IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402, p- ISSN: 2319-2399.Volume 18, Issue 12 Ser. 2 (December 2024), PP 14-22
www.iosrjournals.org

Biodegradation Kinetics Of Hydrocarbons In Polluted Soils As A Function Of Nitrogen And Potassium Content

Kiele Molingo Mbemba*, Gabriel Judicael Okeni-Boba, Jean Maurille Ouamba
Université Marien NGOUABI, Unité De Chimie Du Végétal Et De La Vie, Faculté Des Sciences Et Techniques, B.P. 69, Brazzaville, Congo

Abstract:
This work made it possible to monitor changes in hydrocarbon content in polluted soils as a function of nitrogen and potassium content during bioremediation treatments. Samples were taken from four types of garage soil selected from the Ouenzé, Talangai and Djiri arrondissements in the north of Brazzaville. Four treatments were carried out using bioremediation while optimising the physico-chemical parameters using Bioaugmentation, Biostimulation and a combinaison of Bioaugmentation and Biostimulation. We observed a reduction of between 25% and 85% in hydrocarbon content in hydrocarbon-polluted soils. Soils treated with Bioaugmentation and the Bioaugmentation-Biostimulation mixture had the most optimal nitrogen and potassium content for bioremediation of hydrocarbon-polluted soils. The greatest reduction (75%-85%) in hydrocarbon content was observed for the Bioaugmentation treatment (Bacteria alone) and the Biostimulation and Bioaugmentation combination (NPK+Bacteria). The smallest drop (25%-43%) in hydrocarbon content was observed for soils that had undergone natural attenuation.
Mots clés: *Bacteries, Hydrocarbures, sol, pollution, bioremediation, NPK*

Date of Submission: 04-12-2024 Date of Acceptance: 14-12-2024

I. Introduction

The use of petroleum products has led to significant risks of natural environments contamination [1]. These substances share toxic properties at low doses that threaten the health of organisms and ecosystems [2]. As the world's population grows, there is a concomitant increase in demand for petroleum products that the production could accidentally be a source of environmental pollution, particularly of soil. Indeed, the increase in oil industry activity generates significant economic spin-offs. Over and above economic profitability, these oil activities have a significant impact on the components of the environment because they can modify the composition of soils by releasing inorganic substances such as heavy metals (Cd, Hg, Pb) and metalloids or organic substances, including pesticides, hydrocarbons, PCBs and BTEX, which can degrade the soil, which is an alteration layer covering a rock originating from the bedrock and evolving according to environmental factors [3]. However, soil pollution by hydrocarbons is often accidental or of industrial origin, except in the case of deliberate spraying of hydrocarbons for experimental purposes. Given the complexity and adiversity of soils, the sustainable management of polluted soils is a challenge that involves processes aimed at reducing the target pollutants bioavailability by preventing their transfer to other environments.

Numerous studies around the world have already shown that microbial degradation of hydrocarbons is an ideal process for decontaminating sediments and surface soils, as these compounds can be mineralised or partially transformed either by a particular microorganism or by any other community. Biodegradation of hydrocarbons by bacteria is indisputably one of the most successful developments in the degradation of hydrocarbon pollutants [4]. Due to the lack of commercialised technologies for treating polluted soils, treatment trials have been undertaken based on the use of micro-organisms [5] which remains the best-known method thanks to its low impact on the environment, workers and the population, as well as its reasonable cost of application and durability [6]. Bioremediation is unique in that it approaches the problem of remediating hydrocarbon-polluted soils from the angle of soil-microorganism interactions (bacteria) coupled with the optimisation of physico-chemical parameters [7]. Several bacteria have already been used in numerous applications in the bioremediation of hydrocarbon-polluted environments.The Gram-positive bacterium M. luteus plays an important role in bioremediation because it is able to tolerate and use toxic organic molecules as a source of carbon, and combines these activities with tolerance to heavy metals [8]. It is often isolated from contaminated soils, hydrocarbons and sludge. M. luteus can degrade hydrocarbons and olefinic compounds [9].

