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EFFECT OF BACTERIAL SEEDING AND SOIL AMENDMENT ON PERCENT BASE SATURATION OF CRUDE OIL POLLUTED ULTISOL OF SOUTHERN, NIGERIA.

[1.]Etukudoh, N. E., [2.]Roland Gbarabe [3.]John, Chukwumati A. [4].Dickson A. A.

(1) Department of Soil Science, Agric. Faculty, Akwa Ibom State University, Obio Akpa Campus

(2) Department of Agric. Econ. and Extension, Cap tain Elechi, Amadi Polytechnic, Romuola, PH

(3) Department of soil Science, University of Port Harcourt

(4) Department of Crop/Soil Science, Faculty of Agric., Niger Delta University, Amasoma Wilberforce Island, Bayelsa State

Abstract

A pot experiment was conducted Green House of Agric. Faculty, at the Rivers State University of Science and Technology, Port Harcourt, Nigeria to evaluate the effect of bacterial seeding, Urea and Poultry manure (PM) on percent base saturation (% BS) on crude oil polluted Ultisol of Southern Nigeria. Each port weighting 3kg was contaminated to 2, 5 and 10% (W/W) with Bonny Light crude oil of 0.835 relative density(specfic gravity). Seven (7) days after, each level was amended with Urea and PM, thereafter the pots were seeded with Acinetobacter clavatus, Bacillus subtilis, Pseudomonas aeruginosa and Conynebacterium diptheriae. Unamended and unseeded soils were also included to serve as controls. Treatments were replicated three times, completely randomized and arranged in a Green house. Results showed that % BS decreased with increased in crude oil percent applied. Higher % BS values were observed in the PM amended soil as compared to Urea and the unamended soil treatment options. At week 9, soil inoculated with Pseudomonas aeruginosa had higher %BS in all treatment options in 0-10% pollution levels, Thus, Pseudomonas aeruginosa biostimulated with PM are recommended as suitable agents for improving % BS of crude contaminated Ultitsol in Soutern Nigeria.

Key words: Biostimulated, Bacterial seeding Amended, Bonny Light.

Introduction

Contamination of agricultural land with crude oil is known to have serious implication on it productivity. Amund O. O. and Nwokoye N. (2012) reported that the occurrence of oil pollution on land is of major concern as it would result in the depression of microbial populations and activities. Odu, 2013 also reported that the immediate effect of crude oil spill in an area is that of fire outbreak, followed by the destruction of plants, animals and microbiota. In 1984, Bosert and Bartha

reported that crude oil spill in soil environment reduce oxygen tension and increase anaerbosis. Soil quality is affected through change in soil air, pH, temperature, soluble nutrients and disruption of metabolic process. Soil microorganisms are geometrical in nature and sudden change in soil conditions adversely after the mineralization of organic carbon, nitrogen, sulphur and phosphorus for the growth of higher plans and animals (Moller, 2013). It therefore means that the present and availability of basic cations in the soil is affected byoil spill, thus, the soil fertility. This therefore mean that a number of important agriculturalproperties of depend on the nature and relative proportion of the bases present. Soil percent base saturation is one of the important chemical properties in the soil and is closely related to the soil fertility.

Bases are considered any cations exception hydrogen and aluminium' as well as iron and manganese which are non-base. Percent base saturation means the amount of the cationexchange capacity not holding potential acidity Isirimah (2013) Soil with high basecation exchange capacity and high base saturation are fertility unless they are saline orcontain heavy metals lsirimah (2013).

The important of soil exchange capacity in soil management cannot be over emphasized. Soil exchange capacity indicates the nutrient holding capacity of a soil; it also determines how often and how much lime to be applied to the soil. A higher soil cation exchange capacity indicates a near-neutral of soil pH which is the desirable. To raise crude oil polluted soil pH to a near- neutral or a desirable pH, remediation is often recommended. Among various approaches adopted to remedy a polluted soils include Bioremediation, Bioremediation uses microorganisms to degrade organic contaminants in soil, sludge, and solids either excavated or in situ, This study aims at using crude oil utilizing soil microorganisms stimulated or without nutrient resources (organic or inorganic) to assessed effects to lest for their degradative ability on polluted Ultisol in Southern Nigeria %BS.

Materials and Methods.

