Algerian Journal of Environmental Science and Technology
December edition. Vol.6. Nº4. (2020)
ISSN : 2437-1114
www.aljest.org

Influence of C/N/P ratio evolution on biodegradation of petroleum hydrocarbons-contaminated soil

H. Ouriache*, I. Moumed, J. Arrar, A. Namane, H. Lounici

Laboratoire des Sciences et Techniques de l'Environnement [LSTE], Ecole Nationale Polytechnique, 10 Avenue Hassen Badi, Belfort, El-Harrach, 16110 Alger, Algeria

*Corresponding author: hadjer.ouriache@g.enp.edu.dz ; Tel.: +213 776 64 16 32; Fax: +213 26 35 23

ARTICLE INFO

Article History:

Received ***: 31/12/2019***
Accepted ***: 01/06/2020***

Key Words:

Petroleum hydrocarbons
Soil bioremediation
Biostimulation
Biomass evolution.

ABSTRACT/RESUME

Abstract: *The highest biodegradation efficiency of petroleum hydrocarbons 50% was observed for a C:N:P ratio of 100:10:1 while it reached 28% for a C:N:P ratio of 60:2:1 and moisture of 15% for both of them. For a higher N:P molar ratio greater than or equal to 10, the recorded depletion and consequently biodegradation ratios decreased significantly. The monitoring of temporal evolution of the biomass and C:N:P ratios highlighted that the N: P molar ratio may be a factor limiting which concurs with several studies. In addition to the ability of the indigenous microbial population to oxide petroleum hydrocarbons to a large extent, the results highlighted the remediation feasibility in solid-phase bioreactors.*

I. Introduction

Petroleum products and oils are among the most frequent soil and groundwater contaminants and pose a strong environmental problem. They find their way into the environment as a result of pipeline and underground storage tank leakages, improper were disposal practices, or transportation accidents. Many of these contaminants are toxic, mutagenic, and carcinogenic [1].

Bioremediation is a viable option for remediation of oil-contaminated sites and offers an economical and ecological alternative to traditional physical-chemical and thermal technologies [2,3] Bioremediation either in situ or ex-situ accelerates the naturally occurring biodegradation under controlled conditions [4].

The results under aerobic conditions are considerably better than those under anaerobic conditions [5,6] Its application may be further limited by several factors such as temperature, pH, moisture, nutrients [7] electron acceptors availability and soil type [8-10 and by degradation rates often requiring several weeks to several months to reduce the soil contamination to acceptable levels.

In this work, we have focused on treatment in solid-phase bioreactors on a laboratory scale; which involves mainly contacting contaminated soil with water, nutrients, oxygen, microorganisms to enhance the biodegradation rate.

Solid-phase bioreactors require less energy for forced aeration so this makes them cost-effective even when long treatment durations are required. However, non-uniform contaminant removal and low rates and extent of degradation; attributed to a non-homogeneous distribution of nutrients, electron acceptors, and micro-organisms in the soil; often limit the efficiency of solid-phase treatment such as biopiles [11].

The importance of nutrients to microbial processes has long been known in most cases, the treatment of oil-contaminated environments involves the biostimulation; where the activity of the indigenous bacteria is stimulated by adding nutrients and/or by aeration; as an effective approach to enhance oil biodegradation. The requirements for nitrogen and phosphorous vary with bacteria type, carbon source metabolized, and environmental habitat. It depends on the bacterial specific growth rate, the elemental microbial cell composition, and the maximum carbon conversion efficiency [12].

The recommended carbon: nitrogen: phosphorous mole ratios for enhanced biodegradation commonly cited as optimal C:N:P targets are in the range of 100:10:1 to 100:1:0.5 or of 106:16: 1 [Redfield ratio] [13-17]

Other ratios have been used and considered as suitable or 'optimal' for biodegradation. Ruberto et al. [2003] suggested that the ratio of C:N:P needs to be maintained at 100:12:3 to enable microbial growth and activity to occur. Other studies highlighted that biodegradation can be inhibited by nutrient addition [18-20]. So it is important to ensure an adequate amendment in nutrients and soil moisture levels.

In general, the nutrients amendment required for optimal biodegradation varies with the contaminant and soil type The soil moisture is a very significant parameter and is essential to the development and growth of the micro-organisms, and the various transfers. So it is essential to maintain sufficient water content as recommended, the optimum soil moisture range for microbial activity are 5-15% [21] Several studies suggest a water content of 60% of the field holding capacity [22-24].

On the other hand and despite the good biodegradability of the majority of petroleum hydrocarbons [25,26] ,their persistence in soils may be attributed to the contaminated soil permeability. Fine-grained soils restrict the oxygen transfer and its bioavailability, decreasing consequently the biodegradation efficiency.

The present experimental work investigated how much nutrients amendment is required in the case of sandy-loam contaminated soil by petroleum hydrocarbons [3%] to achieve higher biodegradation efficiency.

