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EFFECT OF PHYTOREMEDIATION GRASSES ON SOIL MICRO-ORGANISMS IN CRUDE OIL CONTAMINATED SOIL IN SOUTH-SOUTH NIGERIA

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ABSTRACT

Soil micro-organisms (bacteria and Fungi) are known to play major roles in nutrient cycling and fertility status of soil, when soil is polluted with crude oil, it negatively impacts on their viability. Against this backdrop, a field experiment at Rivers State University teaching and Research Farm Port Harcourt was conducted to investigate the effect of phytoremediation grasses on soil micro-organisms in crude oil polluted soil. The soil was contaminated with fresh Bonny light crude oil at 0 and 2% v/w; vetiver (Vetiveria zizanioides), guinea grass (Panicum maximum) amended with organic manures (Poultry and Rabbit) were used to remediate the soil for a period of twelve months in two season (wet and dry) seasons. Poultry and rabbit manures were applied at 0,10,20 and 30 tons/ha respectively two weeks after contamination, two weeks later, vetiver and guinea grass splits were planted at a spacing of 20×30cm and 30×30cm respectively. A total of 24 treatment combinations were laid in a factorial fitted into a split plots randomized complete block design. Results showed the population of total heterotrophic bacteria (THB) total heterotrophic fungi (THF) in the soil ranged from $2.3\times10^5$to 4.4×10^6 cfu/gand 1.6×10^4 to 1.64×10^5cfu/g respectively for dry season while hydrocarbon utilizing bacteria (HUB) and fungi (HUF) were $1.3\times10^4$to 8.8×10^5 cfu/g respectively. Conversely, the microbial count for rainy season increased from 2.8×10^5 to 2.19×10^8cfu/g and 1.06×10^4 to 1.48×10^5cfu/g for THB and THF respectively while that of HUB and HUF were 3.2×10^4 to $7.2\times10^6$and 7.0×10^3to4.2×10^4cfu/g respectively. There was significant decrease in microbial activities in contaminated soil for THB, HUB, THF and HUF over control in both seasons. Thereafter, the population of the micro-organisms increases more in contaminated over those of control plots especially in rainy season. Remediation of the soil with the grasses increased the proliferation of hydrocarbon degrading organisms. The population of THB and HUB were more than those of THF and HUF in both seasons. Higher population of micro-organisms were observed in rainy season over dry season period. Amendment of the soil with organic manures significantly increased the population of the micro-organisms. The higher the rate of amendment, the higher the microbial population. The number of microbial population were observed to be higher on soil amended with poultry than rabbit manure.

KEYWORDS: crude oil, pollution, micro-organisms, vetiver, guinea grass, poultry, rabbit manures.

INTRODUCTION:

Active interaction exists between the roots system of most plants and other components of soil biota, especially soil microbes. Kilham, (1994) implicated soil microbes as constituting the driving force for most terrestrial ecosystem as they control the rates of turnover and mineralization of organic substrates. The major group of these are bacteria, fungi, actinomycets and algae.

Heterotrophic bacteria play active role in breakdown of plant, animal and microbial residues and some autotrophic bacteria fix atmospheric nitrogen. Hina et al., (2017) inferred that several micro-organisms present in soil ecosystem can decompose organic carbon fraction present in soil organic matter. This decomposition they noted are mostly microbial mediated, though the rate and extent of decomposition are influenced by environmental variables.

Exploration, exploitation, refining, transportation and storage of petroleum products have resulted to environmental pollution due to oil leaks, accidental spills and illegal activities of man (Hussain and Gondul, 2008; Kvenvolden and Cooper, 2003). Crude oil consists of hydrocarbon (aliphatic and aromatic) among others. These hydrocarbon contains sufficient carbon which are nutritionally poor to sustain micro-organisms (Abbassian et al., 2016; Sathiskumar et al., 2008).

