American Journal of Bioscience and Bioengineering
2022; 10(3): 48-52
http://www.sciencepublishinggroup.com/j/bio
doi: 10.11648/j.bio.20221003.12
ISSN: 2328-5885 (Print); ISSN: 2328-5893 (Online)

Studies of Lodge Bacteria on *Lablab purpureau* and *Pennisetum hybridum* for Potential Degradation of Polycyclic Aromatic Hydrocarbons

Abdullahi Yusuf Bello[1, *], Abubakar Muazu Jodi[2], Abubakar Sanusi[3], Ridwan Abdulsalam Hussein[1], Asabe Danjuma[1]

[1]Department of Basic Science and General Studies, Federal College of Forestry Mechanization, Afaka, Nigeria

[2]Department of Microbiology, Usmanu Danfodiyo University, Sokoto, Nigeria

[3]Department of Microbiology, Kebbi State University of Science and Technology, Aliero, Nigeria

Email address:

abdullahi.belloyusufgwdsok@gmail.com (A. Y. Bello)

[*]Corresponding author

To cite this article:

Abdullahi Yusuf Bello, Abubakar Muazu Jodi, Abubakar Sanusi, Ridwan Abdulsalam Hussein, Asabe Danjuma. Studies of Lodge Bacteria on *Lablab purpureau* and *Pennisetum hybridum* for Potential Degradation of Polycyclic Aromatic Hydrocarbons. *American Journal of Bioscience and Bioengineering*. Vol. 10, No. 3, 2022, pp. 48-52. doi: 10.11648/j.bio.20221003.12

Received: April 18, 2022; **Accepted**: May 7, 2022; **Published**: May 31, 2022

Abstract: A plants tissue lodge bacteria that do not harm plants body but supports its living, these are endophytic bacteria. Studies on *Pennisetum hybdridum* and *Lablab purpureau* endophytic bacteria for degradation of Polyaromatic hydrocarbons (PAHs) were carried out. These bacteria were enumerated, isolated conventionally, screened and determine their degradation potential of diesel and kerosene (PAHs). The total culturable bacterial count on *Lablab Purpureus* (HyB) was 2.0×10^4 cfu/g and *Pennisetum hybridum* (KgG) 3.2×10^5 cfu/g wet plant. About nine (9) bacteria from *Lablab purpureau* and ten (10) bacteria from *Pennisetum hybdridum* were isolated by standard plate techniques. These bacteria were screened using minimal salt agar amended with varied concentrations of kerosene and diesel (PAHs). The bacteria KgG-1, *Bacillus* sp. presented sufficient growth on minimal salt agar plates at 0.8% diesel and 1.0% kerosene. Optimal growth was studied using glucose (0.2 to 1.0 % w/v) as energy and carbon source and cow urine (0.2 to 0.8% v/v) as nitrogen source. The conditions used for optimal growth determination were incubation temperature 36±1°C and incubation period 12 hours interval from 0 to 72 hours. The growth was measured using spectrophotometer 600 nm absorbance. The highest observed growth after addition of glucose (1.0% w/v) in the presence of diesel was 0.53 for a period of 60 hours. Similarly, maximal growth after added kerosene, 0.8% w/v glucose was 0.635 for 60 hours. The cow urine 0.4% v/v in the presence of diesel after 48 hours was maximal 0.20. Also, in the presence of kerosene, the highest growth was 0.235 at 0.4%v/v cow urine for 60 hours. The degradation by *Bacillus* sp was performed on minimal salt broth contained with 0.8% diesel, 1.0% kerosene per 1000 ml, 1.0% glucose w/v, 0.4% cow urine v/v added with 1 ml bacterial suspension. Control set does not carry bacterial suspension. Optical density was determined from 0 to 96 hours at an interval of 24 hours. For degradation of PAHs, the highest optical density for *Bacillus* sp. was 0.471 at 72 hours in the presence of kerosene while for diesel 0.532 at 96 hours. Increasing incubation period increases growth rate of *Bacillus* on PAHs.

