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Abstract: In this research work the variation in the characteristics of some of the physicochemical properties of the swampy soil environment induced with elephant grass mixed with yeast and NPK was examine for various reactors with variation in volume of crude oil contamination. The research was experimentally based and the parameters studied are pH, electrical conductivity, total nitrogen content, total phosphorus content, total potassium content, density and total organic carbon. The combination of these bio-stimulants enhanced remediation of the crude oil contaminated swampy soil environment with favourable microbial growth and nutrient that catalyse the reaction process for effective degradation. The increased in characteristics of the physicochemical properties as well as the decreased favours the bioremediation of the crude oil without inhibiting the activities of the microbes present in each of the bioreactor. This research work demonstrates the significance of this biostimulants combination in enhancing effectiveness in bioremediation programme.

Keywords: Characteristics, physicochemical parameters, elephant grass, mixture, remediation, crude oil, polluted, swampy soil

1. INTRODUCTION

The research work will address the significance of characteristics of physicochemical properties of elephant grass (*pennisetum purpureum*) in remediation of crude oil polluted swampy soil environment. The application of the inorganic substance as well as the yeast will improve the remediation process [1-5]. However, it is seen that due to increase in crude oil exploration, exploitation, processing, as well as transportation through different mechanism has led to constant pollution in the area of Niger Delta area of Nigeria and other region where these processes take place [6-8].

Indeed the soil pollution for now is a major issue to the world and the need for good innovation on how to handle the menace without contaminating the environment is a welcome idealogy in this research work. However, this research work justified such idealogy by ensuring that the elephant grass (*pennisetum purpureum*) and the addition of the inorganic substance does not contaminate the environment at the end of the process. Indeed the application of elephant grass (*pennisetum purpureum*) including the addition of the inorganic NKP and yeast improved the remediation process as well as the end products obtained are environment friendly substance [9-12].

Thoroughly, the significance of the study has been achieved through this mechanism and is recommended to the scientist, engineers, environmentalist and other science oriental discipline.

2. MATERIAL AND METHODS

2.1. Study Area

This study focused on Eminigboko Community of Emughan Clan in Abua/Odual Local Government Area of the Rivers State, Nigeria. Rivers State is one of the States in the Niger Delta region of Nigeria. This region contributed to more than 98% of Nigeria's current economic mainstay. The Nigeria Delta region, though, is blessed with abundant of crude oil and natural gas, yet the people are very poor. Eminigboko Community is located on Latitude 4° 57' 15" N and Longitude 6° 35' 29" E with Elevation

of 11 meters, while the climate type is described as tropical monson climate. The community is a host to SPDC, and as a result, it witnesses occasional oil spillage due to leakage of crude oil pipelines, thereby polluting the soil environment. The Map of Rivers State showing the study area is shown in Figure 1.



Figure1. Map of Rivers State showing the Study Area [13]

2.2. Materials

The following materials were used for this study: crude oil, swampy soil, elephant grass, , oven, beakers, weighing balance, funnel, measuring cylinder, pH meter, pH paper, containers with cover (reactor), stirrer, electrical weighing balance, Gas Chiromatography (GC), Atomic Adsorption Spectrophotometer (AAS), thermometer, crushers (Jaw and Ball mail), detergent, hand glove, nose mask, plastic rubber, trowel and nylon bag. The following chemical reagent were used: sodium sulphate, sulphuric acid, copper sulphate, 50m of sodium hydroxide 0.2m of hydrochloric acid, mixing indicator (methyl red and methylene blue), HCl and sodium hexametaphosphate [14].

2.3. Methods

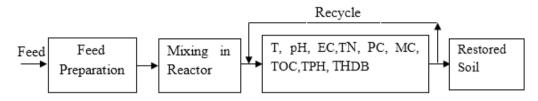
2.3.1. Raw Materials Preparation

The elephant grass was obtained from the Rivers State University Agric farm, while the swampy soil was obtained from Eminigboko Community of Emughan Clan in Abua/Odual Local Government Area of Rivers State. 20 litres of crude oil was obtained from a certified crude oil production company in Rivers State. All the collected samples were transported to the laboratory for preparation and analysis. The treatments elephant in powdered form, yeast and NPK in swampy soil) were washed with distilled water. Upon washing, they were first sun dried for about 3 days and then further dried in the oven at 60 – 70°C for about 2 hours, to expel moisture content. The dried samples were crushed and sieved to uniform particle sizes of 2mm. The sieved particles of elephant grass were divided into five (5) portions with weights 20, 40, 60, 80 and 100g respectively.

