American Journal of Environmental Protection
2023; 12(5): 138-149
http://www.sciencepublishinggroup.com/j/ajep
doi: 10.11648/j.ajep.20231205.12
ISSN: 2328-5680 (Print); ISSN: 2328-5699 (Online)

Biodegradation by Landfarming On-Site of Petroleum Waste from Refining at Pointe-Noire (Republic of Congo)

Vitaline Vanessa Morabo Okoletimou[1, *], Jean de Dieu Nzila[2], Noël Watha-Ndoudy[2, 3, *], Augustin Aimé Lebonguy[4]

[1]Industrial Process Engineering Laboratory, National Higher Polytechnic School, Marien Ngouabi University, Brazzaville, Congo

[2]Geosciences and Environment Research Laboratory (LARGEN), Higher Teachers' College (ENS), Marien Ngouabi University, Brazzaville, Congo

[3]Geosciences Laboratory, Faculty of Sciences and Technology, Marien Ngouabi University, Brazzaville, Congo

[4]National Institute of Exact and Natural Sciences, Brazzaville, Congo

Email address:

nwathandoudy@gmail.com (Noël Watha-Ndoudy), morabovitaline@gmail.com (Vitaline Vanessa Morabo Okoletimou)

[*]Corresponding author

To cite this article:

Vitaline Vanessa Morabo Okoletimou, Jean de Dieu Nzila, Noël Watha-Ndoudy, Augustin Aimé Lebonguy. Biodegradation by Landfarming On-Site of Petroleum Waste from Refining at Pointe-Noire (Republic of Congo). *American Journal of Environmental Protection*. Vol. 12, No. 5, 2023, pp. 138-149. doi: 10.11648/j.ajep.20231205.12

Received: September 12, 2023; **Accepted**: October 23, 2023; **Published**: November 11, 2023

Abstract: This work aimed to evaluate the treatment by landfarming *ex-situ* on site of waste from the oil refining activity in Pointe-Noire. Three types of hydrocarbon-polluted soils were used for this study: Soil 1 (polluted by crude oil by-products), Soil 2 (polluted by crude oil by-products associated with grease) and Soil 3 (polluted by crude oil by-products associated with tank bottom sludge). These soils were treated in the ponds by mixing them with molasses and inoculum in the following proportions: 5400 kg soil, 5.5% molasses and 0.9% inoculum for pond 1 (Soil 1) and 27000 kg soil, 1.1% molasses and 0.185% inoculum for pond 2 (Soil 2) and pond 3 (Soil 3). Total petroleum hydrocarbons (TPH) and trace metal elements (TME) during processing were determined by EPA 3510C + EPA 8015D-2003 and UNI ISO 17294-2-2016, respectively. pH, humidity, temperature, carbon and organic matter were determined by AOAC methods. The results obtained show a decrease in TPH content in Soil 1, Soil 2 and Soil 3, with degradability rates of 67.19, 52.75 and 9.18% respectively. As, Ba, Cd, Co, Cr, Hg and Mo remain below 0.5 mg/kg. Zn levels decrease in Soil 2 (12 to 0.9 mg/kg) and increase in Soil 1 (10 to 15 mg/kg) and Soil 3 (20 to 23 mg/kg). Cu levels increase in Soil 1 (5 to 10 mg/kg) and Soil 2 (12 to 19 mg/kg). In Soil 3, Pb levels dropped from 18 to 12 mg/kg, while Ni levels rose from 3mg/kg to 7mg/kg. Concentrations of these metals (Pb and Ni) in Soil 1 and Soil 2 remained unchanged. pH varied from 4.52 to 8.38, humidity from 2.25 to 22.92%, temperature from 21 to 34°C, air content from 0.04 to 27.71%, carbon from 0.11 to 11.84% and nitrogen between 0.088 and 0.203% in all three soils during treatment. These results show that treatment had a significant impact on TPH.

Keywords: Biodegradation, Landfarming, Petroleum Waste, *Ex Situ* Treatment, Total Petroleum Hydrocarbons

1. Introduction

Since the beginning of the last century, there has been a steady increase in the exploitation of oil deposits. However, the extraction, transport and use of this energy source entail risks of environmental pollution that can influence the ecological balance and sometimes lead to the destruction of ecosystems, as this resource produces huge quantities of waste products [1-3]. In the oil industry, crude oil and petroleum products are stored in metal tanks. During the storage period, due to corrosion caused by petroleum products, the tanks can deteriorate and the stored petroleum products spill onto the ground. The more or less prolonged stagnation of these contaminants causes them to migrate deep into the soil, making them very difficult to remove [4]. These pollutants spilled on the ground percolate and contaminate the subsoil, surface water and groundwater, altering the sites' fauna and microflora. These areas become and remain highly polluted by

organic and metallic compounds from these petroleum products [4]. Among petroleum compounds are polycyclic aromatic hydrocarbons (PAHs), which are toxic, mutagenic and carcinogenic [5, 6]. Thus, environmental pollution by these or complex mixtures of hydrocarbons has been recognized as one of today's most crucial problems [6]. Two types of pollution can be characterized: one is punctual, localized, brutal and intense, and the other diffuses over large areas of land by small quantities of pollutants over a long period [7]. To combat this global problem, one of the proven responses is to clean up polluted environments. This involves a variety of decontamination techniques: some are referred to as "*ex-situ*" since they can treat material "*on-site*" or "*off-site"*, while others are referred to as "*in-situ"* since they enable contaminated material to be treated on-site without having to be excavated [7].

Several authors describe physical, chemical, thermal and biological methods for remediating environments polluted by waste from the oil industry. Among these methods, bioremediation is considered to be environmentally friendly and less costly to apply [5, 7, 8-11].