Keywords: Endophytes, Bacteria, Plants, *Lablab purpureau*, *Pennisetum hybridum*

1. Introduction

Research has been on ground for environmental clean, safe and healthy from various angles. The whole scientific world agrees that using living organisms like plants, bacteria, fungi or algae are the most economical and safer for use. Bacteria are ubiquitous; living in soil, plants, animals, water, air and others. Bacteria colonizing internal tissue parts of plants without causing harm to their host are otherwise regarded as normal flora of plants called endophytes [1]. Over a long evolutionary route, they have

produced pleasant relationship with plants such like symbiotic, mutualistic and others but do not causes harm [2]. Endophytic bacteria function in plants growth stimulation, encourage organic nitrogen fixation, fights/control pathogenic actions and safeguard plants from insensitive external environments. There has been several information concerning bacterial endophytes found in plants grown in contaminated soil contained with varied pollutants. Efforts have been made to search for bacterial endophytic distribution, ecology and diversity and to isolate those endophytes that can breakdown pollutants by cleaning plants internal environments [3-6].

Polycyclic aromatic hydrocarbons (PAHs) are diverse group of organic compounds containing carbon and hydrogen with more than one aromatic ring. They are pollutant surrounding everywhere in the environments; present in air, water or soil as a result of combustion of natural organic materials either via day to day human habits like transportation, refuse burning, industrial production, gasification and or others or natural episodes like volcanic eruptions, forest fires and others [7-9]. According to the European Union and United State Environmental Protection Agency (USEPA) PAHs are highly toxic, mutagenic and or carcinogenic involving one of the top level pollutants that have been related to cardiovascular and respiratory diseases [10, 9] more so, pollutants of such characters require more scientific explanations.

Degradation of PAHs may be found through chemical oxidation, soil adsorption, photo-oxidation, volatilization and bioaccumulation hence, microbial degradation and transformation are regarded as the most important elimination processes [11]. Degradation of PAHs has been successfully achieved by microbes especially bacteria, as a routes for cleaning the contaminated sites and many PAHs degraders have been isolated including *Bacillus*, *Mycobacterium*, *Novosphingobium*, *Nocardioides*, and *Rhodococcus* species [11-14].

2. Materials and Methods

2.1. Sample Collection

Plant samples were collected in the farm field of different area; Ruggar liman and Road Block of kware Local Government area, Sokoto state. Hyacinth bean (*Lablab purpureus*) was from Ruggar liman, and king grass (*Pennisetum hybridum*) was from Buddau area. The plants samples were collected at Rainy season. They were collected at the same day and time and immediately transported to the laboratory.

2.2. Sample Preparation and Processing

Following the method described by [15], the plants samples leafs and stems were washed separately under running tap water. These were also soaked and drained using distilled water. Starting with surface sterilization, the plants leaf and stems was dipped into ethanol 70% for 60sec each. Following separate treatment, each was dipped into sodium hypochlorite; leafs and stems (4% for 5mins) and ethanol 70% treatments to all for 30sec. the samples were then washed with sterile distilled water. Sterile paper towels were used and dried the samples. The surface-sterile leafs and stems 1g of each in 9ml sterile distilled water, were macerated using sterile pestle and mortar [16, 17].

2.3. Isolation and Identification of Endophytic Bacteria

Tissue extracts of each plant were then serially diluted up to 10^{-6} [16]. About 0.1ml of 10^{-3} was spread plated in duplicate on sterile nutrients agar plates. The plates were incubated at 36±1°C for 72 hours [18]. The colonies were counted and expressed as colony forming units per gram of plant (CFU/g). The colonies were sub-cultured again and again in order to obtain pure cultures. Obtained pure isolates were maintained at 4°C in slant tubes until use.

The bacteria were identified based on cultural characteristics, gram's reaction, microscopic and biochemical characteristics. The cultural characteristics such as colony color, form, elevation, margin, diameter, surface, opacity, and texture was observed and also, Shapes, sizes and arrangements of bacteria were used for characterization of bacteria [19]. Pure colonies were gram stained, viewed under the microscope and subsequent biochemical reactions were observed [20]. The strains prototype phenotypic features were compared to the Bergey's manual of Determinative Bacteriology [21, 18].

