Scientia Africana, Vol. 23 (No. 1), February, 2024. Pp 59-72
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EFFICIENCY OF COW HORN-POWDER IN THE REMEDIATION OF CRUDE OIL IMPACTED SOIL.

***Kpakol, N. N., Osasere-Omoruyi,T., Belonwu, D. C. and Okoro, S. E.**
Department of Biochemistry, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria.
Email: nkpakol@gmail.com

Received: 09-11-2023
Accepted: 21-12-2023

https://dx.doi.org/10.4314/sa.v23i1.5

Journal Homepage: http://www.scientia-african.uniportjournal.info
Publisher: *Faculty of Science, University of Port Harcourt.*

ABSTRACT

Efficiency of Cow-Horn Powder in the Remediation of Crude Oil Impacted Soil was evaluated. Five beds of 40cm by 40cm with depth 30cm, partitioned on 220cm^2 plot of land in Aluu, Port Harcourt were used for the study. A volume, 0.9 liters of Crude Oil was used to contaminate 4 beds of partitioned soil per 0.18m^2 and left undisturbed for 14 days. Three of the partitioned contaminated beds (XgCH, YgCH and ZgCH) were thereafter bio-stimulated with 50g, 75g and 100g respectively of Cow Horn-Powder. The 4th served as negative control and the 5th, neither stimulated nor contaminated. Soil samples in triplicates, collected from depth 0-25cm from various beds at day zero soil contamination, Biostimulation Week 6 and 8 respectively were homogenized and analyzed for relevant Hydrocarbon indices, Heavy Metals and Physico-chemicals using standard methods. The highest remediation concentrations of the TPH which ranged between 652.11±0.0050 mg/kg and 893.20±0.005 mg/kg and PAH (92.99±0.001 mg/kg and 54.08±0.001 mg/kg) after eight weeks of remediation yielded 59.1% and 42.0% respectively. Degrees of reductions which ranged between 0.10±0.01 and 26.0±0.05 were recorded in the values of the Heavy Metals (Ni, Pb, Cr, Mn, Cu, Zn, Fe and Cd). Accordingly, significant differences ($P \leq 0.05$) were noticed between the values of TPH, PAH and Heavy Metals of the impacted soils and controls. Results suggest that Cow Horn-Powder is potent in remediating Crude Oil impacted Soils.

Key Words: Efficiency, Cow Horn-Powder, Remediation, Crude Oil, Impacted Soils.

INTRODUCTION

Nigeria is one of the countries that has great challenges with Crude Oil Pollution. Although, petroleum is one of the major drivers of the Economy of Nigeria since its commercial exploration started in the year 1958 (Steyn, 2009), the pollution associated with it has negatively affected Land and water ways. This has cumulated to the lands becoming less productive (Ihekeet *al*., 2019). In addition, the waters for fishing and the Creeks have eventually become polluted (Osuagwu, 2018). A highly-recognized region in Nigeria that has experienced unprecedented crude oil pollution on lands and water bodies is the Niger Delta. Unwarranted destruction and improper management of oil pipelines by the people and operational oil companies respectively are some major causes of crude oil pollution. Many civil unrests have been witnessed in the Niger Delta Region. This is because of the harmful effects that pollution from crude oil has had on the surrounding ecosystem (Inoniet *al*., 2006).

The need to adequately restore crude oil contaminated soils, as to give support to the growth of plants and other services of the environment cannot be overemphasized. Biological, chemical and physical methods are used to remediate contaminated soils. Some of these techniques come with a lot of challenges, for instance, not been friendly. Thus, the need to come up with techniques that are more friendly (Njoku, 2008). According to Azubuike*et al*. (2016), some remediation techniques on the environment (for example, chemical and mechanical) come with a lot of consequences. This makes the biological or biodegradation method a more welcome method of remediation. Although huge economic profits are obtained from the exploration and exploitation of crude oil, the environment needs not to be destroyed. It is therefore pertinent to come up with methods of restoring the crude oil contaminated environment. Petroleum hydrocarbons may be degraded by microorganisms (bacteria and fungus), as reported by Ogbonna*et al*. (2012). Many bioremediation methods that include soil biostimulation have been implemented, and this has aided much in the recovery of our environment. One strategy for speeding up the microbial degradation of crude oil is to enhance the population of soil microorganisms by adding fertilizers to improve the nutrients of soils, particularly nitrogen and phosphorus (Sing, 2018). According to Yakubu (2007), the above method is highly efficient, vast, economical and is friendly environmentally.

Remediation with Cow Horn-Powder on a crude oil impacted Soil is yet to be studied in detail. In doing this, heavy metals, total petroleum hydrocarbons, and polycyclic aromatic hydrocarbon concentrations in crude oil-affected soils bio-stimulated with Cow horn-powder must be assessed.

Therefore, this study was designed to ascertain the efficacy and bio stimulation potentials of cow horn-powder in remediation of crude oil impacted soils.

MATERIALS AND METHODS

Sources of Materials

The Cow Horns used for this study were obtained from an Abattoir located at Aluu Town in Ikwerre Local Government Area of Rivers State, Nigeria. The light crude oil was collected by vertical drilling and pumping from Shell Petroleum Development Company (SPDC), Port Harcourt and stored in a big plastic rubber. The soil samples used were obtained from the experimental plot of land at Aluu Town in Ikwerre Local Government Area of Rivers State, Nigeria.

Equipment:

Varian CP 3800 Fid Gas Chromatography (Italy), Varian 240 FTS Atomic Absorption (Fulltech, Italy), Spectroscopy (Italy), Ultra violet/Visible (UV/Vis) Spectrophotometer (Fultech, Italy), Hanna 7864 Multipara meter Insitu meter (Romania), 250D Lab tech Oven Fultech (Italy).

Apparatus, Reagents and Chemicals

Apparatus:

Digestive tube, manual hammering hallow metal pipe, measuring cylinder, microwave digester, retort stand, round bottom flask, Thermometer, crucible with lid (50ml), Petrich dishes.