Toxicity of these hydrocarbon to micro-organisms in crude oil contaminated soil exposes the microbial species to drastic changes to their population and abundance (Abbassian et al., 2015, Megharaj et al., 2011). Contamination of agricultural land with crude oil has been implicated to have serious implication on their productivity. Amund and Nwokoye, (1993) reported that occurrence of oil pollution on land is a major concern to the environment as it results in the depression of microbial population and their activities.

Mchill, (1976) and Rowell, (1977) in their study noted that soil micro-organisms are in dynamic equilibrium with the soil system and are sensitive to changes in soil condition in a way that their numbers increases rapidly in several fold if any carbon source such as crude oil is introduced either deliberately or accidentally into the soil. Their work showed that there is an initial lethal effect on certain micro-organisms, followed by an increase in their numbers. This they attributed to the fact that petroleum degraders exist in every soil, introduction of crude oil into the soil bring about selective enrichment of its members thus enabling them to increase in number at rates higher than unpopulated soil (Odu, 1981).

Elevated concentration of organic materials due to crude oil pollution results to increased water holding capacity of the soil, decrease mineralization, death of soil micro-organism due to toxic chemicals, retarded growth of nitrifies and reduction in some available nutrients (Amadi et al., 1993).

Study carried out by ITRC WG, (2001) showed that some plants aid microbial biodegradation by increasing the size of the microbial population in soil surrounding the roots (rhizosphere) or mycorrhizal (association of fungi and plant roots), loosening the soil and transporting oxygen and water into the rhizosphere (a symbiotic relation between the plants and soil microbes.

There is therefore need to decontaminate the soil to enhance nutrient and biological recovering and restore the nutrient status of the soil and reduce the negative effects on soil microbial and physiological properties of the soil (Rafael et al., 2020).

Studies have implicated vetiver (Vetiveria zizanioides) and guinea grass (Panicum maximum) as effective in remediation of crude oil polluted soil (Chukwumati, J.A and Omovbude,S 2020), Obire and Akinde, 2004). Effectiveness of these grasses may be attributed to their ability to reestablish strong symbiotic association with wide range of soil microbes in the rhizosphere thus providing nutrients for the plant growth and microbes to thrive (khan, 2005), thus degrading the contaminants.

This study is aimed at investigating the effect of crude oil contamination on soil micro-organisms (bacteria and Fungi) and to examine the effectiveness of these grasses amended with organic manure in enhancing these micro-organisms.

MATERIALS AND METHODS

The study was carried out at Rivers State University, Nkpolu, PortHarcourt teaching and research farm. The site is situated at latitude $4^{0}5^{1}$Nand longitude $7^{0}0^{1}$E with an elevation of 18m above sea level (FAO, 1984). Mean annual rainfall ranges from 3000 to 4000mm (FAO, 1984). Annual temperature varies between 22 to 31^{0}C (FDRD, 1981), while the relative humidity (RH) is between 35 to 90% depending on the particular period of the year.

SOIL OF THE STUDY SITE

The soil was from coastal plain sands geomorphic region. It is typically sandy loam (typic paleudult) formed over sedimentary rocks and belongs to the ultisol order of the United State Soil Taxonomy (Soil Survey Staff, 1975).

SOURCES OF CRUDE OIL

Nigerian Bonny light crude oil (fresh) obtained from shell Petroleum Company Nigeria limited, Bayelsa State flow station was used and a concentration of 0 and 2% was used in the studied area. Each of the experimental plots (3 × 4m) with exception of the control plot was treated with crude oil from a watering can; evenly sprayed and worked into the soil with garden fork.

AMENDMENT MATERIALS

Poultry and rabbit manures were used as amendment materials. The organic manures (poultry and rabbit) were applied unto the soils with the exception of control plots two weeks after contaminating the soil with crude oil. The organic manures were broadcast and worked into the soils at the rate of 0, 10, 20 and 30tonnes per hectare, respectively.

PREPARATION AND PLANTING OF PLANT MATERIALS

The site of the study has been under continuous cultivation with different crops, the last being cassava and maize.