II. Materials and methods

II.1. Soil

Contaminated soil was collected, according to AFNOR X31-100 [27], from a site with a history of petroleum contamination over years, located at 36° 11'26,01 North, 3° East 11'08,0 in Dar El Beida. The soil samples were taken depth of 0-20cm.

The soil was air-dried at room temperature, sieved at 0.8mm, homogenized according to the quartering method; this method is repeated twice and then stored at 4°C until used.

Soil characterization study focused on the determination of pH according to ISO 10390 [28], size and texture by sieving and sedimento-metry, organic matter by calcinations, organic carbon according to NF T90-102 [29], nutrients after removal of the soil matrix, after reducing nitrates to nitrites, nitrates and nitrites were determined by diazotization reactive method, and Ammonia nitrogen was determined by the Nessler method according to ISO 14256-1 [30], and phosphorous ions were determined according to the standard NF EN 1189 [31].

II.2. Total petroleum hydrocarbons

The measurement of soil total petroleum hydrocarbons content was based on the gravimetric determination. Petroleum hydrocarbons were extracted from 2g of soil; mixed and crushed with 2.5g of anhydrous sodium sulfate; using the ultrasonic extraction method with dichloromethane as solvent. Soil samples were extracted two times [15min for each extraction] with 18ml of solvent. The concentrated extract was weighed just after filtration and solvent rotary evaporation at 40°C to determine the residual petroleum hydrocarbons contents. These contents were revised to dry weight content obtained from the weight loss of soil dried at 105°C.

II.3. Total heterotrophic microorganisms

To enumerate the heterotrophic microbial population, 1g of soil was serially diluted and spread on nutrient agar. Plates were incubated for 24-48 hours at 37°C before counting units forming colonies [UFC]. Biomass was revised also to dry weight content.

Experiments were carried out in aerobic conditions in microcosms containing 210 g of polluted soil.

Two molar ratios of C:N:P of 100:10:1 [A] and 60:2:1 [B] and water content of 15% w/w dry weight were used to investigate the extent of bioremediation of the contaminated soil. Additional microcosms without the addition of inorganic nutrients [NH_4Cl and KH_2PO_4] and water was used as control [C], whereas microcosms sterilized with 2g of $HgCl_2$/kg of dried soil according to Nam et al. [2001], moistened to 15% and amended with inorganic nutrients according to the C:N:P ratios of 100:10:1 [A'] and 60:2:1 [B'] acted as abiotic controls. Nutrients were added only at the beginning of biostimulation experiments. All microcosms, except C, were covered with aluminum foil to avoid light and incubated at 27°C for 9 weeks.

The soil moisture was controlled and adjusted up 15% by spraying water, and aeration was achieved periodically by mixing thoroughly contaminated soil.

The total petroleum hydrocarbons contents, total microbial cell numbers, nutrients contents, pH, moisture, and temperature in the reactors were periodically monitored in both abiotic and biotic systems for 9 weeks.

The total petroleum hydrocarbons removal and biodegradation ratios were estimated from equations [1] and [2]:

$$\text{Petroleum removal ratio} = \frac{[TPH]_{\circ} - [TPH]bio}{[TPH]_{\circ}} \quad [1]$$

$$\text{Petroleum biodegradation ratio} = \frac{[TPH]abio - [TPH]bio}{[TPH]_{\circ}}$$

[2]With:

$[TPH]_0$ = initial concentration of petroleum hydrocarbons;

$[TPH]_{bio}$ = concentration of total petroleum hydrocarbons at time "t" for biotic system;

Algerian Journal of Environmental Science and Technology
December edition. Vol.6. Nº4. (2020)
ISSN : 2437-1114
www.aljest.org

$[TPH]_{abio}$= concentration of total petroleum hydrocarbons at time "t" for abiotic system.

III. Results and discussion

II..1. Soil characterization

The analysis soil studied reveals a sandy loam texture with [15%] of clay, [35.75%] of silt and [49.25%] of sand as consequence about the texture triangle [31] and a fine-grained soil [160µm].
The results of measurements of permeability show a permeable soil for transfer between the various phases and good oxygenation. The main characteristics of the studied soil, are given in table 1. Soil pH was in the range for the growth of microorganisms degraders of hydrocarbons [32] .
Contaminated soil samples reveal a TPH content of [31±1]g/kg dried soil and the determination of C:N:P molar ratio [100:0.07:0.002] highlighted the amendment of the contaminated soil is required. The microbial analysis confirmed the presence of indigenous microorganisms able to initiate and maintain diesel oil degradation [33]. After amendment soil contaminated, this number would increase according to several authors [4]

