

# Physicochemical parameters monitoring during bioremediation of soils polluted by hydrocarbons in Brazzaville (Congo)

**Abstract:** This work aimed to treat soils polluted by hydrocarbons using the bioremediation technique. Soils samples were taken from four (04) garages chosen in some districts of Brazzaville, namely Ouenzé, Mfilou, Djiri and Talangai. Four treatments were carried out for bioremediation treatment while monitoring physicochemical parameters by bioaugmentation, biostimulation and mixture of bioaugmentation and biostimulation. Several physicochemical parameters were monitored during the different treatments: The ranges of physicochemical parameters values are respectively between 23.3 and 28.4°C for temperature, between 38,6 and 5266,01 μS/cm for conductivity, between 6.01 and 7,5 for pH, between 0 and 70% for the maximum water retention capacity, between 1 and 10% for humidity, between 0 and 88 mg/Kg for nitrogen and between 0 and 298.93 mg/Kg for potassium. The Total Petroleum Hydrocarbons (TPH) concentrations in polluted soils decreased significantly throughout the treatments with a drop between 25% and 85%. The greatest drop (75%-85%) in hydrocarbon concentrations is observed for Bioaugmentation (Bacteria) and Biostimulation+Bioaugmentation (NPK+Bacteria) combination treatments. Thus, bioremediation by adding Bacillus genus bacteria would be an alternative way for the depollution of soils polluted by hydrocarbons.

**Keywords:** *Bacteria, hydrocarbons, soil, pollution, bioremediation*

## 1. Introduction

Congolese oil landscape expansion, with the discovery of significant hydrocarbon deposits, constitutes an undeniable economic opportunity. Alongside this expansion, we are seeing accidental oil spills, particularly from onshore oil installations. These areas with rich ecosystems, located near onshore oil installations, can suffer oil spill hazards which can pollute their soils, their rivers, their plantations as well as groundwaters, causing risks for the environment and the human health. Recognized as a major environmental problem, oil pollution is the subject of intensive research. Indeed, the spread of hydrocarbon molecules in soil and the real risk of their transfer to groundwater and into food chain have made the decontamination of contaminated sites a major concern [1]. Furthermore, chemical and physical remediation methods have shown their limits due to their cost and their secondary impact on environment. Also, the increasingly restrictive evolution of environmental legislation has sparked a

environmental protection and sustainable development. It is for this purpose that bioremediation or biological depollution, one of the most used methods for purifying environments contaminated by hydrocarbons, has become, over recent decades, one of the most developed research themes. In addition, bioremediation is an interesting alternative to other depollution processes (physicochemical, thermal, etc.) due to its reduced environmental impacts and its low cost [2]. Bioremediation has the particularity of addressing the soil polluted by hydrocarbons remediation problem from the angle of soil-microorganisms (bacteria) interactions coupled with the optimization of physicochemical parameters. The biodegradation of hydrocarbons by bacteria is undeniably one of the most successful processes in the elimination of hydrocarbon pollutants [3]. Indeed, in 2005, nine (9) cyanobacteria, one hundred and three (103) fungi and fourteen (14) algae were identified as being capable of degrading hydrocarbons and seventy-nine (79) bacterial genera capable of using hydrocarbons as a sole source of carbon and energy. But, it has been established that bacteria are qualitatively and

Several bacteria have already been used in numerous applications in bioremediation of environments polluted by hydrocarbons. The Gram-positive bacterium *M. luteus* has an important role in bioremediation because it has abilities to tolerate and use toxic organic molecules as a carbon source and combines these activities with tolerance to heavy metals [4]. It is often isolated from contaminated soils, hydrocarbons and sludge. *M. luteus* can degrade hydrocarbons and olefinic compounds [5]. Several aerobic bacterial strains participate in the degradation of Polycyclic Aromatic Hydrocarbons (PAHs). The molecules of naphthene, fluorene, anthracene, pyrene and benzo(a)anthracene are degraded by bacterial strains of *Pseudomonas*, *Rhodococcus* or even *Mycobacterium* sp [6]. Several metabolic ways degradation of branched hydrocarbons have been demonstrated for 2-methyl octene, 3,6-dimethyl octane and 2,6-dimethyl decane using *Pseudomonas citronellolis* bacterial strains [7]. *Pseudomonas aeruginosa* strains have been shown to be effective in the n-alkanes degradation, but cannot degrade either branched or aromatic alkanes [8]. *Bacillus* genus strains have been used in several studies showing the biodegradation effectiveness on soils polluted by hydrocarbons. On the other hand, very few bioremediation studies have been carried out with indigenous *Bacillus* genus strains obtained in Congo-Brazzaville. This work sets itself the objective of monitoring physicochemical parameters of soils polluted by hydrocarbons during soil bioremediation using *Bacillus* bacterial strains.

## 2. Material and Methods

### 2.1. Study Site

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 km<sup>2</sup>. 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 [9]. This study was carried out in four (04) garages located in four different 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.

### 2.2. Soil Sampling

Soil samples polluted by hydrocarbons were taken from the following four (4) garages, namely: Ewoulama Garage (Garage A), Dubai Garage (Garage B), Moise Garage (Garage C) and Prince Garage (Garage D). Figure 2 shows an Ewoulama garage (Garage 1) 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

**Table 1:** 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 1: Brazzaville Map indicating the localisation of the four (04) garages chosen for this study [10]



Figure 2: Ewoulama Garage (Garage A)

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 15m<sup>2</sup> 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 boxes measuring 13m by 23.5m. The soils collected are placed in sterilized glass jars and covered with aluminum foil (Figure 3). Then, they were sent to the laboratory where they were 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 3:** Conditioning of soil samples polluted by hydrocarbons for transport to laboratory

### 2.3. Bacterial strains

Bacteria sought were those belonging to the *Bacillus* genus obtained after isolation. These bacteria were isolated in Irsen laboratory in Brazzaville by using suspension dilution method [11]. Bacterial strains are inoculated into a BH type mineral

15 days.

### 2.4. Inoculation tests

Four (4) plastic boxes containing polluted soils 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 NPK nutrients in order to stimulate biostimulation.

### 2.5 Physicochemical parameters of soil samples

Temperature and electrical conductivity were measured using a multi-parameters such as Water Quality Meter (EZ9908), according to ISO-11265 standard distilled water in the ratio 1/5 (w/v). The pH was measured with a Water Quality Meter type pH meter (EZ9908) according to the ISO-10390 standard which consists of measuring a suspension of soil in distilled water according to the ratio 1/5 (w/v). The maximum water retention capacity (MWRC) was determined under the action of gravimetry according to a distilled water-soil ratio 1/1 (w/v) [12]. Humidity, nitrogen and potassium were obtained potentiometrically by directly immersing the humidity tester probe or the NPK fertilizer detector soil tester of the Sonkir type multi-parameter directly into the soil.