Remediation by biological treatment or bioremediation is distinguished by the fact that it exploits the ability of endogenous and/or exogenous living organisms, such as microorganisms and plants, to biodegrade or inactivate pollutants such as hydrocarbons under aerobic or anaerobic conditions [12]; This process involves activating the natural ability of many microorganisms to break down pollutants into inert compounds, such as water and carbon dioxide. It requires a good knowledge of the environment and its physico-chemical parameters, such as oxygen, humidity, pH and nutrients (N, P, C), which influence the microbial degradation of specific contaminants [5, 7, 13]. Thus, bioremediation is an attractive technology for tackling the rehabilitation of sites polluted by petroleum products [14, 15]. Landfarming" is one such bioremediation technique that enables surface bioremediation of soils contaminated by petroleum waste [13, 16].

Internationally, as well as in some African countries, several studies have been carried out on hydrocarbon biodegradation [5, 7]. In Congo-Brazzaville, a developing country, hydrocarbons are one of the main sources of energy. They are used to power motor vehicles, industry and, in particular, electricity production and distribution plants. The Congo is therefore faced with the problem of hydrocarbon spills in various ecosystems [2]. Unfortunately, in Congo-Brazzaville, data in this field are fairly recent and very little research has been carried out [1, 2, 17].

Congolaise de raffinage (CORAF) discharges liquid, solid and gaseous waste into the environment. This results in soil and environmental pollution. Nevertheless, CORAF subcontracts the management of its waste, and in particular its disposal, to a company called Green Service, which handles waste management, collection, treatment and industrial cleaning on behalf of local manufacturers. The company has installed treatment basins on the CORAF site. The present study consisted to evaluate the *onsite landfarming* technique initiated by this company in the Congolese coastal region (Pointe-Noire, Congo). The aim was to monitor the degradation kinetics of petroleum hydrocarbons, the evolution of heavy metal content and physico-chemical parameters in spoil (sludge and mixed soil) during landfarming.

2. Materials and Methods

2.1. Location of Study Area

The study area is located in Pointe-Noire, arrondissement 5 Mongo-Mpoukou (Figure 1), precisely within the CORAF compound (latitude 4°44'24.57816 S, longitude 11°5046.4244' E, altitude 38m) (figure 1). It is located in a coastal sedimentary basin of Tertiary (Pliocene) age. The site's geological substratum consists of the cirque series, with clayey sandstones, sands and clays [18-20].

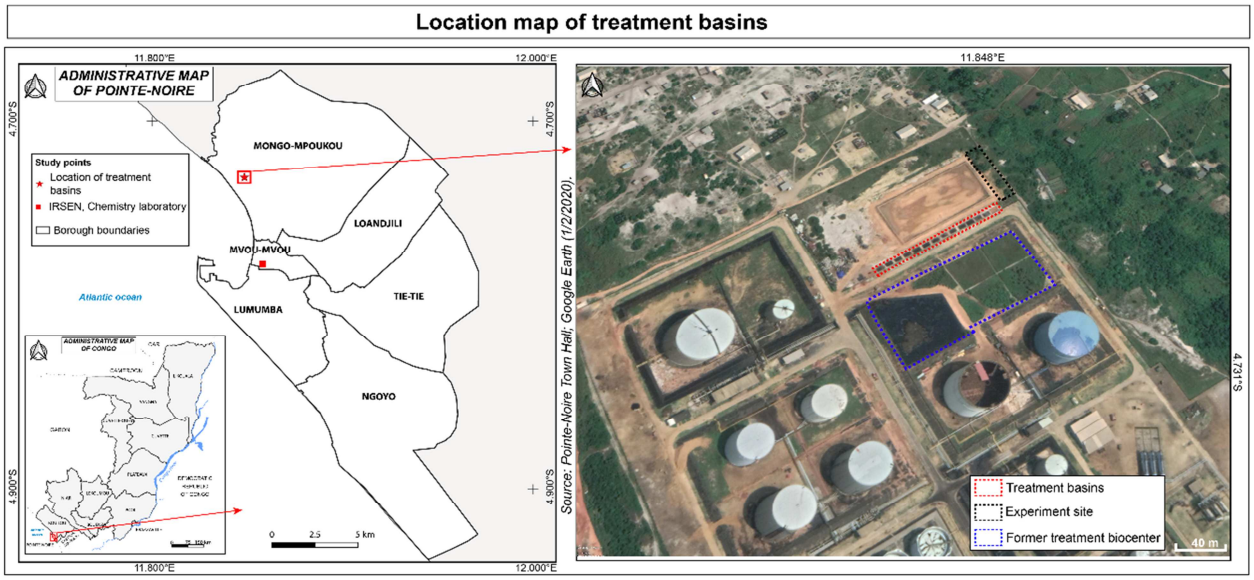

Figure 1. *Location of treatment basins on the CORAF site.*

2.2. Study Material

Three types of soil were considered in this study (Figure 2):

1. Soil polluted by crude oil by-products (Sol 1);
2. Soil polluted by the mixture of petroleum sludge (from tank bottoms) with grease (Sol 2);
3. Soil polluted by a mixture of tank bottom sludge and crude oil by-products (Sol 3).

Figure 2. *Polluted soils analyzed.*

2.3. Methods

2.3.1. Experimental Device and Process for Landfarming Ex Situ on Site

The treatment basins used are rectangular in shape, with the following dimensions: 7m long, 4m wide and 50cm deep. These open-air basins all have the same dimensions. The quantities of materials brought in were distributed as follows: 5,400 kg of polluted soil, 5.5% molasses and 0.9% inoculum for Soil 1; 27,000 kg of soil, 1.1% molasses and 0.185% inoculum for Soils 2 and 3. After the reception of the polluted soils contained in big bags of known mass, excavators are used to spread the soils in basins previously covered with polyethylene plastic sheeting to ensure the impermeability of the basins. A physico-chemical characterization was then carried out at the IRSEN laboratory before the landfarming treatment process was launched. At the start of the treatment process, the polluted soil and the soil improvers were mixed, and then left for 24 hours for manual turning. Then, every week, molasses, water and inoculum were added and aerated by manual turning after 24 hours. Treatments lasted 133 days for Soil 1, Soil 2 and Soil 3 respectively. Figure 3 summarizes the entire treatment process.