Table 1. *Soil chemical and biological characteristics*

D_{60} [µm]	160	pH	7.21
D_{10} [µm]	15	Humidity[%]	2.46
Cu [D_{60}/D_{10}]	10.66	Organic matter[%]	5.97
β[m/s]	$5.56*10^{-5}$	Organic carbon[%]	2.08
Clay [%]	15	NO^{-}_{2} [g/g]	$0.98*10^{-6}$
Silt [%]	35.75	NO^{-2}_{3} [g/g]	$1.020*10^{-4}$
Sand [%]	49.25	NH_4^+ [g/g]	$0.600*10^{-6}$
Texture	Sandy loam	P[g/g]	$1.625*10^{-6}$
ρ[g/cm^3]	1.61	C :N*10^2 :P*10^3	100 :7 :2
Porosity	0.41	Biomass[CFU/g soil]	$98.4*10^3$

III.2 Biological treatment

III.2.1 Total petroleum hydrocarbons

The results of measurements of the total petroleum hydrocarbons residual [TPH] in systems A and B highlighted negligible removal rate the first two weeks, not exceeding 4%, the same as those observed in the case of the natural attenuation [C]. This can be attributed to the age of the pollution and the nature of petroleum hydrocarbons in the presence but also has the inadequacy of degrader microorganisms able to initiate and support TPH degradation. Indeed, biodegradation did not occur when the population of indigenous microorganisms capable of degrading the target contaminants was less than 10^5 CFU/g of soil.
After that, similar profiles of TPH removal [figure 1a] were observed for both systems A and B, and TPH contents decreased significantly. This depletion is, however, more important in the case of C:N:P molar ratio of 100:10:1 from the 3rd week. An increase in TPH degradation was observed from the 3rd week for systems A and B, to reach the end of treatment TPH removal ratio of 62% and 39% respectively. TPH removal was significantly important for C:N:P molar ratio of 100:10:1 up to 62% compared to C:N:P molar ratio of 60:2:1.
After a period of adaptation, biostimulation resulted in rapid degradation of TPH in the early stage [within 6 weeks] to reach at the end of treatment for systems A and B, total removal efficiencies of 62% and 39% respectively.
The C:N:P molar ratio of 100:10:1 improves the removal performance of 59% compared to the C:N:P molar ratio of 60:2:1.
In the case of natural attenuation [C], TPH removal did not exceed 15% due probably to abiotic processes such as volatilization, and/or photo-oxidation.
TPH removal in abiotic systems [A' and B'] was in the 10-11 % range due to the volatilization and evaporation process after 9 weeks of incubation.
Over the whole treatment period, the evolution of total petroleum hydrocarbons biodegradation followed similar profiles that those of TPH removal.
After two weeks, TPH biodegradation ratios increased from 3rd and 5th weeks for systems A and B respectively, and seemed to stabilize after the 7th week. This would correspond to the depletion of nutrients and/or biodegradable hydrocarbons fraction and the presence of recalcitrant hydrocarbons and toxic metabolites.
The optimum TPH biodegradation ratios varied between 50% and 28% at the end of treatment for systems A and B respectively

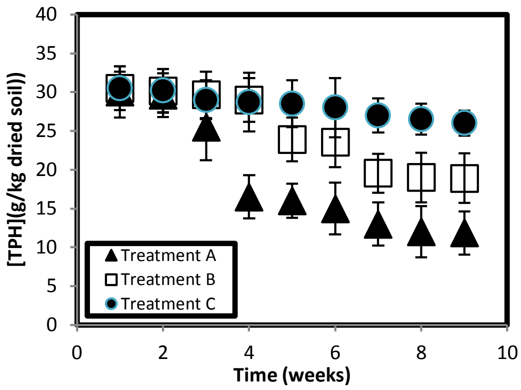

Figure 1a. *Temporal evolution of total petroleum hydrocarbons removal*

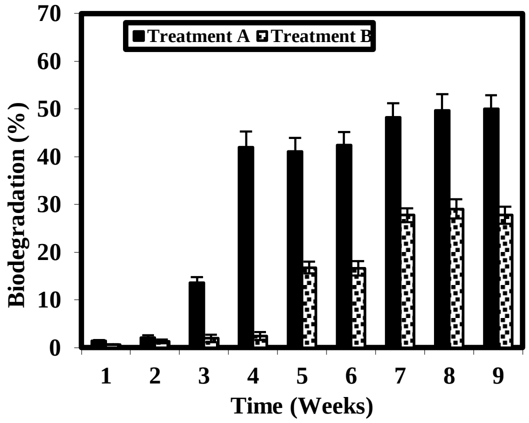

Figure 1b. *Temporal evolution of total petroleum hydrocarbons biodegradation*

III.2.2 Biomass

Biostimulation treatments increased significantly total heterotrophic microorganisms regardless C:N:P molar ratios studied after 1 week of treatment. The same trend of biomass evolution was observed for both systems A and B [figure 2b]. The biomass seemed constant in the 1st week, in the order of 10^5 CFU/g of dried soil for both systems, which could explain the low biodegradation ratios rates.