### 2.6 Total petroleum hydrocarbons (TPH) concentrations 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

soil samples. The total petroleum hydrocarbons (TPH) from the hydrocarbon-polluted soils were extracted by Soxhlet during the treatments (Figure 4). 10g of soils wrapped in Wattman filter paper were introduced into the cartridge and hydrocarbons were extracted with dichloromethyl at 40°C for 6 hours.



**Figure 4** : Extraction of hydrocarbons by Soxhlet

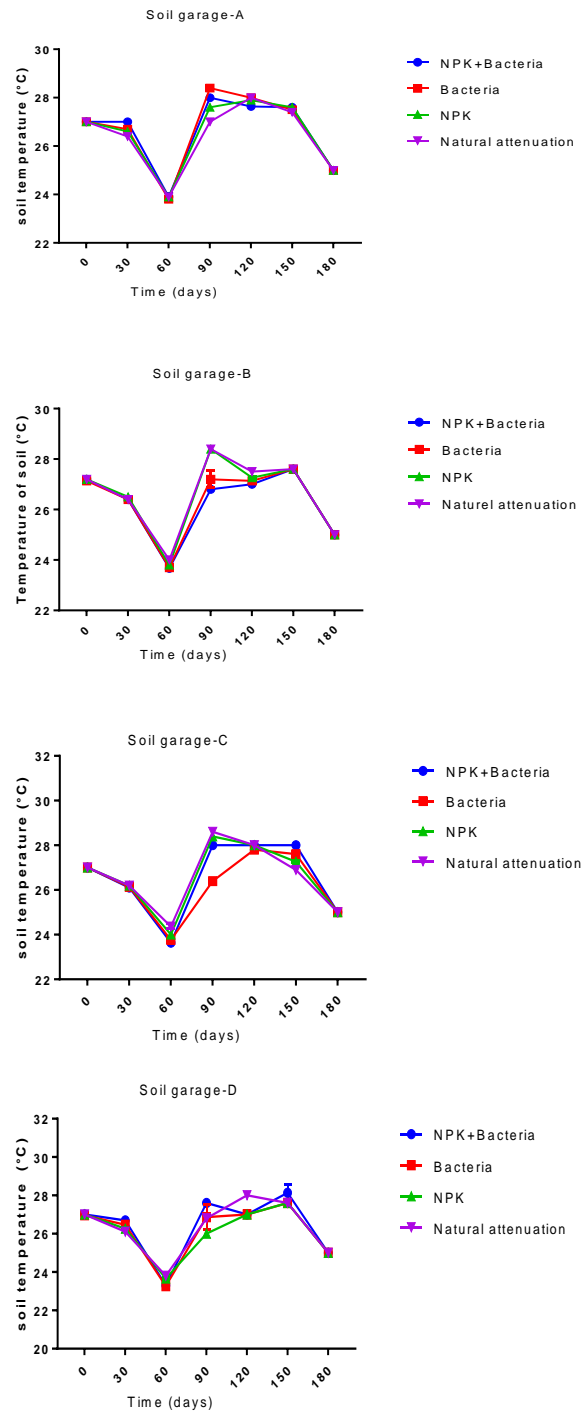
The soils were dried at ambient temperature of 25°C and the masses were measured every 24 hours until a stable mass was obtained. The total petroleum hydrocarbon (TPH) rate was calculated according to the following formula:

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

### 3. Results and Discussion

#### 3.1 Evolution of different garages soil temperature depending on treatments and time

Figure 5 shows the evolution of the different garages (A, B, C, D) soil temperature as a function of treatments and time. We note that during the 180 days, whatever the treatments, temperature values oscillate between 23.3 °C and 28.0 °C for soil of Garage-A, 23.6 °C and 28.6 °C for soil of Garage-B, 23.8 °C and 28.4 °C for soil of Garage-C and 23.7 °C and 28.4 °C for soil of Garage-D. For all garages and whatever the treatments, a drop in temperature is observed until the 30th day before going back up to the 90th, then a new drop in temperature is observed up to 180 days. Temperature peaks are observed around 28°C. The recorded temperatures fit well within the temperature range which determines the conditions required for good microbial degradation [13; 14]. Indeed, the optimal growth temperature and temperature range that a microorganism can tolerate determine its survival and the role it will play in hydrocarbons degradation [15]. The temperature is proportional to bioremediation speed. In general, a decrease in temperature is accompanied by a



**Figure 5** : Evolution of garage (A, B, C, D) soils temperature depending on treatments and time

temperatures have the effect of increasing the rate of biodegradation [16;17]. In our work, hydrocarbons degradation is optimal around 28°C.

#### 3.2 Evolution of different garages soil electrical conductivity depending on treatments and time

Figure 6 shows the evolution of the different garages (A, B, C, D) soil electrical conductivity depending on treatments and time.

of time. We can note a variation in soil electrical conductivity between 66.41 and 2600.01  $\mu\text{S}/\text{cm}$  for soil of Garage A; 38.6 and 5266.01  $\mu\text{S}/\text{cm}$  for soil of Garage B; 110 and 3633.67  $\mu\text{S}/\text{cm}$  for soil of Garage C and 144.26 and 4162  $\mu\text{S}/\text{cm}$  for soil of Garage D. All curves evolve in a similar way with much higher conductivities with the NPK and NPK+Bacteria treatments. The increase in conductivity is due to the overdose of nutrients in the NPK and NPK+Bacteria treatments. The low conductivities of soil treated only with bacteria or under natural attenuation can be explained by the fact that soils sampled are ferrallitic soils which are naturally poor in mineral elements [18].

The depth of sampling is a factor also contributing to the drop in conductivity values because soil collected beyond 15 cm depth reduces excess salts to 80% [19]. Furthermore, these soil electrical conductivity values are in accordance with the Agronomy Momento in which it is demonstrated that limit values for degradation of hydrocarbons in soil are between 0 and 6000  $\mu\text{S}/\text{cm}$  [20]. Although after 180 days, we see a drop in electrical conductivity in all treatments. These electrical conductivity values are well within the recommended range for the degradation of hydrocarbons.