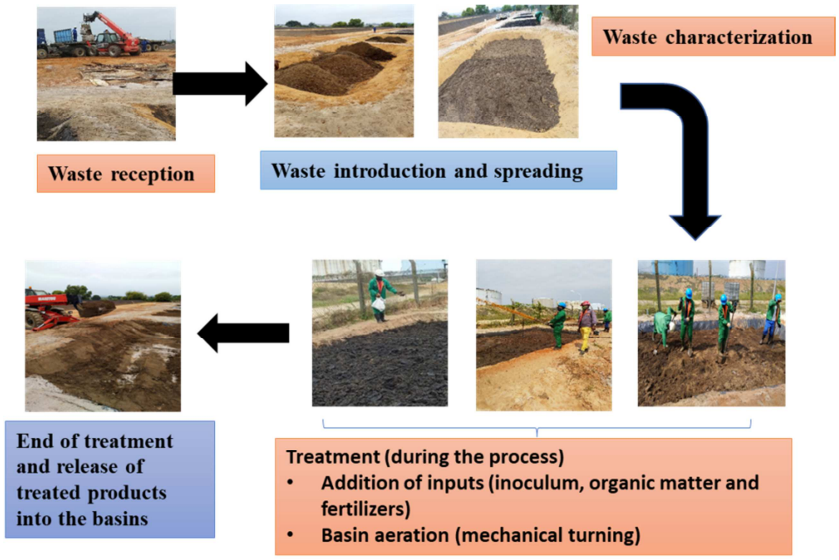

Figure 3. *Treatment process.*

2.3.2. Sample Collection and Conditioning

Samples of the soils to be analyzed were taken at the start of the treatment and once a week. They were taken systematically at five points using an auger at a depth of 30 cm, and the samples obtained were mixed to form a composite sample (Figure 4). The average spacing between samples was five (5) meters long and three (3) meters wide. For each sample, 1 kg of soil was taken and placed in a sealed glass vial. The vials were then stored in a refrigerator at a temperature of 4°C for subsequent analysis. A total of 52 samples were taken.

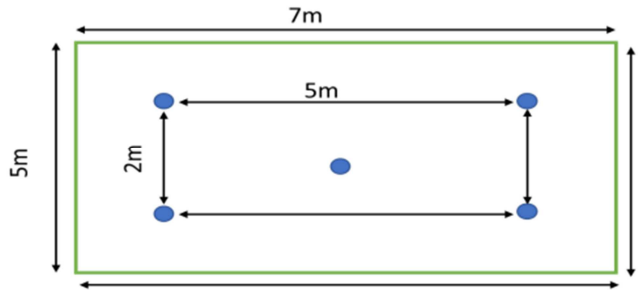

Figure 4. *Sampling points in a treatment basin.*

2.3.3. Physico-Chemical Analysis of Samples

The various physico-chemical analyses (humidity, temperature, pH, air content, porosity, density, loss on ignition, organic matter, carbon, nitrogen and phosphorus) of the soil samples were carried out weekly at the analytical chemistry laboratory of the National Institute of Exact and Natural Sciences (IRSEN) in Pointe-Noire. However, for the determination of total hydrocarbons content (THC), analyses were carried out every 15 days. These analyses were carried out to monitor the evolution of THC degradation during treatment, and to estimate the optimum duration of treatment.

(i). Temperature

The operation involves taking in situ measurements once a week using a WESTON model 226 thermometer, by inserting the thermometer probe into the pond and taking the reading.

(ii). pH

pH is measured using a benchtop perimeter meter from Hanna Instruments (HI), model 9321. A suspension of 20 g of soil dissolved in 50 ml of distilled water is mixed for 30 min using a SCHOTT GERATE model 7M 120 Bioblock electronic magnetic stirrer. After stirring, the mixture is left to stand for 5 minutes and the pH meter electrode is immersed in the solution to read the pH value directly on the pH meter display [21].

(iii). Humidity

The method used is the gravimetric method (Baize, 1988), which consists of determining the wet weight (P1) and dry weight (P2) of a soil sample after drying at 105°C. The sample is placed in a Bioblock Scientific Salvis oven at 105°C until it reaches constant weight, after which the moisture content is calculated using the following formula [22, 23]:

$$\text{Humidity } \% = \frac{P_1 - P_2}{P_1} \times 100$$

With: P_1: initial wet weight
P_1: Initial weight dried at 105°C

(iv). Organic Carbon

Carbon is determined based on the relationship between carbon and organic matter. The transition from total organic matter content to total organic carbon was obtained by dividing the total organic matter content by the coefficient of 1.72 [24, 25].

$$\% C = \% OM/1.72$$

(v). Organic matter (OM)

After removal of soil moisture, organic matter (OM) is determined by loss on ignition after calcination in a Thermolyne Barnstead type 48000 furnace model F48055, (Fisher Scientific, France) at 1100°C for two hours (2h). The weight lost represents the weight of organic matter existing in the soil [22, 26]. Organic matter was calculated using the following formula [22, 26]:

$$\text{MO } \% = \frac{P_1 - P_{1100°C}}{P_1} \times 100$$

$P_{1100°C}$: weight loss of a sample after calcination at 1100°C
P_1: Initial air-dried weight

(vi). Air Content

The notion of aeration controls the penetration of air (oxygen) into the soil. It is determined by the following relationship [27]:

$$Air\ Content = \%P - \%H$$

The notion of porosity (P) is linked to that of density (apparent and absolute) by the following relationship [27]:

$$D_{app} = D_{abs} \times (1 - P)$$

(vii). Total Nitrogen Dosage

Total nitrogen was determined by the Kjeldahl method. This consists of mineralizing 2g of soil organic matter cold for 30 minutes with concentrated sulfuric acid (10mL) in the presence of a mineralization catalyst, then hot for 2h on the thermostat. The nitrogen recovered as ammonium is treated with 20mL of distilled water and 30mL of sodium hydroxide 400g/L, then distilled as ammonia before being determined volumetrically [21, 28].