The area was ploughed, harrowed with tractor, marked and pegged. Vetiver grass (Vetiveria zizanioides) and guinea grass (Panicum maximum) collected from National Root Research Institute Umudike, Abia State and Rivers State University Teaching and Research Farm, respectively were planted two weeks after amendment materials were added at a spacing of 20 by 30cm and 30 by 30cm for vetiver and guinea grasses respectively. A total of 24 treatment combinations (Table 1) below were laid out in a factorial fitted into a split plot randomized complete block design with contaminated and uncontaminated as the main plots, other factors served as sub plots. All the treatments were replicated three times making a total of 72 plots.

EXPERIMENTAL DESIGN

TABLE 1: TREATMENT COMBINATIONS ARE AS FOLLOWS:

TREATMENTS	CODE	KEY
C0P0	T1	Control, planted with vetiver grass, no organic manures, no crude oil
C0P2	T2	No crude oil, 20 tons/hectare poultry manure, vetiver planted
C0R0	T3	No crude oil, no rabbit manure, veyiver planted
COR2	T4	No crude oil, 20 tons per hectare rabbit manure, vetiver planted
C1P0	T5	Contaminated with crude oil, no poultry manure, vetiver planted
C1P1	T6	Contaminated with crude oil, amended with 10tons/ha poultry, vetiver planted

C1P2	T7	Contaminated with crude oil, amended with 20tons/ha poultry, vetiver planted
C1P3	T8	Contaminated with crude oil, amended with 30tons/ha poultry, vetiver planted
C1R0	T9	Contaminated with crude oil, no rabbit manure, vetiver planted
C1R1	T10	Contaminated with crude oil, amended with 10tons/ha rabbit, vetiver planted
C1R2	T11	Contaminated with crude oil, amended with 20tons/ha rabbit, vetiver planted
C1R3	T12	Contaminated with crude oil, amended with 30tons/ha rabbit, vetiver planted
C0P0	T13	No crude oil, no poultry manure, guinea grass planted
C0P2	T14	No crude oil, amended with 20tons/ha poultry, guinea grass planted
C0R0	T15	No crude oil, no rabbit manure, planted with guinea grass
C0R2	T16	No crude oil, amended with 20tons/ha rabbit manure, guinea grass planted
C1P0	T17	Contaminated with crude oil, no poultry manure, guinea grass planted
C1P1	T18	Contaminated with crude oil,amended with 10tons/ha poultry, guinea grass planted
C1P2	T19	Crude oil contaminated, amended with 20tons/ha poultry, guinea grass planted
C1P3	T20	Crude oil contaminated, amended with 30tons/ha poultry, guinea grass planted
C1R0	T21	Crude oil contaminated, no rabbit manure, guinea grass planted
C1R1	T22	Crude oil contaminated, amended with 10tons/ha rabbit, guinea grass planted
C1R2	T23	Crude oil contaminated, amended with 20tons/ha rabbit, guinea grass planted
C1R3	T24	Crude oil contaminated, amended with 30tons/ha rabbit, guinea grass planted.

3.2.2 Determination of Bacterial/Fungi Count

Total heterotrophic bacteria and fungi count was determined to assess the rate of biodegradation of crude oil.

Serial ten-fold dilution (Ofunne, 1999) was used for the isolation and enumeration of bacteria and fungi in soils. Appropriate decimal dilutions of samples were plated onto manitol-soil extract agar (Obire and Wemedo, 1996). Nutrient agar (Oxiod cm 13) was used for bacteria while Potato Dextrose Agar was used for fungi. A volume of cycloheximide was incorporated into mannol-soil extract agar to suppress fungi growth while same equal volume of streptomycin solution was added unto Potato Dextrose Agar to suppress bacterial growth.

Spread plate technique was used for all inoculations and the inoculated plates were incubated at 28^0C for 3-5 days.

Discrete colonies that developed were counted as the total viable counts for heterotrophic bacteria on viable counts for fungi in the soil sub culturing purified all the isolates.

Pure bacterial isolates were identified on the basis of their cultural and morphological characteristics and by reference to Buchanan and Gibbons, (1974) and Barnet and Hunter, 1972 for bacterial and fungal, respectively.