Indeed, Pelaez et al. [35] reported that the guidelines of the USEPA suggest that bioremediation was feasible when there was at least 10^3 CFU/g soil of the microbial population according to Lin et al., 2010 and when indigenous microorganisms capable of degrading the pollutant are less than 10^5 CFU/g of soil, bioremediation will not occur at a significant rate according to Forsyth et al., 1995.

The number of heterotrophic microorganisms increased to reach its maximum in the 4th week of $7.5*10^{10}$ CFU/g soil and $8.0*10^9$ CFU/g soil, corresponding to biodegradation ratios of 42% and 2% for systems A and B respectively. Kumar et al. [36] showed that a better soil biodegradation, with a moisture of 15%, pH 8, a temperature of 28°C and inoculums concentration of 10^6 CFU/g soil. The low biodegradation ratio for higher C: N ratio [B] despite the content of $8.0*10^9$ CFU/g soil could be probably due to the use of another substrate in the organic matter [6%] than petroleum hydrocarbons. It is generally recognized that microorganisms require about 10 parts of carbon and 1 part of nitrogen for efficient biodegradation. If this ratio becomes higher the growth and utilization of carbon sources will be retarded [37].The other hand, the addition of nutrients according to the C:N:P ratio of 60:2:1 affect the community dynamics of heterotrophic microbial populations and as consequence affect the rate and the extent of biodegradation according to [37,38]

After 7 weeks of treatment, total microorganisms decreased to reach between $1.2*10^3$-$2.2*10^3$ CFU/g soil for treatments A and B, due probably to nutrients depletion. For natural attenuation [C], total bacterial counts decreased until it becomes negligible from the 3rd week. Stimulation of microorganisms improved microbial growth microbial in comparison to natural attenuation [C] while but did not enhance biodegradation efficiency according to C:N:P ratio. At the end of the essays, the number of microorganisms was at the same order of magnitude in both treatments.

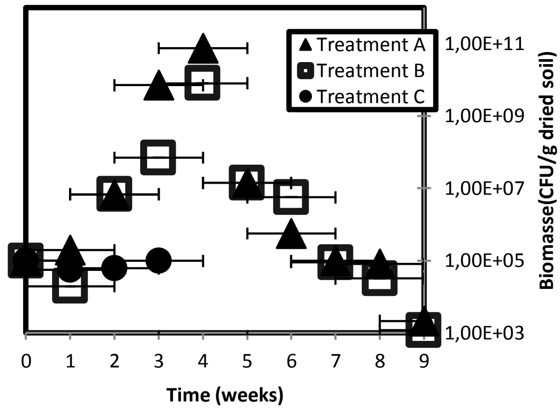

Figure 2. *Biomass temporal evolution for Biotic systems*

IV. Discussion

Contaminated soils poor in nitrogen [N] and phosphorous [P] contents require the addition of nutrients to allow a sufficient increase in biomass and therefore significant degradation rates of petroleum hydrocarbons. These must be used at appropriate concentrations in a certain ratio of carbon/nitrogen/phosphorous [C:N:P], which is the aim of our study. In general, the molar ratios C: N ranging from 14:1 to 560:1 have been proposed as a suitable or optimum for biodegradation.

Petroleum hydrocarbons degradation in both biostimulation treatment A and B showed a similar trend but different biodegradation ratios depending on the C:N:P ratio. The addition of nutrients

Algerian Journal of Environmental Science and Technology
December edition. Vol.6. Nº4. (2020)
ISSN : 2437-1114
www.aljest.org

enhanced oxidation of petroleum hydrocarbons up 62% and 39% for C: N: P ratios 100:10:1 and 60:2:1 respectively.

During the first two weeks of treatments, the C:N:P molar ratios seemed constant, negligible TPH biodegradation occurred [≤2%] and an increase in heterotrophic microorganisms in the order of 10^5 CFU/g soil for both systems was observed. These results confirmed the use of microorganisms of another substrate in organic matter than petroleum hydrocarbons.

After that, an increase in heterotrophic microorganisms to reach their maximum of $7.5*10^{10}$ CFU/g at the 4th-week soil was followed by a microbial activity with 42% biodegradation efficiency although the C:N:P molar ratio increased from 100:10:1 to 125:7:1 [figure 3a].

TPH and nutrient contents analysis highlighted both microbial growth and microbial activity for C:N:P ratios between100:10- 180:10. In the case of C:N:P molar ratio of 60:2:1 [B], an increase in heterotrophic microorganisms number after the second week of treatment to reach a maximum of $8*10^9$ CFU/g soil was not followed by an increase in biodegradation efficiency [3%] [Figure3b].