(viii). Total Hydrocarbons and Heavy Metals Content

Heavy metals were determined at the laboratory in San Marino, Italy, using the UNI en ISO 17294 -2-2016 method. The analysis of total hydrocarbon content was carried out in the "Regional Laboratory Cluster of Excellence" located within the Faculty of Sciences and Technology of the Marien Ngouabi University of Brazzaville. The method used was EPA 3510C + EPA 8015D 2003. The standard test for the determination of total petroleum hydrocarbons in soils is by Soxhlet extraction using an organic solvent [5, 8, 16, 29]. The total percentage content of total hydrocarbons (THC) was calculated using the following formula [17, 23]:

$$THC = \frac{w_2 - w_0}{w_1} \times 100$$

3. Results and Discussion

3.1. Heavy Metals

The results of heavy metal analysis reveal that all heavy metal concentrations are below the limit value set in Italian standard UNI in ISO 17294 -2-2016 (Table 1). However, there are variations in Ni, Pb, Cu and Zn concentrations. These

variations may be due to the variation in pH in the different studied environments, as according to the findings of Ros *et al.*, and USEPA [30, 31] the degradation of organic and inorganic compounds is controlled by pH, nutrients (phosphorus and nitrogen), aeration and humidity. Indeed, several studies have shown that the mobilization and availability of mineral elements in most soils are favored not only by high pH and low ionic strength but also by other factors such as cation exchange capacity (CEC), organic matter content and biological activities [30, 32-39]. These authors point out that the increase in heavy metals observed in the media may also be due to the redox potential since low redox potential values promote the dissolution of hydroxides and lead to an increase in metals associated with the components. Variations in redox potential can modify the degree of oxidation of ligands or metal-binding elements, directly influencing the solubility of heavy metals.

The very low Cd content (<0.5) can be justified by the fact that it is a very rare element in its natural form. This metal can be found in the form of Cadmium sulfide (CdS) or carbonate ($CdCO_3$) and is more often associated with Zn, Cu and Pb in the form of sulfide [40]. This may also be because Cd is much more mobile than Zn, particularly around pH 4.5-5.5 than above pH 7.5 [37, 40]. Its divalent form is soluble but can be complex with organic and oxidized forms [32, 34, 35, 37, 38].

In addition, heavy metal concentrations and requirements may also depend on microorganisms, since to generate energy, many microorganisms depend on metal compounds as electron donors for metabolism [35]. It is therefore common to find metals on the surface of bacteria since they are important components of bacterial walls [35, 37, 40]. Some of these metals or their ions are toxic. Despite the presence of these, some microorganisms can survive in environments polluted by them at low concentrations of heavy metals, because in large quantities, these high levels inhibit the metabolic activity of cells. Metal compounds have both a direct and indirect impact on the rate of degradation [35, 41]. When in high concentrations, the toxic nature of certain contaminants can have toxic effects on microorganisms and slow down decontamination. The degree and mechanisms of toxicity vary according to the specific toxic substances, their concentration and the micro-organisms exposed [41]. Some organic and inorganic compounds are toxic to target life forms. Overall, heavy metals are generally toxic to soil microbial flora [42]. According to Ledin (2000) [43], microorganisms, particularly bacteria, can interact with metals via various mechanisms [35]. These interactions between bacteria and metals generally modify the toxicity and mobility of the original metal. The production by microorganisms of substances such as organic compounds or sulfides, for example, can also modify the solubility, and therefore the mobility, of metals. In addition, through their participation in biogeochemical cycles, microorganisms modify the characteristics of the organic matter in their environment, which can alter the behavior of metals via chelation or complexation mechanisms [35]. Microbes are capable of using an almost infinite combination of electron donors and acceptors to fuel their metabolism. In addition to these redox reactions (oxidation/reduction), they have also developed a myriad of other strategies enabling them to detoxify their environment [39]. In addition, bacteria and fungi can indirectly influence metal mobility by modifying the environment through reactions, such as acidification [44-46]. According to Berthelin [45] once acids are excreted into soils, they can play a part in solubilizing mineral elements and dissolving minerals. Microorganisms are well known for their ability to decompose a wide range of organic compounds and absorb inorganic substances [39]. Studies made by Chibuike and Obiora [47] revealed microorganisms capable of reducing and extracting heavy metals through reactions taking place in soils.

Furthermore, to explain the non-evolution of certain trace metal elements (As, Ba, Cd, Co, Cr, Hg, Mo) in soils during treatment, [35] have shown that heavy metals can be absorbed and immobilized by clay minerals or also complexed by soil organic matter, thus forming an organometallic complex. Carbonated, clayey or organic matter-rich soils that are generally negatively charged tend to retain metal cations, making them less available [35, 40, 44, 46]. Under reducing conditions, heavy metals can be immobilized in the form of insoluble sulfides or ferric oxides; ferric oxides are virtually traps for all trace elements, which can be dissolved in the form of ferrous iron [48].

Table 1. *Heavy metal content (mg/kg) of polluted soil samples before and after treatment.*

Element	Content (mg/kg)						Limit values
	Initial state			Final state			
	Soil 1	Soil 2	Soil 3	Soil 1	Soil 2	Soil 3	
Arsenic	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5	29
Barium	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5	160
Cadmium	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5	0,8
Cobalt	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5	9
Chrome	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5	100
Mercury	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5	0,3
Molybdenum	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5	0,5
Nickel	< 0,5	< 0,5	3	< 0,5	< 0,5	7	35
Lead	17	20	18	17	20	12	85
Copper	5	5	12	10	10	19	36
Zinc	10	12	20	15	0,9	23	140

About zinc, the results of [36] showed that micro-organisms play a role in the decontamination of Zn-contaminated soil. They can mobilize Zn from the soil solution of polluted soil and only yield a minimal portion or release a minimal portion into this solution. According to these authors, Zn concentration decreases with increasing pH. This indicates that Zn is adsorbed by soil constituents. According to Bouzabata & Djamaa and Maynaud [37, 40], Zn is in divalent form and not very mobile at acid pH (4.5-5.5), whereas at higher pH (6.5-7.5), Zn is more available thanks to the solubilization of its mineral and organic forms. Thus, under ordinary and slightly basic conditions, Zn is one of the most mobile elements [48]. This was observed in Soil 1 and Soil 3. On the other hand, in Soil 2, a significant decrease in Zn was observed. This can be explained by the fact that the bioavailability of this element largely depends on the presence of micro-organism activity [32].