RESULTS AND DISCUSSION

Results of the microbiological status of the soil on both dry and rainy seasons are presented in tables 2 and 3 below. The population of total heterotrophic bacteria and fungi (THB and THF) of the soil in dry season ranged from 2.3×10^5 to 4.4×10^{6cfu}/g and 1.6×10^{4cfu}/g to1.64×10^{5cfu}/g for THB and THF respectively while the population of hydrocarbon utilizing bacteria and fungi was $1.3\times10^4$to 8.8×10^{5cfu}/g for HUB and HUF respectively. Conversely, the microbial population of the soil for rainy season increased from 2.8×10^{5cfu}/g to 2.19×10^{8cfu}/g and 1.06×10^4 to 1.48×10^{5cfu}/g for THB and THF respectively, while that of utilizing

bacteria and fungi were 3.2×10^{4cfu}/g to 7.2×10^{6cfu}/g and $7.0\times10^{3}$to 4.2×10^{4cfu}/g for hydrocarbon utilizing bacteria and fungi respectively. The results revealed that the activities of total heterotrophic and hydrocarbon utilizing bacteria were more in rainy season than in dry season.

Table 2: Total microbial population in Treated soil in dry season

TreatmentDepth	THB cfu/g	HUB cfu/g	%HUB	THF cfu/g	HUF cfu/g	%HUF
COPO 0–15 cm)	4.8 x 10^5	1.9 x10^5	3.96	1.0 x10^5	9.0 x10^4	9.00
15-30	3.6 x 10^5	1.2 x10^5	3.33	1.62 x10^5	4.2 x10^4	2.59
COP2 0 – 15	6.5 x 10^5	1.9 x10^5	2.92	1.54 x10^5	7.3 x10^5	4.74
15-30	4.4 x 10^6	8.8 x10^5	1.35	9.2 x10^4	2.9 x10^4	3.15
COR2 0 – 15	3.6 x 10^5	1.3 x10^4	3.61	1.18 x10^5	3.2 x10^4	2.71
15-30	3.7 x 10^5	1.46 x10^5	3.95	1.64 x10^5	1.7 x10^4	1.04
CIPO 0 – 15	3.7 x 10^5	1.7 x10^4	4.63	4.8 x10^4	2.3 x10^4	4.79
15-30	2.3 x10^5	1.7 x10^5	7.39	3.1 x10^4	1.1 x10^4	3.55
CIPI 0 – 15	3.7 x10^5	2.5 x10^5	6.76	1.22 x10^5	1.2 x10^4	9.84
15-30	2.4 x10^5	7.0 x10^4	2.92	4.2 x10^4	1.7 x10^4	4.05
CIP2 0 – 15	2.8 x10^5	1.9 x10^5	6.79	8.8 x10^4	1.6 x10^4	1.82
15-30	6.1x10^5	8.3 x10^4	1.36	9.3 x10^4	2.8 x10^4	3.01
CIP3 0 – 15	5.1 x 10^5	2.7 x10^5	5.29	3.3 x10^4	1.8 x10^4	5.46
15-30	4.5 x10^5	1.4 x10^5	3.11	1.6 x10^4	9.0 x10^3	5.63
CIR1 0 – 15	2.8 x10^5	2.4 x10^5	7.14	7.4 x10^4	1.1 x10^4	1.49
15-30	2.9 x10^5	1.6 x10^5	5.52	1.48 x10^5	1.8 x10^4	1.22
CIR2 0 – 15	1.84 x10^6	8.3 x10^5	4.51	5.6 x10^4	2.2 x10^4	3.93
15-30	5.2 x10^5	2.18 x10^5	4.19	8.7 x10^4	1.4 x10^4	1.61
CIR3 0 – 15	4.2 x10^5	3.9 x10^5	9.29	1.8 x10^4	7.0 x10^3	3.89
15-30	3.2 x10^5	1.13 x10^5	3.53	3.5 x10^4	2.8 x10^4	8.00

