DOI: 10.26730/2618-7434-2022-1-16-30

UDC 332.368:504.53.062.4

BIOREMEDIATION OF CRUDE OIL CONTAMINATED SOIL USING BLENDED MIXTURES OF FISH AND PIGGERY WASTES AS BIO-STIMULATING AGENTS

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Abstract.
The present study investigated the potentials of two blended organic manures (Fish and pig wastes) as biostimulating agents in restoring induced crude oil pollution in soil samples. 200g of soil sample was obtained and polluted with 10% (w/w) of Bonny crude. 30g of the blended mixture of the organic manure (fish and pig wastes) were added to the polluted soil samples. The bioremediated soil was subsequently analyzed for physicochemical properties, total Petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAH), heavy metals and endogenous oil degrading fungi populations on weekly basis for a period of five weeks. From the results obtained, bioremediation with the blended organic manure significantly improved the levels of nitrogen, P, K and organic matters when compared with the control. The concentrations of PAH and TPH were significantly reduced following treatment with the organic remediants in a time dependent manner. The results also revealed that there was a significant increase ($P<0.05$) in the fungal load on the amended soil sample following the five weeks treatment plan. Thus, the study suggests that a combination of fish and pig wastes could serve as an excellent biostimulating agent for the remediation of crude oil polluted soils.

Article info
Received:
22 February 2022

Revised:
23 March 2022

Accepted:
30 March 2022

Keywords: hydrocarbons, bioremediation, organic manure, pollution, crude oil

For citation: Elebo A., Onyekwere O., Hassan N.M., Ottah S.O., Bando D.C., Tutuwa A.J., Ugochukwu V.U., Anigbo G.C., Nuhu I. (2022) Bioremediation of crude oil contaminated soil using blended mixtures of fish and piggery wastes as bio-stimulating agents, *Journal of mining and geotechnical engineering*, 1(16):16. DOI: 10.26730/2618-7434-2022-1-16-30

1 Introduction

Despite the numerous government legal framework at protecting the Nigeria environment and ecosystem, there has been a geometric increase in the challenges facing the environment especially in the Niger delta region where environmental degradation occasioned by constant hydrocarbon production, oil spillages and gas flaring still persist (Leke and Leke, 2019). Crude oil pollution has become a serious problem confronting industry stakeholders, scientists and regulators. In Africa, Nigeria remains one of the largest crude oil producers and with a sharp rise in urbanization,

DOI: 10.26730/2618-7434-2022-1-16-30

environmental pollution arising from petroleum hydrocarbon has increased greatly, hence the need for remediation (Xu et al, 2018). Undoubtedly, the anthropogenic activities of man is evidently relied on oil in meeting its energy demands, which has continued to boom the petrochemical industry and has further led to the deterioration of the environment (Xue et al, 2015). It is reported that several million barrels of crude oil was spilled into the ecosystem in Nigeria with an over four thousand incidents occurring in the Niger delta region. These discharges and spills of hydrocarbon occur in the process of production, storage, transportation, refining, processing, leakage accidents, and during overhauls of refineries. (Chaerun et al, 2004; Chen et al, 2015). The disturbing cases of crude oil spillage and its attendant consequences on agricultural soils, aquatic lives and underground water have been previously reported (Sikkema et al, 1995). These hydrocarbons contain hundreds of substances that includes heavy metals, polycyclic aromatic hydrocarbons and most of these chemicals have been listed as priority pollutants which have the tendency to persist in the environment over a long period of time. Ameliorating crude oil polluted soil has become a complex project. Several remediation techniques have been deployed by experts to clean-up polluted sites in other to attain a sustainable environment. The composition, complexity, high refractory index and insolubility of crude oil have greatly increased the difficulties in remediation. At present, various physicochemical methods deployed in remediating crude oil polluted sites such as base catalyzed dechlorination, oxidation and high temperature incineration. However, these methods are effective in remediating an expansive polluted soil quickly but are costly and have the tendency to result in secondary pollution. Bioremediation have shown to be eco-friendly and cost effective in the restoration of crude oil polluted sites (Osazee et al, 2005). Bioremediation technology utilizes biological agents as biostimulants to degrade pollutants. Crude oil pollution tends to increase the soils' carbon content and depletes the nitrates and phosphorus levels, destroys soil microbes and prevents the exchange of oxygen between the soil and atmosphere [Okolo et al, 2005; Adedokun and Ataga, 2007; Onuoha et al, 2003). Organic manure improves soil fertility by adding nutrients to the soil and it has been reported to boost plant growth in crude oil polluted soils and as such serves as a better alternative bioremediation method (Okolo et al, 2005). Organic manure such as fish wastes serves as biostimulating agents by utilizing the resident microbial populations to remediate a contaminated soil by catalyzing the natural attenuation process. In view of these challenges, this study was designed at investigating the potentials of a blended mixture of fish and piggery wastes as bioremediants in restoring crude oil polluted soil.