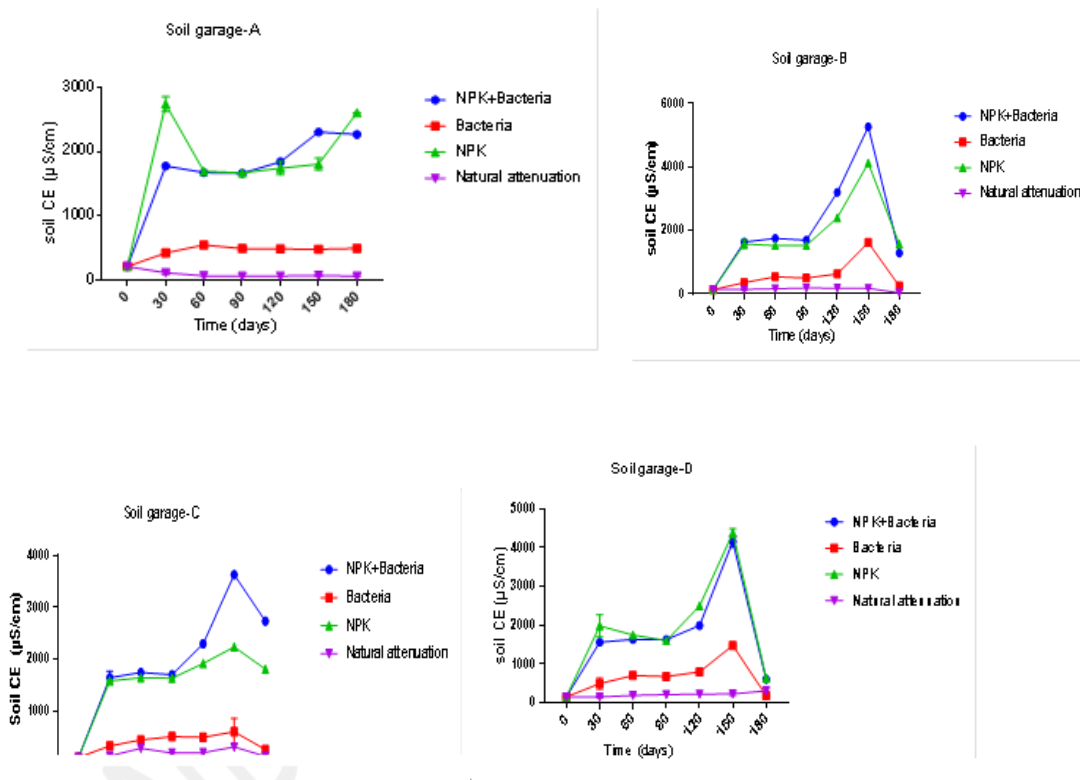


Figure 6

:Evolution of garage (A, B, C, D) soil electrical conductivity depending on treatments and time

### 3.3 Evolution of different garages soils pH depending on treatments and time

Figure 7 illustrates the variation of garages (A, B, C, D) soils pH during the different treatments as a function of time. We note that at start of all treatments, pH values are neutral around 7.50 in soil of Garage A, 7.10 in soil of Garage B, 7.15 in soil of Garage C and 7.15 in soil of Garage D. For soil having undergone natural alteration, a slight increase in pH is observed up to 90 days with values of up to 7.5. Then, we observe a drop up to 180 days with pH values

in ranges of 7 to 7.5. At 180 days, the pH values of all soils are around 7. The soil curves (A, B, C, D) treated with NPK and NPK+Bacteria evolve in a similar way and are almost superposable. The general trend of these curves shows a drop in pH up to 6. Thus, unlike soils (A, B, C, D) having undergone natural attenuation or treated only with bacteria whose pH values remain around the neutrality whatever the soils and treatments duration, pH of the soils (A, B, C, D) treated with NPK and NPK+Bacteria tend towards acidification the further we advance in treatments. This acidity could be explained by the degradation of hydrocarbons by microorganisms due to the

as CO<sub>2</sub> and H<sub>2</sub>O [21; 22]. Thus, soil polluted by hydrocarbons becomes slightly acidic due to the appearance of acids in small quantity resulting either from the n-alkanes oxidation or from compounds

resulting from the breakdown of aromatic nuclei [23]. It should be emphasized that these pH values are in the optimal range favorable to hydrocarbons biodegradation.

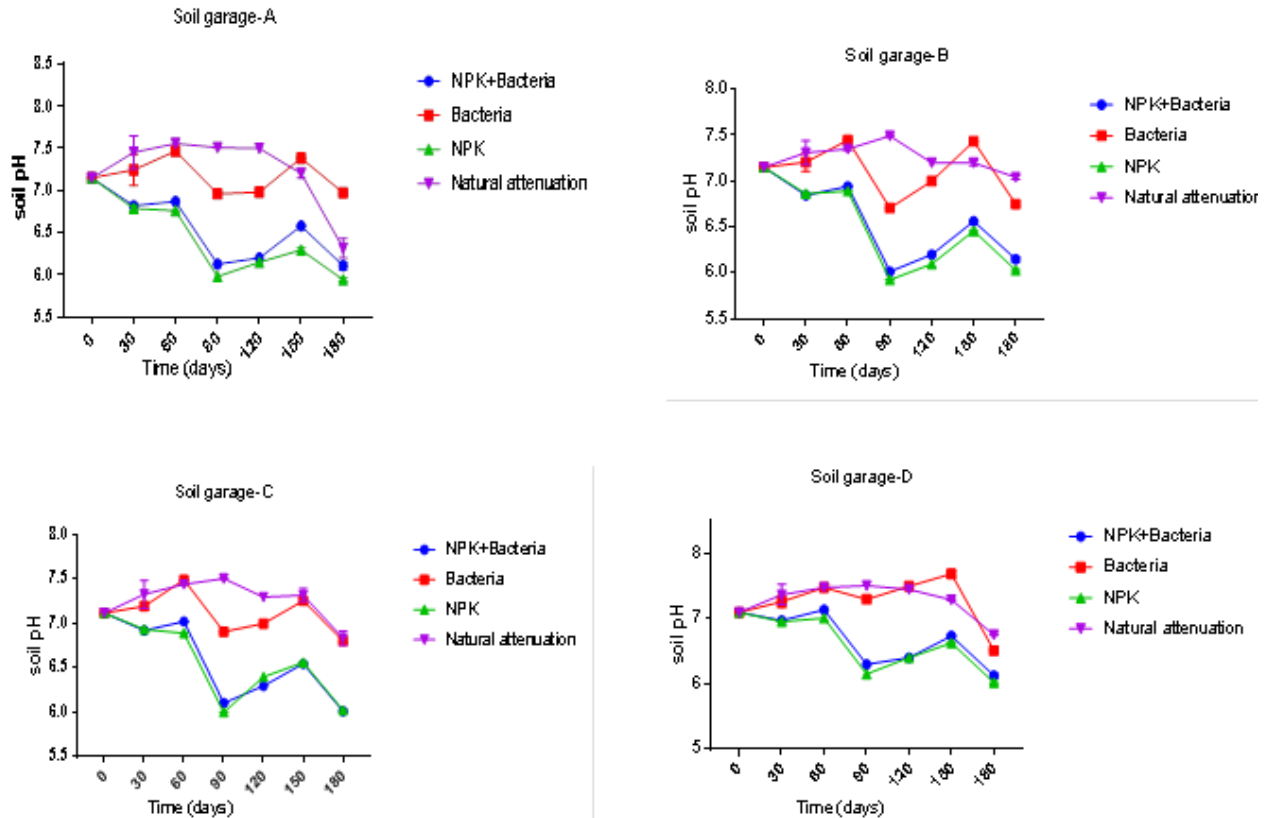


Figure 7 :Evolution of garage (A, B, C, D) soil pH depending on treatments and time

