUSSION

The results of investigations as shown in Table 1 indicate the physiochemical constituent of the various part of a fresh Water Hyacinth plant

Table 1: Physicochemical constituent of Fresh Water Hyacinth

Parameters	Stem	Root
Nitrogen (%)	2.78±0.02^{b}	2.19±0.01^{a}
Phosphorus (%)	4.94±0.02^{b}	4.27±0.01^{a}
Potassium (%)	31.96±0.02^{b}	17.52±0.02^{a}
TOC (%)	10.54±0.01^{b}	8.74±0.03^{a}
TOM (%)	18.11±0.11^{a}	14.85±0.05^{a}
Cellulose (wt.%)	8.97±0.03^{a}	13.89±0.02^{c}
Lignin (wt.%)	3.55±0.03^{a}	7.25±0.03^{a}
Hemicellulose (wt.%)	12.82±0.04^{a}	20.60±0.17^{b}
Reducing Sugar (mg/L)	9.70±0.01^{c}	6.20±0.03^{a}
Wax (wt.%)	18.40±0.16^{c}	11.88±0.02^{a}

Values are mean ± standard deviation (M±S.D) of triplicate determinations (n=3).
Values bearing the different superscript letter (a, b) are significantly different (P<0.05)

Table 2 show the proximate analysis of various parts of fresh water Hyacinth

Table 2: Proximate composition of Fresh Water Hyacinth

Parameter	Stem	Root
Moisture (%)	96.46±0.91	91.4±1.35
Ash (%)	17.02±0.15	38.52±0.42
Crude Lipid (%)	2.99±0.81	1.22±0.63
Crude Fibre (%)	21.18±0.88	12.16±1.00
Crude Protein (%)	11.39±0.90	6.10±1.01

The result of the total heterotrophic count and hydrocarbon utilization bacterial count are shown in table 3 below

Table 3: Monitoring of bacterial growth during enrichment for bacterial isolation

Days	Total heterotrophic bacterial count. (CFU/g)	Hydrocarbon utilizing bacterial count (CFU/g)
5	2.92±0.098 x10^{6}	1.68±0.1 x 10^{6}
10	2.6±0.28 x 10^{6}	1.42±0.06 x10^{6}
15	2.10±0.25x10^{6}	8.0±0.7 x 10^{5}
20	1.87±0.09 x10^{6}	6.8±0.18 x10^{5}

Key: CFU = Colony forming unit

The physicochemical studies of the polluted soil are shown below in table 4

Table 4: Baseline Properties of the polluted soil sample

Parameter	Value
pH	4.94
Electrical Conductivity (µS/cm)	156.72
Total organic carbon TOC (%)	1.44
Total Nitrogen (%)	0.13
Potassium (ppm)	0.31
Phosphorus (ppm)	19.43
Iron (ppm)	1.278
Cadmium (ppm)	3.031
Lead (ppm)	17.521
Zinc (ppm)	0.728
Particle size, sand, clay and silt (%)	86, 10, 4
TPH (ppm)	30,560.06
PAH (ppm)	21,161.88

Key: Data represents average value of a duplicate.

Table 5: Physicochemical of the polluted soil sample analyzed on day zero of bioremediation.

Parameters	Control soil	WHC	SMC	SMC+WHC
pH	6.10±0.01^{c}	6.31±0.01^{b}	5.36±0.03^{a}	6.42±0.014^{d}
E C (µS/cm)	110.30±0.03^{c}	127.36±0.06^{d}	143.98±2.15^{e}	12.04±0.04^{b}
Nitrate	1.16±0.01^{a}	0.94±0.03^{b}	0.68±0.01^{a}	1.26±0.03^{d}
Total Nitrogen (%)	2.27±0.01^{c}	2.07±0.07^{b}	0.19±0.01^{a}	2.72±0.01^{d}
Phosphate (ppm)	68.15±0.03^{c}	63.50±0.03^{b}	61.24±0.04^{a}	75.63±0.01^{d}
Iron (ppm)	1.03±0.01^{b}	1.15±0.01^{c}	1.22±0.01^{d}	1.01±0.10^{b}
Cadmium (ppm)	2.09±0.01^{c}	2.38±0.02^{d}	2.87±0.37^{e}	1.95±0.01^{b}
Lead (ppm)	12.45±0.03^{c}	13.12±0.05^{d}	15.94±0.01^{e}	10.18±0.01^{b}
Zinc (ppm)	0.51±0.01^{c}	0.52±0.00^{c}	0.67±0.01^{d}	0.47±0.01^{b}
Phosphorus (ppm)	22.24±0.02^{c}	20.71±0.01^{b}	19.98±0.02^{a}	24.67±0.01^{d}
Potassium (ppm)	0.85±0.01^{c}	0.64±0.01^{b}	0.49±0.00^{a}	1.04±0.01^{d}
TOC(%)	3.26±0.01^{c}	34.00±0.02^{c}	42.00±0.01^{c}	75.00±0.01^{c}