Five hundred and forty (540) Kilograms of top soil (0-15cm) was excavated from the Rivers state University of Sciences and Technology Teaching and Research Farm for the experiment. The soil was sieved, harmonized and divided into four (135 kg each) and respectively polluted to 0, 2, 5 and 10% (w/w) with light crude oil of 0.83 5 specific gravity (Relative density). Each pollution level (135kg) was further divided into three (3) (45kg) and amended with I0tha^{-1} Urea, 10tha^{-1} poultry manure and left unamended. Three (3) kilograms of each amended soil were transferred polybags (5) measuring 30 x25cm^2 and seeded with 24m1 of 24hours nutrient both culture of *A. clavatus, B. subtillis, P. aeruginosa* , *C. diptheriae* while one (1) bags was left without bacterial seeding to served as control. The treatments were repeated three times, completely randomized and arranged in a Greenhouse. After sixty-three (63) days

sampling was done, During the study period, the experiment was kept moistened at field capacity using water can Akpan and Ekpo, (2014).

Isolation, Identification and Cultivation of the Hydrocarbon Utilizing Bacteria (HUB)

Bacteria used in this study were the indigenous hydrocarbon utilizing bacteria. The study soil was contaminated with the Bonny light crude seven (7) days after the soil was collected, sieved for the purpose of isolation and identification of hydrocarbon utilizing bacteria (HUB). One(1) gram of previously sieved soil sample was thoroughly shaken in 9m1 of sterile distilled water to give 10^{-1} dilution. One milliliter (lml) of the 10^{2} dilution was transferred into the next test tube containing 9m1 normal saline (diluents) and diluted serially in one-tenth stepwise up to 10^{3}dilution. From the dilutions of 10^{3} of each soil sample, zero point one millilitre (ft imi) aliquot was transferred aseptically on to freshly prepared oil agar medium and spread with a sterile glass rod. The inoculated plates were incubated at 37°C for 24 hours after which the plates were examined for growth.

For the purpose of characterization and identification of bacteria. Cultures of bacteria were obtained by aseptically streaking representative colonies of different cultural types which appeared on the agar plates on to freshly prepared nutrient agar plates and incubated at 28°C for 24 hours. These nutrient agar plates were stored in the refrigerator and served as pure stock cultures for subsequent characterization and identification tests. The following biochemical tests were performed: gram staining, motility, catalase, coagulase, methyl red, Vogues-proskaver, indole, citrate utilization and sugar fermentation. The pure cultures were identified on the basis of their cultural, morphological and physiological characteristics.

Seeding of Bacteria into the Soil

Four (4) most numerous species were isolated from the studied soil for the research. These were Acinetobacter clavatus, Bacillus sub tilis, *Pseudomonas aeruginosa* and *Corynebacterium diphtheriae* having (1.80×10^{4}, 1.35×10^{4}, 2.00×10^{4}, cfu/g soil) counts respectively.

Nutrient broth culture of all the hydrocarbon utilizing genera isolated from the polluted soil were prepared and incubated for 48 hours. Nutrient broths were supplemented with Bonnylight crude oil to optimize the organisms (Science Guardian, June 18, 2010). These nutrient broth cultures served as inocula for the treatment units. Twenty (20)millilitres (130 1/ha) of the 24 hours nutrient broth culture of each of the test organism were introduced into the respective units (Ekpo and Udofia, 2016).

Analytical methods for Soil

The soil samples were processed for mechanical and chemical analyses. The soil samples were air-dried, crushed and passed through a 2mm sieve. For the determination of organic C, total N, Ca, K, P, Na and Mg,

the samples were further ground to pass through a 100-mesh sieve.

* pH was determined in water with glass electrodes in the1:2.5 soil water ratio.

* Organic matter was determined by wet oxidation method of Nelson and Summers .

* Total nitrogen was determined using macrokjeldahl digestion and distillation method of Jackson.

* Exchangeable bases (Ca, Mg,) were extracted with molar ammonium acetate, while K and Na concentration were determined by flame photometry and

* Magnesium and Sodium were determined by EDTA titration.

* Exchangeable acidity (Al plus H) was extracted with molar KCL solution and acidity determined by titration.

* Effective cation exchange capacity (ECEC) was taken as the sum of individual exchangeable bases plus exchange acidity.

* Available P was determined by methods described by Sparks (2018).

* Mechanical analysis was carried out by hydrometer procedures as described by Klute.

Results.