### 3.4 Evolution of different garages soils maximum water retention capacity (MWCR) depending on treatments and time

Figure 8 shows the evolution of the maximum water retention capacity (MWCR) of garage soils during the different treatments as a function of time. The initial values of MWCR are around 35%. We note a progressive decrease in the maximum retention capacity for all soils during the first 150 days, with values oscillating between 0 and 20% whatever the treatment. On the other hand, from 180 days onwards, the MWCR of all soils increases very sharply, reaching values between 50% and 70%. At 180 days:

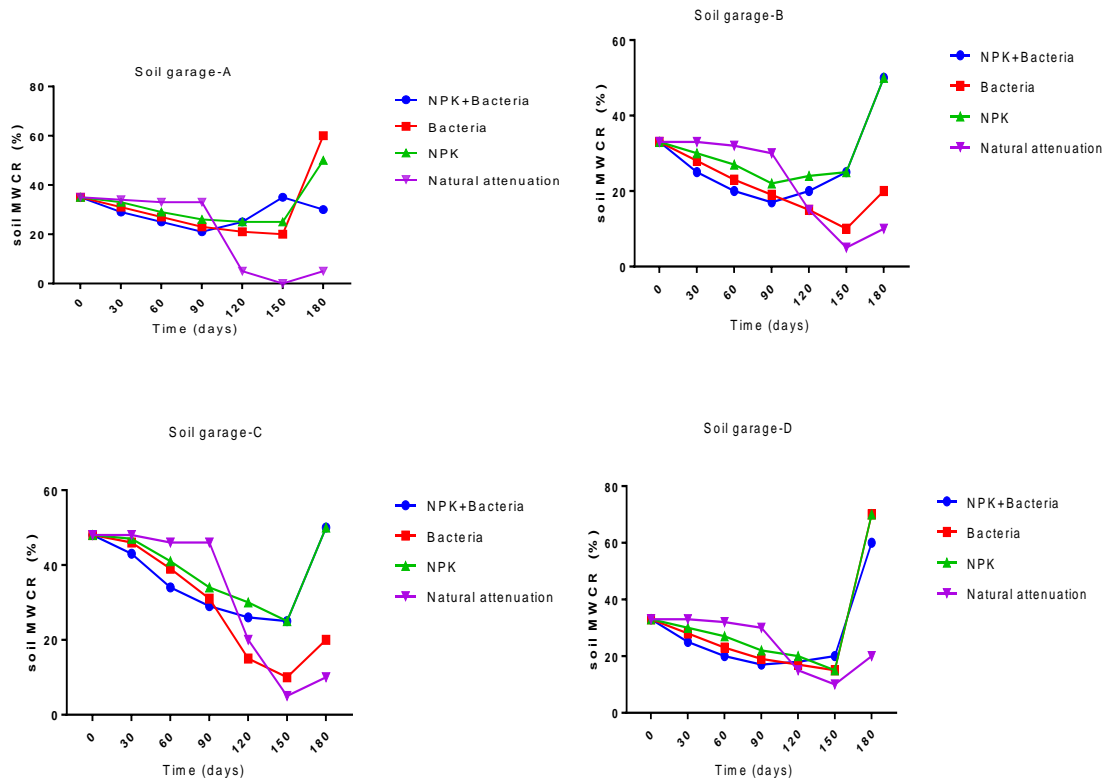
- the soil of garage A has better MWCR (around 60%) with treatments based on bacteria and NPK,
- the soils of garages B and C have better MWCR (around 50%) with the NPK and NPK+bacteria treatments.

- soil D presents better MWCR (around 65-70%) with treatments based on bacteria, NPK and NPK+bacteria.

This sharp decrease in MWCR over the first 150 days is due to soil pollution degree because the more the soil is polluted, the more hydrocarbons will prevent water from being retained in the soil [12]. In fact, the water retention capacity is significantly reduced by hydrocarbons, which hinders the circulation of air and water in soil matrix and therefore reduces water infiltration into the soil [24]. However, the sharp increase between 150 and 180 days in all designated treatments is synonymous with the reduction in pollution and therefore a progressive degradation of hydrocarbons. Indeed, the reduction of hydrocarbons in the soil frees soil pores and allows, under the effect of gravity, water infiltration into soil interstices [25]. Between 50 and 85% of the retention capacity (maximum quantity that soil can store),

activity is maximum; it decreases from 85% due to local anaerobiosis (reduction of oxidative processes) and below 50% due to lack of water [26]. All MWCR values obtained from 180 days are between 50% and

70% while those between 0 and 150 days are between 0 and 20%. The optimal MWCR values for hydrocarbon degradation are from 180 days.



**Figure 8 :** Evolution of garage (A, B, C, D) soils MWCR (maximal water capacity retention) depending on treatments and time