2 Materials and method

2.1 Sample collection

Soil sample was collected from the demonstration farm of Bioresources Development Centre Ubulu-uku, in Aniocha North Local government area of Delta state, Nigeria. 0-15 cm of the top soil was collected using a sterile manual shovel, a method described by (Ogbonna et al, 2007). The soil samples were passed through a 2.0 mm sieve (pore size) and conveyed in a black sterile perforated polythene bags for aeration to the laboratory for analysis. The crude oil used for the contamination of the soil sample was Bonny light crude obtained from the analysis Laboratory of the Nigerian National Petroleum Cooperation (NNPC), Moscow road, Port Harcourt, Rivers State Nigeria.

The fish wastes were collected from Harimon fish farm situated at No. 4 Federal housing estate road off Okpanam road, Asaba, Delta state. It was dried for two weeks, while the piggery wastes was collected from Prince Manner's farm Off Asaba-Ibusa road. All the reagents for the experiments were procured at Reigneth stores Intl Ltd., Mushin, Lagos and were of analytical grade. About 100 g each of the fish and pig wastes were air-dried for two weeks, grounded and mixed with a mechanical mixer in the ratio of 1:1. The control, polluted and amended soil samples were analyzed for their physicochemical properties.

2.2. Sample preparation

Debris, stones and coarse gravels were removed from the collected soil samples and was ground using a mortar and pestle and then passed through a 2.0 mm stainless steel sieve (pore size). The soil

DOI: 10.26730/2618-7434-2022-1-16-30

samples were air-dried at room temperature of between 25°-36°C and relative humidity of 20 to 50% for seven days and was conveyed in a black sterile perforated polythene bags for aeration to the lab for analysis of specific physicochemical properties as described below.

2.3 Physicochemical analysis

2.3.1 Analysis of heavy metals

1 gram of each soil sample was weighed out and transferred into an empty beaker of 250 ml. To this soil sample, 15 ml of a mixture of HNO_3, H_2SO_4, and $HClO_4$ in a ratio of 5:1:1 was added. The mixture was gently stirred and placed on a heating mantle up to a temperature of 800° C until a clear solution was obtained. The mixture was then cooled and made up to 30 ml with 2% HNO_3 and filtered. The concentrations of the heavy metals (Cd, Cr, As, Pb, Cu) were obtained using an atomic absorption spectrophotometer (Shimadzu AA-670, Japan) after the preparation of a reference solution.

2.3.2 Analysis of polycyclic aromatic hydrocarbons

2 g of the soil samples were weighed into a clean extraction container (50 ml beaker). 10 g of sodium sulphate was added and mixed together with the sample. 10 ml of dichloromethane was used as the extraction solvent. The mixtures were carefully filtered into clean solvent rinsed extraction bottle, using filter paper fitted into Buchner funnels. The extraction was concentrated to 2 ml and then transferred for clean-up/separation. The dichloromethane extract was cleaned- up by passing through a column packed with anhydrous Na_2SO_4 salt. The resulting extract was concentrated on a rotary evaporator to give an oily residue which was again dissolved in 1 ml CH_2Cl_2 and 1 μL was injected into the GC for analysis.

2.3.3 Analysis of pH

About 20 g of soil samples were weighed and transferred into a 100 ml beaker. Into this sample, 40 ml of distilled water was added and stirred properly with a glass rod and was allowed to stand for 30 mins with intermittent stirring. The pH meter was calibrated with a buffer solution with a pH value of 7 and then adjusted to known pH buffer solutions of 4.0 and 9.3. To the soil water suspension, an electrode was immersed and pH value was estimated from the readings on the meter.