Several aerobic bacterial strains are involved in the degradation of Polycyclic Aromatic Hydrocarbons (PAHs). Naphthene, fluorene, anthragene, pyrene and benzo(a)anthracene molecules are degraded by bacterial strains of pseudomonas sp, rhodococcus sp and mycobacterium sp [10]. Strains of pseudomonas aeruginosa have been shown to be effective in degrading n-alkanes, but cannot degrade branched alkanes or aromatics [11]. Strains of the genus Bacillus have been used in several studies showing the efficiency of biodegradation of soils polluted by hydrocarbons.The aim of this work is to monitor the elimination kinetics of hydrocarbons in polluted soils during different biotreatments as a function of nitrogen and potassium content.

II. Material And Methods

Study Site

This study was carried out at Brazzaville in the Republic of Congo. The Republic of Congo covers an area of 342,000 km2.It is located in Central Africa, straddling the equator between latitudes 3°30' North and 5° South and longitudes 11° and 18° East. The agglomeration of Brazzaville covers an area of nearly 265 km2. Brazzaville is located in the southern part of Congo, between 4°6'15" and 4°22'30" of southern latitude and between 15°6'0" and 15°19'15" east longitude [12]. This study was carried out in four (04) garages located in fourth differents districts of Brazzaville city in Republic of Congo (Figure 1): Ewoulama Garage or Garage A in Djiri district, Dubai Garage or Garage B in Ouenzé district, Moise Garage or Garage C in Mfilou district and Prince Garage or Garage D in Talangai district.

Soil Sampling

Soil samples polluted by hydrocarbons were taken from the following fourth (4) garages, namely: Ewoulama Garage (Garage A), Dubai Garage (Garage B), Moise Garage (Garage C) and Prince Garage (Garage D). picture. The geographical coordinates of these different garages concerned by the study are summarized in Table 1. The sampling method chosen is systematic random. The samples taken by garage are composite type, resulting from a mixture of sub-samples series taken in accordance with the systematic random sampling strategy (ISO-18400-102, 2017). For this, a rectangular area polluted by hydrocarbons was chosen in each garage and was demarcated over an area of 10 to 15m2 with a square mesh. In each demarcated area, 5 sub-samples were taken from the four corners of the demarcated area as well as in the center. The 5 sub-samples were taken from a depth of 20 cm using an auger. The 5 sub-samples were mixed equally in order to constitute the most representative composite sample. Each composite sample is made up of 10 kg of contaminated soil resulting from the mixture of 5 sub-samples. Then, composite samples from each garage were placed in plastic box measuring 13m by 23.5m. The soils collected are placed in sterilized glass jars and covered with aluminum foil (Figure 2). Then, they were sent to the laboratory where they have been dried for three (03) days at ambient temperature of 25°C, then sieved on 2mm mesh sieves (ISO-23266, 2020). Finally, these soil polluted composite samples were weighed and then packaged in 1000mL glass bottles.

Figure 1 : Map of Brazzaville indicating the locaction of 04 garages chosen for this study. (Brazzaville carte » Vacances - Arts- Guides Voyages, 2018)

Tableau I: Geographic coordinates of the 04 districts concerned by the study

Garages (codes)	Geographic coordinates		Districts
	Latitude	Longitude	
Ewoulama (Garage A)	4°11'24"S	15°15'18"E	Djiri
Dubai (Garage B)	4°14'6"S	15°18'0"E	Ouenzé
Moise (Garage C)	4°13'12"S	15°16'12"E	Mfilou
Prince (Garage D)	4°13'12"S	15°17'6"E	Talangai

Figure 2 : Conditioning of soil samples polluted by hydrocarbons for transport to laboratory

Bacterial strains

Bacteria sought are those belonging to the Bacillus genus obtained after isolation. These bacteria were isolated in Irsen laboratory in Brazzaville by using suspension dilution method [13]. Bacterial strains are inoculated into a BH type mineral medium. The bacterial culture is kept in an incubator at 37°C for 15 days.