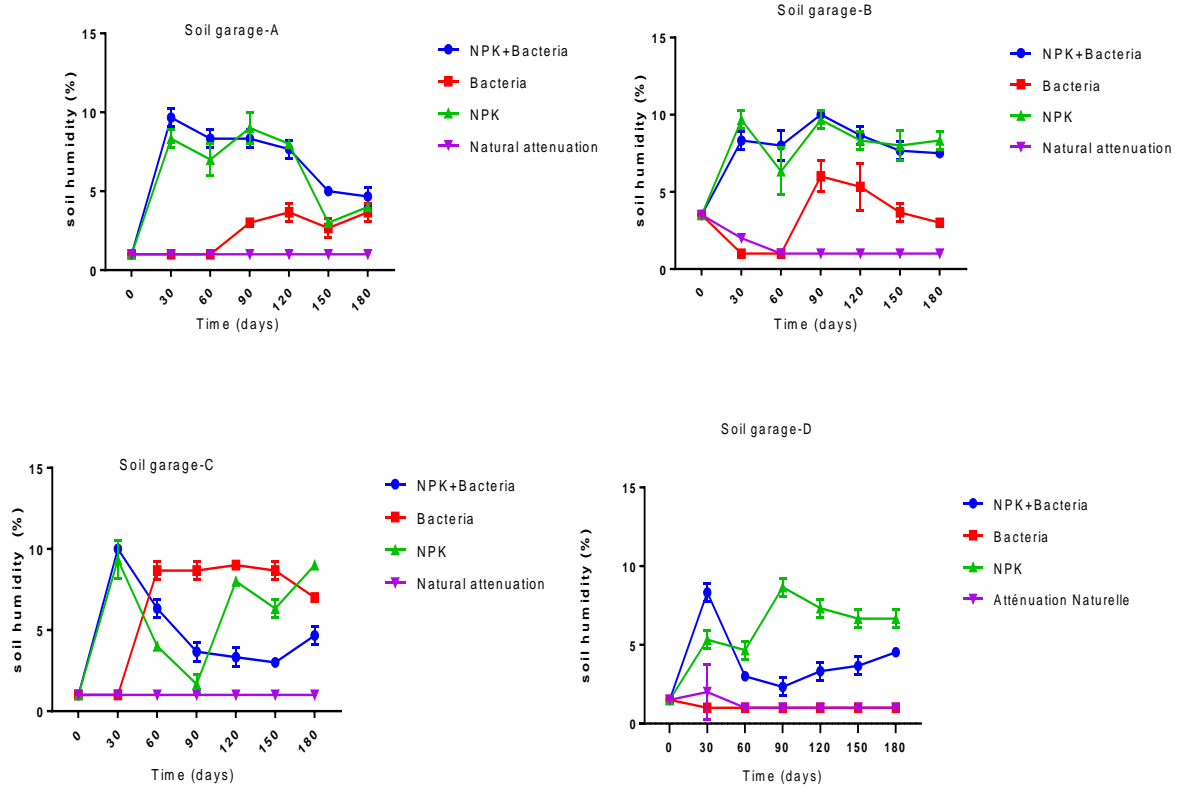
### 3.5 Evolution of different garages humidity depending on treatments and time

Figure 9 shows the evolution of soils garages (A, B, C, D) humidity during the different treatments as a function of time. In general, the results obtained show that humidity varies between 1 and 10% for all soils. An increase in humidity is observed in the different treatments during the 180 days due to optimization by adding water into the medium. This increase can be explained by the reduction of water evaporation and permeability caused by hydrocarbon hydrophobicity [27], which would increase soil water retention. The reduced transpiration and reduced rate of water absorption into soil result in increased soil humidity. These values remain in favorable conditions for hydrocarbon degradation [14] even if humidity below 2% would limit good bacterial growth for hydrocarbon degradation [28]. The low value in natural attenuation treatments could be explained by the climatic conditions of the sampling site or the texture and the effect of oil pollution as highlighted by the work carried out [29]. However, soil humidity is higher (close to 10%) for soil treated with NPK and

NPK+Bacteria.

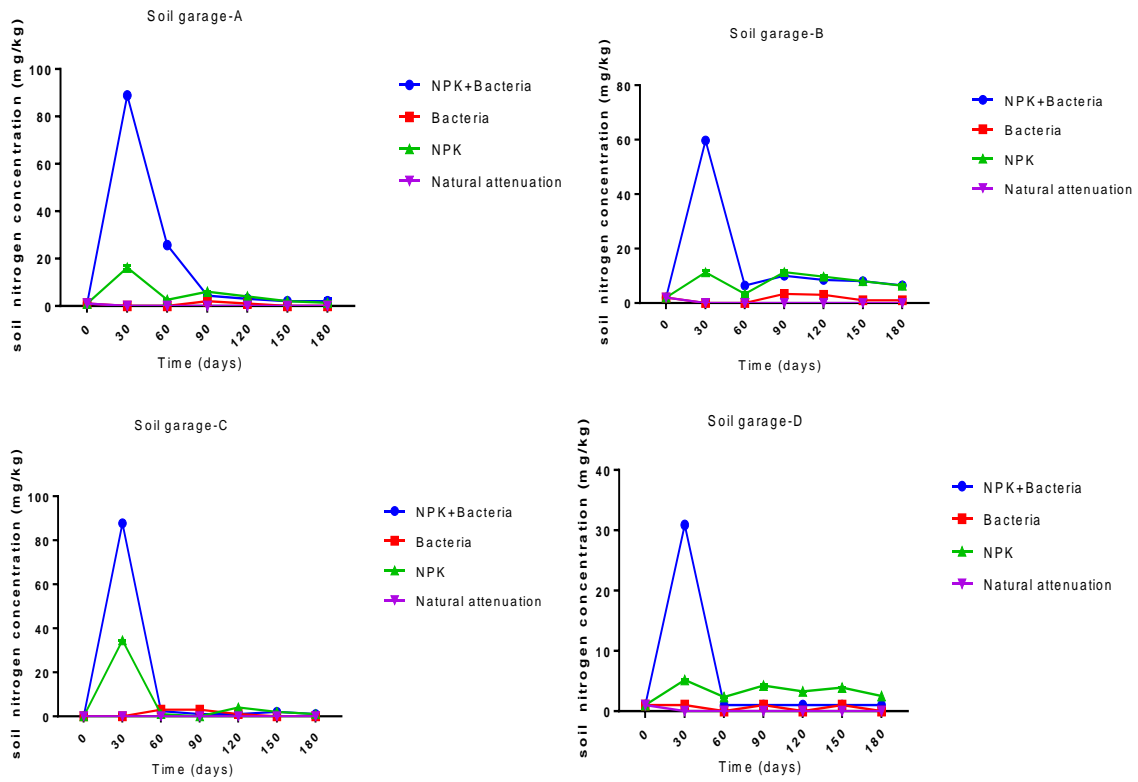
### 3.6 Evolution of different garages nitrogen concentration depending on treatments and time

Figure 10 shows the evolution of soils garages (A, B, C, D) nitrogen concentrations during the different treatments as a function of time. We can see that at the start of all treatments, the nitrogen concentrations are very low and almost zero for all soils. These low values show the poverty of soil nitrogen in environments polluted by hydrocarbons. This nitrogen deficit in hydrocarbon-polluted soils is due to organic carbon increase, thus causing high biological activity while decreasing the nitrogen concentration [30; 31]. During the first 30 days of NPK+Bacteria and NPK treatments, nitrogen concentrations were significantly high, varying from 0 to 88 mg/kg for soil of Garage A; from 0 to 59.68 mg/kg for soil of Garage B; from 0 to 87.68 mg/kg for soil of Garage C and from 0 to 30.67 mg/kg for soil of Garage D. The nitrogen concentrations increase is only observed in soil treated with NPK+Bacteria and NPK.