2.3.4 Analysis of total nitrogen

Kjeldahl method (Jackson, 1973) was deployed with slight modifications. 20 g of soil sample was transferred to Kjeldahl flask and mixed with 20 ml distilled water. The flask was swirled for about 2 mins and allowed to stand for 30 mins. To the soil water suspension, 10 ml of a mixture of K_2SO_4, $FeSO_4$ and $CuSO_4$ was added in the ratio of 10:1:0.5 respectively. 30 ml of concentrated H_2SO_4 was added to the above mixture and swirled for 2mins. The flask was heated for about 15 min and rotated intermittently. The flask was allowed to cool and about 100 ml of distilled water was added to the mixture to avoid coagulation. This process was followed by distillation, the liquid portion of the mixture in the Kjeldhal flask was transferred to a distillation flask leaving behind the soil in the flask. The soil residue was washed for six times with 50 ml of distilled water and the liquid transferred to a distillation flask after allowing soil residues to settle for few seconds each time. Few pieces of glass bids and granulated zinc was added to the flask to ensure effective boiling process. The flask was then placed on a distillation stand and fitted to a condenser. 25 ml of 4% H_3BO_4 was poured in a 250 ml beaker with 3 drops of a mixed indictor and was placed under the condenser. (The beaker tip should be deep in the boric acid solution). The distillation flask was held at an angle of 45° and 100 ml of 10M NaOH was added to it. The distillation flask was connected with the condenser by splesh head and the contents of both flasks was carefully swirled followed by heating of the flask. 150 ml of the distillate was collected with absorption of ammonia. The liberation of ammonia was tested by holding wet red litmus paper to the tip of the condenser. The boric acid was back titrated with standard H_2SO_4 until the blue color disappears. Blank sample was running simultaneously. Total Nitrogen in the soil was calculated with the below expression:

$$\text{Total Nitrogen}\left(\text{TN}\right)=\frac{\left(V_s-V_B\right)\cdot M\cdot 0.014\cdot 100}{W},$$

DOI: 10.26730/2618-7434-2022-1-16-30

where V_S is the vol. of H_2SO_4 for sample titration, V_B is the vol. of H_2SO_4 for blank titration, M is th3.5 molarity of H_2SO_4 and W is the weight of soil.

2.3.5 Analysis of total petroleum hydrocarbon

Total petroleum hydrocarbon was determined by gravimetric process as described by [13].

100g of the soil samples was refluxed with a mixture of 3 g KOH and 100cm³ methanol for 2h. The mixture was filtered and the filtrate extracted twice with 25 cm³ redistilled hexane. The combined extract was evaporated to about 1.0 cm³ and was subjected to clean-up with a short silica gel column. After elution with purified n-hexane, the oil was isolated for weighing by evaporation of the hexane. Coefficient of variation for the triplicate determination of each sample was of the order of 20-30%.

2.3.6 Determination of organic matter

The soil organic matter was estimated using the loss on ignition LOI procedure as described by (Zhang and Wang, 2014). 10 g of the soil samples were placed in a 35 ml porcelain crucible and dried in an oven at 105°C. It was later placed in a desiccator to cool. This process was repeated until a constant mass for the soil and crucible was obtained. A muffle furnace was preheated to 360°C and soil samples were heated in the furnace at that temperature for 2h. After 2h, the crucible with soil was removed from the furnace and placed in a desiccator to cool before being weighed. The organic matter was evaluated as the difference between the mass of the crucible and the soil before and after heating.

2.3.7 Analysis of total potassium and phosphorus

Phosphorus was extracted according to method described by Olsen using a 0.5M $NaHCO_3$ solution adjusted to a pH of 8.5 (Olsen, 1954). Total Phosphorus was determined using colorimetric method. Phosphomolybedenum blue and ascorbic acid (reducing agent) and Sb was added to sample to give a stable Mo-P-Sb compound. After 10 mins, the color intensity was read at 882 nm and recorded using spectronic 20 colorimeter. Total Potassium was analyzed using atomic absorption spectrophotometric method after extraction with 1N NH_4OAc with the pH adjusted to 7.0. Standard stock was prepared by dissolving KCl in H_2O. Sodium was added to the final standard and test sample to reduce ionization interference. Potassium was determined at 7665Å wavelength

2.3.8 Determination of fungal population using potato dextrose agar

The Potato Dextrose Agar (PDA) plates were prepared according the manufacturer's specifications. About 1.0 g of each soil samples was serially diluted up to ten folds dilution. 0.1 ml (at 10^{-4} dilution) of the polluted crude oil soil solution was added onto PDA plates each for determination of viable fungal cell counts. These plates were incubated at room temperature (31±3°C) for 5 days. The counts obtained were multiplied by the dilution factor to obtain the fungal cell counts per gram of soil.