Inoculation tests

Four (4) plastic box containing soil polluted by hydrocarbons were treated, respectively:

- Box 1: Polluted soil + Natural attenuation (no amendment), this box contains only polluted soil,
- Box 2: Polluted soil + Consortium (Bacteria+NPK), this box is made up of polluted soil and a mixture of nutrients NPK + Bacillus type bacteria,
- Box 3: Polluted soil + Bacteria (Bioaugmentation), this box contains polluted soil with a medium of Bacillus type bacteria,
- Box 4: Polluted soil + nutrients NPK (Biostimulation), this box contains polluted soil with NKP nutrients in order to stimulate bio-stimulation.

Nitrogen and Potassium content in soils

Nitrogen and potassium were obtained potentiometrically by directly immersing the humidity tester probe or the NKP fertilizer detector soil tester of the Sonkir type multi-parameter directly into the soil.

Hydrocarbon content in soils

The total petroleum hydrocarbons (TPH) were extracted by Soxhlet according to Environment Protection method 3540 Agency (EPA). EPA Method 3540 is based on liquid-solid extraction by continuous recirculation on the boiled and decondensed solvent sample. The total petroleum hydrocarbons (TPH) from the hydrocarbon-polluted soils were extracted by Soxhlet during the treatments (Figure 3). 10g of soil wrapped in Wattman filter paper are introduced into the cartridge and hydrocarbons are extracted with dichloromethyl at 40oC for 6 hours.

Figure 3 : Extraction of hydrocarbons by Soxhlet

The soil is dried at ambient temperature of 25°C and the mass is measured every 24 hours until a stable mass is obtained. The total petroleum hydrocarbon (TPH) rate is calculated according to the following formula:

$$\% \text{ TPH} = \frac{\text{initial mass} - \text{final mass}}{\text{initial mass}} X\, 100$$

III. Results And Discussion

Evolution of hydrocarbon biodegradation in polluted soil A as a function of nitrogen and potassium content

Figure 4 shows the biodegradation of hydrocarbons in polluted soil A as a function of nitrogen and potassium content during the different biotreatments of soil A. The following can be noted:

- In soil A that has undergone natural attenuation, the hydrocarbon content (from 13.12% to 10% between 0 and 180 days) decreases over time, while the nitrogen and potassium contents remain very low up to 90 days, then become zero from 120 days.
- In soil A treated with Bacteria, the hydrocarbon content (from 13.12% to 1.62% between 0 and 180 days) fell over time, while the nitrogen and potassium contents were virtually zero up to 30 days. At 60 days, there was a sharp increase in nitrogen (90 mg/kg) and potassium (80 mg/kg) levels. From 90 days, there was a sharp drop in nitrogen content. The potassium content began to fall slightly from 150 days. At 180 days, the nitrogen content is 1 mg/kg and the potassium content is 80 mg/kg,
- In soil A treated with NPK, the hydrocarbon content (from 13.12% to 3.89% between 0 and 180 days) fell over time, while the nitrogen and potassium contents were zero up to 30 days, then increased at 60 days (400 mg/kg for nitrogen and 1200 mg/kg for potassium) before falling again. At 180 days, the nitrogen content is 28 mg/kg and the potassium content is 2 mg/kg,
- In soil A treated with NPK-Bacteria, the hydrocarbon content (from 13.12% to 1.88% between 0 and 180 days) fell over time, while the nitrogen and potassium contents were zero up to 30 days, then increased at 60 days (500 mg/kg for nitrogen and 1200 mg/kg for potassium) before falling again. At 180 days, the nitrogen content is 100 mg/kg and the potassium content is 308 mg/kg.

Evolution of hydrocarbon biodegradation in polluted soil B as a function of nitrogen and potassium content

Figure 5 shows the biodegradation of hydrocarbons in polluted soil B as a function of nitrogen and potassium content during the different biotreatments of soil B. The following can be noted:

- In soil B that had undergone natural attenuation, the hydrocarbon content (from 11.86% to 10.5% between 0 and 180 days) fell over time, while the nitrogen and potassium contents remained very low and became zero after 90 days,