The low biodegradation efficiencies were probably due to the high level of C: N: P between 88:2:1- 537.5: 1.3: 1. Thus, the microbial growth would be the result of the used substrate in organic matter. For this latter C:N:P ratio, a biodegradation efficiency of total hydrocarbons of 82% was obtained in the case of artificially diesel-oil contaminated soil [results no shown] while it doesn't exceed 29% in this study for an aged contaminated soil.

After the 4th week, we observed a decrease in heterotrophic microorganisms to reach at the end of treatment $2.2*10^3$ CFU/g soil and $1.2*10^3$ CFU/g soil corresponding to TPH removal of 62% and 39% and C:N:P molar ratio of 85:5:1 and 300:2:1 for treatments A and B respectively. These results would correspond to nutrients depletion and/or biodegradation of most of the recalcitrant hydrocarbons and the presence of toxic metabolites.

The highest degradation rate has been obtained with the first one, which was 62% for 9 weeks of treatment. This ratio was frequently reported as a baseline for approaches biostimulation [39] and it was considered as optimal [40]

Ghazali et al. [17] reported that the ratio of C:N:P needs to be maintained at 120:10:1 to enable microbial growth and activity to occur for better biodegradation of hydrocarbons according to Thomas et al. [1992]. Cerqueira et al. [2014] demonstrated that nutrient ratios of C:N:P of 100: 0.4: 0.2 and 100:10:1 were to be effective for hydrocarbon-degrading. Although it was generally accepted that optimal C: N ratio for bioremediation in the soil is 10, Børresen et al. [42] highlighted optimal C: N ratios between 11 and 27 [on the weight basis].

Trindade et al. [2005] reported that no nitrogen addition was necessary, as the nitrogen concentration in soil was sufficient to maintain the C:N:P ratio to 100:1.25:1.

Venosa et al. [43] obtained a removal efficiency of petroleum hydrocarbons of 90% for alkanes and 80% for aromatics for a ratio of 150: 10: 3. Sanscarta et al. [2009] used a ratio of 100:7.5:0.5 to obtain a diesel removal efficiency of 97%.

Grace et al. [44] study consisted of the use of three different C:N:P ratios 100: 27: 6.5, 100: 11: 3.7 and 100: 4.6: 3.1. Successful stimulation to the communities was achieved with 100:11:3.7 corresponding to TPH removal > 80%; this ratio was close to the nutrients required for the recommended C:N:P ratio [100:10:1] for biopiles operation [USEPA, 2002] and also the desired ratio [100:15:1] forex bioremediation [45].

V. Conclusion

This study reported the ability of biostimulation to eliminate 62% and 39% of petroleum hydrocarbons in a long-term contaminated soil for molar ratio C:N:P 100:10:1 and 60:2:1 respectively.

The addition of sources of nitrogen and phosphorous was necessary to enhance the biodegradation of petroleum hydrocarbons from the fact that intrinsic contents of the soil nutrients essential for the activity and growth of microorganisms are very low. Abiotic loss played an important role in petroleum hydrocarbons reduction.

The oxidation of petroleum hydrocarbons in different systems showed a similar trend but different biodegradation ratios depending on the C:N:P molar ratio 100:10:1, resulted in enhanced oxidation of petroleum hydrocarbons. The highest biodegradation ratio obtained was 50%. In addition to the ability of the indigenous microbial to oxide petroleum hydrocarbons to a large extent, the results highlighted that the microbial activity remains for C:N:P molar ratio such as 220:4:1, 538:1:1, 967:2:1and poor in nitrogen content.