**Figure 9:** Evolution of garage (A, B, C, D) soil humidity depending on treatments and time

UNDER PEER



**Figure 10:** Evolution of garage (A, B, C, D) soils nitrogen concentration depending on treatments and time

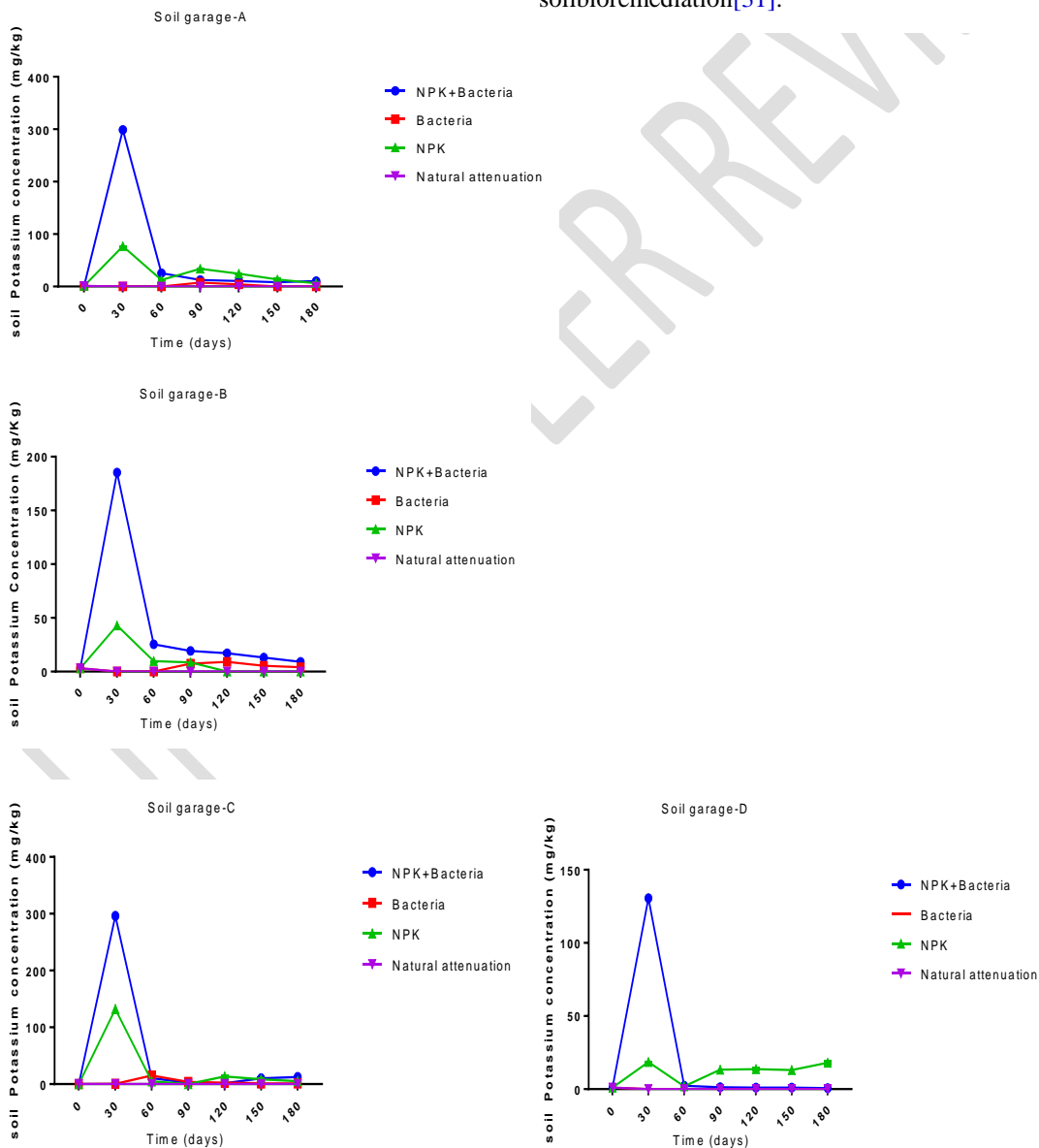
UNDER PEEL

This increase is caused by the supply of nutrients in both treatments (NPK+Bacteria and NPK). However, after 30 days of treatments (NPK+Bacteria and NPK), the nitrogen concentrations gradually decreased, signaling the consumption of nutrients by the microorganisms. However, soil treated with NPK+Bacteria and NPK have nitrogen contents suitable for soil bioremediation [31].

### 3.7 Evolution of different garages potassium depending on treatments and time

Figure 11 shows the evolution of soils garages (A, B, C, D) potassium concentrations during the different treatments as a function of time. At the start of all treatments, potassium concentrations are very low and almost zero. Potassium levels in

undergone natural attenuation and treatment with only bacteria remain very low. In soil treated with NPK+Bacteria and NPK, the potassium concentrations increase up to 30 days only varying from 0 to 300 mg/kg for soil of Garage A; from 0 to 200 mg/kg for soil of Garage B; from 0 to 300 mg/kg for soil of Garage C and from 0 to 150 mg/kg for soil of Garage D. Between 30 and 180 days, the potassium concentrations in soil treated with NPK+Bacteria and NPK gradually decrease until they reach closer to zero. For soil treated with NPK+Bacteria and NPK, the increase in potassium concentrations between 0 and 30 days is due to the addition of nutrients in the treatments. The drop in potassium levels from 30 days is due to the fact that it has been consumed by the bacteria for their growth and survival [32]. Soil treated with NPK+Bacteria and NPK have potassium concentrations suitable for soil bioremediation [31].



**Figure 11** :Evolution of garage (A, B, C, D) soils potassium

### 3.8 Evolution of different garages total petroleum hydrocarbon (TPH) concentration depending on treatments and time

Figure 12 shows the evolution of soils garages (A, B, C, D) total petroleum hydrocarbon (TPH) concentrations during the different treatments as a function of time. The initial content of total hydrocarbons in soils of garages A, B, C and D are respectively 8.19%, 8.29%, 10.76% and 7.07%. The total petroleum hydrocarbon concentrations varies as follows:

- for soil having undergone natural attenuation: we observe a slight drop in total petroleum hydrocarbon concentrations from 8.19% (soil of Garage A) or 8.29% (soil of Garage B) to 6%, i.e. a yield of 25% for the soils of garages A and B. No drop in total petroleum hydrocarbon concentrations is observed for soil of Garage C, the concentration in TPH in soil remains stable at 10.76%. For the soil of Garage D a drop the hydrocarbon concentrations is observed, going from 7.07% to 4%, i.e. a yield of 43.4%,
- for soil having undergone the 3 treatments (Bacteria, NPK, NPK+Bacteria), we observe a drastic drop in the hydrocarbon concentrations of 8.19% (soil of Garage A) or 8.29% (soil of Garage B) or 10, 76% (soil of Garage C) to less than 2%, i.e. a yield greater than 75% for the soils of Garages A, B and greater than 81% for the soil of Garage C. For the soil of Garage D, we observe a drastic drop in the total petroleum hydrocarbon concentrations

up to 1% only in soils treated with bacteria alone and with NPK+Bacteria, i.e. a yield greater than 85%, while a drop in the total petroleum hydrocarbon concentrations up to 4% is recorded only in soils treated with NPK with a yield of only 43%.