OM(%)	15.00±0.02^{c}	10.00±0.03^{c}	5.00±0.03^{c}	2.00±0.01^{c}
TPH	19850.06±0.49^{c}	22721.16±0.57^{d}	24948.39±1.01^{e}	18986.76±0.78^{b}

Key: Data represents duplicate Mean± Standard Error; Superscripts reflect homogenous subsets, Columns with similar superscripts are significant at $p<0.05$ and otherwise are not significant at $p>0.05$

Table 6: Physicochemical properties of the polluted soil sample analyzed after 60 days of bioremediation

Parameters	**Control soil**	**WH**C	**SMC**	**SMC+WCH**
pH	6.76±0.02^{c}	6.53±0.15^{b}	6.14±0.03^{a}	6.92±0.25^{d}
E.Conductivity (µS/cm)	118.45±0.03^{c}	103.76±0.25^{b}	83.87±0.29^{a}	124.56±0.43^{d}
Nitrate	2.18±0.01^{c}	1.37±0.01^{b}	1.18±0.03^{a}	2.84±0.02^{d}
Total Nitrogen (%)	2.97±0.01^{b}	2.63±0.11^{b}	1.58±0.10^{a}	3.74±0.16^{c}
Phosphate (ppm)	8.24±0.03^{c}	83.45±0.5^{b}	70.7±0.52^{a}	127±0.41^{d}
Iron (ppm)	0.73±0.50^{a}	0.93±0.02^{b}	1.02±0.02^{c}	0.71±0.20^{a}
Cadmium (ppm)	1.35±0.01^{c}	1.92±0.30^{d}	2.02±0.11^{e}	1.22±0.51^{b}
Lead (ppm)	9.85±0.50^{c}	10.15±0.40^{d}	10.58±0.01^{e}	7.97±0.02^{b}
Zinc (ppm)	0.31±0.10^{b}	0.47±1.00^{c}	0.48±0.04^{c}	0.27±0.15^{ab}
Phosphorus (ppm)	32.08±0.04^{c}	27.25±0.03^{b}	22.91±0.01^{a}	41.82±0.29^{d}
Potassium (ppm)	4.97±0.02^{c}	3.86±0.02^{b}	2.40±0.10^{a}	6.56±0.30^{d}
TOC(%)	4.20±0.02^{c}	36.00±0.02^{c}	47.00±0.01^{c}	82.00±0.01^{c}
OM(%)	17.00±0.01^{c}	11.00±0.031^{c}	8.00±0.03^{c}	5.00±0.02^{c}
TPH	4313.41±0.59^{c}	5543.24±0.76^{d}	7593.36±1.20^{e}	3297.32±2.70^{b}

Key: Data represents duplicate Mean± Standard Error; Superscripts reflect homogenous subsets, Columns with similar superscripts are significant at $p<0.05$ and otherwise are not significant at $p>0.05$

Discussion

Unpolluted soil (control), contaminated soil treated with water hyacinth compost (WHC), spent mushroom compost (SMC), and spent mushroom compost + water hyacinth compost (SMC+WHC) all had baseline physicochemical contents. Crude oil significantly changed the test soil parameters, according to the pH of the experimental setup upon contamination (Table 5 and 6). The hydrophobic nature of crude oil and its capacity to produce dryness in soil surface layers were probably the causes of the large increase in soil pH. This might worsen salinization and raise the soils' pH levels (Collins, 2009; Njoku et al., 2009). Additionally, polluted soils had considerably higher TOC and TPH levels ($p<0.05$). OM, however, dropped dramatically ($p < 0.05$). A decrease in the exchangeable anion/cation acidity was probably the source of this (Ikhajiagbe et al., 2019). According to David et al. (2019), a decrease in OM was also noted in soils with a high pH of 6.42, which was linked to a decrease in the activity and aggregation of beneficial rhizopheric bacteria. impact of diesel pollution on the pH of the soil during bioremediation Table 5 shows that the pH value of the soil in CS was as high as 6.10 and substantially higher than the WHC of 6.31 ($p < 0.05$).