Reagents and Chemicals

All the reagents and chemicals were of the grade gotten from BDH chemicals limited and Hopkins and Williams Essex all in England. The following were the reagents and chemicals used: Acetone (C_3H_6O), Acetic acid ($C_2H_4O_2$), Ammonia (NH_3), Ammonium hydroxide (NH_4OH), Argon (Ar), Boric acid (H_2BO_4), Calcium Chloride ($Ca\ (Cl)_2$), Concentrated Hydrochloric acid (HCl), and Water (H_2O).

Baseline Studies of Experimental Oil

Soil samples werethoroughly homogenized and collected from 0-25cm depth using a hoe. The soil samples were prepared and analyzed from all the plots for Polycyclic Aromatic

Scientia Africanica, Vol. 23 (No. 1), February, 2024. Pp 59-72
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Hydrocarbon (PAH), Total Petroleum Hydrocarbon (TPH), Heavy Metals (V, Fe, Cr, Cd, Ni, Mn, Cu, Zn, and Pb). This was done to know the true state of the experiment site.

Crude Oil Contamination of Soil

Zero point nine (0.9) liters of crude oil was used to artificially contaminate the Soil per 0.18m^2. As described by Ayotamuno*et al*. (2006), a plastic can that had holes was used. The oil was driven into the soil using fork. The plot designated Normal Control (NC) was not contaminated.

The contaminated plots were left for fourteen days. The plots were then tilled systematically through homogenization, ploughing and windrows construction to bring about good surface areas for breaking down of microbes, weathering, vaporization, temperature etc. This was done after every two days.

Preparation of Bio stimulating Agent (Cow Horn-Powder)

Thirty- four (34) pieces of Cow Horns were purchased from an Abattoir in Aluu-Town, Ikwerre Local Government Area of Rivers State and taken to the laboratory, washed thoroughly with deionized water to remove dirt and impurities from the surfaces. They were cut into pieces and air-dried for 72 hours. They were ground with electric grinder and finally applied to the soil.

Soil Biostimulation

After two weeks of impacting the soils with crude oil, soil samples were collected and analyzed.

Thereafter, the partitioned plots encoded XgCH, YgCH and ZgCH were biostimulated with 50g, 75g and 100g respectively of ground Cow Horn samples.

Experimental Design

The Experimental design used in this study was carefully done to encompass the collection, measurement and analysis of data. Application of randomization was done. In order to have an excellent finalization of results, quantitative experiment was taken into consideration.

The study was carried out on a plot of land by size 220m^2. The plot was divided into five sub plots, separated apart by two meters. The sub-divided plots were plot 1 (Normal Control), plot 2 (Negative Control), plot 3 (Treated Plot), plot 4 (Treated Plot) and plot 5 (Treated plot). The plots were respectively coded NC, -Ve C, XgCH, YgCH and ZgCH.

While NC received no contamination and biostimulation, -Ve C was contaminated but not biostimulated. The remaining three plots were contaminated and treated (bio stimulated using Cow Horns).

The partitioned plots were made into beds of 40cm by 40cm with depths of 30cm. This was done to mitigate against contaminants flowing to nearby plots; especially if/ when it rains.

Soil Samples Collections

The soil samples were collected from 0-25cm depth, and were thoroughly homogenized. Soil samples were collected as follows: before soil contamination, before soil biostimulation, on the 6th and 8th weeks respectively. The soil samples were collected and put in plastic bags that have been sterilized, in alignment with the opinion of Kale *et al*. (2018). The samples were immediately transferred to the laboratory for analyses.

Soil Sample Preparation

The soil samples were left undisturbed overnight, haven been soaked with deionized water. They were subsequently pounded in an agate mortar and with the aid of plastic vial, got filtered.

Soil Sample Clean-up

In order to remove polar hydrocarbon, moisture, interferences, colour and any possible impurity, the sample clean-up was done. This was done by filtering the extract through a layer (dual) of six meal glass Florisil R / Na_2SO_4SPE tube 2g/2g under an applied pressure.

Digestion of Soil

Soil digestion was done using a mixture of nitric acid and hydrochloric acid.

Instrumentation and Methods of Assay

Determination of Extractable Total Petroleum Hydrocarbon (ETPH)

Soxhlet method was used for the extraction of Total Petroleum Hydrocarbon (TPH) from the soils.

Following the extraction procedures described in ASTM D 5569 method (ASTM., 2005), the extraction of TPH from soil samples was done in a Brinkman Buchi 461 automated extraction apparatus. In line with the method of Schwartz *et al*. (2012), the Total Petroleum Hydrocarbon content of the soil samples were estimated.

Determination of Polycyclic Aromatic Hydrocarbon (PAH)

Following the extraction procedures outlined in ASTM: D 4657-92, the extraction of PAH from soil samples was done.

Determination of the Concentration of Heavy Metals in the Soils using AAS

The method of Mielke*et al*. (2004) was followed in the extraction of metals. One molar solution of nitric acid using a ratio of 5:1 was used to extract the metals in contaminated and uncontaminated samples of soil. The concentrations in the samples were extrapolated from the standard graphs plotted against absorbance.

Statistical Analysis

Statistical package for the Social Sciences (SPSS) was used to determine the differences among treated and control groups. Comparison among groups were done using one way ANOVA. Significant differences between control and treated were assessed by the least significant difference (LSD). All data were expressed as mean ± Standard error of the mean; p- values less than or equal to 0.05 were considered to be significant (Igwe*et al*.,2020).

RESULTS AND DISCUSSION

Baseline Properties of the Clean Experimental Soil

The baseline studies of theheavy metals, Total Petroleum Hydrocarbon (TPH) and Polycyclic Aromatic Hydrocarbon (PAH) from the research study site are presented in table 1 below.