2.3.2. Experimental Procedure

Each of the swampy soil was sieved to a uniform particle size using a sieve of 2mm mesh size, and divided into eleven (11) portions of samples with an average weight of 1kg. The weighed soil samples were measured into 11 reactors followed by addition of 100ml of crude oil sample into each of the reactor containing the soil and then, mixed properly with the aid of a stirrer. The stirred samples were left for three day to settle without disturbance before commencing the soil treatment. After the three days of soil stabilization, 20g, 40g, 60g, 80g and 100g of elephant grass particles were added into the reactors with swampy soil. One reactor each of swampy soil was not added with the treatment, which serves as control sample. The aim was to ascertain if there is significant difference between the performance of the treatment options and natural on the physicochemical properties of the selected

parameters as well as the degradation (natural attenuation). The reactors containing swampy soil with elephant grass treatment were labelled SCM in powdered form; yeast and NPK in Swampy Soil were labelled SCEG and CEG respectively. Every two days, the reactors were stirred to make sure there is uniform distribution of the treatment. Every two weeks (14 days), about 10g of each soil sample in three separate sample bottles were collected for Laboratory analysis. The laboratory analysis lasted for a period of 84 days. The soil physiochemical analysis includes pH, moisture content, total organic content (TOC), total nitrogen, phosphorus content and electrical conductivity. Also, the Total Petroleum Hydrocarbon (TPH) and Total Hydrocarbon Degrading Bacteria (THDB) were analysed for every 14 days. The description of the process analyses is given in Figure 2.



2.3.3. Soil pH

The pH of soil before and during treatment was determined in the laboratory. 10% (w/v) of air dried soil suspension for each sample was prepared in de-ionized water, and allowed to settle for about one hour. Thereafter, it was filtered through Whatman filter paper, while the pH of the filtrate was determined via calibrated pH meter called Hanna HI 2211 pH/ORP meter [16].

2.3.4. Electrical Conductivity

The moisture content (MC) of samples was determined following method described by some research groups. Thus, 10g of each soil sample was weighed into crucible and heated in an oven at 105°C for 24 hours to dry off water content [17]. Thereafter, the dried soil samples were cooled in desiccator for 30 minutes. On cooling, the samples were reweighed to constant weight. The percentage moisture content was calculated according to equation (1).

$$MC (\%) = \frac{w_1 - w_2}{w_1} \times 100 \%$$
(1)

Where MC = Moisture content (%), $w_1 =$ Initial weight of soil sample (g) and $w_2 =$ Weight of dried soil sample (g).

2.3.5. Total Organic Content

The total organic content (TOC) was determined using the method in by research groups. 1.0g of crushed fine representative soil samples was weighed in duplicate into 250ml beaker. 10ml of potassium dichromate solution was pipette into beakers and then, rotated gently to completely wet the soil sample, followed by the addition of 20ml of concentrated H_2SO_4via an automatic pipette, directing the stream into the suspension. The beaker wasinitially gently rotated to obtain a uniform mixture and then, vigorously in the next one minute for effective and more complete oxidation before being allowed to stand for 30 minutes on sheet of asbestos [18]. On settling, 100ml of distilled water was added followed by addition of 3-4 drops of 0.5 ml diphenylamine indictor. The solution was titrated against 0.5N ferrous sulphate solution. The process was repeated using distilled water (blank titration) in absence of soil to standardize the dichromate. The TOC would was calculated using the formula.

$$TOC = Blank - \frac{volume \ of \ soil \ sample \ titre \times 0.195}{weight \ of \ soil \ sample} \times 100 \ \%$$
(2)

2.3.6. Nitrogen Content

Nitrogen content was determined according to APHA standard method, using APHA 4500-NO₃B.