2.4. Screening of Bacterial Endophytes on PAHs

The identified bacteria were screened using minimal salt media (MSM) agar: g/l KH_2PO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; $NaH_2PO_4 \cdot H_2O$, 0.5; NH_4Cl, 0.5; NaCl, 4; $NaHCO_3$, 0.5; and Na_2CO_3, 0.5; $FeCl_2 \cdot H_2O$, 1.5; NaCl, 9; $MnCl_2 \cdot 4H_2O$, 0.197; $CaCl_2$, 0.9; $CoCl_2 \cdot H_2O$, 0.238; $CuCl_2 \cdot H_2O$, 0.017; $ZnSO_4$, 0.287; $AlCl_3$, 0.05; H_3BO_3, 0.062; $NiCl_2 \cdot 6H_2O$, 0.024; agar-agar, 18; [18] on plates amended with different concentrations of PAHs (0.4 to 1.2% per litre). The plates were incubated at 36 ± 1°C for 72 hours for growth of proficient effective bacterial isolate potentially to be used for further degradation of PAHs.

2.5. Optimal Growth Characteristics

Optimization for the study was carried out using Glucose 0.4 to 1.0% w/v as a source of carbon and energy and Cow urine 0.2 to 0.8% v/v, as a source of nitrogen. These were added in Erlenmeyer's flask 250ml containing MSM broth 100ml, best concentrations for growth on PAHs (0.8% diesel and 1.0% kerosene) and inoculated with 24 hour fresh bacteria colony. The flasks were incubated on a rotary shaker 150rpm at 36±1°C for 72 hours [22]. The growth was observed at 0, 12, 24, 36, 48, 60 and 72 hours using spectrophotometer (LW-V-200 RS UV/VIS, Germany) 600nm absorbance.

2.6. Potential Degradation of PAHs by Endophytes

The degradation by endophytic strain KgG-1 (*Bacillus* sp.) was performed under growth conditions pH 7.0, 36± 1°C. The MSM broth containing 0.8% per litre diesel and 1.0% per litre kerosene supplemented with 1.0% (w/v) glucose and 0.4% (v/v) cow urine in Erlenmeyer flasks 250 ml was used [23]. About 1ml of bacterial suspension was added to the prepared flask. Control flask does not contained bacterial inocula rather, all similar concentrations with PAHs and other sources. All the flasks were incubated at 36±1°C on a rotary shaker. These were subjected for OD determination at 600nm spectrophotometer (LW-V-200 RS UV/VIS, Germany) at a difference of 24 hours for 96 hours [24]. Duplicate set for each treatment were carried out.

2.7. Statistical Analysis

The results are presented in bars and line graph as mean and standard deviations are error bars.

3. Results and Discussion

3.1. Isolation and Screening of Bacterial Endophytes

The total culturable heterotrophic bacterial counts in *Lablab Purpureus* (HyB) and *Pennisetum hybridum* (KgG) reveal the presence of 2.0×10^4 and 3.2×10^5 cfu/g wet plant respectively. About nine (9) bacteria from HyB and ten (10) bacteria from KgG were isolated. Among the growing endophytic bacteria, only few exhibited growth at higher concentrations (0.4 to 1.2% per litre) of PAHs. A total of seven (7) bacteria from HyB and five (5) bacteria from KgG were found growing in the presence of both diesel and kerosene used as sole carbon and energy sources. The twelve (12) bacterial isolates were subjected to cultural, microscopic and biochemical characterizations to further confirm the bacteria at conventional level (Table 1). After screening, *Bacillus* specie from *Pennisetum hybridum* was found growing efficiently on minimal salt agar plates. In Previous reports *Bacillus* species are particularly isolated as endophytic bacteria [25, 15]. The bacteria *Bacillus* sp. presented growth most efficiently above 0.7% per litre of both diesel and kerosene and *Bacillus* sp. were selected for the study. Previous studies have shown that, *Bacillus* species [26] was found growing and tolerant to the PAHs containing habitat.