Table 1: Baseline Properties of the Clean Experimental Soil Compared with WHO and DPR Level

S/N	PARAMETERS	UNITS	VALUES	WHO std.	DPR std.
1	pH	-	4.26 ± 0.10	5.5-7.5	6.0-7.5
2	Moisture content	%	32.30 ± 0.44	50-55	50-60
3	Total Nitrogen	mg/kg	5.04 ± 0.03	10-50	40
4	Soil Organic Matter (SOM)	mg/kg	13.10 ± 0.07	57-110	2-50
5	Phosphorus	mg/kg	0.35 ± 0.003	25-50	25-50
6	Conductivity	µs/cm	38.70 ± 1.16	110-570	110-570
7	Ni	mg/kg	9.00 ± 0.001	0.07	35.0
8	V	mg/kg	ND	0.05	0.05
9	Cd	mg/kg	0.40 ± 0.00	0.003	0.8
10	Pb	mg/kg	20.20 ± 0.27	0.01	85.0
11	Cr	mg/kg	32.30 ± 0.03	0.05	100.0
12	Mn	mg/kg	88.90 ± 0.05	0.4	437.0
13	Cu	mg/kg	10.60 ± 0.06	0.2	36.0
14	Zn	mg/kg	23.40 ± 0.01	0.2	140
15	Fe	mg/kg	431.80 ± 0.008	0.3	140.0
16	Co	mg/kg	ND	0.01	20
17	TPH	mg/kg	0.66 ± 0.001	50	50
18	PAH	mg/kg	0.94 ± 0.001	1.0	1.0

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Values are means of triplicate determinations ± standard deviation.
TPH = Total Petroleum Hydrocarbon. PAH = Polycyclic Aromatic Hydrocarbon.
ND = Not Detected.

Effects of Enhanced Bioremediation on Soil Heavy Metals

The results of Nickel (Ni), Vanadium (V), Cadmium (Cd), Lead (Pb), Chromium (Cr), Manganese (Mn), Copper (Cu), Zinc (Zn), Iron (Fe) and Cobalt (Co) from all the impacted plots and control are clearly presented in Table 2.

Table 2 (a): Effects of Enhanced Bioremediation on Soil Heavy Metals

Parameters	NC	-Ve C	XgCH	YgCH	ZgCH
Ni (mg/kg)					
Day 0	4.10 ± 0.00^{cde}	4.70 ± 0.00^{cde}	3.44 ± 0.00^{abde}	4.70 ± 0.00^{ace}	3.00 ± 0.00^{abcd}
Week 6	4.50 ± 0.00^{qy}	1.40 ± 0.00^{qy}	5.00 ± 0.00^{xz}	5.00 ± 0.00^{xz}	3.80 ± 0.00^{xz}
Week 8	7.50 ± 0.00^{qy}	6.60 ± 0.00^{qy}	3.00 ± 0.00^{xz}	3.40 ± 0.00^{xz}	5.50 ± 0.00^{xz}
V(mg/kg)					
Day 0	ND	ND	ND	ND	ND
Week 6	ND	ND	ND	ND	ND
Week 8	ND	ND	ND	ND	ND
Cd (mg/kg)					
Day 0	0.10 ± 0.00^{qy}	0.70 ± 0.00^{qy}	0.20 ± 0.00^{xz}	0.10 ± 0.00^{xz}	ND
Week 6	0.20 ± 0.00^{qy}	0.10 ± 0.00^{qy}	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
Week 8	ND	ND	ND	ND	ND
Pb(mg/kg)					
Day 0	6.07 ± 0.12^{cde}	9.01 ± 0.02^{cde}	6.47 ± 0.46^{abde}	8.00 ± 0.00^{abce}	7.04 ± 0.06^{abcz}
Week 6	14.00 ± 0.00^{cde}	1.00 ± 0.00^{cde}	12.00 ± 0.00^{abde}	8.00 ± 0.00^{abce}	10.01 ± 0.02^{abcd}
Week 8	2.00 ± 0.00^{cde}	9.00 ± 0.00^{cde}	8.00 ± 0.00^{xz}	ND	1.00 ± 0.00^{xz}
Cr(mg/kg)					
Day 0	4.47 ± 0.03^{cde}	5.17 ± 0.03^{cde}	3.57 ± 0.03^{abde}	3.87 ± 0.03^{abce}	2.77 ± 0.03^{abcd}
week 6	4.67 ± 0.03^{cde}	4.67 ± 0.03^{cde}	3.77 ± 0.03^{abde}	2.27 ± 0.03^{abce}	5.17 ± 0.03^{abcd}
week 8	10.00 ± 0.03^{cde}	13.10 ± 0.03^{cde}	5.17 ± 0.03^{abe}	5.17 ± 0.03^{abe}	7.47 ± 0.03^{abcd}

Values are means of triplicate determinations ± standard deviation.

ND = Not Detected.
NC = Normal Control
-Ve = Negative Control
XgCH = Treated Plot (50g of Cow Horn powder)
YgCH = Treated Plot (75g of Cow Horn powder)
ZgCH = Treated Plot (100g of Cow Horn powder)