2.3.7. Phosphorous Content

Phosphorus content was determined according to APHA standard method 4500 \cdot PO $\frac{3}{4}$.

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3. RESULTS AND DISCUSSION

3.1. Physicochemical Properties of Soil under Treatment

The performance of the contaminated swampy soil after application of the investigated treatments was studied by observing the variations in the swampy soil physicochemical properties with time. However, the soil classification based on the its particle size distribution percentage is shown in Table 1, while the physicochemical properties of the soils recorded before the soil was contaminated and after contamination prior before treatment addition are shown in Table 2. Also, Figures .1 to 9 show the variations in physicochemical properties of swampy soil with time as a result of treatment application.

 Table1. Soil Classification

Soil Sample	Bulk Weight (g)	Sandy (g)	Swampy (g)	Clay (g)
Swampy Soil	500	35.5 (7.1%)	243.5 (48.7%)	221.0 (44.2%)

Table 1 demonstrates the soil classification of sandy swampy soil with bulk weight of 500g each. From the bulk weight of the soil samples, the analysis revealed that soil sample collected from the swampy and water logged forest contains sand weighing 35.5g, representing 7.1%,. Thus, from this percentage composition, the soil is therefore classified as swampy soil according to USDA guidelines [19].

Table2. Results Analysis of Soils before and after Contamination
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Parameters	Swampy Soil		
	Before Pollution	After Pollution	
pH	6.52	6.21	
EC (µS/cm)	108.31	481.57	
TOC (%)	2.13	6.37	
N (%)	1.032	0.094	
P (%)	1.49	0.0029	
K (%)	18.40	1.48	
Density (g/ml)	1.37	1.86	
TPH(ppm)	122.89	46530.21	
TBC (cfu/ml)	1.76 x 10 ³	$1.84 \ge 10^2$	

Table 2 shows the physicochemical properties of swampy soil soil before and after crude oil contamination. Thus, pH, electrical conductivity (EC), total organic carbon (TOC), nitrogen content (N), phosphorus content (P), potassium (K) Total Petroleum Hydrocarbon (TPH) content and Total Bateria Counts(TBC) of the soils before and after pollution show variations. The changes observed in the properties of the soil after contamination with crude oil is an indication that crude oil has great impact on the soil. From the result obtained the swampy soil. Nitrogen plays more active role then TOC, P, K and other parameter as discussed in this research. However, the nutrients and TBC population reduced immediately as the crude oil was added this is illustrated in Table 1.

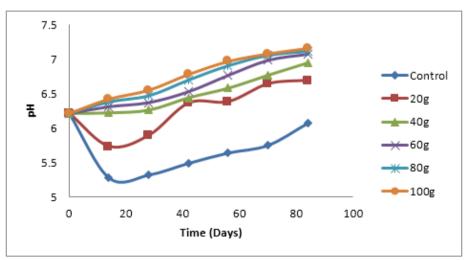


Figure3. Variation of pH in Swampy Soil against Time for the Mixture of Elephant Grass in Powdered Form, Yeast and NPK

Figure 3 demonstrates the variation of pH in swampy soil on impact of time for bioremediatiant mixture of elephant grass in powdered form, yeast and NPK in Swampy Soil, yeast and NPK in crude oil degradation of various mass of 20, 40, 60, 80 and 100g for elephant grass in powdered form, yeast and NPK in swampy soil and equal mass of 40g for yeast and NPK each introduced into each of reactor. Lag phase was observed for some of the reactors before sudden increase in the pH value from more acidic to less acidic, before attaining neutral and alkaline state, which enhance microbial growth in the bioreactors as well as increased petroleum hydrocarbon degradation. The variation in the pH value can be attributed to the variation in the period of exposure (time) as well as the environmental factors [20]. The pH of the soil decreased from its initial condition of 6.21 to 5.28 and further to 5.28 at the 14th day, but thereafter, increased to 6.09, 6.62, 6.89, 7.04, 7.11 and 7.18 in control, 20g, 40g, 60g, 80g and 100g elephant grass in powdered form, yeast and NPK in swampy soil samples at the 84th day respectively. Again, the soil pH increases as the weight of elephant grass in powdered form, yeast and NPK in swampy soil was increased, which indicated that high treatment weight favours pH of the soil.