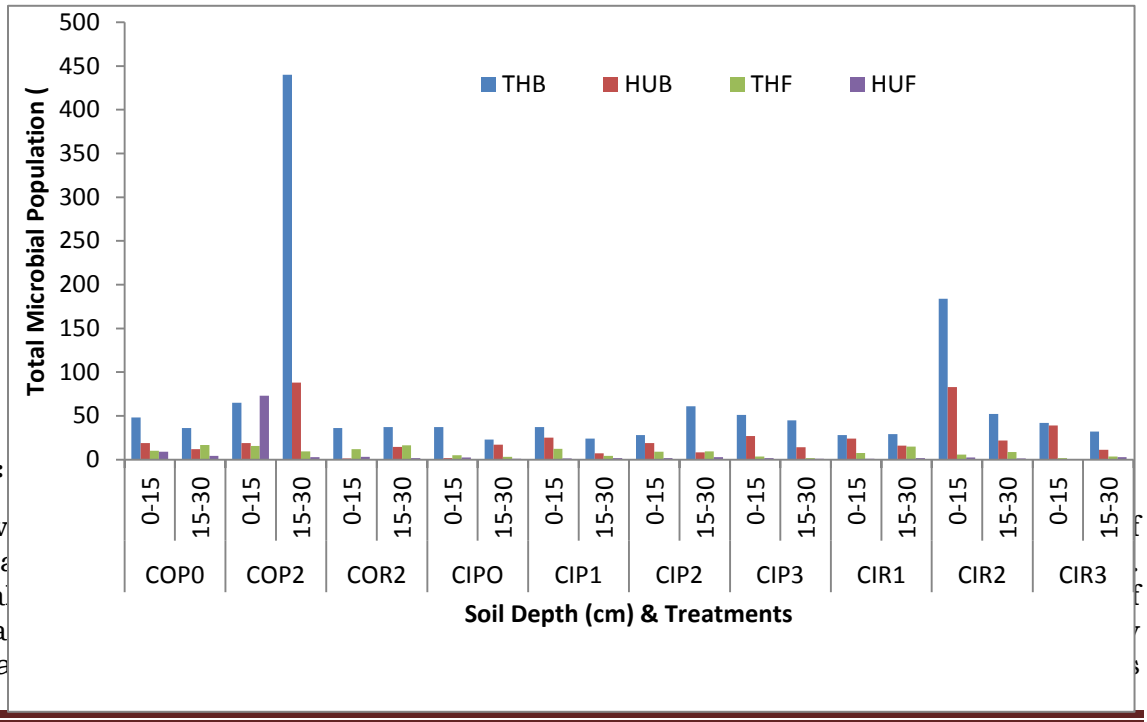

Figure 1:

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in the soil immediately after contamination.

Thereafter, the population increases more in contaminated over those of control plots especially in rainy season. This finding agrees with the report of Raghad and Mehjin, (2019) who observed a significant decrease in count of heterotrophic bacteria and an increase in hydrocarbon utilizing bacteria after some weeks of contamination. This observation tally with the findings of Odu, (1981) who inferred that contamination