Thus, we note yields between:

- 75% and 85% for soils treated with Bacteria alone and with NPK+Bacteria,
- 43% and 75% for soils treated with NPK alone,
- 25% and 43% for soils having undergone natural attenuation.

Treatments with the addition of bacteria showed strong degradation compared to other treatments. The decrease in total petroleum hydrocarbons (TPH) is due to good optimization of the physicochemical and microbiological parameters, the supply of distilled water and aeration by turning the soil, thus promoting good degradation of hydrocarbons by bacteria. Considerable decrease in total petroleum hydrocarbons (TPH) in soil would be caused by the assimilation of microorganisms and the good maintenance of conditions based on physicochemical and microbiological parameters optimization [33]. Several studies corroborate with our present work hydrocarbon rates after 30 days with reduction rates between 56% and 85% by using bacterial strains of the genus *Bacillus* [11; 34].

concentration depending on treatments and time



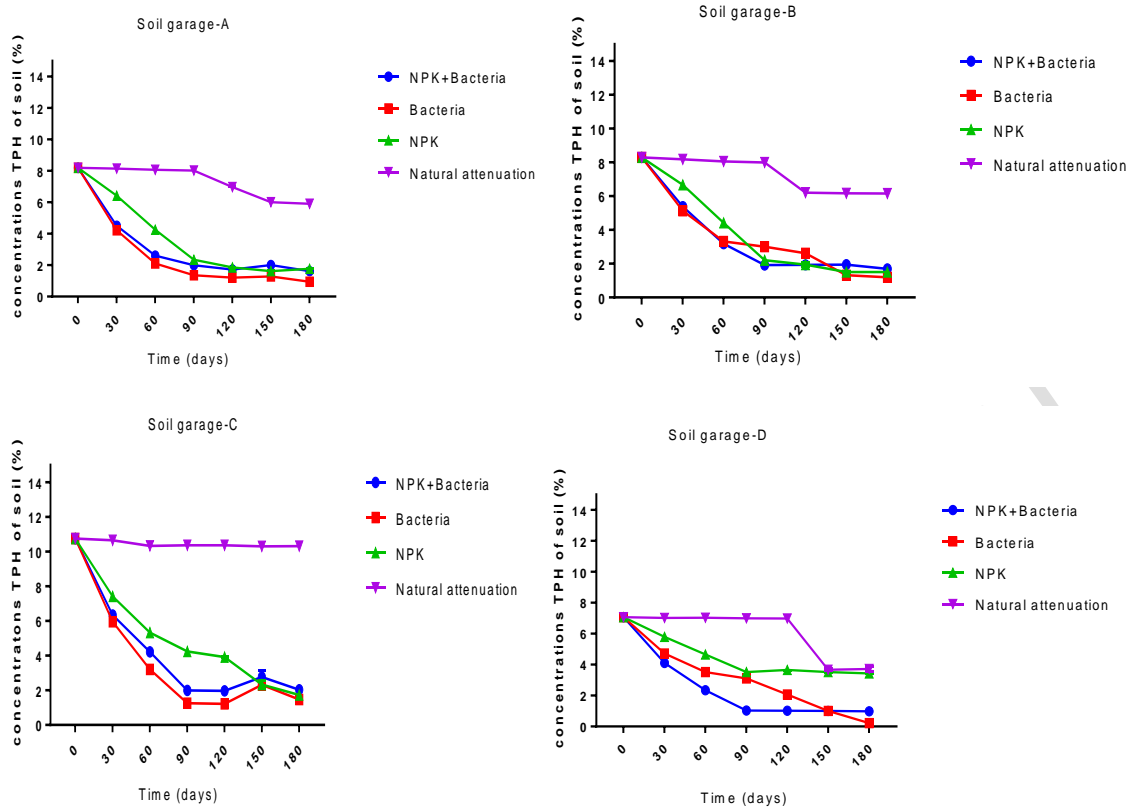


Figure 12: Evolution of garage (A, B, C, D) soil hydrocarbon (TPH) concentrations depending on treatments and time

UNDER PEER

#### 4. Conclusion

The aim of this work was to decontaminate soils polluted by hydrocarbons using the bioremediation technique. For this, four treatments were applied to soil samples taken from different garages in the city of Brazzaville while monitoring the physico-chemical parameters by bioaugmentation, biostimulation and mixture of bioaugmentation and biostimulation.

Physicochemical parameters study was carried out to better understand the factors that could influence microbial metabolism during the degradation of total petroleum hydrocarbons in the soil. The results obtained showed that the physicochemical parameters are optimal during organic degradation processes. Indeed, the temperatures recorded, oscillating between 23.3 and 28.4°C, fit well within the range of temperatures required for good microbial degradation. The values of electrical conductivity of soils, between 38.6 and 5266.01  $\mu\text{S}/\text{cm}$ , are well within the range of optimum degradation of hydrocarbons. Likewise, we note that pH values oscillating between 6.01 and 7.5 are well within the range favorable to the biodegradation of hydrocarbons. On the other hand, maximal water retention capacities (MWRC) are optimal for the degradation of hydrocarbons only from 180 days with values between 50% and 70%. The values relating to humidity remain in favorable conditions for the degradation of hydrocarbons, even if the most favorable conditions (close to 10%) are attributed to soil treated with NKP and NKP+Bacteria. Soil treated with NKP+Bacteria and NKP have the most optimal nitrogen and potassium concentrations for bioremediation of soils polluted by hydrocarbons. We observe a drop between 25% and 85% in the hydrocarbon concentrations in soils polluted by hydrocarbons. The greatest drop (75%-85%) in the hydrocarbon concentrations is observed for the Bioaugmentation treatments (Bacteria alone) and the Biostimulation and Bioaugmentation combination (NKP+Bacteria). The smallest drop (25%-43%) in hydrocarbon concentrations is observed for soil that have undergone natural attenuation. Consequently, bioremediation by adding *Bacillus* genus bacteria would be a good alternative way for the depollution of soils polluted by hydrocarbons.