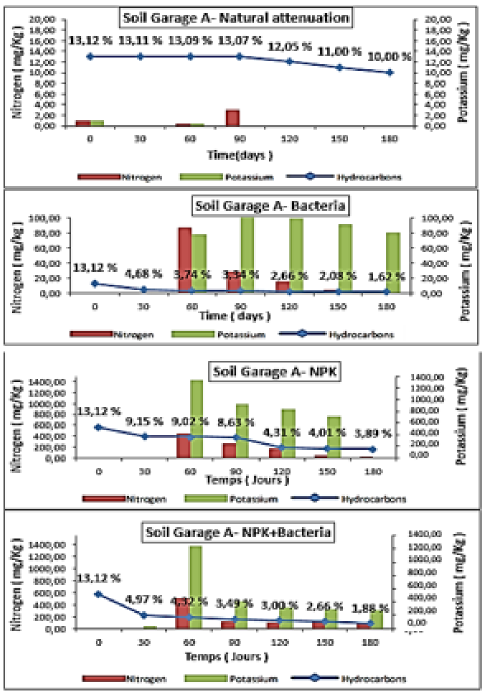

Figure 4: Evolution of hydrocarbon content in soil A treated (Natural attenuation, Bacteria, NKP, NKP-Bacteria) as a function of nitrogen and potassium content

- In soil B treated with bacteria, the hydrocarbon content (from 11.86% to 1.91% between 0 and 180 days) fell over time, while the nitrogen and potassium content was virtually zero up to 30 days. At 60 days, there was an increase in nitrogen (110 mg/kg) and potassium (40 mg/kg). From 90 days, there was a sharp drop in nitrogen content. The potassium content began to fall slightly from 120 days. At 180 days, the nitrogen content was 51 mg/kg and the potassium content was 20 mg/kg,
- In soil B treated with NPK, the hydrocarbon content (from 11.86% to 2.17% between 0 and 180 days) fell over time, while the nitrogen and potassium contents were virtually zero up to 30 days. At 60 days, an increase in nitrogen (300 mg/kg) and potassium (1000 mg/kg) was observed. From 90 days, there was a sharp drop in nitrogen and potassium levels. At 180 days, the nitrogen content is 190 mg/kg and the potassium content is 65 mg/kg,
- In soil B treated with NPK-Bacteria, the hydrocarbon content (from 11.86% to 2% between 0 and 180 days) fell over time, while the nitrogen and potassium contents were virtually zero up to 30 days. At 60 days, an increase in nitrogen (500 mg/kg) and potassium (1700 mg/kg) was observed. From 90 days, there was a sharp drop in nitrogen and potassium levels. At 180 days, the nitrogen content was 193 mg/kg and potassium 40 mg/kg.

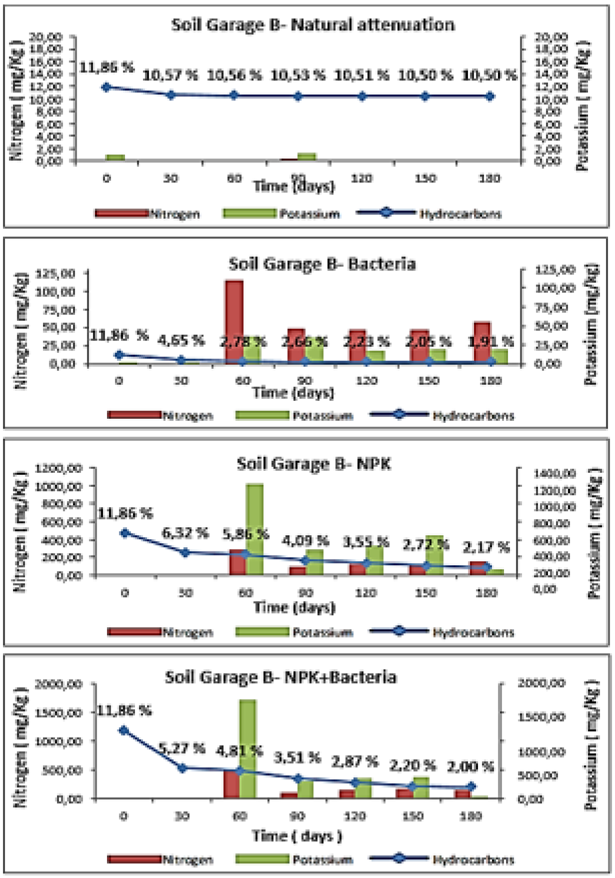

Figure 5: Evolution of hydrocarbon content in soil B treated (Natural attenuation, Bacteria, NKP, NKP-Bacteria) as a function of nitrogen and potassium content