Table 2 (b): Effects of Enhanced Bioremediation on Soil Heavy Metals

Parameters	NC	-Ve C	XgCH	YgCH	ZgCH
Mn(mg/kg)					
Day 0	3.13±0.05^{cde}	57.5±0.05^{cde}	34.4±0.05^{abde}	37.5±0.05^{abce}	20.2±0.05^{abcd}
Week 6	49.10±0.05^{cde}	33.4±0.05^{cde}	48.0±0.05^{abde}	24.2±0.05^{abce}	44.3±0.05^{abcd}
Week 8	17.00±0.00^{cde}	24.0±0.05^{cde}	33.0±0.05^{abde}	28.0±0.05^{abce}	26.0±0.05^{abc}
Cu (mg/kg)					
Day 0	3.10±0.00^{qy}	6.20±0.00^{qy}	0.70±0.00^{xz}	2.30±0.00^{xz}	1.80±0.00^{xz}
Week 6	3.43±0.06^{cde}	10.00±0.00^{cde}	0.93±0.06^{abde}	4.93±0.06^{abce}	3.23±0.06^{abcd}
Week 8	1.80±0.00^{qy}	ND	2.50±0.00^{xz}	1.90±0.00^{xz}	3.00±0.00^{xz}
Zn (mg/kg)					
Day 0	3.20±0.03^{cde}	5.89±0.01^{cde}	6.00±0.00^{abde}	4.43±0.00^{abce}	3.25±0.00^{abcd}
Week 6	5.62±0.00^{cde}	2.51±0.01^{cde}	8.33±0.00^{abde}	3.88±0.06^{abce}	8.71±0.05^{abcd}
Week 8	6.09±0.05^{cde}	8.14±0.05^{cde}	7.00±0.05^{abde}	7.00±0.05^{abc}	7.00±0.05^{abc}
Fe (mg/kg)					
Day 0	429.4±0.008^{cde}	410.8±0.008^{cde}	1503.7±0.008^{abde}	1518.7±0.008^{abce}	1510.8±0.008^{abcd}
Week 6	408.2±0.008^{cde}	392.5±0.008^{cde}	863.0±0.008^{abde}	1083.1±0.008^{abce}	1137.0±0.008^{abcd}
Week 8	430.0±0.008^{cde}	384.1±0.008^{cde}	903.0±0.008^{abde}	1222.0±0.008^{abce}	1291.1±0.008^{abcd}
Co (mg/kg)					
Day 0	ND	ND	ND	ND	ND
week 6	ND	ND	ND	ND	ND
week 8	ND	ND	ND	ND	ND

Values are means of triplicate determinations ± standard deviation.

ND = Not Detected.
NC = Normal Control
-Ve = Negative Control
XgCH = Treated Plot (50g of Cow Horn powder)
YgCH = Treated Plot (75g of Cow Horn powder)
ZgCH = Treated Plot (100g of Cow Horn powder)

Effects of Enhanced Bioremediation on Total Petroleum Hydrocarbon (TPH) in Soil

The analytical results of the Total Petroleum Hydrocarbon (TPH) from all the impacted beds and controls are summarized and presented in table 3.

Table 3: Effects of Enhanced Bioremediation on Total Petroleum Hydrocarbon (TPH)(mg/kg) in Soil

Experimental Groups	NC	-Ve C	XgCH	YgCH	ZgCH
Day 0	0.697±0.005^{cde}	1940.5±0.005^{cde}	2003.5±0.005^{abde}	1835.3±0.005^{abce}	1594.2±0.003^{abcd}
Week 6	1.446±0.005^{cde}	1066.0±0.005^{cde}	1622.2±0.005^{abde}	1088.2±0.005^{abce}	783.10±0.0050^{abcd}
Week 8	0.3090±0.005^{cde}	1073.0±0.005^{cde}	893.20±0.005^{abde}	752.10±0.005^{abce}	652.11±0.0050^{abcd}

Values are means of triplicate determinations ± standard deviation.
NC = Normal Control
-Ve = Negative Control
XgCH = Treated Plot (50g of Cow Horn powder)
YgCH = Treated Plot (75g of Cow Horn powder)
ZgCH = Treated Plot (100g of Cow Horn powder)

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(a) Efficiency of Reduction of TPH after six weeks of Remediation

The efficiency of reduction of Total Petroleum Hydrocarbon (TPH) after six weeks of remediation are presented in figure 1.2 (a) below.

The figure shows clearly the efficiency of Cow Horn powder in reducing the concentrations of Total Petroleum Hydrocarbon (TPH) following Crude Oil contamination.

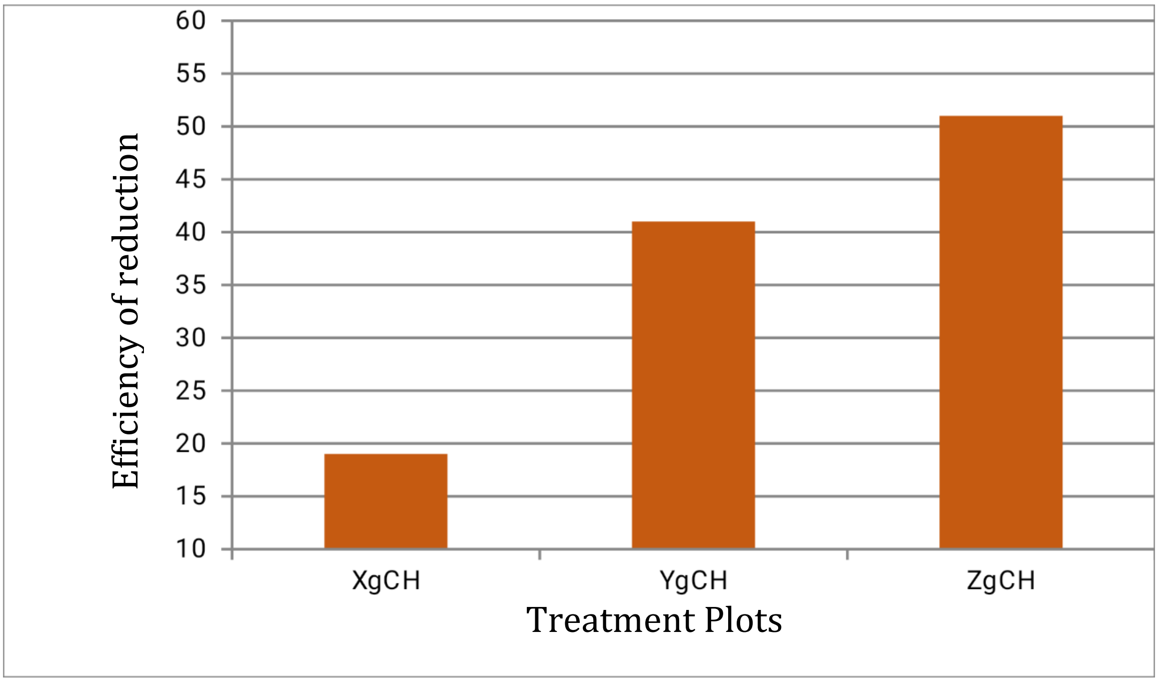

Figure 1.2(a): Efficiency of Reduction of TPH after six weeks of Remediation.