3.2. Optimal Growth Characterization

Optimization was based on two different sources; glucose and nitrogen using varied concentrations. Glucose addition to a medium was said to increase energy for available microbes. The microbes become activated thus, accelerating the conversion of compounds or substrate in a medium [27]. Increase growth rate of *Bacillus* sp. in the presence of glucose as an additional carbon and energy source was observed during the study. The maximal growth was above 59 hours (0.53 absorbance) at a concentration of 1.0% w/v glucose in the presence of diesel (Figure 1). The maximal growth rate of *Bacillus* sp. when glucose was added as a carbon and energy source reaches 0.635 absorbance at concentration 0.8% w/v for 60 hours in the presence of kerosene (Figure 2). The glucose addition for bacterial growth in this study may follow the phenomenon that microbes require their own separate substrate for growth but do not utilized carbon and energy while transformation reaction processes [28].

Table 1. *Conventionally Identified Endophytic Bacteria from Lablab purpureau and Pennisetum hybridum.*

Isolate code	Potential Bacteria
HyB-1	*Bacillus* sp.
HyB-2	*Micrococcus* sp.
HyB-3	*Pseudomonas* sp.
HyB-4	*Clavibacter* sp.
HyB-5	*Pseudomonas* sp.
HyB-6	*Bacillus* sp.
HyB-7	*Microbacterium* sp.
KgG-1	*Bacillus* sp.
KgG-1	*Bacillus* sp.
KgG-2	*Enterobacter* sp.
KgG-3	*Bacillus* sp.
KgG-4	*Bacillus* sp.
KgG-5	*Micrococcus* sp.

Key: HyB: Lablab purpureau, KgG: Pennisetum hybridum.

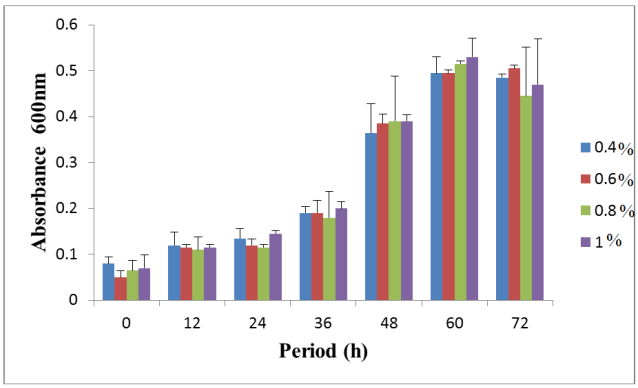

Figure 1. *Growth Effects of Bacillus sp. on diesel and different concentrations of Glucose (as an energy source) at 12 hour time intervals (Error bars are standard deviation).*

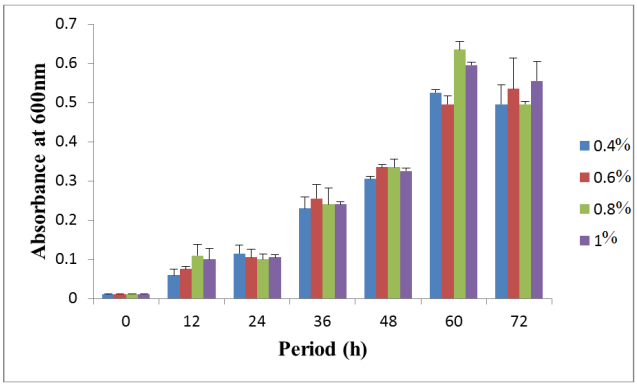

Figure 2. *Growth Effects of Bacillus sp. on Kerosene and different concentrations of Glucose (as an energy source) at 12 hour time intervals (Error bars are standard deviation).*

Application of nitrogen to a medium or microbial niche was said to stimulate or shaped the bacterial community structure [29]. The response of growth after the added cow urine (as nitrogen source) was fabulous may be because it include certain inorganic substances [22]. The growth of *Bacillus* sp. hit the highest point, 0.2, at a concentration of 0.4% v/v cow urine for 48 hour in the presence of diesel (Figure 3). The highest growth observed on cow urine for *Bacillus* sp. was at 0.4% v/v for 60 hours (0.235 absorbance at 600nm) in the presence of kerosene (Figure 4).