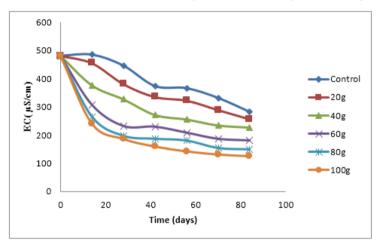


Figure4. Variation of EC in Swampy Soil against Time for the Mixture of Elephant Grass in Powdered Form, Yeast and NPK

Figure 4 shows the variation of Electrical Conductivity (EC) in polluted swampy soil with elephant grass in powdered form, yeast and NPK in swampy soil treatment. Like earlier stated, the initial concentration of EC in swampy soil increased after contamination. However, as the bioremediation time progresses, EC values decreased, and the rate of decrease were more obvious in samples with treatment. Meanwhile, the rate of EC reduction increases with increase in the weight of elephant grass in powdered form, yeast and NPK in swampy soil in the sample.

Thus, EC in swampy soil after pollution was 481.57μ S/cm, but at the 84^{th} day, it decreased to 281.79μ S/cm, 256.43μ S/cm, 227.58μ S/cm, 180.68μ S/cm, 149.25μ S/cm and 124.74μ S/cm for samples with elephant grass in powdered form, yeast and NPK in swampy soil containing 0g (control), 20g, 40g, 60g, 80g and 100g weight respectively in the samples.

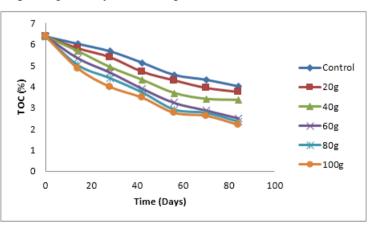


Figure5. Variation of TOC in Swampy Soil against Time for the Mixture of Elephant Grass in Powdered Form, Yeast and NPK

Figure 5 shows the variation of Total Organic Carbon (TOC) with time for swampy soil treated with elephant grass in powdered form, yeast and NPK in swampy soil treatment. Again, as shown in the Figure 5, TOC decreases with time across the treatment options. However, the percentage of TOC in the control soil sample was higher than samples treated with elephant grass in powdered form, yeast and NPK in Swampy Soil. In earlier study, the decreasing rate of TOC with time recorded for the treatment options was attributed to the utilisation of TOC for energy by micro-organism.

In the analysis, TOC in swampy soil before pollution was 2.13%, but increased to 6.37% after contamination, and then, decreased further to 4.01%, 3.74%, 3.36%, 2.51%, 2.36% and 2.19% for samples with elephant grass in powdered form, yeast and NPK in swampy soil containing 0g (control), 20g, 40g, 60g, 80g and 100g weight respectively at the 84th day. it was observed that the percentage of TOC in soil after the period of 84 days of treatment, especially with treatment weights of 60 to 100g, the reduction in TOC of the soils were close to the recorded TOC before contamination (Table 1).

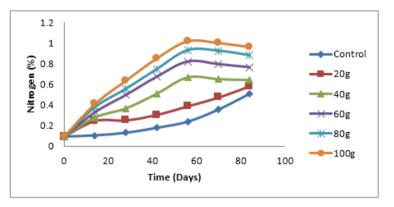


Figure6. Variation of Total Nitrogen in Swampy Soil against Time for the Mixture of Elephant Grass in Powdered Form, Yeast and NPK

The profiles of total nitrogen variation in swampy soil treated with elephant grass in powdered form, yeast and NPK in Swampy Soil are shown in Figure 6. Thus, total nitrogen increases with both time and treatment weight. It was also observed that total nitrogen increased steadily up to the 56th day, and thereafter, remained relatively constant for samples containing 40g treatment weight and above. This implied that the addition of treatment produced more nitrogen in the soil that was utilised by hydrocarbon degrading bacteria to breakdown TPH content in the soil.

Total nitrogen recorded in Swampy Soil before pollution was 1.032%, but decreased to 0.094% after contamination. However, at the end of the treatment, total nitrogen increased to 0.507%, 0.582%, 0.644%, 0.766%, 0.884% and 0.963% in control (0g elephant grass in powdered form, yeast and NPK in swampy soil), 20g, 40g, 60g, 80g and 100g samples respectively.