Pb levels did not vary in Sol 1 and Sol 2 samples. This may be due, on the one hand, to the presence of sulfates reduced to sulfide, which traps it and, on the other hand, to the fact that lead is difficult to destroy and non-degradable [34]. On the other hand, a decrease in lead was observed in Sol 3. This may be because it is not very mobile that soluble organic complexes have been formed and/or that the soil has exceeded its absorption capacity for Pb, as it is mainly bound either to clays, iron oxides and hydroxides, or to organic matter [34].

We also note an increase in Cu content, which may be due to the variation in pH that makes it available in the medium as a result of microbial activity. When pH decreases by one unit, the concentration of free metal cations increases by about a factor of 2 in the soil solution of the medium [45, 48]. Also, certain chemolithotrophic bacteria can oxidize the reduced forms of iron and sulfur contained in sulfides, producing sulfuric acid that can dissolve silicates, phosphates, oxides and sulfides, thus releasing heavy metals. This acidification also promotes the mobility of other trace elements [32, 34, 35, 37, 38, 45, 48]. This is also observed for Ni in Sol 3, where an increase in concentration is observed because the pH of the medium is not very acidic. However, an acid pH would favor the solubilization of heavy metals, which are highly toxic to bacteria [32, 34, 35, 37, 38, 45, 48, 49].

3.2. pH

The evolution of pH throughout the experiment is shown in Figure 4. The pH increases progressively during treatment of the three polluted soils (Figure 4). In the treatment basins, pH rises from 4.52 to 6.02 at the end of treatment for Soil 1, from 4.99 to 7.32 for Soil 2 and from 6.32 to 7.86, tending towards alkalinity for Soil 3. This increase is certainly due to the microbial activity that takes place during the treatment of polluted soil, through the synthesis of metabolites, as alkaline pH increases the sorption and bioavailability of nutrients for the development of microbes [50]. Indeed, it has been shown that a decrease or increase in pH can indicate acidification or alkalinization of the medium due to the activity of microorganisms with different metabolism [32, 34-36, 38]. According to Dommergues, microflora influences soil pH through their metabolic products, which can be acidifying (mineral or organic acids) or alkalinizing (ammonia, for example) [44]. To this end, Hadjou and Rabhi have shown that the colonization and growth of bacteria and fungi responsible for the degradation of HPT are more favorable at pH levels between 5 and 9 [10]. The results obtained in this study fall within the range where microbial activity is favorable [31, 33].

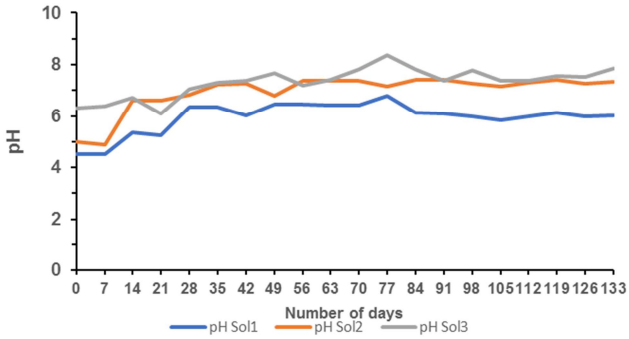

Figure 5. *pH versus time.*

3.3. Humidity

At the start of treatment of the polluted soils, the moisture content was higher in the Sol 3 samples (22.92%) than in the Sol 2 (8.72%) and Sol 1 (4.72%) samples. This is due to the difference in the degree of pollution in the three soils, the nature of the permeability of the waste and, above all, the amount of water added to each basin. At the end of treatment, higher moisture levels were observed in samples Sol 1 and Sol 2, with values of 17.01% and 12.10% respectively, while in Sol 3 a decrease in moisture content was observed, with a value of 10.94% (Figure 6). Throughout the treatment, moisture levels varied considerably from one week to the next; this could be explained by the fact that water was added every week. Nevertheless, average moisture levels were 17.44% for Soil 1, 11.51% for Soil 2 and 13.52% for Soil 3, with an increasing trend in moisture levels for Soil 1 and 2 samples and a decreasing trend for Soil 3 values. These results are similar to those obtained by Bouderhem and Goma-tchimbakala *et al.* [17, 49], which obtained moisture contents ranging from 6% to 17% and from 13% to 26% respectively. The moisture contents obtained are significantly lower than those obtained by Rajaona Rafihavanana [11], whose results show that soil moisture content at these sites is high (33% to 37%). Indeed, for landfarming treatment, ideal soil moisture is between 40% and 85% of the soil's water retention capacity (field capacity), or between 12% and 30% of its weight [31].

It was noted that at t=28 days of Sol 2, the moisture content is at 2.04%, this could be due to aeration, as the latter causes the moisture to be lost and the pollutant to dry out, consequently reducing the activity of microorganisms [51, 52]. According to Lukić *et al.* [52] low humidity levels (<2%) have a negative effect. They affect the proper increase of microorganisms and soil aeration, reducing the availability of

oxygen essential for aerobic metabolic processes. Thus, low moisture levels limit the rate of PHC degradation by influencing microbial activity while limiting microorganism/pollutant contact and inhibiting the enzymatic degradation process [49]. On the other hand, excessively high moisture levels will affect soil gas permeability and generate conditions that restrict oxygen transfer, thus limiting aerobic microbial metabolism [49].

Micro-organisms need water to be able to move and develop properly, and to be able to desorb pollutants through their metabolism. Water enables the mobility of micro-organisms in treatment basins. It also serves as a transport medium through which numerous nutrients and organic compounds diffuse to the microbial cell, and through which metabolic waste is eliminated [51]. Thus, this rate must be controlled, as excessive soil moisture restricts air movement in the subsoil, reducing the availability of oxygen, which is also necessary for the metabolic processes of aerobic bacteria [29].