2.3.9 Determination of hydrocarbon utilizing fungal population

0.1 ml of the crude oil polluted soil sample was infused onto a modified mineral salt medium (Mills, et al, 1978). Sterile filter paper was soaked in a filter and was aseptically placed onto the cover of the inverted plate and incubated for 6 days at 37°C, a vapor phase transfer method (Okpokwasili and Amanchukwu, 1988). The experiment was carried out *n* triplicates and the average mean counts of colonies were recorded and used to the calculation of colony forming unit multiplied by the dilution factors for hydrocarbon utilization within the fungal population. The isolated colonies of mineral salts medium were further purified by sub culturing onto PDA medium to obtain a pure culture.

2.3.10 Bioremediation experiment design

The bioremediation process was carried out for 5 weeks between January – May, 2020. The soil samples were divided into 6 treatment reactors as shown in Fig. 1.

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C (CONTROL): SOIL WITHOUT BLENDED AMMENDMENT AGENT	**A1 (FOR 1 WEEK):** POLLUTED SOIL + 30 g OF BLENDED AMENDMENT AGENT (BAA) + 50% DISTILLED WATER	**A2 (FOR 2 WEEKS):** POLLUTED SOIL + 30 g OF BLENDED AMENDMENT AGENT (BAA) + 50% DISTILLED WATER
A3 (FOR 3 WEEKS): POLLUTED SOIL + 30 g OF BLENDED AMENDMENT AGENT (BAA) + 50% DISTILLED WATER	**A4 (FOR 4 WEEKS):** POLLUTED SOIL + 30 g OF BLENDED AMENDMENT AGENT (BAA) + 50% DISTILLED WATER	**A5 (FOR 5 WEEKS):** POLLUTED SOIL + 30 g OF BLENDED AMENDMENT AGENT (BAA) + 50% DISTILLED WATER

Figure 1. Dividing soil samples into 6 treatment reactors (illustrative representations of the experimental setup).
Рис. 1. Разделение образцов почвы на 6 реакторов для обработки (иллюстративное представление экспериментальной установки).

2.3.11 Bioremediation study

120 g of each soil sample was measured into plastic containers labeled C, A1, A2, A3, A4 and A5 respectively. Sample C was used as the control while sample A1, A2, A3, A4 and A5 represents different time intervals of 1, 2, 3, 4 and 5 weeks respectively as indicated in figure 1.0. The soil sample in each plastic container was contaminated with 10% (w/w) Bony light crude oil and thoroughly mixed together to achieve severe contamination because above 3% concentration oil has been reported to be increasingly deleterious to soil biota and crop growth (Osuji and Onojake, 2004). The samples were allowed for one week for proper infiltration after which 30 g of blended amendment agent was added to A1, A2, A3, A4 and A5 representing each time interval.

Each container except C (control) was made up to 50% volume by distilled water for proper percolation. Water was sprinkled on A1, A2, A3, A4 and A5 sporadically when the water level gets low, in line with the published work (Odukuma, 2003; Odukuma, 2006).

2.3.12 Determination of crude oil bioremediation

The method of (Nrior and Echezolom, 2016)] was used in calculating the percentage of bioremediation in the experiment. The process followed the steps stated below:

- **STEP 1:** The amount of pollutant remediated equals to initial concentration of pollutant (week 1) minus the final concentration of pollutant at the end experiment (Last week).

- **STEP 2:** The percentage (%) of bioremediation equals amount of pollutants divided by the initial concentration of pollutant (Week 1) multiply by 100.

$$AR\ (_{TPH)}) = I_C - F_C \quad (1)$$

$$\%\ B\ (_{TPH/PAH)}) = \frac{AR}{IC} \times 100 \quad (2)$$

where AR is the amount remediated; % B is the percentage bioremediation; I_C is the initial concentration of pollutant; F_C is the final concentration of pollutant.

2.3.13. Statistical analysis

The experiment was carried out in triplicates and data analyzed using Statistical Package for Social Sciences (SPSS), incident 21.0 software. The results were expressed as mean ± standard error

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of mean, the comparison for significance between the control and the experimental group was analyzed using analysis of variance (ANOVA) and the level of significance was $P < 0.05$.

3 Results and discussion

The result of the physicochemical analysis of the soil samples are presented in Table 1.