Table 1. Physico-chemical Properties of the Soil used for the study before the addition of treatments

Parameter Measured	Values
pH(H_2O) 1:2.5	5.80
Organic Carbon (%)	3.91
Total N (%)	0.12
Available Phosphorus (mg kg^{-1})	267
Exchangeable bases	
Ca (cmol kg^{-1}	3.07
Mg (cmol kg^{-1})	1.30
Na(cmol kg^{-1})	0.73
K(cmol kg^{-1})	0.86

Exchangeable acidity (cmol kg^{-1})	1.00
ECEC (cmol kg^{-1})	6.96
BS (%)	83.22
Sand(%)	85.82
Silt (%)	8.42
Clay(%)	5.76
Textural class	Sandy loam

Physicochemical property of the soil used for the study are presented in Table I. the soil was acidic in nature with mean pH value of 5.80. The exchangeable bases: Ca, Mg, K and Na means values were 3.07, 1.30, 0.73 and 0,86cmol/kg respectively with cation exchange capacity of 6.96cmol kg^{-1} soil and 83.22 percent base saturation. The proportion of the sand,silt and clay were 861.80, 849.4, 863.00 and 87.10, 83.90, 81.60 and 58.30, 5.56 with means values of 858.20, 84,20 and 57.60 respectively. Texturally, the soil was predominantly sandy loam.

Properties Poultry Manure Chemical

Chemical properties of poultry manure used as soil amendment are presented in

Table 2. The poultry manure was rich in exchange bases with a 6.99 mean pH.

Table 2. Chemical Properties of Poultry Manure

Parameter Measured	Values
pH(H_20 1.2.50)	6.99
Total N (%)	2.5 9%
Phosphorus (mg kg^{-1})	2.18%
Calcium	6.20%
Magnesium	2.00%
Sodium	0.70%
Potassium	3.30%

Inoculation resulted in a decrease in %BS. Thus, %BS and ECEC were higher in the uninoculated soil as compared to the inoculated soil in all the treatment options. soil without bacterial inoculation, application of crude oil increased %BS from 83.22 — 84% at 2% pollution level and further decreased to 83.29% at 5% pollution level and to 69.15% at 10% pollution level in the unamended soil. In the soil amended with urea, %BS increased from 86.72 to 91,55, 96.61% and decreased to 74.34% at 2, 5 and 10% pollution levels respectively, in the uninoculated soil.

Table 3.Soil Percent Base Saturation of the Studies Soil. Table 4.9: Effect of treatments on soil base saturation (%) at zero and at week nine

	Day 1			**Week 9 (Day 63)**		
Crude Oil Level	**Amendments**			**Amendments**		
/Bacteria	**0**	**1**	**2**	**0**	**1**	**2**
0%B_0	83.22	86.72	92.73	62.96	83.63	88.33
0%B_1	76.10	85.00	88.81	29.82	56.46	63,17
0%B_2	71.56	78.89	81.40	36.26	58.96	65.63
0%B_3	74.04	81.99	89.30	33.33	61.62	66.66
0%B_4	77.58	81.20	88.80	41.49	51.62	63.23
2%B_0	84.00	91.55	92.12	66.67	82.08	85.36
2%B_1	73.90	90.00	90.50	65.34	79.78	82.14
2%B_2	72.00	87.70	88.90	58.84	70.35	73.25
2%B_3	69.10	89.90	90.60	63.89	75.53	81.86
2%B_4	72.30	89.00	90.00	64.29	69.70	77.21
5%B_0	83.29	96.61	94.71	57.08	65.80	68.02
5%B_1	70.40	85.40	87.77	51.05	61.93	65.48
5%B_2	68.70	82.60	84.30	52.48	63.00	65.41
5%B_3	73.56	84.70	86.63	56.02	65.24	69.08
5%B_4	72.90	79.64	87.74	52.72	62.36	68.30
10%B_0	69.15	79.34	84.50	33.08	48.62	49.17
10%B_1	47.98	65.56	77.00	25.54	44.11	46.69
10%B_2	55.87	65.60	65.07	24.85	43.79	48.69

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10%B_3	66.18 71.07 75.70	32.42 47.37 55.25
10%B4	58.07 63.84 75.11	27.17 38.61 50.21
	15.23 16.82 13.45	**15.28 14.67 14.89**
LSD (0.05)		

Key: B_0, B_1, B_2, B_3, B_4, Soil without inoculation, soil inoculated with *A. clavatus, B. subtilis, P. aeruginosa and C. diphtheriae* respectively.: 0, 1, 2, = Soil without amendment, soil amended with urea and PM respectively.