3.3. Degradation of PAHs by Bacterial Endophytes

There was increasing degradation potential observed in both the PAHs by endophytic *Bacillus* species with respect to time. Similarly, *Bacillus* specie growth in the presence of PAHs was most effective at 72 and 96 hours of incubation. The *Bacillus* specie effective optical density observed on kerosene and diesel was 0.471, 0.443 and 0.49, 0.532 absorbance at 72 and 96 hours respectively (Figure 5). The bacteria *Bacillus* specie presented higher degradation ability of kerosene at 72 hours. Similarly, increased incubation period showed increased growth rate by *Bacillus* and thus, the bacteria may highly be recommended for degradation of PAHs.

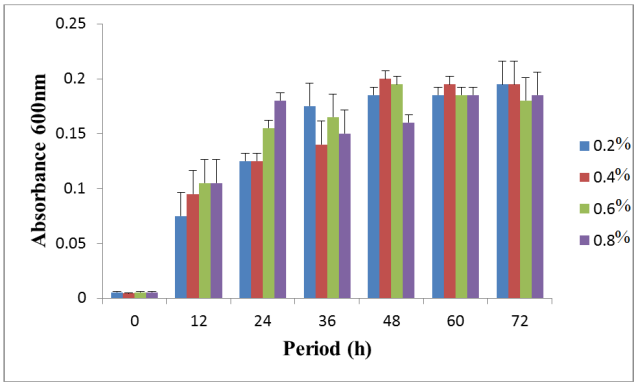

Figure 3. *Growth Effects of Bacillus sp. on diesel and different concentrations of Cow urine (as a Nitrogen source) at 12 hour time intervals (Error bars are standard deviation).*

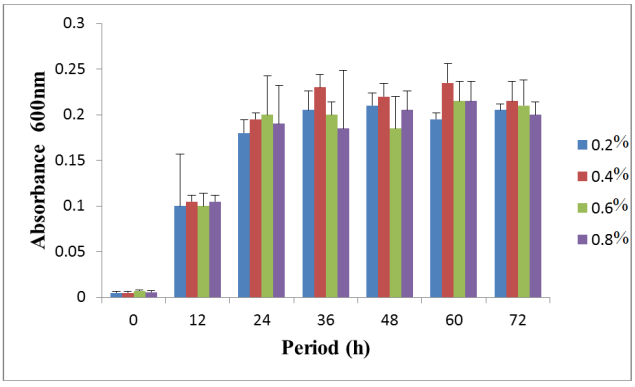

Figure 4. *Growth Effects of Bacillus sp. on kerosene and different concentrations of Cow urine (as a Nitrogen source) at 12 hour time intervals (Error bars are standard deviation).*

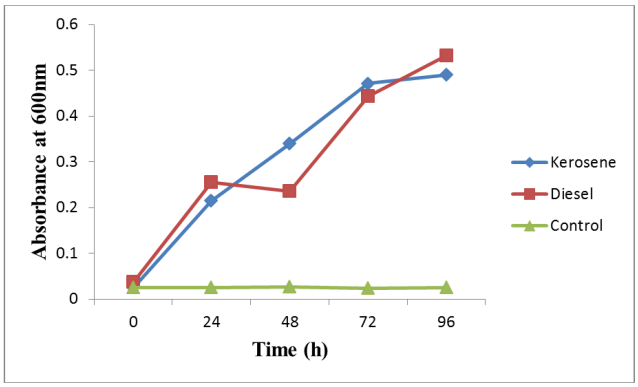

Figure 5. *Potential Degradation of Polycyclic Aromatic Hydrocarbons by Bacillus sp.*

4. Conclusion

The study confirmed there are bacteria found on the tissue of plants, *Pennisetum hybdridum* and *Lablab purpureau* called endophytes. These bacteria have the mechanism to degrade polycyclic aromatic hydrocarbons (diesel and kerosene). Among the plants lodge bacteria, *Bacillus* sp. was the most effective bacteria in degradation of diesel and kerosene. Hence, further studies may determine the kind of mechanisms, requirements and other appropriate conditions for degradation of diesel and kerosene.

References

[1] Schulz, B., and Boyle, C. (2006). What are endophytes? Microbial Root Endophytes (Schulz BJE Boyle CJC Sieber TN, eds), pp. 1-13.