Table 1. Physicochemical properties of soil sample (control), crude oil polluted soil sample and blended amended soil sample
Табл. 1. Физико-химические свойства образца почвы (контрольный образец), образца почвы, загрязненного сырой нефтью, и образца почвы с добавлением смеси

Soil parameters	**Soil sample (Control)**	**Soil sample + Crude oil**	**Soil sample + Crude oil + Blended manure**
Total nitrogen (%), (TN)	0.24	0.15	0.28
Organic matter (%), (OM)	7.18	5.54	7.14
Total Potassium, (TK) mg/kg	0.47	0.21	0.43
Total Phosphorus, (TP) mg/kg	0.53	0.29	0.49
pH	6.26	5.38	6.53
Sand (%)	9.16	8.24	8.06
Silt (%)	14.34	18.50	19.13
Clay (%)	76.50	73.26	72.81
Moisture content (%)	10.51	10.26	10.43

The analysis revealed that TN, OM, TK and TP decreased as the soil samples were polluted with crude oil. After, bioremediation with the blended amendment agents, the values increased significantly when compared side by side with when the soil was polluted which suggests a gradual restoration of the soil. TN was seen to increase from 0.15% to 0.28 after remediation, 0.49 mg/kg respectively. The pH of the crude oil polluted and bioremediated soil samples revealed to be slightly acidic. The soil samples showed high clayey composition. On the other hand, the moisture content was seen to have decreased after the soil sample were polluted with crude oil and increased after remediation (10.26% to 10.43 %) when compared with the control. (10.51%)

Table 2. Fungal population (10^{-4} cfu/g)
Табл. 2. Популяция грибов (10^{-4} КОЕ/г)

Soil sample/ week	**0**	**1**	**2**	**3**	**4**	**5**
Control	23.12 ±2.98	22.93 ±1.57	20.91 ±1.92	20.23 ±1.09	19.34 ±2.09	17.01 ±1.23
Blended amended sample	31.32 ±2.17*	36.18 ±2.04*	38.12 ±2.10*	44.10 ±2.93*	47.23 ±1.98*	55.23 ±1.23*

Values are expressed as mean ± standard deviation.
Values in asterisk are considered statistically significant at $P < 0.05$.

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The fungal populations in the soil samples are presented in Table 2. The values of the control soil samples revealed that the fungal populations decreased from 23.12±2.98 (10^{-4} cfu/g) to 17.01±1.23 (10^{-4} cfu/g)**.** On the other hand, the fungal populations of the blended amended samples increased from 31.32±2.17 to 55.23±1.23 (10^{-4} cfu/g) as the study period increased. On weekly basis, the fungal load increased throughout the duration of the study.

The hydrocarbon utilizing fungal populations in the soil samples were represented on Table 3. The result revealed that the fungal load decreased significantly in the control soil sample throughout the duration of the study. After remediation, the fungal load was seen to have increased as the week progresses.

Table 3. Hydrocarbon utilizing fungal population (10^{-4} cfu/g)
Табл. 3. Популяция грибов, использующих углеводороды (10^{-4} КОЕ/г)

Soil sample/ week	**0**	**1**	**2**	**3**	**4**	**5**
Control	15.12 ±0.32	13.27 ±0.56	12.07 ±1.43	10.56 ±1.64	8.76 ±0.78	5.63 ±1.23
Blended amended sample	20.57 ±0.53*	24.56 ±0.64*	25.12 ±1.10*	28.82.10 ±1.93*	31.67 ±0.95*	34.06 ±1.61*

Values are expressed as mean ± standard deviation.
Values in asterisk are considered statistically significant at $P < 0.05$.

The result of the analysis revealed that copper (Cu) had the highest concentration when the soil sample was polluted with crude oil followed by lead (Pb). The values of Cu and Pb increased significantly when compared with the control soil sample ($P < 0.05$). There is a slight increase in the concentrations of Cd, As and Cr when compared with the control. The heavy metal values in the polluted soil samples ranged from between 2.34±0.24 to 24.16±1.65 mg/kg. After remediation, it can be seen that the levels of lead and copper were significantly reduced on application of the blended amendment agent.

Table 4. Concentrations of heavy metals
Табл. 4. Концентрации тяжелых металлов

Heavy metals	**Soil sample (Control) [mg/kg]**	**Soil sample + Crude oil [mg/kg]**	**Soil sample + Crude oil + BBA [mg/kg]**
Cd	0.67±0.14	2.28±0.43	1.18±0.12
Pb	5.05±1.48	20.65±0.52*	13.86±0.22*
Cr	0.98±0.23	2.34±0.24	1.16±0.11
Cu	4.91±1.60	24.16±1.65*	11.23±1.10*
As	0.05±0.01	2.98±0.06	1.86±0.01

Values are expressed as mean ± standard deviation.
Values in asterisk are considered statistically significant at $P < 0.05$.

The concentrations of TPH and PAH in the soil samples are presented in Table 5. Results revealed that TPH and PAH concentrations decreased in a time dependent manner; the values of the concentrations of TPH ranged from 18360 to 10056 mg/kg and PAH ranged from 47.11 to 4.5 mg/kg.