(b) Efficiency of Reduction of TPH after Eight weeks of Remediation

Figure 1.2 (b) below shows the efficiency of reduction of Total Petroleum Hydrocarbon (TPH) after eight weeks of remediation. The figure reveals the potency of Cow Horn Powder in causing reductions in the concentrations of Total Petroleum Hydrocarbon (TPH) following Crude Oil contamination.

Efficacy of reduction of TPH at Week 6 (Figure 1.2a) and Week 8 (Figure 1.2b) shows an enhancement in reduction profile of TPH.

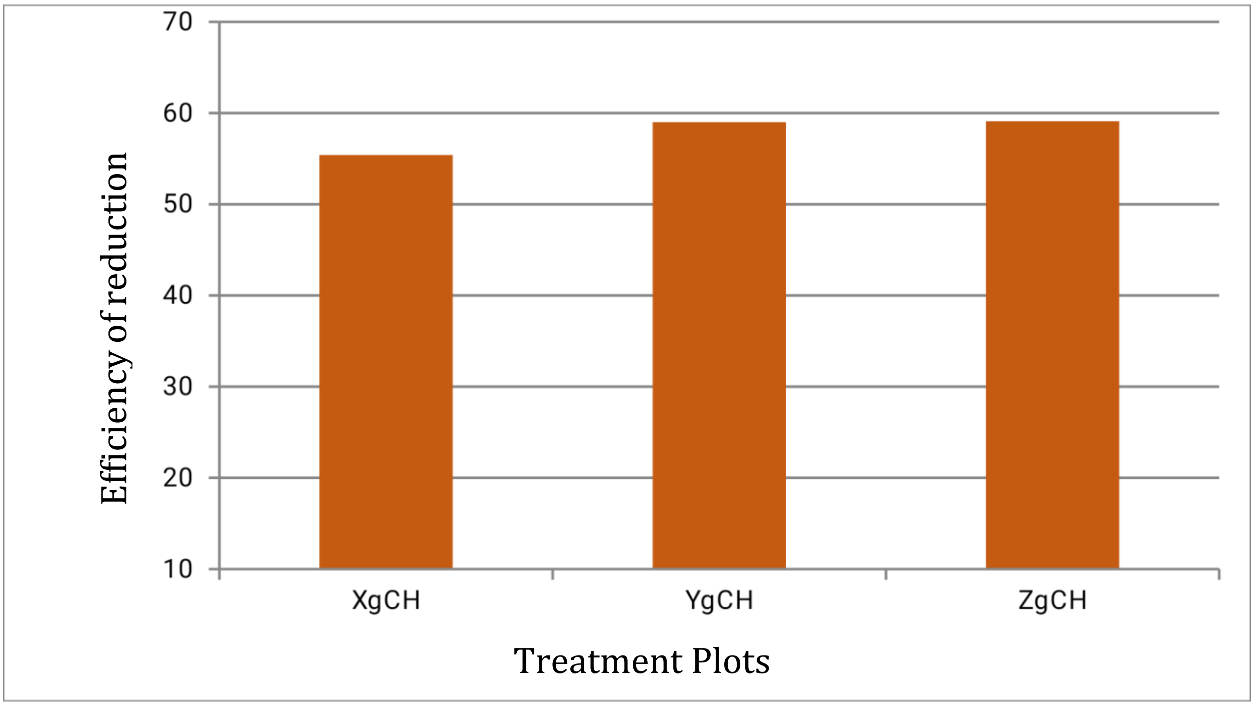

Figure 1.2 (b): Efficiency of Reduction of TPH after Eight weeks of Remediation

Effects of Enhanced Bioremediation on Polycyclic Aromatic Hydrocarbons (PAH) in Soil

Succinctly presented in table 4 are the PAH analyzed results of the impacted Crude Oil and Cow Horn-Powder remediated soils with controls.

Table 4(a): Effects of Enhanced Bioremediation on Polycyclic Aromatic Hydrocarbons (PAH)(mg/kg) in Soil

Parameters	NC	-Ve C	XgCH	YgCH	ZgCH
Anthracene					
Day 0	0.02±0.01^{cde}	148.9±0.001^{cde}	140.0±0.001^{abde}	159.3±0.001^{abce}	89.29±0.001^{abcd}
Week 6	0.552±0.001^{cde}	143.0±0.001^{cde}	98.77±0.001^{abde}	129.0±0.001^{abce}	89.03±0.001^{abcd}
Week 8	0.409±0.001^{cde}	139.0±0.001^{cde}	88.62±0.001^{abde}	92.99±0.001^{abce}	61.92±0.001^{abcd}
Benzo (a)pyrene					
Day 0	0.03±0.01^{cde}	142.3±0.001^{cde}	138.2±0.001^{abde}	150.2±0.001^{abce}	87.55±0.001^{abcd}
Week 6	0.559±0.001^{cde}	140.0±0.001^{cde}	93.20±0.001^{abde}	122.8±0.001^{abce}	80.40±0.001^{abcd}
Week 8	0.400±0.001^{cde}	138.1±0.001^{cde}	77.60±0.001^{abde}	90.10±0.001^{abce}	59.70±0.001^{abcd}
Benzo (b)fluoranthene					
Day 0	0.02±0.01^{cde}	150.0±0.001^{cde}	143.9±0.001^{abde}	156.3±0.001^{abce}	90.00±0.001^{abcd}
Week 6	0.601±0.001^{cde}	142.2±0.001^{cde}	99.50±0.001^{abde}	126.5±0.001^{abce}	79.20±0.001^{abcd}
Week 8	0.403±0.001^{cde}	137.3±0.001^{cde}	82.30±0.001^{abde}	92.80±0.001^{abce}	60.55±0.001^{abcd}
Acenaphthene					
Day 0	0.04±0.01^{cde}	148.0±0.001^{cde}	133.4±0.001^{abde}	160.1±0.001^{abce}	89.20±0.001^{abcd}
Week 6	0.553±0.001^{cde}	141.3±0.001^{cde}	92.31±0.001^{abde}	127.1±0.001^{abce}	90.10±0.001^{abcd}
Week 8	0.399±0.001^{cde}	140.0±0.001^{cde}	80.22±0.001^{abde}	91.70±0.001^{abce}	59.20±0.001^{abcd}
Acenaphthylene					
Day 0	0.03±0.01^{cde}	140.0±0.001^{cde}	145.3±0.001^{abde}	158.4±0.001^{abce}	88.23±0.001^{abcd}
week 6	0.555±0.001^{cde}	142.4±0.001^{cde}	97.44±0.001^{abde}	128.3±0.001^{abce}	85.70±0.001^{abcd}