In the soil amended with PM, % BS decreased from 92.73 — 92.12% at 2% pollution level,increased to 94.7 1% at 5% pollution level and further decreased to 84.50% at 10% pollution level.

Inoculation resulted in the decreased of %BS from 83.22 to 76.10, 71,56, 74.04, 77.58% and from 84 to'73.90, 72, 69.19, 72.30% and from 83,29% to 70.40, 68.70, 73.56, 72.90% and from 69.15 to 47.98, 55.87, 5S.00, 56.30 in soil without inoculation, soil inoculated with A. clavatus, *B.subtilis, P.aeruginosa* and *C. diphtheriae* at 0, 2, 5 and 10% pollution levels respectively, In the unamended soil. Similar decreases in %BS as a result of inoculation were also noted in the mended soil treatments across all pollution levels.

Percent base saturation decreased during the study period. After treatment applications at week nine, % BS decreased from 83.22% to 62.96, 76.10 to 29.82. 71.56 to 3626, 74.04 to 33.33, 77.58 to 41.49% and from 84% to 66.67, 73.90 to 65.34, 72 to 58.84, 70.40 to 51.05,68.70 to 52.48, 73.56 to 5602, 72.90 to 52.72% and from 69.15 to 33.08, 47.98 to 25.54,55.87 to 24.85, 66.18 to 32.42, 58.07 to 27.17% in the soil without inoculation, soil inoculated with *A. clavatus, B.subtilis, P.aeruginosa and C. diphtheriae* at 0, 2, 5 and 10% pollution levels in the unamended soil. The decrease was applicable to both amended and unamended soil. Effective cations exchange capacity in the soil also changed i the similar manner.

In the inoculated soil, highest %BS ranged between (77.58 and 41.49%, 73.90 and 65.34%, 71.07 and 56.02% and 66.70 and 32.42%) were obtained in the soil inoculated with *A. clavatus*, and *Paeruginosa*. at 0, 2 and 5, 10% pollution levels respectively, in the unamended soil after treatments at week nine of the study period. In soil amended with urea, the ranges were between 81.99 and 61 .62%, 90 and 79.78%, 84.70 and 65.24%, 71.07 and 47.37% were obtained as the highest %BS ranges recorded in the soil inoculated with P. aeruginosa at 0 — 2% and 5 — 10% pollution levels respectively. In soil amended with PM,the highest %BS ranges were 89.30 and 66.66%, 90.60 and 8 1.86%, 86.63 and 69.08%, 75.70 and 55.25% also obtained in the soil inoculated with *P.aeruginosa* at 0, 2, 5 and 10%pollution levels respectively.

Al the end of the study (week nine), PM amended soil had the highest ECEC and %BS bllowed by the urea amended soil. Finally, %BS values as compared to uninoculated soil in iH the pollution levels. Significant differences ($P < 0.05$) were observed in the %BS values 1beween the amended and the unamended soil.

Discussions

Higher percent base saturation values were observed in uninnoculated soil treatment options 0-10% pollution levels. This suggests that

inoculation may have resulted in the increased in microbial population, thus subsequent assimilation and immobilization of nutrients in the icu1ated soil as early reported by Essien et al (2012). Higher percent base saturation was noted in the poultry manure amended as compared to other soil treatments option after treatment. This therefore means that poultry manure is a better soil amendment material as compound to area.

Decreased in percent base saturation with increased in percent crude oil applied may be due to the inhibition of some microbial vital functions in the presence and toxicity of high crude oil concentration as earlier reported by Xu and Schurri (2019).

In week 9 (day 63), higher percent base saturation values were observed in the inoculated as compared to uninoculated soil treatment options. This may have been as a result of improvement and changes in soil aeration and biochemical condition as earlier reported by Jones and Margression (2014) observations that plants nutrients are mineralized out of hazardous substances with time. It may also be that other soil microbes which were attractedto the polluted and amended soil died with time and their cell materials become organicsubstrate that increased percent base saturation of the soil.

Conclusion and Recommendations

Since in week 1 and in week 9, percent base saturation values were higher in the P. aeruginosa inoculated soil amended with poultry manure as compared as compared to other treatment options, crude oil polluted Ultisol should be inoculated with P. aeruginosa and amended with poultry manure to increase its nutrients holding capacity.

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