The individual PAH in blended amended soil sample are presented in Table 6. The concentration of the individual PAHs was seen to decrease as the amended soil samples were treated with the

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bioremediants within the duration of study. Naphthalene was seen to have the most significant decrease in concentration at 4 and 5 weeks witnessing BDL values.

Table 5. Concentration of TPH and PAH
Табл. 5. Концентрации ОНУ и ПАУ

Sample	**Time (Week)**	**TPH (mg/kg)**	**PAH (mg/kg)**
C (control)	0	27550	47.11
A1	1	18360	15.76
A2	2	15175	13.43
A3	3	12340	9.17
A4	4	11118	6.06
A5	5	11056	4.52

Table 6. Concentrations of Polycyclic Aromatic Hydrocarbon
Табл. 6. Концентрация полициклических ароматических углеводородов

PAHs, mg/kg	**Week:**					
	0	**1**	**2**	**3**	**4**	**5**
2-methylenaphthlene	1.10	0.29	0.26	0.12	0.09	0.06
Acenaphthene	0.95	0.46	0.38	0.24	0.13	0.11
Acanapthylene	0.26	0.81	0.62	0.43	0.25	0.21
Anthracene	3.56	1.04	0.75	0.41	0.24	0.20
Benzo (a) anthracene	2.78	0.77	0.68	0.33	0.17	0.13
Benzo (a) pyrene	2.37	1.05	0.88	0.53	0.32	0.28
Benzo (b) fluoranthene	4.15	0.91	0.85	0.71	0.36	0.34
Benzo (g,h,i) prylene	2.98	0.86	0.58	0.32	0.10	0.099
Benzo (k) fluoranthene	2.36	1.16	1.04	0.74	0.64	0.58
Chrysene	2.98	1.14	1.10	0.94	0.46	0.42
Dibenzo (a,h) anthracene	2.31	1.15	1.11	0.94	0.72	0.69
Fluoranthene	7.10	2.12	1.48	0.84	0.62	0.59
Fluorene	2.01	0.61	0.58	0.32	0.20	0.18
Indeno (1,2,3) pyrene	2.14	0.12	0.09	0.04	0.01	BDL
Naphthalene	0.92	0.05	0.03	0.01	BDL	BDL
Phenanthrene	2.04	1.09	0.98	0.52	0.31	0.23
Pyrene	5.10	2.13	2.02	1.73	1.44	1.41

* BDL: Below Detectable Limit

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The concentration of TPH in the soil samples is presented in Figure 2. Results revealed that TPH concentration decreased in time dependent manner; the values of the concentrations of TPH ranged from 18,360 to 10,056 mg/kg.

The concentration of PAH in the soil samples are presented in Figure 3. Results revealed that PAH concentrations decreased in time dependent manner; the values of the concentrations of PAH ranged from 47.11 to 4.5 mg/kg.

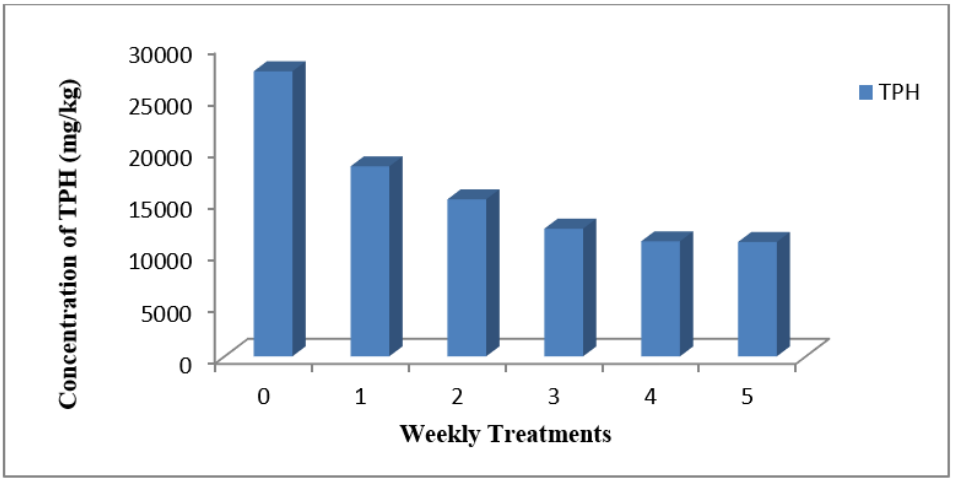

Figure 2. Concentration of Total Petroleum Hydrocarbon (TPH) versus weekly treatment.
Рис. 2. Концентрация общих нефтяных углеводородов (ОНУ) в зависимости от еженедельной обработки.