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week 8	0.410±0.001^{cde}	139.0±0.001^{cde}	86.50±0.001^{abde}	90.99±0.001^{abce}	58.33±0.001^{abcd}
Naphthalene					
Day 0	0.03±0.01^{cde}	143.0±0.001^{cde}	148.0±0.001^{abde}	155.2±0.001^{abce}	86.57±0.001^{abcd}
Week 6	0.558±0.001^{cde}	139.7±0.001^{cde}	95.00±0.001^{abde}	125.5±0.001^{abce}	81.00±0.001^{abcd}
Week 8	0.408±0.001^{cde}	137.3±0.001^{cde}	89.00±0.001^{abde}	89.50±0.001^{abce}	61.72±0.001^{abcd}
Phenanthrene					
Day 0	0.04±0.01^{cde}	149.2±0.001^{cde}	136.3±0.001^{abde}	159.0±0.001^{abce}	91.15±0.001^{abcd}
Week 6	0.554±0.001^{cde}	145.2±0.001^{cde}	98.61±0.001^{abde}	130.1±0.001^{abce}	85.02±0.001^{abcd}
Week 8	0.422±0.001^{cde}	142.4±0.001^{cde}	87.31±0.001^{abde}	92.75±0.001^{abce}	54.99±0.001^{abcd}
1, 2-Benzanthracene					
Day 0	0.02±0.01^{cde}	152.3±0.001^{cde}	144.2±0.001^{abde}	162.0±0.001^{abce}	85.23±0.001^{abcd}
Week 6	0.557±0.001^{cde}	146.3±0.001^{cde}	93.80±0.001^{abde}	129.7±0.001^{abce}	0.406±0.001^{cde}
Week 8	0.406±0.001^{cde}	141.1±0.001^{cde}	88.72±0.001^{abde}	91.11±0.001^{abce}	56.82±0.001^{abcd}

Values are means of triplicate determinations ± standard deviation.

NC = Normal Control
-Ve = Negative Control
XgCH = Treated Plot (50g of Cow Horn powder)
YgCH = Treated Plot (75g of Cow Horn powder)
ZgCH = Treated Plot (100g of Cow Horn powder)

Table 4(b): Effects of Enhanced Bioremediation on Polycyclic Aromatic Hydrocarbons (PAH)(mg/kg) in soil

Parameters	NC	-Ve C	XgCH	YgCH	ZgCH
Pyrene					
Day 0	0.02±0.01^{cde}	147.8±0.001^{cde}	140.4±0.001^{abde}	157.3±0.001^{abce}	84.30±0.001^{abcd}
Week 6	0.551±0.001^{cde}	140.8±0.001^{cde}	94.49±0.001^{abde}	128.4±0.001^{abce}	84.60±0.001^{abcd}
Week 8	0.390±0.001^{cde}	136.3±0.001^{cde}	84.66±0.001^{abde}	91.50±0.001^{abce}	54.40±0.001^{abcd}
Chrysene					
Day 0	0.03±0.01^{cde}	145.1±0.001^{cde}	141.1±0.001^{abde}	161.2±0.001^{abce}	88.40±0.001^{abcd}
Week 6	0.556±0.001^{cde}	143.1±0.001^{cde}	96.33±0.001^{abde}	126.8±0.001^{abce}	85.00±0.001^{abcd}
Week 8	0.398±0.001^{cde}	135.3±0.001^{cde}	89.23±0.001^{abde}	88.60±0.001^{abce}	60.80±0.001^{abcd}
Fluorine					
Day 0	0.04±0.01^{cde}	151.8±0.001^{cde}	143.5±0.001^{abde}	159.7±0.001^{abce}	83.22±0.001^{abcd}
Week 6	0.550±0.001^{cde}	146.2±0.001^{cde}	97.20±0.001^{abde}	129.6±0.001^{abce}	81.72±0.001^{abcd}
Week 8	0.415±0.001^{cde}	139.9±0.001^{cde}	88.70±0.001^{abde}	89.30±0.001^{abce}	57.22±0.001^{abcd}
Indeno (1,2,3) pyrene					
Day 0	0.02±0.01^{cde}	148.7±0.001^{cde}	146.0±0.001^{abde}	160.4±0.001^{abce}	84.30±0.001^{abcd}
Week 6	0.499±0.001^{cde}	145.4±0.001^{cde}	100.0±0.001^{abde}	127.3±0.001^{abce}	88.33±0.001^{abcd}
Week 8	0.403±0.001^{cde}	140.7±0.001^{cde}	90.56±0.001^{abde}	90.55±0.001^{abce}	59.50±0.001^{abcd}
Benzo (k) fluoranthene					
Day 0	0.04±0.01^{cde}	144.5±0.001^{cde}	139.3±0.001^{abde}	158.5±0.001^{abce}	87.50±0.001^{abcd}
week 6	0.500±0.001^{cde}	141.3±0.001^{cde}	93.87±0.001^{abde}	124.5±0.001^{abce}	90.28±0.001^{abcd}
week 8	0.402±0.001^{cde}	132.4±0.001^{cde}	84.40±0.001^{abde}	92.00±0.001^{abce}	60.70±0.001^{abcd}
Dibenz (a, h) antracene					
Day 0	0.03±0.01^{cde}	146.3±0.001^{cde}	142.0±0.001^{abde}	159.2±0.001^{abce}	80.36±0.001^{abcd}
Week 6	0.549±0.001^{cde}	142.2±0.001^{cde}	101.1±0.001^{abde}	121.9±0.001^{abce}	81.40±0.001^{abcd}
Week 8	0.401±0.001^{cde}	140.5±0.001^{cde}	87.60±0.001^{abde}	89.70±0.001^{abce}	54.08±0.001^{abcd}
Fluoanthene					
Day 0	0.02±0.01^{cde}	144.2±0.001^{cde}	140.2±0.001^{abde}	158.3±0.001^{abce}	87.53±0.001^{abcd}
Week 6	0.559±0.001	143.3±0.001^{cde}	95.65±0.001^{abde}	123.6±0.001^{abce}	86.92±0.001^{abcd}
Week 8	0.412±0.001	141.0±0.001^{cde}	83.51±0.001^{abde}	91.60±0.001^{abce}	58.90±0.001^{abcd}