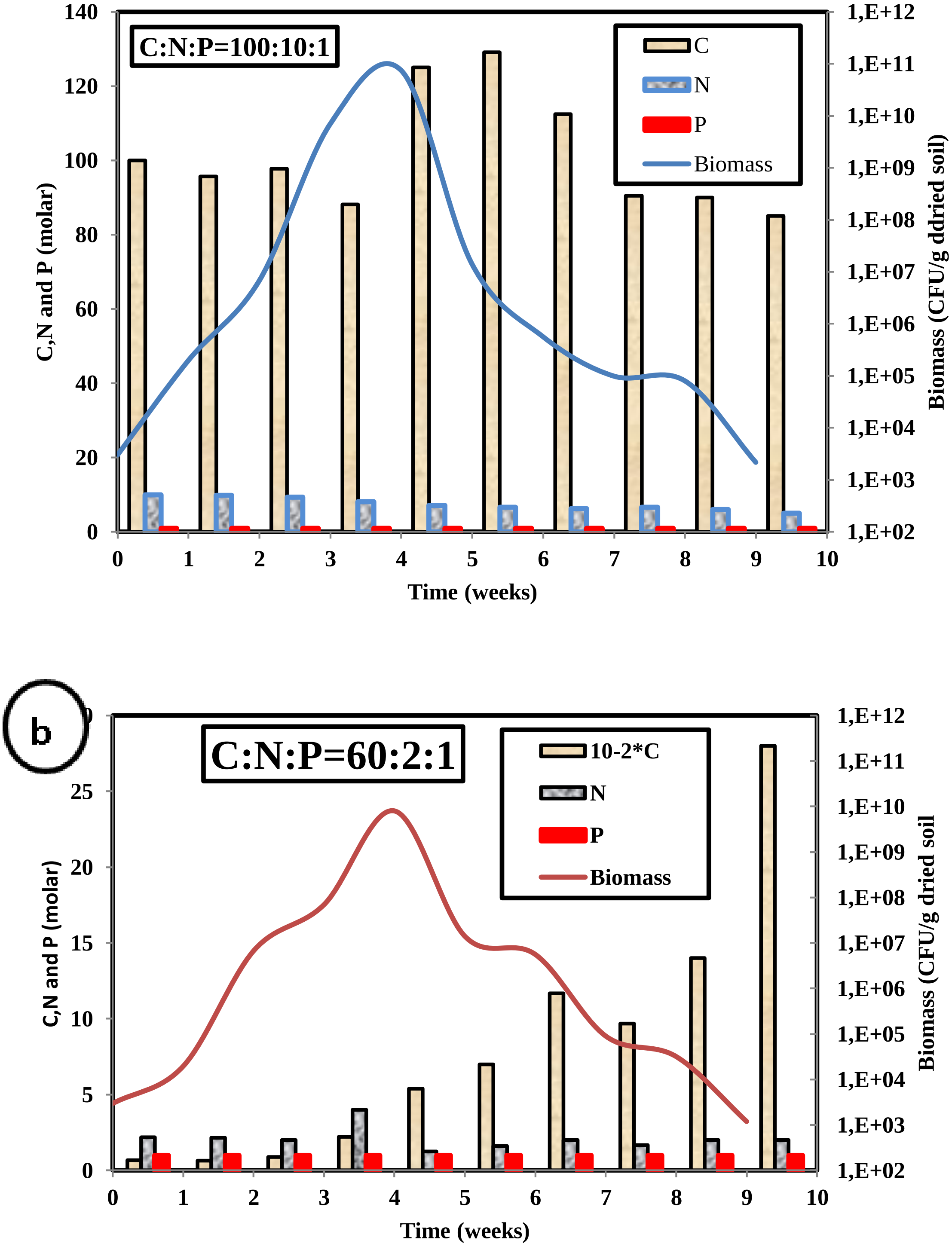

Figure 3. *Temporal evolution of the biomass and C:N:P molar ratio*
a]. C :N :P 100 :10 :1 b]. C:N:P 60:2:1