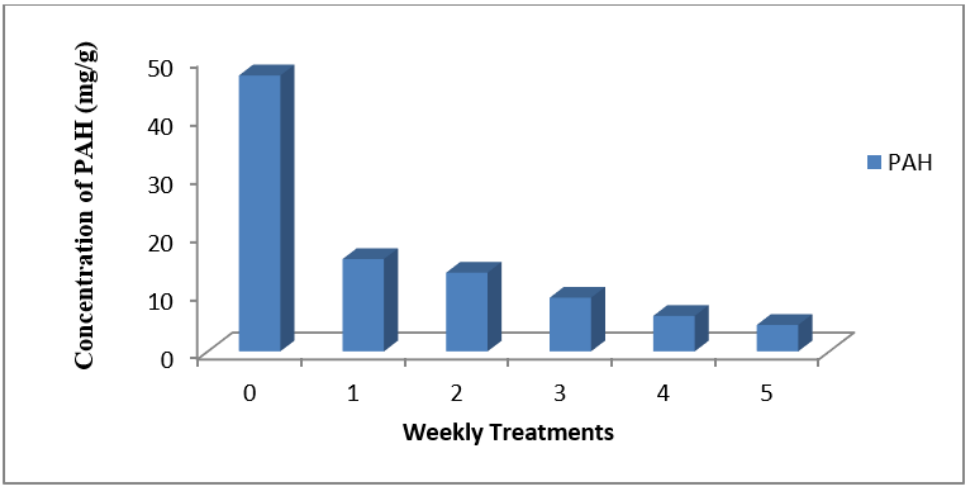

Figure 3. Concentration of Polycyclic Aromatic Hydrocarbon (PAH) versus weekly treatment.
Рис. 3. Концентрация полициклических ароматических углеводородов (ПАУ) в зависимости от еженедельной обработки.

Crude oil pollution is an increasing global problem affecting both developed and developing countries. Crude oil is a mixture of wide varieties of constituents consisting primarily of hydrocarbons, heavy metals and aromatic hydrocarbons.

These constituents have been shown to be toxic to plants and the soil ecosystem (Onuh et al, 2008). From the results of the study there was an appreciable decrease in the levels of PAHs after the first week of remediation with the blended mixture of the organic manure. There was a 90% reduction in the concentrations of PAHs; this is in agreement with work reported by (Kelechi et al, 2019). This

DOI: 10.26730/2618-7434-2022-1-16-30

can be attributed to the fact that microbes utilize the chemical contaminants resident in the soil as a source of energy and through a redox reaction chain, degrades the target contaminants in the crude oil and into a useable energy source for the microbes (Osazee et al, 2019). Similarly, (Hill, and McCarty, 1967) reported that microbial flora are capable of degrading hydrocarbons in soil composted mixture. The physicochemical properties such total nitrogen, phosphorus ion, potassium ion, organic matter and pH were analyzed before and after remediation with the amendment agent. From the results, the levels of total nitrogen, potassium ions and phosphorus ions increased significantly for the soil samples polluted with crude oil. The high concentrations of TK and TP could be attributed to the rapid decay and mineralization of organic and mineral materials in the soil; these processes lead to the release of the elements (Nnaji et al, 2005). The decrease in total nitrogen and available nitrates in the soil sample polluted with crude oil can be attributed to temporal immobilization of these nutrients by these microbes (Jong, 1980). Nitrogen and phosphorus are among the most important mineral elements needed for growth and productivity in plant. It was reported (Ogboghodo et al, 2004). The same where he stated there was a decrease in nitrogen and pH value the in crude oil polluted soil. This work is also in agreement with the work of (Alfreda and Ekene, 2011) where they observed an increase in the level of total nitrogen, TK and TP after the application of poultry manure in a crude oil polluted soil. After the first of remediation, it can be seen from the result that the levels of TN, TK and TP increased significantly in the soil sample polluted with crude oil. This could be attributed to the fact that organic manure has a balanced supply of nutrients including micro nutrients. There is also an increased soil nutrients availability due to increased soil microbial activities in organic manures. Organic manure has the potential of decomposing harmful element, improve soil structure and soil water availability (Si et al, 2011). According to (Onuh et al, 2008), the level of TN was increased significantly on the application of poultry manure and cow dung on crude oil polluted soil. The work is also in agreement with (Kelechi et al, 2019) where they reported that TN, TP and TK increased as bioremediants was added to the polluted soil sample.