Evolution of hydrocarbon biodegradation in polluted soil C as a function of nitrogen and potassium content

Figure 6 shows the biodegradation of hydrocarbons in polluted soil C as a function of nitrogen and potassium content during the different treatments of soil C. The following can be noted:

- In soil C that had undergone natural attenuation, the hydrocarbon content (from 12.83% to 9.0% between 0 and 180 days) fell over time, while the nitrogen and potassium contents remained very low and became zero after 30 days,
- In soil C treated with bacteria, the hydrocarbon content (from 12.83% to 1.47% between 0 and 180 days) fell over time, while the nitrogen and potassium content was virtually zero up to 30 days. At 60 days, an increase in nitrogen content was observed. At 180 days, the nitrogen content was 50 mg/kg and the potassium content was 77 mg/kg,

- In soil C treated with NPK, the hydrocarbon content (from 12.83% to 2.25% between 0 and 180 days) fell over time, while the nitrogen and potassium contents were zero up to 30 days. At 60 days, an increase in nitrogen (400 mg/kg) and potassium (1100 mg/kg) was observed. From 90 days, there was a sharp drop in nitrogen and potassium levels. At 180 days, nitrogen and potassium levels are 1 mg/kg,
- In soil C treated with NPK-Bacteria, the hydrocarbon content (from 12.83% to 1.41% between 0 and 180 days) fell over time, while the nitrogen and potassium contents were virtually zero up to 30 days. At 60 days, an increase in nitrogen (600 mg/kg) and potassium (1800 mg/kg) was observed. From 90 days, there was a sharp drop in nitrogen and potassium levels. At 180 days, the nitrogen content is 16 mg/kg and the potassium content is 48 mg/kg.
-

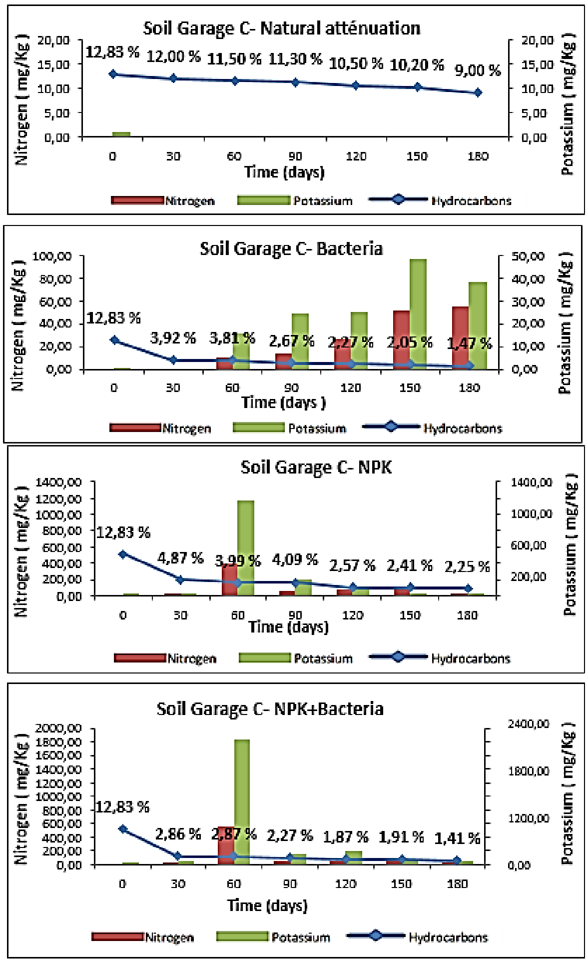

Figure 6: Evolution of hydrocarbon content in soil C treated (Natural attenuation, Bacteria, NKP, NKP-Bacteria) as a function of nitrogen and potassium content

Evolution of hydrocarbon biodegradation in polluted soil D as a function of nitrogen and potassium content

Figure 7 shows the biodegradation of hydrocarbons in polluted soil D as a function of nitrogen and potassium content during the different treatments of soil C. The following can be noted:

- In soil D that had undergone natural attenuation, the hydrocarbon content (from 8.29% to 6.15% between 0 and 180 days) fell over time, while the nitrogen and potassium contents were 2 mg/kg and 3 mg/kg respectively at the initial stage. They then become zero up to 180 days,
- In soil D treated with bacteria, the hydrocarbon content (from 8.29% to 1.19% between 0 and 180 days) fell over time, while the nitrogen and potassium contents were 2 mg/kg and 3 mg/kg respectively at the initial stage. These levels then dropped to zero up to 60 days. An increase in these levels was observed from 90 days. A drop in these levels was observed from 150 days. At 180 days, the nitrogen content was 1 mg/kg and the potassium content was 4 mg/kg,
- In soil D treated with NPK, the hydrocarbon content (from 8.29% to 1.50% between 0 and 180 days) fell over time, while the nitrogen and potassium contents were 2 mg/kg and 3 mg/kg respectively at the initial stage. At 30 days, nitrogen (10 mg/kg) and potassium (40 mg/kg) levels increased. From 60 days, there was a sharp drop

in nitrogen and potassium levels. At 180 days, nitrogen and potassium levels were 6.5 mg/kg and 0 mg/kg respectively,

- In soil D treated with NPK-Bacteria, the hydrocarbon content (from 8.29% to 1.69% between 0 and 180 days) fell over time, while the nitrogen and potassium contents were 2 mg/kg and 3 mg/kg respectively at the initial stage. At 30 days, there was an increase in nitrogen (60 mg/kg) and potassium (170 mg/kg). From 60 days, there was a sharp drop in nitrogen and potassium levels. At 180 days, nitrogen and potassium levels were 6.5 mg/kg and 9 mg/kg respectively.

Discussion on evolution in hydrocarbon content as a function of nitrogen and potassium content in the different soil (A, B, C, D) treatments

Figures 3 to 6 show the evolution of hydrocarbon content as a function of nitrogen and potassium content during the different treatments of soils A, B, C and D. The results clearly show that there is little or no presence of nitrogen and potassium nutrients in the soils initially polluted by hydrocarbons. Soils that had undergone natural attenuation were devoid of nutrients throughout the treatments. This is a limiting factor for biodegradation [14]. The results showed that hydrocarbon content fell sharply when bacteria and NPK nutrients were added to hydrocarbon-polluted soils. The 3 different soil treatments (Bacteria, NPK and NPK-Bacteria) can be classified according to their effectiveness in degrading hydrocarbons by comparing the initial hydrocarbon content (THC-0) with the remaining hydrocarbon content in the soil after 180 days of treatment (THC-180) in the different soils. The results are as follows:

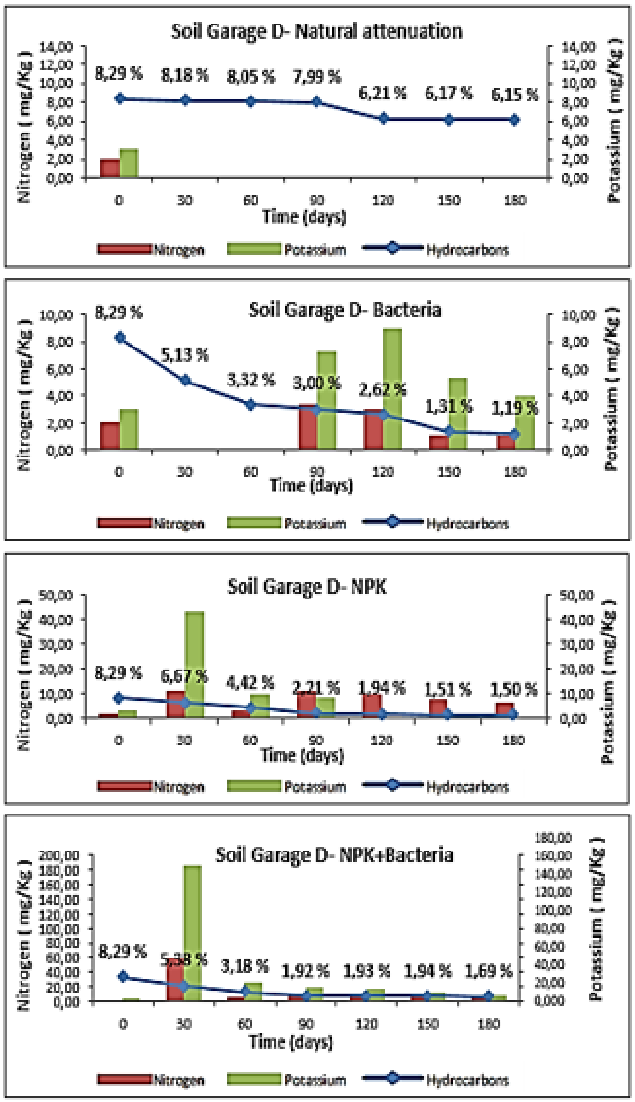

Figure 7: Evolution of hydrocarbon content in soil D treated (Natural attenuation, Bacteria, NKP, NKP-Bacteria) as a function of nitrogen and potassium content

- Soil A (THC-0=13,12%) : THC-180 Bacteria (1,62%)< THC-180 NKP-Bacteria (1,88%) < THC-180 NKP (3,89%),
- Soil B (THC-0=11,86%) : THC-180 Bacteria (1,91%)< THC-180 NKP-Bacteria (2%)< THC-180 NKP (2,17%),
- Soil C (THC-0=12,83%): THC-180 Bacteria (1,47%)< THC-180 NKP-Bacteria (1,41%)< THC-180 NKP (2,25%),
- Soil D (THC-0=8,29%) : THC-180 Bacteria (1,19%)< THC-180 NKP (1,5%)< THC-180 NKP-Bacteria (1,69%).

It can therefore be deduced that all the soils treated with bacteria have the best hydrocarbon removal rates compared with those treated with NPK alone. Soils treated with NPK-Bacteria show better hydrocarbon removal than soils treated with NPK alone for 3 soils (A, B, C). Hydrocarbons in soil D are therefore better degraded by bacteria alone, which metabolise petroleum hydrocarbons as an energy source [15]. The NPK nutrients used to grow the bacteria should accelerate their growth and therefore the rate of hydrocarbon degradation [16].This was clearly not the case in our study. This means that there may have been a sufficient number of micro-organisms in the soil because the number of hydrocarbon-degrading organisms in the contaminated soil determines the rate of degradation and a lack of these microbes leads to a reduced rate of hydrocarbon degradation [17]. or that the quantity of nutrients injected into the soil was insufficient. Optimisations can be made to improve our results

IV. Conclusion

The aim of this study was to monitor evolution of hydrocarbon content in polluted soils as a function of nitrogen and potassium content during bioremediation treatments. To do this, four treatments were applied to soil samples taken from different garages in the city of Brazzaville, while optimising the physico-chemical parameters by bioaugmentation, biostimulation and a combinaison of bioaugmentation and biostimulation. Nitrogen and potassium levels were monitored to better determine their effect on microbial metabolism during hydrocarbon degradation in the soil. Soils treated with NPK+Bacteria and NPK had the most optimal nitrogen and potassium levels for bioremediation of hydrocarbon-polluted soils. A drop of between 25% and 85% in hydrocarbon content was observed in hydrocarbon-polluted soils. The greatest reduction (75%-85%) in hydrocarbon content was observed for the Bioaugmentation (Bacteria alone) and combined Biostimulation and Bioaugmentation (NPK+Bacteria) treatments. The smallest reduction (25%-43%) in hydrocarbon content was observed for soils that had undergone natural attenuation. As a result, bioremediation using bacteria of the Bacillus genus could be an alternative way of cleaning up hydrocarbon-polluted soils.

Conflicts Of Interest

The authors declare no conflict of interest or personal relationship that could have appeared to influence the work of this article.

Acknowledgements

Authors would like to thank SGS laboratory in Pointe-Noire, Republic of Congo, for their cordial support in carrying out this study.