V. References

1. Yen, C.-H.; Chia-Hsien, Y.; Ku-Fan, Ch; Chih-Ming, K.; Shu-Hao, L.; Ting-Yu, C. Application of persulfate to remediate petroleum hydrocarbon-contaminated soil: Feasibility and comparison with common oxidants. *Journal of Hazardous Materials*,18(2011)pp. 2097–2100.
2. Mrayyan, B.; Batikhi, M. N . Biodegradation of total organic carbon [TOC] in Jordanian petroleum sludge. *Journal of Hazardous Materials* B,120(2005)pp.127 - 134.
3. Coulon, F.; Pelletier, E.; Gourhant, L. ; and Dellile, D. Effects of nutrient and temperature on degradation of petroleum hydrocarbons in contaminated sub-Antarctic soil. Chemosphere, , 58(2005)pp.1439 - 1448.
4. Delille, D.; Coulon, F.; and Pelletier, E. Effects of temperature warming during a bioremediation study of natural and nutrient-amended hydrocarbon contaminated sub-Antarctic soil. Cold Regions Sciences and Technology, ,40(2004)pp.61 – 70.
5. Mukherji, S.; Jagadevan, S., Mohpatra, G., and Vijay, A.Biodegradation of diesel oil by an Arabian sea sediment culture isolated from the vicinity of an oil field. Bioresource Technology,95(2004)pp.281 – 286.
6. Zappi, M. E.; Rogers, B.A.; Teeter, C.L., Gunnison, D.; and Bajpai, R. Bioslurry treatment of soil contaminated of total petroleum hydrocarbons, *Journal of Hazardous Materials* ,46(1996)pp.1 - 12.
7. Margesin, R.; Schinner, F . Bioremediation [natural attenuation and biostimulation] of diesel-oil-contaminated soil in an alpine glacier skiing area. Applied Environmental Microbiology,67(2001)pp.3127 - 3133.
8. Antizar-Ladislao, B.; Lopez-Real, J.; and Beck A. J. .Investigation of organic matter dynamics during in-vessel composting of an aged coal-tar contaminated soil using fluorescence excitation-emission spectroscopy. Chemosphere, ,64(2006)pp.839 - 847.
9. Troquet, J.; Larroche, C.; Dussap, C.-G .Evidence for the occurrence of an oxygen limitation during soil bioremediation by solid-state fermentation. Biochemical *Engineering Journal*,13(2003)pp103 – 111.
10. Mukherji, S.; and Vijay, A. Critical issues in bioremediation of oil and tar contaminated sites. In: Proceedings of the International Conference on Advances in Civil Engineering, Civil Eng. Dept., IIT Kharagpur, India, 3–5 January, (2002) 507 - 516.
11. Nano, G.; Borroni, A.; Rota, R .Combined slurry and solid-phase bioremediation of diesel contaminated soils. Journal of Hazardous Materials,100,(2003)pp.79 - 94.
12. Leys, N.M.; Bastiaens, L.; Verstraete, W.; Springael, D. Influence of the carbon /nitrogen /phosphorus ratio on polycyclic aromatic hydrocarbon degradation by Mycobacterium and Sphingomonas in soil. Applied Microbiol Biotechnol, 66(2005)pp.726 – 736.
13. Gibb, A.; Chu, A.; Wong, R. C. K.; Goodman, R.H. Bioremediation kinetics of crude oil at 5°C.*Journal of Environmental Engineering*,2001,pp.818 – 822.
14. Ferguson, S.H.; Franzmann, P. D.; Revill, A. T.; Snape, I.; Rayner, J. L .The effects of nitrogen and water on mineralisation of hydrocarbons in diesel-contaminated terrestrial Antarctic soils.Cold Regions Science and Technology, 37, (2003),pp.197 – 212.
15. Trindade, P. V. O.; Sobral, L. G.; Rizzo, A. C. L.; Leite, S. G. F.;. Soriano, A. U. Bioremediation of a weathered and a recently oil-contaminated soils from Brazil: a comparison study. Chemosphere, 58(2005) pp.515 – 522.
16. Kwock, C.; Loh, K. Effects of Singapore soil type on bioavailability of nutrients in soil bioremediation. Advances in Environmental Research, ,7(2003)pp.889 - 900.
17. Ghazali, F. M.; Rahman, R. N. Z. A.; Salleh, A. B. ; Basri, M. Biodegradation of hydrocarbon in soil by microbial consortium. International Biodeterioration & Biodegradation, , 54(2004)pp.61 - 67.
18. Manilal, V. B.; Alexander, M. Factors affecting the microbial degradation of phenanthrene in soil. Appl. Microbiol. Biotechnol ,35(1995),pp. 401 - 405.
19. Ruberto, L.; Vazquez, S. C.;. Cormack, W. P. M. Effectiveness of the natural bacterial flora, biostimulation and bioaugmentation on the bioremediation of a hydrocarbon contaminated Antarctic soil. International Biodeterioration & Biodegradation, ,52(2003) pp.115 – 125.
20. Walworth, J. L.; Woolard, C. R.; Braddock, J. F.; Reynolds, C. M . Enhancement and inhibition of soil petroleum biodegradation through the use of fertilizer nitrogen: an approach to determining optimum levels. Journal of Soil Contamination,6(1997),pp.465 - 480.
21. Garcia, C.; Hernandez, T.; Roldan, A.; Albaladejo, J.; Castillo, V. Organic amendment and mycorrhizal inoculation as a predice in afforestation of soils with Pinus halepenis Miller: effect on their microial activity.Soil Biol. Biochem, ,32(2000) pp.1173 – 1181.
22. Sabaté, J.; Vinãs, M.; Solanas, A.M . Laboratory-scale bioremediation experiments on hydrocarbon-contaminated soils. International Biodeterioration and Biodegradation, ,54(2004)pp.19 - 25.
23. Shkidchenko, A. N.; Kobzev, E. N.; . Petrikevich, S. B.; Chugunov, V. A.; Kholodenko, V. P.; Boronin, A.M . Biodegradation of black oil by microflora of the Bay of Biscay and biopreparations. Process Biochemistry, ,39(2004)pp.1671 – 1676.
24. Sanscartier,D.;Zeeb,B.;Koch,I.;Reimer,K.Bioremediation of diesel-contaminated soil by heated and humidified biopile system in cold climates. Cold Regions Science and Technology,55(2009)pp.167-173.
25. Nocentini, M.; Pinelli, D.; Fava, F . Bioremediation of a soil contaminated by hydrocarbon mixtures: the residual concentration problem.Chemosphere, ,41(2000)pp.1115 – 1123.
26. 26. Lee, M.;. Kim, M. K.; Singleton, I.; Goodfellow, M.; Lee, S.T . Enhanced biodegradation of diesel oil by a newly identified Rhodococcus baikonurensis EN3 in the presence of mycolic acid. Journal of Applied Microbiology ,100(2006)pp.325 – 333.
27. AFNOR [1992].AFNOR X31-100 .Qualité des sols - Échantillonnage - Méthode de prélèvement d'échantillons de sol.
28. ISO [2005]. ISO 10390.Soil quality -- Determination of pH. ISO/TC 190/SC 3. Chemical methods and soil characteristics.
29. AFNOR [1997].NF T 90-102 : Essais des eaux. Guide pour la détermination du carbone organique total [COT]. NF EN 1484 [7.97].
30. 30. ISO [2003]. ISO/TS 14256-1.Qualite du sol -- dosage des nitrates, des nitrites et de l'ammonium dans les sols bruts par extraction au moyen d'une solution de chlorure de potassium.
31. AFNOR [1997].NF EN 1189.Qualité de l'eau - Dosage du phosphore - Dosage spectrométrique à l'aide du molybdate d'ammonium.
32. Nam, K.; Rodriguez, W.; Kukor, J.J. Enhanced degradation of polycyclic aromatic hydrocarbons by biodegradation combined with a modified Fenton reaction. Chemosphere, ,45(2001)pp.11–20.