However, as SMC increased, a modest drop in soil pH was observed in the CS. This implies natural attenuation that is in line with Njoku et al. (2009)'s research. Additionally, as SMC increased, E. crassipes dramatically reduced soil pH in WHC ($p<0.05$). This is probably because E. crassipes produces low molecular weight organic acids that increase soil fertility by enhancing nitrification and pollutant breakdown while lowering soil pH (Tang and Angela, 2019). Our findings support those of Ayotamuno et al. (2006), who demonstrated that in a model soil contaminated by crude oil, higher levels of organic matter had a negative impact on soil pH. The WHC that was treated with 100 g of fresh E. crassipes had the lowest pH, almost matching the CS. This implies that fresh E. crassipes is probably a potent biostimulant that can be applied to contaminated soils to improve their characteristics and lower their pH. The WHC had a considerably greater TOC concentration than the CSS ($p<0.05$; Figure 2). Following E. crassipes amendment, TOC dramatically dropped as WHC rose. One hundred grams of fresh E. crassipes. In comparison to the CS at 90 WHC+SMC, WHC had the lowest TOC (24.12), which was noticeably higher. When fresh and powdered E. crassipes were compared on TOC, the concentration of fresh E. crassipes-amended soils was significantly lower than that of the powdered form across all WHC. Compared to the processed version, fresh organic matter-amended soils have been found to have greater TOC (Waleed et al., 2020).

However, elevated soil organic carbon (TOC) may result from soil polluted by derived hydrocarbon compounds. This outcome is in line with research by Devatha et al. (2019), which found that diesel exposure raised TOC levels dramatically by raising TPH. Additionally, a decrease in soil TOC may indicate the biodegradation of soil hydrocarbons, according to Musa et al. (2018). Total petroleum hydrocarbon (TPH) during bioremediation as a result of diesel contamination

TPH was not found in the CS, however the concentration of TPH in the WHC for the three analyzed WHCs was considerably greater than the CS ($p<0.05$). As SMC increased, the concentration of TPH decreased marginally. Natural attenuation could be the cause of this occurrence. When E. crassipes was added to the soil, TPH dramatically dropped while WHC rose for all treatments. While TPH was highest in WHC at 1 WHC, it was lowest in soil that had been treated. This finding suggests that E. crassipes can help reduce TPH when used to treat diesel-contaminated soils. Additionally, across all WHCs, TPH was considerably lower in soils treated with fresh E. crassipes than in soils treated with powdered E. crassipes.

The active living matter in fresh E. crassipes, as opposed to powdered E. crassipes, is probably the cause of this. This outcome is in line with research by Udeh et al. (2013), which found that fresh E. crassipes is a useful biostimulant for treating soils contaminated by hydrocarbons. TPH dropped dramatically. The maximum TPH loss was seen in contaminated soils supplemented with 100 g of fresh E. crassipes. Contaminated soils were subsequently corrected. The least amount of TPH was lost in diesel-contaminated soil that was left untreated when compared to other treatments. Natural attenuation can lower soil TPH, however it takes longer than using organic matter as stimulants. The findings of Akpe et al. (2015), who showed significant TPH reduction in hydrocarbon-polluted soils within 60 days following biostimulation with plantain peels and guinea corn shaft, are supported by our conclusion. The CSS did not detect TPH. Additionally, the percentage TPH loss with the new amendment types

E. crassipes was substantially more prevalent than in the powdered form. This is probably because fresh E. crassipes uses a lot of organic carbon. This work is comparable to that of Swamps (2013), who evaluated bioremediation in petroleum-polluted mangrove swamps in the Niger Delta using powdered E. crassipes and found significant TPH reduction after 70 days. They proposed that the presence of nutrients from organic waste was the cause of the high TPH loss that was observed.

CONCLUSION

According to this study, fresh E. crassipes is probably a more effective biostimulant than its powdered form when it comes to cleaning up soils contaminated with petroleum fuel. The substantial decline in soil pH, total organic carbon (TOC), and total petroleum hydrocarbon (TPH), along with the large loss of TOC and TPH, supported this. Because of the timeframe of this study and limitations with the microbial assessment of fresh organic matter, further work is required to isolate the microbes that aid fresh E. crassipes in biodegradation for improved remediation strategies. The research is necessitated by the need to proffer an appropriate solution to the menace of Water Hyacinth (*Eichhornia crassipes*) in Nigerian fresh waters. In addition, this research will address the insufficient supply of biofertilizer to improve crude oil impacted sites in the Niger Delta.

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