References

[1] Roudi M. (2014). Biodiesel Production From Microalgae-Chlorella Sorokoniana. Australian Journal Of Basic And Applied Sciences, 8(3), 140-145.
[2] Priyadarshanee, M., Mahto, U., And Das, S. (2022). Mechanism Of Toxicity And Adverse Health Effects Of Environmental Pollutants. In Microbial Biodegradation And Bioremediation (Pp. 33-53). Elsevier.
[3] Samanth, M. (2024). An Inclusive Evaluation Of Soil Pollution And Its Remediation By Chemical, Physical And Biological Methods. Ijcs, 12(4), 05-17.
[4] Baltaci, M. O., Omeroglu, M. A., Ozkan, H., Taskin, M., And Adiguzel, A. (2024). Enhanced Biodegradation Of Crude Oil Contamination By Indigenous Bacterial Consortium Under Real Conditions. Biocatalysis And Biotransformation, 42(1), 56-67.
[5] Simon, M., And Joshi, H. (2021). A Review On Green Technologies For The Rejuvenation Of Polluted Surface Water Bodies: Field-Scale Feasibility, Challenges, And Future Perspectives. Journal Of Environmental Chemical Engineering, 9(4), 105763.
[6] Safwat, S. M., And Matta, M. E. (2021). Environmental Applications Of Effective Microorganisms: A Review Of Current Knowledge And Recommendations For Future Directions. Journal Of Engineering And Applied Science, 68(1), 48.
[7] Jamil, N., Kumar, P., And Batool, R. (Eds.). (2020). Soil Microenvironment For Bioremediation And Polymer Production. John Wiley & Sons.
[8] Das, R., And Kazy, S. K. (2014). Microbial Diversity, Community Composition And Metabolic Potential In Hydrocarbon Contaminated Oily Sludge: Prospects For In Situ Bioremediation. Environmental Science And Pollution Research, 21, 7369-7389.
[9] Mussa, E. S. E. S., Hewait, H. M., And El-Sharnouby, S. F. (2024). Diesel Biodegradation Capacities Of Bacillus Subtilis Or632422, Micrococcus Luteus Or632421 Isolated From Petroleum-Contaminated Soil. Egyptian Journal Of Soil Science, 64(4), 1537-1547.
[10] Feng, S., Gong, L., Zhang, Y., Tong, Y., Zhang, H., Zhu, D., ... And Yang, H. (2021). Bioaugmentation Potential Evaluation Of A Bacterial Consortium Composed Of Isolated Pseudomonas And Rhodococcus For Degrading Benzene, Toluene And Styrene In Sludge And Sewage. Bioresource Technology, 320, 124329.

[11] Bekenniche N. (2014). Cracterization Of Hydrocarbon Biodegradation Activities By Different Microbial Genera Isolated From Contaminated Sites. Doctoral Thesis Of University Of Oran, Algiers.
[12] Kimbatsa F.G., Mahoungou E., Berton Ofouemé Y. (2018). The Importance Of Horticulture In The Fight Against Food Insecurity, Poverty And Environmental Protection In Brazzaville (Republic Of Congo). Openedition Journals Caribbean Studies. 38-40, Pp. 1-47
[13] Elenga Wilson P. S., Okeni-Boba J. G., Kayath A. C, Mbemba K .M, Nguimbi E., Ahombo G. (2022). Qualitative And Quantitative Assesment Of A Surfactin Biosurfactant In The Bioaugmentation Of Crude-Oil Contaminated Soil In Garages In The Republic Of Congo. 10, 1-11.
[14] Benguenab A. (2022). Bioremediation Of Hydrocarbon-Polluted Soils With Telluric Fungi. Doctoral Thesis In Applied Microbiology. Université D Ibn Badis Mostaganem. Algérie.
[15] Fingas M. (2013). The Basic Of Oil Spill Cleanup. Troisième Edition, Boca Raton. Pp 1-266.
[16] Neebee, E., Nkwocha, E. E., And Oguzie, E. E. (2019). Effectiveness Of Npk Fertilizer-Saw Dust Amendment On Biodegradation Of Crude Oil In Polluted Soil.
[17] Koshlaf, E., And Ball, A. S. (2017). Soil Bioremediation Approaches For Petroleum Hydrocarbon Polluted Environments. Aims Microbiology, 3(1), 25.