33. Gray, R. H.; Bebbington, K. J. Environmental accounting, managerialism and sustainability: Is the planet safe in the hands of business and accounting; Advances in Environmental Accounting and Management,1(2000)pp. 1-44.
34. Abu, G. O., Dike, P. O. A study of natural attenuation processes involved in a microcosm model of a crude oil-impacted wetland sediment in the Niger Delta. Bioresource Technology, 99(2008)pp 4761 – 4767.
35. Pelaez, A. I.; Lores, I.; Sotres, A.; Mendez-Garcia, C.; Fernandez-Velarde, C.; Santos, J. A.; Gallego, J. L. R.; & Sanchez, J. Design and field-scale implementation of an "on-site" bioremediation treatment in PAH-polluted soil. Environmental Pollution,181(2013) pp.190–199.
36. Kumar, M.; Gupta, S.K.; Garg, S.K.; Kumar, A. Biodegradation of hexachlorocyclohexane isomers in contaminated soils. Soil Biology & Biochemistry,38(2006)pp. 2318-2327.
37. Cerqueira, DM.; Tran, U.; Romaker, D.; Abreu, JG.; Wessely, O. Sterol carrier protein 2 regulates proximal tubule size in the xenopus pronephric kidney by modulating lipid rafts. Dev Biol,394[1] (2014)PP.54-64.
38. Das, K.; Mukherjee, AK. Crude petroleum-oil biodegradation efficiency of Bacillus subtilis and Pseudomonas aeruginosa strains isolated from a petroleum-oil contaminated soil from North-East India. Bioresource Technology, ,98[7] (2007)PP.1339-1345.
39. Cheng, H.; Mulla, DJ. The Soil Environment in: Bioremediation of contaminated soils. Adriano, D.C.
40. Sabaté, J.; Vinãs, M.; and Solanas, A.M. Laboratory-scale bioremediation experiments on hydrocarbon-contaminated soils. International Biodeterioration and Biodegradation , 54(2004)pp.19 - 25.
41. Susan, H.; Fergusona,B.; Peter, D.; Franzmannc, Andrew, T.; Revilld, Ian Snapeb.; John, L.; Raynerc. The effects of nitrogen and water on mineralisation of hydrocarbons in diesel-contaminated terrestrial Antarctic soils.Cold Regions Science and Technology, 37(2003)pp. 197– 212.
42. Børresen, M.; Breedveld, G.D.; Rike, A.G. Assessment of the biodegradation potential of hydrocarbons in contaminated soil from a permafrost site.Cold Regions Science and Technology,37(2003)pp.137–149.
43. Venosa, A. D.; Lee, K.; Suidan, M. T.; Gercia-Blanco, S.; Cobanli, S.; Moteleb, M., Haines, J. R.; Tremblay, G.; Hazelwood, M. Bioremediation and biorestoration of Crude oil contaminated Freshwater wetland on the St. Lawrence River. *Bioremediation Journal*,6(2002) pp. 261-281.
44. Liu, W. ; Grace et al. Bioremediation of petroleum hydrocarbon contaminated soil: Effects of strategies and microbial community shift. International Biodeterioration & Biodegradation,65 (2011)pp.1119-1127.
45. Philp, J.C.; Atlas, R.M. Bioremediation of contaminated soils and aquifers. In: Atlas, R.M., Philp, J. [Eds.], Bioremediation: applied microbial solutions for realworldenvironmental cleanup. ASM Press: Washington, DC, (2015)pp. 211-235.

Please cite this Article as:

Ouriache H., Moumed I. , Arrar J., A. Namane A; , Lounici H., Influence of C/N/P ratio evolution on biodegradation of petroleum hydrocarbons-contaminated soil., ***Algerian J. Env. Sc. Technology**, **6**:**4 (2020) 1604-1611**