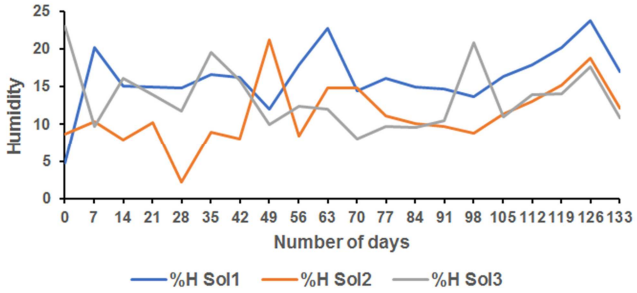

Figure 6. *Humidity trends over time.*

3.4. Air Content

The results obtained (figure 6) show a fluctuation in air content throughout the treatment, with a downward trend for Soils 1 and 2, and an upward trend for Soil 3. Throughout treatment, average air contents were 13.83%, 17.62% and 9.26% for Soils 1, 2 and 3 respectively. During treatment, air content varied between 2% and 28% for Soil 1, between 4% and 30% for Soil 2, and between 0% and 15% for Soil 3. These variations depend on the porosity and humidity of the medium. Air content varies according to the treatment carried out and the degree of soil pollution. Decreases in air content can be justified by the high moisture content of the water in the treatment basins. According to Or *et al.* [53] when water content decreases, soil air content and water-gas interfacial area increase, which favors gas diffusion and improves gas exchange with the atmosphere. When humidity exceeds its optimum, porosity and air content fall, while on the contrary, they increase [54]. This parameter is very important, as aeration allows oxygen to penetrate the pond bottom. Several studies have shown that the bioremediation of aromatic hydrocarbons under aerobic conditions is achieved by microorganisms (prokaryotes or eukaryotes) possessing different enzyme systems, such as dioxygenases, monooxygenases and lactases [39, 52, 55, 56]. These enzymes use oxygen in their reaction [52, 57]. Thus, oxygen is an essential element for aerobic degradation and serves as a limiting factor in biodegradation. Its absence could asphyxiate microorganisms, block microbial activity and render oxygenase enzymes ineffective and therefore unavailable. The effectiveness of oxygenase enzymes in breaking down hydrocarbons depends largely on the quantity of oxygen molecules [52].

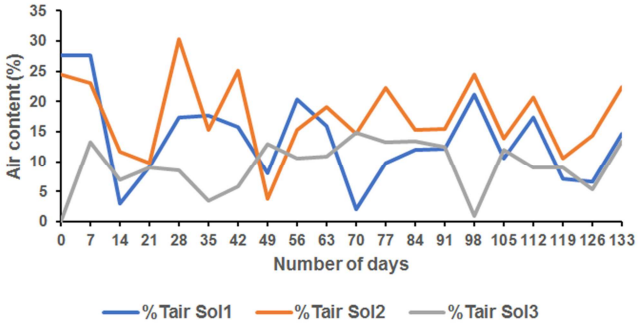

Figure 7. *Changes in air content during treatment of the three polluted soils.*

3.5. Temperature

Figure 7 shows the temperature evolution of the polluted soils and the ambient environment during landfarming. In the treatment basin containing Sol 1, ambient temperature and temperature varied during treatment. As soil temperature varies according to ambient temperature, we note that temperature evolves according to climatic and geographical conditions [31]. It should be noted that the ambient (or air) temperature as well as that of each soil treatment basin varied between 21 and 35°C. From these results, it can be seen that during treatment, the temperature varied from 21 C to 28°C in Soil 1; from 30°C to and 27.5°C respectively in Soils 1, 2, and 3 and at the end it was then from 26°C to 35°C in Soil 2, and from 27°C to 35°C in Soil 3. This seems logical, given their different degrees of pollution. These variations observed during treatment may prove the presence of microbial activity in basins containing polluted soils. Microbial activity can increase the temperature of the environment [29, 33, 56]. Indeed, temperature, like other physico-chemical parameters, can promote good microbiological activity in the medium and a reduction in total hydrocarbon levels during the bioremediation period [7]. As a result, during biodegradation treatment, the biological activity of organic matter is highly sensitive to temperature variations. Indeed, ambient temperature has a major influence, as the growth, selection and activity of microorganisms in soils or sludges depend on the temperature of the medium, which is influenced by ambient temperature [52]. The biodegradation temperature varies between 20 and 37°C, but its maximum is around 35°C. Below 10°C, it no longer occurs [31]. Results obtained during this period reveal that temperature values are within the range of temperature intervals favorable to the growth of microorganisms [11]. These results are in line with those of Ouédraogo *et al.* [7], and Zairi, *et al.* [58], whose values oscillated around 15 and 25°C respectively, then 16 and 39°C during the experiments.

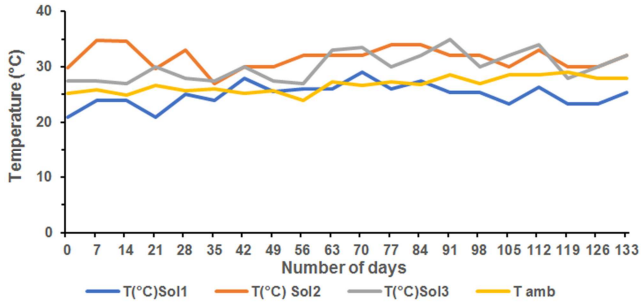

Figure 8. *Evolution of soil temperature and ambient temperature during treatment.*

Thus, some authors have highlighted a causal principle between the rate of organic substrate and the heat generated, but also with the rate of biodegradation of petroleum hydrocarbons [15, 59, 60]. The temperature rise is at the origin of physico-chemical and biological reactions (metabolic and/or cometabolic) which, through them, promote the intimate rapprochement of matrices (soil/substrate) and consequently the solubility of petroleum hydrocarbons: an ideal condition for making the latter bioavailable and biodegradable by microorganisms [15, 33]. This analysis is in line with the thinking of some researchers that "Temperature is a key factor in the biodegradation of recalcitrant hydrocarbons such as PAHs" and/or others when they state that "Petroleum hydrocarbons, despite their hydrophobicity, gradually solubilize in water as a function of increasing temperature" [15]. Several authors quoted by Ouédraogo *et al.* [7] have concluded that when temperatures are higher, with a maximum between 30 and 40°C, the activity rate of microorganisms is higher, so the rate of hydrocarbon biodegradation increases. In our study, the rate of pollutant biodegradation could be reduced during periods when temperatures were below 30°C. However, this could not prevent a significant drop in total hydrocarbon levels over the months of biotreatment. These results concur with the work of Ouédraogo *et al.* [7].