[2] Lodewyckx, C., Vangronsveld, J., Porteus, F., Moorea-Edward, R. B., Taghavi, S. (2002). Endophutic bacteria and their potential application. *Critical Review in Plant Science*, 24: 583-606.

[3] Shim, H., Zhuang, X., and Chen, J. (2007). New advances in plant growth-promoting rhizobacteria for bioremediation. *Environment International Journal*, 33: 406-413.

[4] Peng, A., Juan L., Yanzheng., and Gap, Z. (2013). Distribution of endophytic bacteria in alopecurus aequalis sobol and oxalic noriculata from soil contaminated by polycyclic aromatic hydrocarbon, *PLOs ONE*, 24: 539-548.

[5] Anna, G and Rafal G. (2015). Phytoremediation of polycyclic aromatic hydrocarbon in soils artificially polluted using plant-associated endophytic bacteria and Dactylis glomerata as the bioremediation plant. *Polish Journal of microbiology,* 65 (1): 295-3.

[6] Moore, F. P., Barac, T., Borremans, B., Vangronsveld, J., Vander, L. D., Campbell, C. D and Moore, E. R. (2006). *Journal of Systematic and Applied Microbiology*. 29 (7): 539-556.

[7] Mrozik, A., Poitrowska-seget, Z., and Abuzek, L. (2003). Bacterial degradation and bioremediation of polycyclic aromatic hydrocarbon. *Polish Journal of Environmental Studies,* 121: 15-25.

[8] Yang, D. (2013). Levels, sources and potential risks of polycyclic aromatic hydrocarbons (PAHs) in multimedia environment along the Jinjiang River mainstream to Quanzhou Bay, China. *Marine Pollution Bulletin*, 76: 298–306.

[9] Byambaa, B., Yang, L., Matsuki, A., Nagato, E. G., Gankhuyag, K., Chuluunpurev, B., Banzragch, L., Chonokhuu, S., Tang, N. and Hayakawa, K. (2019). Sources and characteristics of Polycyclic Aromatic Hydrocarbons in Ambient Total Suspended Particles in Ulaanbaatar City, Mongolia. *International Journal of Environmental Research and Public Health*, 16: 1-16.

[10] Khalili, N. R., Scheff, P. A. and Holsen, T. M. (1995). Polycyclic Aromatic Hydrocarbon source fingerprints for coke ovens, diesel and gasoline engines, highway tunnels, and wood combustion emissions. *Atmospheric Environment*, 29: 533–542.

[11] Lyu, Y., Zheng, W., Zheng, T. and Tian, Y. (2014). Biodegradation of Polycyclic Aromatic Hydrocarbons by *Novosphingobium pentaromativorans* US6-1. *PLoS ONE*, 9 (7): 1-8.

[12] Kanaly, R. A. and Harayama, S. (2009) Advances in the field of high-molecular-weight polycyclic aromatic hydrocarbon biodegradation by bacteria. *Microbiology and Biotechnology*, 3: 136–164.

[13] Florian, A. H., Arne, B., Marcia, D., Dietmar, H. P. and Hans, H. R. (2013) Elucidation of in situ polycyclic aromatic hydrocarbon degradation by functional metaproteomics (protein-SIP). *Proteomics*, 13: 2910–2920.

[14] Maiti, A., Das, S. and Bhattacharyya, N. (2013). High gelatinase activity of a newly isolated polycyclic aromatic hydrocarbon degrading bacteria *Bacillus weihenstephanensis* strain AN1. *Journal of Pharmaceutical Resources*, 6: 199–204.

[15] Jabborova, D., Annapurna, K., Fayzullaeva, M., Sulaymonov, K., Kadirova, D., Jabbarov, Z. and Sayyed, R. Z. (2020). Isolation and Characterization of Endophytic Bacteria from Ginger (*Zingiber officinale* Rosc). *Annals of Phytomedicine*, 9 (1): 116-121.