Values are means of triplicate determinations ± standard deviation.

NC = Normal Control
-Ve = Negative Control
XgCH = Treated Plot (50g of Cow Horn powder)
YgCH = Treated Plot (75g of Cow Horn powder)
ZgCH = Treated Plot (100g of Cow Horn powder)

DISCUSSION OF FINDINGS

Presented in Table 1 are the baseline results of the clean experimental soil. The pH of the soils (4.26±0.10) was acidic. This is normal for an agricultural soil. This finding was in consonance with the work of Belonwu*et al*. (2007) which established pH from baseline studies to be acidic. Gray *et al*. (1998) documented that the pH of soils has a complementary relationship with the nutrients available in soils to plants.

The values of Pb, Ni, Cd, Mn, Cr, Cu, Zn and Fe were found to be higher than the recommended values by the WHO. Oviasogie andNdiokwere (2008) had opined that, soils that are low in pH would bring about higher concentrations of heavy metals in soils.

The mean values of 0.65±0.001mg/kg and 0.934±0.001mg/kg for TPH and PAH respectively were within the recommended range by DPR for soils of agricultural purposes. This was a clear indication that the research site had no crude oil contamination to its effect.

The none detection of Vanadium and cobalt in the soil samples may be adduced to the fact that, minerals in the soils that contained vanadium were deficient. Also, the activities of humans that should have brought about higher concentrations of V and Co in the soils were minimal (Cappuyns&Slabbinck, 2012).

Table 2 is the results of enhanced bioremediation on soil heavy metals. It reveals 16.1% decrease, 13.0% increase and 27% decrease in Ni from the contaminated beds (XgCH, YgCH&ZgCH) respectively after soil contamination. Statistical differences were notably observed between the impacted beds and control bed values. These findings are in tangent with the report of Nwaichi*et al.* (2016), who documented that oil pollution results in the contamination of the environment with Ni.

Conversely, Vanadium (V) and Cobalt (Co) were not detected. These may be attributed to minimal or insignificant presence of the materials in the polluted soils that would have caused the detection of V and Co.

The table (2) reveals low mean concentrations of Cadmium (Cd) in the soils (0.20±0.000mg/kg, 0.10±0.00mg/kg and none detection) respectively for XgCH, YgCH and ZgCH after soil pollution. No significant differences were observed between the impacted soils and controls.

It was observed that Pb, Cr, Cu, Mn, Zn & Fe were present in the impacted soils. While statistical differences were observed in the values of all the metals (Cr, Mn, Pb, Zn and Fe), no statistical difference was observed in the values of Cu.

The result obtained in this present study is also in consonance with the documentations of Essiett*et al.* (2010) who carried out a study to assess the concentrations of heavy metals in crude oil polluted soils.

14.3%, 43.0% and 40.0% reductions were observed in the lead (Pb) content of soils from XgCH, YgCH and ZgCH after six weeks of remediation. Statistical differences were recorded between values from the remediated plots and controls. In the same vein, after one month of remediation, 75% reduction was observed from the XgCH plot, with a statistical difference to the control. None detection of Pb was recorded for the YgCH soils. 50.0% reduction was recorded from the ZgCH bed, with a statistical difference when compared with the control. The general reductions in the lead content of crude oil

Scientia Africana, Vol. 23 (No. 1), February, 2024. Pp 59-72
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polluted soils remediated with cow horn powder corroborate the findings of Essien*et al.* (2015) who recorded that augmentation of cow dung to a crude oil contaminated soil is efficient in the reduction of lead. Vanadium (V) and Cobalt (Co) were not detected after the sixth week and one month of remediation respectively. The reason for the none-detection of V & Co may be due to the insignificant concentrations of the two elements in the impacted soils.

A calculated decrease of 19.3%, 51.4% & 10.0% were observed in chromium content of soils from XgCH, YgCH and ZgCH respectively, after the sixth week of remediation. Statistical differences were recorded between the various remediated plot values and control. Also, 49.0%, 49.0% and 25.3% decreases were made after the eight week of remediation. Significant differences were also seen between the values from the remediated plots and controls. This result is in tandem with the work of Nwaichi*et al.* (2021) who recorded over 60% Cr reduction in soils after remediation.