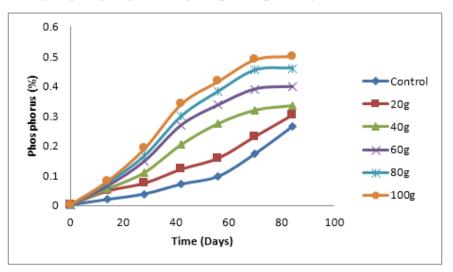


Figure7. Variation of Phosphorus in Swampy Soil against Time for the Mixture of Elephant Grass in Powdered Form, Yeast and NPK

Figure 7 shows the profiles of phosphorus content versus time for elephant grass treatment in swampy soil. Again, phosphorus content increases with increase in time, and also as treatment weight was increased, and was least recorded in the control sample. Again, between 70 and 80 days of remediation, increase in phosphorus was relatively seemed to retards though, it may completely deplete if the analysis continues beyond 84 days as it is being consumed by the microorganisms.

Hence, from the analysis, the phosphorus content in swampy soil before pollution was 1.49%, but decreased to 0.0029% after contamination, and thereafter increased to 0.264%, 0.303%, 0.335%, 0.398%, 0.460% and 0.501% respectively in control, 20g, 40g, 60g, 80g and 100g samples treated with elephant grass at the 84th day.

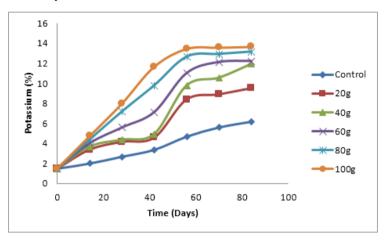


Figure8. Variation of Potassium in Swampy Soil against Time for the Mixture of Elephant Grass in Powdered Form, Yeast and NPK

Figure 8 shows the profiles of potassium content versus time for swampy soil samples treated with elephant grass. Again, the concentration of potassium content recorded in samples treated with elephant grass was higher than those of nitrogen and phosphorus, but the percentage of potassium content increases with increase in time and treatment weight.

However, as recorded from the analysis, potassium content in swampy soil before pollution was 18.40%, but decreased to 1.48% after contamination, and thereafter increased to 6.16%, 9.51%, 11.92%, 12.22%, 13.16% and 13.66% respectively in control, 20g, 40g, 60g, 80g and 100g samples treated with elephant grass at the 84th day.

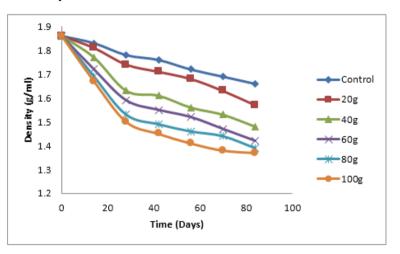


Figure9. Variation of Density in Swampy Soil against Time for the Mixture of Elephant Grass in Powdered Form, Yeast and NPK

Figure 9 shows the profiles of soil density versus time for swampy soil treated with elephant grass. Soil density as a measuring index for bioremediation studies was rarely investigated. However, there were changes in soil density across the treatment samples as recorded in this study. Thus, density increases with time and weight of treatment.

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Again, the density of swampy soil before pollution was recorded as 1.37g/ml, but increased to 1.86g/ml after contamination, and thereafter decreased to 1.66g/ml, 1.57g/ml, 1.48g/ml, 1.42g/ml, 1.39g/ml and 1.37g/ml respectively in control, 20g, 40g, 60g, 80g and 100g samples treated with elephant grass at the 84th day.

4. CONCLUSION

The following conclusion was drawn from the research work, as stated below:

- The combination of elephant grass, yeast and NPK is a good mixture of bio-stimulant for effective remediation of contaminated soil environment.
- The mixture is capable of inhibiting the functional parameters the influence bioremediation.
- The mixture solution increased the microbial build up in the bioreactor.
- Increased in nutrient observed with decrease in crude oil concentration as well as increased in microbial growth.

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