From the result of the study, the fungal load was seen to have decreased in the crude oil polluted soil sample when compared with the control. The decreased was consistent from week 0 through to week 5.

This observation is in agreement with (Atlas, et al, 2011), who reported that the drop in the total heterotrophic fungal count in the contaminated soil in the first week can be attributed to selective inhibition of members of the microbial community as a result of the toxic component of petroleum and also as a result, reduced aeration and upset of carbon/inorganic nutrient balance for the indigenous population caused by the present of petroleum. After adding bioremediants the fungal population increased from week 0 to week 5, which could be attribute to increased microbial activity. The result of the analysis showed that the pH values of the soil sample in both polluted and amended soil samples ranged from 5.38 – 6.53. This indicated that the pH of the soil sample was suitable for the growth of the fungi. According to (Ogbonna, et al, 2007), bacteria can increase rapidly in the soil with pH value ranging from 5.0 to 8.0.

Conclusion

The study was designed to test the efficacy of a blended mixture of organic manure (fish and piggery waste) as potential biostimulating agent in restoring an induced crude oil polluted sample**.** The result of the analysis reviewed that the bioremediants was able to degrade TPH and PAH by 60% and 90% respectively. Similarly, the blended organic manure was able to increase the levels of TN, TP, TK, moisture content, organic matters and fungal load in the amended soil sample. Thus, the study suggests that a blended mixture of fish and pig wastes can serve as excellent bioremediation agent against a crude oil polluted soil.

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DOI: 10.26730/2618-7434-2022-1-16-30

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Conflict of interest

The authors declare that there is no conflict of interest.

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DOI: 10.26730/2618-7434-2022-1-16-30

УДК 332.368:504.53.062.4

БИОРЕМЕДИАЦИЯ ПОЧВЫ, ЗАГРЯЗНЕННОЙ СЫРОЙ НЕФТЬЮ, С ИСПОЛЬЗОВАНИЕМ ОТХОДОВ РЫБНОЙ И СВИНОВОДЧЕСКОЙ ПРОМЫШЛЕННОСТИ СМЕШАННОГО СОСТАВА В КАЧЕСТВЕ БИОСТИМУЛИРУЮЩИХ АГЕНТОВ

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Аннотация.
В данном исследовании изучалась возможность потенциального использования двух смешанных органических удобрений (отходы пищевой и рыбной промышленности) в качестве биостимулирующих агентов при восстановлении образцов почвы, загрязненных сырой нефтью. Для анализа было взято 200 г образца почвы, загрязненного 10% (масс.) сырой нефти Bonny. 30 г смеси органического навоза (рыбные и свиные отходы) было добавлено к загрязненным образцам почвы. После этого биоочищенная почва анализировалась на физико-химические свойства, общее содержание нефтяных углеводородов (ОНУ), полициклических ароматических углеводородов (ПАУ), тяжелых металлов и популяции эндогенных грибов, разлагающих нефть, еженедельно в течение пяти недель. Согласно полученным результатам, биоремедиация с использованием смешанного органического навоза значительно повысила уровни азота, фосфора, калия и органических веществ по сравнению с контрольными цифрами.
Концентрации нефтяных углеводородов и полициклических ароматических углеводородов значительно снизились после обработки органическими препаратами в зависимости от времени. Результаты также показали значительный рост ($P<0,05$) грибковой загрузки на образце почвы с внесенными изменениями после пятинедельной обработки. Таким образом, исследование показывает, что комбинация рыбных и свиных отходов может служить отличным биостимулирующим агентом для восстановления загрязненных сырой нефтью почв.

Информация о статье
Поступила:
22 февраля 2022 г.

Рецензирование:
23 марта 2022 г.

Принята к печати:
30 марта 2022 г.

Ключевые слова:
углеводороды, биоремедиация, органический навоз, загрязнение, сырая нефть, биостимулирующий агент

Для цитирования: Элебо А., Ониеквере О., Хассан Н., Оттах С., Бандо Д., Тутува А., Угочукву В., Анигбо Г., Нуху И. Биоремедиация почвы, загрязненной сырой нефтью, с использованием отходов рыбной и свиноводческой промышленности смешанного состава в качестве биостимулирующих агентов // Техника и технология горного дела. – 2022. – № 1 (16). – С. 16-30. – DOI: 10.26730/2618-7434-2022-1-16-30

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Conflicts of Interest

The authors declare no conflict of interest.

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Авторы заявляют об отсутствии конфликта интересов.

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