#### DATA AVAILABILITY

The experimental data used to support the findings is included within the article.

#### REFERENCES

- [1] Gabet A. (2004). Remobilization of polycyclic aromatic hydrocarbons (PAHs) present in contaminated soils using a surfactant of biological origin. Doctoral thesis n°12-2004 from the University of Limoges, specializing in water chemistry and microbiology. 177.
- [2] Daverey A., Pakshirajan K. (2011). Pretreatment of synthetic dairy wastewater using the sophorolipid producing yeast *Candida bombicola*. *Appl. Biochem. Biotechnol.* 163:720-728.
- [3] Sauret C. (2011). Ecology of marine bacterial communities subjected to oil pollution. Doctoral thesis from Pierre and Marie Curie University.
- [4] Olaniran A.O., Balgobind A., Pillay B. (2013). Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies. *Int. J. Mol. Sci.*, 14, 10197-10228.
- [5] Zhuang W.Q., Tay J.H., Maszenan A.M., Krumholz L. R., Tay S. T-L. (2003). Importance of Gram-positive naphthalene-degrading bacteria in oil-contaminated tropical marine sediments. *Lett. Appl. Microbiol.* : 251-270.
- [6] Premnath N., Mohanrasu K., Guru Raj Rao R., Dinesh G.H., Siva Prakash G., Ananthi V., Kumar Ponnuchamy, Govarthanan Muthusamy, Arun A. 2021. A crucial review on polycyclic aromatic hydrocarbons - Environmental occurrence and strategies for microbial degradation, *Chemosphere*, Volume 280.
- [7] Alvarez H.M. (2003). Relation ship between  $\beta$ -oxidation pathway and the hydrocarbon-degrading profile in actinomycetes bacteria. *International Biodeterioration and biodegradation* 52, 35-42.
- [8] Bekenniche N. (2014). Cracterization of hydrocarbon biodegradation activities by different microbial genera isolated from contaminated sites. Doctoral thesis of University of Oran, Algiers.
- [9] 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

- [10] Brazzaville map. (2018). Holidays-Arts-Guides-Travels.
- [11] 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 surfactinbiosurfactant in the Bioaugmentation of crude-oilContaminatedSoil in Garages in the Republic of Congo. *10*, 1-11.
- [12] Kaboré-Ouédraogo P., Savadogo P., Cheik A., Savadogo A., Alfred S. (2010). Study of the bioremediation of soilscontaminated by hydrocarbons in Burkina Faso. 10 p.
- [13] Rad S.N., Ghavidel A., Alikhani, H-A. (2015). Bioremediation of Gasoil by Indigenous Bacterial Strains Isolated from Oil Contaminated Soils. *Journal of Soil Environment* 1, 45-50.
- [14] Kumar V., Shahi, S.K., Singh, S. (2018). Bioremediation: an eco-sustainable approach for the restoration of contaminated sites. In: Singh, J., Sharma, D., Kumar, G., Sharma, N. (eds) *Microbial Bioprospecting for Sustainable Development*. Springer, Singapore.
- [15] Bertrand J-C., Caumette P., Lebaron P., Matheron R., Normand P., Sime-Ngando T. (2015). *Environmental Microbiology : Fundamentals and Applications*. Microbial Ecology. Springer.
- [16] Jasmine J., Mukherji S. (2015). Characterization of oilysludgefrom a refinery and biodegradabilityassessmentusingvariouslydrocarbonegradingstrains and reconstituted consortia, *Journal of Environmental Management*, Volume 149, pp. 118-125.
- [17] Song D., ArataKatayama A. 2010. Approach for estimatingmicrobialgrowth and biodegradation of hydrocarbon contaminants in the subsoilfromfieldmeasurements: 1. Development and verification of the model *Environmental Sciences and Technologies*, 44 (2), 767-773.
- [18] Nzila J-D. (2013). State, Needs and Priorities for Sustainable Land Management in Congo-Brazzaville. pp.5-14.
- [19] Bauder T.A., Waskom R. M., Davis J.G.(2007), Irrigation water quality criteria, *Crop Series Irrigation*, no.0.506.
- [20] Aumar N., Sediri D. (2018). Study of the physicochemical properties of the soils of two service stations Idjeur and Fréha. Master memory. MouloudMammeriTizi-Ouzou University, Biology, Specialty:
- [21] Sakshi, Singh S.K., Haritash A.K. (2019). Hydrocarburesaromatiquespolycycliques : pollution et assainissement des sols. *Int. J. Environ. Sci. Technol.* 16, 6489–6512.
- [22] Sakata, T., Kojima, T., Fujieda, M., Takahashi, M., Michibata, T. (2003). Influences of probiotic bacteria on the production of organic acids by porcine cecal bacteria in vitro. *Proceedings of the Nutrition Society*, 62(1), 73-80.
- [23] Meriem S., Benkhebcheche D.E. (2016). Contribution to the study of the biodegradation of hydrocarbons in drilling muds by bacteria producing biosurfactants. Memory, University of the Mentouri Constantine Brothers, 77p.
- [24] Onojake M.C., Osuji L. C.(2015).Assesmtment of the physico chemical properties of hydrocarbon contaminated soil. *Archives of applied science research*. pp.48-58.
- [25] Szejba D. (2020). Importance of the influence of the retention properties of drained clay soils on the reduction of flood risks. *Some water*; 12(5):1315.
- [26] Langenhoff A. (2007).In situ bioremediation technologies – experiences in the Netherlands and future European challenges, TNO, Complementary report for EURODEMO to the "Status report on technologicalreliability for demonstratedsoil and groundwater management technologies withspecial focus on the situation in Europe (D6.2) part 2", 21 pp.
- [27] Chalghmi H. (2015). Study of marine pollution by hydrocarbons and characterization of their biochemical and molecular effects on the clam *Ruditapes* sp. *Geochemistry*. University of Bordeaux; University of Monastir (Tunisia).
- [28] Belmenai M., Benhafed N. E. (2015). Isolation and characterization of hydrocarbonoclasticbacteria. Memory, University of the Mentouri Constantine Brothers, 66pp.
- [29] Emoyan O.O., Akporido S.O. and Agbaire P.O. (2018), Effects of soil pH, Total Organic Carbon and texture on fate of Polycyclic Aromatic Hydrocarbons (PAHs) in soils, *Global NEST Journal*, 20(X), XX-XX.
- [30] Varjani S.J. (2017). Remediationprocesses for petroleumoilpollutedsoil. *Indian Journal of Biotechnology* n° 16. pp. 157-