Table 3: Total microbial population in treated soils in rainy season

TREATMENT/Depth	THB cfu/g	HUB cfu/g	THF cfug	%HUB	HUF	%HUF
COPO 0 – 15 (cm)	2.18×10^7	7.2×10^4	4.1×10^4	3.30	3.9×10^4	9.51
15-30	6.5×10^5	3.2×10^4	3.8×10^4	4.92	2.1×10^4	5.53
COP2 0 – 15	1.12×10^7	2.0×10^5	2.9×10^4	1.79	1.2×10^4	4.14
15-30	5.7×10^6	7.2×10^6	3.1×10^4	1.26	2.8×10^4	9.03
COR2 0 – 15	8.1×10^6	6.9×10^4	2.3×10^4	8.52	2.1×10^4	9.13
15-30	7.3×10^6	3.4×10^4	1.06×10^4	4.66	9.0×10^3	8.49
CIPO 0 – 15	9.4×10^7	1.43×10^5	2.6×10^4	1.52	1.8×10^4	6.92
15-30	8.6×10^7	1.18×10^5	2.9×10^4	1.37	2.5×10^4	8.62
CIPI 0 – 15	1.14×10^8	1.24×10^5	2.7×10^4	1.09	1.3×10^4	4.81
15-30	7.3×10^7	1.9×10^5	3.5×10^4	2.60	1.5×10^4	4.29
CIP2 0 – 15	2.19×10^8	5.2×10^4	1.2×10^4	2.37	7.0×10^3	5.83
15-30	1.08×10^8	3.6×10^5	2.5×10^4	3.33	1.6×10^4	6.40
CIP3 0 – 15	3.6×10^8	1.12×10^5	7.6×10^4	3.11	4.2×10^4	5.53
15-30	1.1×10^8	7.6×10^4	3.2×10^4	6.91	1.0×10^4	3.13
CIR1 0 – 15	2.8×10^5	2.4×10^5	7.4×10^4	8.57	1.1×10^4	1.49
15-30	2.9×10^5	1.6×10^5	1.48×10^5	5.52	1.8×10^4	1.22
CIR2 0 – 15	1.84×10^6	8.3×10^5	5.6×10^4	4.51	$2.2 \times 10^{4+}$	3.93
15-30	5.3×10^5	2.18×10^5	8.7×10^4	4.11	1.4×10^4	1.61

of soils with crude oil has lethal effect on the soil microbes, as most of the micro-organisms may have been killed by contaminants as it affects their respiration; this is later followed by an increase in their number due to the existence of petroleum degraders in soils that bring about selective enrichment of its members.

The increase in microbial population in contaminated soils could also be attributed to soil micro-organisms in the rhizosphere of plants growing in contaminated soils.