[16] Zinniel, D. K., Lambrecht, P., Harris, N. B., Feng, Z., Kuczmarski, D., Higley, P., Ishimaru, C. A., Arunakumari, A., Barletta, R. G. and Vidaver, A. K. (2002). Isolation and Characterization of Endophytic Colonizing Bacteria from Agronomic Crops and Prairie Plants. *Applied and Environmental Microbiology*, 68 (5): 2198–2208.

[17] Phillips, L. A,, Germida, J. J., Richard E. Farrell, R. E. and Greer, C. W. (2008). Hydrocarbon degradation potential and activity of endophytic bacteria associated with prairie plants. *Soil Biology and Biochemistry*, 40: 3054–3064.

[18] Anyasi, R. O., Atagana, H. I. and Sutherland, R. (2019). Identification and characterization of PAH-degrading endophytes isolated from plants growing around a sludge dam. *International Journal of Phytoremediation*, 21 (7): 672-682.

[19] Nafi'u, A., Usman, M. H., Bello, A. Y., Mustapha, G. and Maiturare, H. M. (2017). Screening and Identification of Cellulase- Producing Bacteria Isolated from Rumen of Camel in Sokoto Main Abattoir. *International Journal of Scientific Research and Management*, 5 (7): 5826-5832.

[20] Bello, A. Y., Nafi'u, A., Haruna, Z. A., Ridwan, A. H., Mohammed, A. and Ibrahim, M. T. (2020). Bacteria Screening with Sufficient Nitrate Extraction from Wastewater. *Journal of Applied Life Sciences International*. 23 (12): 52-58.

[21] Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. and Williams, S. T. (1994). Bergey's manual of determinative bacteriology, 9th edition. Williams and Wilkins, Baltimore.

[22] Tripathi, M., Vikram, S., Jain, R. K. and Garg, S. K. (2009). Isolation and Growth Characteristics of Chromium (VI) and Pentachlorophenol Tolerant Bacterial Isolate from Treated Tannery Effluent for its Possible Use in Simultaneous Bioremediation. *Indian Journal of Microbiology*, 51 (1): 61–69.

[23] Borah, D. and Yadav, R. N. S. (2014). Biodegradation of Diesel, Crude Oil, Kerosene and Used Engine Oil by a Newly Isolated *Bacillus cereus* Strain DRDU1 from an Automobile Engine in Liquid Culture. *Arabian Journal of Science and Engineering*, 39 (7): 5337-5345.

[24] Zhu, X., Ni, X., Waigi, M. G., Liu, J., Sun, K. and Gao, Y. (2016). Biodegradation of Mixed PAHs by PAH-Degrading Endophytic Bacteria. *International Journal of Environmental Research and Public Health*, 13: 1-13.

[25] Iqbal, A., Arshad, M., Karthikeyan, R., Gentry, T. J., Rashid, J., Ahmed, I. and Schwab, A. P. (2019). Diesel degrading bacterial endophytes with plant growth promoting potential isolated from a petroleum storage facility. *3 biotech*, 9: 1-12.

[26] Shahzadi, S., Khan, Z., Rehman, A., Nisar, M. A., Hussain, S. Z., and Asma, S. T. (2019). Isolation and characterization of *Bacillus amyloliquefaciens* 6A: A novel kerosene oil degrading bacterium. *Environmental Technology and Innovation*, *14*, 100359.

[27] Qi, B., Zhang, K., Qin, S, Lyu, D. and He, J. (2022). Glucose addition promotes carbon fixation and bacteria diversity in c-poor soils, improves root morphology, and enhances key N metabolism in apple roots. *PLoS ONE*, 17 (1): 1-26.

[28] Abdullahi, Y. B., Mohammed, S. S. D. and Ijah, U. J. J. (2020). Microbiological and Physicochemical Assessment of Tannery Polluted Soil Inoculated with Pure and Mixed Fungi Slurry. *Fudma Journal of Sciences,* 4 (1): 732 – 743.

[29] Wang, Q., Wang, C., Yu, W., Turak, A., Chen, D., Huang, Y., Ao, J., Jiang, Y. and Huang, Z. (2018). Effects of Nitrogen and Phosphorus Inputs on Soil Bacterial Abundance, Diversity and Community Composition in Chinese Fir Plantations. *Frontiers in Microbiology*, 9: 1-10.