3.6. Nitrogen

The results obtained show that during treatment, nitrogen levels varied between 0.123 and 0.164 (%) for Soil 1; 0.119 and 0.203 (%) for Soil 2 and 0.088 and 0.158 (%) for Soil 3 (figure 9). Nitrogen content in Soil 2 was slightly higher at the end of the treatment than at the beginning. On the other hand, in Soil 3, there was a slight decrease in nitrogen content at the end of the treatment compared with the beginning of the experiment. This is not the case for Soil 1, where the levels at the beginning and end of the experiment are the same. At the start of treatment, nitrogen levels in the three soils (1, 2 and 3) were 0.126%, 0.119% and 0.140% respectively, whereas at the end of treatment, they were 0.132%, 0.153% and 0.113% in these three soils. Generally speaking, the curves show little variation, with a constant trend at the end of the treatment. Overall, the values observed are close to those of Soltani [9]. According to this author, nitrogen sources are always low in treatment media, especially during periods of high photosynthetic activity. At high concentrations, nitrogen becomes a limiting factor for hydrocarbon biodegradation in soils. The form of nitrogen generally used is that directly assimilated by biomass, i e., nitrate [61]. Some specific forms of nitrogen have varying effects on the mineralization of organic compounds. This difference could be attributed to the difference in bioavailability of the two nitrogenous forms: NH_4^+ tends to adsorb onto clay minerals and is therefore less available than the NO_3^- form, which has the particularity of being easily and rapidly assimilated by microorganisms; hence its interest in a large number of studies involving the influence of nutrient inputs on hydrocarbon biodegradation [61]. In addition, these variations may be due to the types of treatment carried out (molasses and inoculum), their frequency and the quantities added to the three soil basins undergoing treatment.

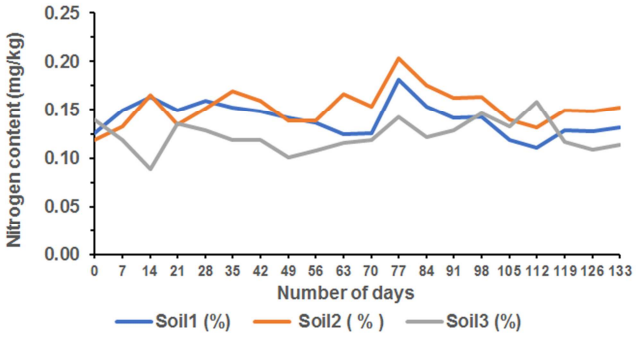

Figure 9. *Changes in nitrogen levels as a function of treatment time.*

3.7. Total Carbon

Figure 10 shows the evolution of total carbon content during landfarming treatment. The total carbon content of Soil 1 was constant for the first 7 days, then varied slightly to reach a value of 2.898% at the end of the treatment. In Sol 2, on the other hand, the total carbon content begins to fall at the start of the second week, only to rise again on the 14^e day of treatment; it then rises again in a sawtooth pattern to reach a value of 3.116% at the end of treatment. Finally, for Sol 3, total carbon content also varied throughout the treatment, with values ranging from 2.19 to 10.14%. The high total carbon content (5.75%) at the start of the experiment decreased to 4.871% at the end of the treatment.

In Sol 1 and Sol 2, the curves show a latent phase during the first week of treatment, followed by a decrease in the second week. This could be due to a phase of adaptation of the microflora present in the medium to the pH of the medium. Indeed, Rajaona Rafihavanana [11] has shown that bioremediation is most effective in the pH range between 5 and 9. The decrease in total carbon content during the second week could be justified by microbial activity, which may be responsible for the decrease in carbon, as Rhbal *et al.* [15], and Wannoussa *et al.* [62] point out that in polluted environments, microorganisms consume more carbon to produce energy.

In Sol 3, the decrease in carbon content began as soon as treatment began. This may be because conditions in the pond were conducive to degradation (pH 6; temperature 27°C). In

Sol 1, the weekly addition of molasses throughout the treatment did not alter the carbon content. It is possible that the low hydrocarbon content compared with the other two soils did not inhibit microbial activity. However, for Sol 2 and Sol 3, the addition of molasses increased the total carbon content in each case. This increase can be explained by the fact that high hydrocarbon contents and the addition of molasses in both soils inhibited microbial activity. According to Malika and Roumaissa [63], the addition of additives (fertilizers, bacteria...) can inhibit the growth of microorganisms as well as their microbial activities. In addition, these authors indicate that the presence of antagonistic interactions can take place between bacteria in soils and thus inhibit the growth of certain microorganisms.