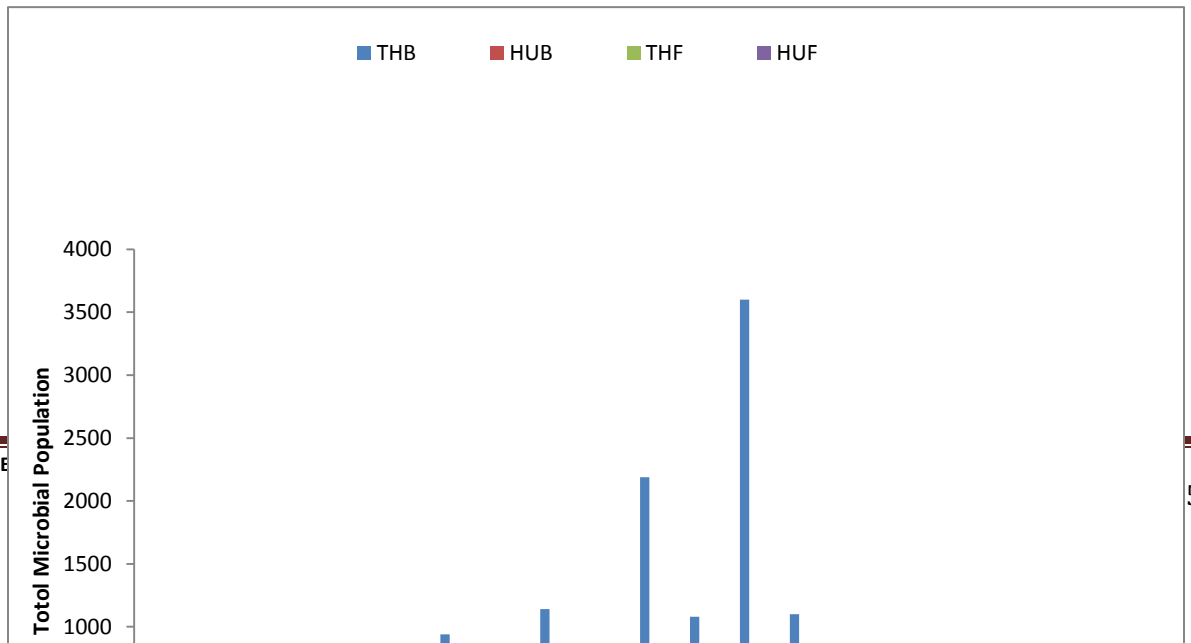

Figure 2: Effect of treatments on microbial popu;lation during rainy season

This finding corroborates with Khan, (2005), Sunanthaposuk, (2000) who reported that vetiver grass has the ability of establishing a strong symbiotic association with a wide range of soil microbes in the rhizosphere that provide nutrients (nitrogen fixing bacteria, phosphate solubilizing bacteria and fungi, mycorrhizal and cellulolytic fungi) and phytohormones (plant growth regulator bacteria) for plant development.

The increase probably could also be due to the presence of the plants (vetiver and guinea grasses) which may have provided optimizing conditions for biodegradation through aeration and addition of nutrients.

Studies have shown that organisms associated with plant roots have the capability of enhancing soil microbial activities for the degradation of contaminants, (by organisms that associate with the roots). The contaminants are broken down through the bioactivity that exists in the rhizosphere (ITRC WG, 2001). This bioactivity according to ITRC WG, is derived from proteins and enzymes that are produced and exuded by plants or from soil organisms such as bacteria, fungi and yeast.

Analysis of the data on microbial population and treatments showed that treatments of the soil had significant effect on the proliferation of hydrocarbon degrading organisms, as the population of the organisms increased after treatment of the soil; (Figure 2).

The results also showed that the population of THB and HUB were more than those of fungi in both seasons and this agrees with the observation of Kerry, (1990). The reason for this increase of bacterial population over fungi could be due to adaptability of microbes to different soil environmental conditions; among which are adequate fertilization, strong symbiotic association between the micro-organisms and the plant, good moisture condition and temperature.

The result of the study also showed higher population of microorganisms in rainy season over dry season. This could probably be due to high moisture content, one of the most decisive factors affecting the life of microorganisms.

Amendment of the soils with organic manures increased the population of the microorganisms, the higher the rate of amendment, the higher the microbial population. Higher numbers of microbial population were observed in soil amended with poultry manure over that of rabbit. The above increase could be attributed to the fact that organic manures serve as a source of energy for microorganisms.

CONCLUSION

Treatment of the crude oil contaminated soil with phytoremediation grasses (Vetiver and Guinea) grasses amended with organic manures restored and increases the soil microbial population and activities. The higher the rate of organic manures application, the increase in the activities and population of heterotrophic and hydrocarbon utilizing bacteria and fungi. The increase was more in poultry than rabbit manures.

REFERENCES

Abbassian, F., Lockington, R., Megharaj, M and Naidu, R. (2016). The biodiversity changes in microbial population of soils contaminated with crude oil. Current Microbiology. 72(6), 663-670.

Abbassian, F., Lockington, R., Mallavarapu, M and Naidu, R. (2015). A comprehensive review of aliphatic

hydrocarbon biodegradation by bacteria. Appl. Biochem. Biotechnol. 17, 670-699.

Amadi, A., Dickson, A and Mate, A. (1993). Remediation of oil polluted soil. Effect of organic and inorganic nutrients supplements on the performance of maize (Zea mays). Water, Air and Soil Pollution. 6, 55-76.

Barnett, H.L and Hunter, B.B. (1972). Illustrated genera of imperfect fungi, (3rd edn). Burgess Publishing Company, USA.

Amund, O. O and Nwokoye, N. (1993). Hydrocarbon degradation bagentid of yeast isolated from polluted lagoon. Journal of Science Resources Development. 1, 65

Buchanan, R. V and Gibbons, N. E. (1974). Begrey's manual of bacteriology (2nd ed). Witkins Co, Bathmidore.

Chukwumati, J. A and Omovbude, S. (2020). The dynamics of vetiver (Vetiveria zizanioides) and guinea grass (Panicum maximum) amended with organic manures in remediation of crude oil contaminated soil in Port Harcourt. Greener Journal of Agricultural Sciences. 10(2), 88-94.