50% decrease was recorded for cadmium (Cd) content of soils after six weeks of remediation from the three remediated plots respectively. No differences significantly were observed. Conversely, no cadmium was found after one month of remediation. This may suggest that, the cow horn powder were effective in removing cadmium from the crude oil impacted soils. This was corroborated by Amos-Tuatua*et al.* (2014) who recorded a mean concentration of < 0.001±0.01 mg/kg for Cd from a municipal dumpsite soils in Yenegoa, Nigeria. This present study further agrees with the findings of Essien*et al.* (2015) who recorded that, addition of cow dung to crude oil impacted soils enhances the removal of cadmium.

After six weeks of remediation, reductions were observed in the values of manganese (Mn) from all the remediated plots, with significant differences to the controls. However, after one month of remediation, increases were observed from the 3 different remediated plots. Statistical differences were recorded when compared to the controls. The initial reductions after six weeks of remediation shows that the cow horn samples were effective in reducing the amount of Mn in soils impacted with crude oil. This finding is in line with the report of Essiett*et al*. (2010) who assessed heavy metal concentration in soils impacted with crude oil. The later increase recorded after one month of remediation may be attributed to soil's near surface chemical reactions, which may have impeded the efficiency of the remediating agent in reducing the amount of manganese (Mn) in the soils (Rasheed *et al.*, 2013).

The Table (2), further reveals a decrease of 75%, 30.4% and 6.0% respectively in the copper (Cu) contents from the remediated plots after six weeks of remediation, with statistical differences to the controls. On the contrary, increases of 28%, 5.3% and 40.0% were observed after one month of remediation. The decreases recorded after six weeks of remediation may be attributed to significant increase in the numbers of microorganisms. This may have placed high demand on the Copper (Cu) generated from the soils, thus, leading to a decrease in the Cu content of the soils (Agbogidi*et al.*, 2007). The general increases in the concentrations of Cu after one month of remediation may be adduced to the fact that, the cow horn powder were potent in improving the soil's nutrient. This is in absolute agreement with the findings of Mbah*et al*. (2009), who documented that, adding organic wastes to oil contaminated soil led to improved agronomic properties of the soils. Although copper is needed in mild quantity in soils for plant's growth, high presence in soil can lead to fatty acid desaturation (Mass & Mason, 1968).

General increases were noticed in the values of Zinc (Zn) from the remediated plots after six weeks and one month respectively. Significant differences were observed when compared to the controls. These results shows the effectiveness of cow horns in adding nutrient (Zinc) to soils impacted with crude oil. The obtained results conforms to the work of

Nigam *et al.* (2014) who opined that manures are needed to increase the concentration of zinc in soil for healthy growth of plants.

Increases of 53.0%, 62.3% and 64.1% were observed in the Iron (Fe) content of soils after six weeks of remediation from the XgCH, YgCH and ZgCH respectively, with significant differences to the controls. Similarly, 52.4%, 65.0% and 67.0% increases were respectively recorded from the remediated plots after one month of remediation. These current study results, agrees with the findings of Akenga, *et al.* (2014) who investigated the concentration of Fe in soils at Kakamega North District, Kenya.

The concentrations of TPH (mg/kg) after six weeks and one month of remediation are also contained in Table 3. Reductions in the content of TPH after six weeks and one month of remediation were observed from the XgCH, YgCH and ZgCH remediated soils. Statistical differences were noticed between the remediated soils and controls. After six weeks of remediation, 19.0%, 41.0% and 51.0% reductions were observed from the remediated plots (XgCH, YgCH and ZgCH). In the same vein, 55.4%, 59.0% and 59.1% reduction were recorded in the concentration of TPH after one month of remediation. This suggest that, cow horns were effective in reducing the TPH content of soils impacted with crude oil. This result obtained corroborate the findings of Okolo*et al.* (2005), who documented that, addition of poultry manure to crude oil polluted soil enhanced degradation of crude oil.

The effects of enhanced bioremediation on Polycyclic Aromatic Hydrocarbons (PAH) as contained in Table 4 shows significant increases from the various contaminated beds (140.0±0.001mg/kg, 159.3±0.001mg/kg and 89.29±0.001mg/kg) for XgCH, YgCH and ZgCH respectively when compared with the controls. This is in tandem with the work of Nwaichi*et al*. (2016); who established that crude oil impaction brings about aliphatic and aromatic hydrocarbons such as PAH and TPH. Essien*et al.* (2015) recorded that petroleum hydrocarbons (PAH) is one of the most detected contaminants of organic origin. The calculated increase in PAH concentration from XgCH, YgCH and ZgCH were respectively 99.3%, 99.4 and 99.4%. These indicate a condition of major spill. Soil high PAH concentrations pose great challenge to the proper yielding of crops (Nwachukwu, 2001).

Also presented in Table 4 are the concentrations of PAH of the crude oil impacted and cow horn-powder remediated soils after six and eight weeks respectively. The table reveals 30.0%, 19.0% and 0.29% decrease in the content of PAH from the remediated beds (XgCH, YgCH and ZgCH) respectively after six weeks of remediation. Significant differences were observed between the remediated plots and controls. Similarly, reductions of 37.0%, 42.0% and 31.0% were recorded respectively from XgCH, YgCH and ZgCH plots, after one month of remediation. Statistical differences were noticed between all the remediated plot values and controls. These results shows that cow horns were efficient in causing reductions in PAH content of soils impacted with crude oil. These findings are in consonance with the research work of Essien*et al*. (2015). Allowing PAH in soils to be high is not conducive for the effective growth of plants.

CONCLUSION AND RECOMMENDATION

Findings from this research work reveals that Cow Horn-Powder are potent in reducing the concentrations of TPH, PAH and heavy metals in crude oil contaminated soil. This research also demonstrated that Cow Horns are good sources of manure. However, it is being recommended here that, a comparative study should be carried out using cow horns (organic manure) and NPK fertilizer (inorganic manure) to ascertain which is more efficient in remediation of petroleum hydrocarbon polluted soils.

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