Federal Department of Rural Development Area Program. (1981). Federal Development of Rural Development Area Program Preparatory Report, Nov, 1981.

Hina, Khatoon., Praveen, S., Maitreyie, N and Lakshmi, T. (2017). Role of microbes in organic carbon decomposition and maintenance of soil ecosystem. International Journal of Chemical Studies. 5(6), 1648-1656).

Food and Agricultural organization. (1984). Food and Agricultural Organization Year Book, FAO, Rome. 4, 122-127.

Hussain, T and Gondal, M. A. (2008). Monitoring and assessment of toxic metals in Gulf war oil spill contaminated soil using Laser induced breakdown spectroscopy. Environmental Monitoring Assessmnt. 391-399.

ITRC, W. G. (2001). Regulatory guidelines. The interstate technology and regulatory cooperation work group. Phytoremediation Work Team.

Kerry, E. (1990). Micro-organisms colonizing plants and soil subjected to different degrees of human activity, including petroleum contamination in the vest fold hills and Mac Robedron land. Antartica Polar Biology10(430), 19.

Khan, A. G. (2005). Role of soil microbes in the rhizospheres of plant growing on trace metal contaminated soils in phytoremediation. Journal of Trace element medical biology. 18, 355-364.

Killham, K. (1994). Soil ecology. Cambridge University Press. 242.

Kvenvolden, K. A and Cooper, C. K (2003). Natural seepage of crude oilinto the marineenvironment. Geo-Marine Letters vol 23, number 3-4, pp 140-146

McGill, W. B. (1976). An introduction to the effects of oil spills on soils of some general restoration and clean up procedures. Alberta Institute of Pedology, Publication. 9, 76.

Megharaj, M., Ramakrishnan, B., Venkateswarlu, K., Sethonathan, N and Naidu, R. (2011). Bioremediation approaches for organic pollutants. A critical perspective. Environmental Internation. 37(8), 1362-1375.

Obire, O and Akinde, S. B. (2004). Poultry manure amendment of oil polluted soils for sustainable development in the Niger Delta.

Journal of Nigerian Environmental Society. 2(2).

Obire, O and Owemedo, S. A. (1996). The effect of oil field waste water on the microbial population of a soil in Nigeria. Niger Delta Biologia. 1(1), 77-85.

Odu, C. T. (1981). Degradation and weathering of crude oil under tropical condition. Proceeding of Seminar of Petroleum Industry and the Nigeria Environment.

Ofunne, J. I. (1999). Bacteriological examination of clinical specimens. Achugo publications, Ama J. K. Recreation Park, Owerri, Nigeria.

Rafael, G. L., Jose, M. B and Carlos, G. (2020). Biological methods of polluted soil remediation, an effective. Journal of Environmental Sciences and Public Health. 4, 112-133.

Raghad, N. H and Mehjin, A. A. (2019). Effect of petroleum hydrocarbon contamination. Raf. Journal of Science. 28(1), 13-22.

Rowell, M. J. (1977). Oil contaminated soils and some aspects of reclamation on soil Science Conservation Remediation Research, Alberta Soils Workshop, Reddeer. Alberta.

Sathishkumar, M., Binupriya, A. R., Baik, S. H and Yun, S. E.(2008). Biodegradation of crude oil by individual bacterial strains and a mixed bacterial consortium isolated from hydrocarbon contaminated areas. Clean- Soil, Air, Water. 36(1), 92-96.

Soil Survey Staff. (1975). Soil taxonomy: A basic system of soil classification for mapping and interpreting soil survey. USDA Agricultural Handbook. 436 Washington D.C, 754.

Sunanthaposuk, V. (2000). Study on soil microbial biodiversity in rhizosphere of vetiver grass in degrading soil. In Abstract of Papers. Proceedings of Second Vetiver Conference, (ICV-2). January 18-22, Phetchaburi, Thailand.