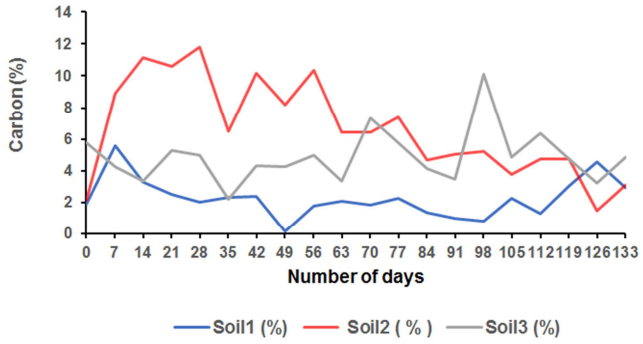

Figure 10. *Evolution of carbon content during processing.*

3.8. Total Hydrocarbon Content

Monitoring of hydrocarbon degradation during treatment showed a considerable decrease in Sol 1, a fairly significant drop in Sol 2 and a very small drop in Sol 3. Indeed, the concentration of total hydrocarbons fell from 2.13% to 0.70% after 91 days, from 9.24% to 4.78% after 133 days and from 8.66% to 7.87% after 147 days for Sol 1, Sol 2 and Sol 3 respectively. Hydrocarbon degradability rates were 67.19%, 52.75% and 9.18% respectively for Soil 1, Soil 2 and Soil 3 (Figure 11). The high rate of pollutant depletion in the Soil 1 and Soil 2 samples could be explained by the fact that petroleum hydrocarbons are used in microbial metabolism [29, 59, 60]. In Sol 3, the slight decrease could be due to high hydrocarbon concentrations, which probably modified metabolism by inhibiting degradation and limiting oxygen utilization, for example [15]. These results also show that the decrease in total petroleum hydrocarbon concentration varies from one soil sample to another, depending on the concentration of the pollutant at the start of treatment.

As the Republic of Congo has no standards for hydrocarbon limit values in soils, a comparison of the results of this study with the standards set by countries such as Quebec, the Netherlands, the USA, Italy, France and Madagascar, shows that the degradation rates obtained are very low, and thus the total hydrocarbon content in treated soils is still well above the standards accepted by these countries [64]. In addition, comparison with the French Impact Value (VCI) values for the remediation of polluted sites and soils shows that the hydrocarbon content for Sol 1 is higher than the VCI for sensitive use soil, but lower than the VCI for non-sensitive use soil, which is 25,000 mg/kg dry matter [65]. On the other hand, THC levels in Soils 2 and 3 were higher than both soil ICVs for all uses. These high values could be due to excess nutrients. Indeed, Gautier [61] points out that excess nutrients limit biodegradation and no longer significantly increase or stimulate hydrocarbon degradation rates. In addition, other parameters may also account for the low level of degradation in Sol 3. The treatment duration was fairly short and probably did not allow complete biodegradation of all hydrocarbon components, since Marin *et al.* [33] achieved a significant 80% reduction in THC only after 11 months of *landfarming* treatment. For these authors, aliphatic hydrocarbons were degraded during the first three months at the start of treatment, while aromatic hydrocarbons, condensed cycloalkanes and other compounds were degraded between 3 and 11 months. A very high THC concentration at the start of the experiment can also influence the rate of degradation. For example, Petavy. *et al.* point out that the effectiveness of biological treatments is limited and insufficient when the hydrocarbon concentration is very high [64]. In the same vein, Wang *et al.* [66] working on samples with an initial THC concentration of 4,100 mg/kg soil, much lower than our study, achieved an 83% reduction in THC after a 175-day *landfarming* treatment. So, to boost degradation, it would be interesting to add nutrients and indigenous microorganisms, as pointed out by Couto *et al.*, and Guarino [67, 68].

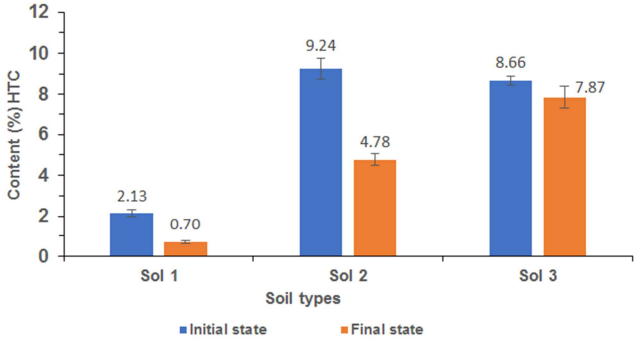

Figure 11. *Hydrocarbon content of soils at the beginning and end of treatment.*

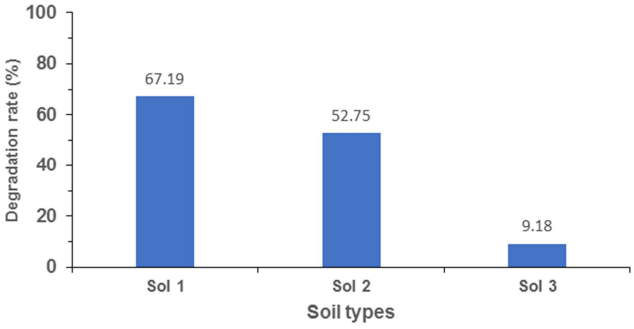

Figure 12. *Soil hydrocarbon degradability rates at the end of treatment.*

4. Conclusion

This study highlighted the biodegradation of hydrocarbons

in polluted soils using the *landfarming* technique. The decrease in hydrocarbon content in soils was shown to be a function of treatment time and input quantity. The main physico-chemical parameters able of influencing microbial metabolism were determined to gain a better understanding of the bioremediation capacity of hydrocarbon-contaminated soils. The parameters studied vary according to the level of pollution and the quantity of inputs. Monitoring of hydrocarbon concentration during treatment showed a greater decline in Soil 1, followed by Soil 2, while hydrocarbon elimination was lowest in Soil 3. However, in all treated soils, total hydrocarbon levels are still higher than the standards taken as a reference. However, time remains a very important and determining factor in the efficiency of the technique applied. Finally, the input must be controlled to avoid inhibiting microbial activity. A combination of oxygenation by turning the soil, bio-augmentation of indigenous microorganisms and nutrient amendment can improve the rate of degradation.

References

[1] Lebonguy, A. A., Goma-Tchimbakala, J., Miamb, i E. et Keleke, S. (2017). «Isolation and characterization of petroleum product emulsifying Pseudomonas strains from a generating set fuel tank». *African Journal of Microbiology Research,* vol. 11, N°122, pp. 920-926.

[2] Goma-Tchimbakala, J., Lebonguy, A. A., & Ponguy Soueli, C. R. (2019). Caractérisation des bactéries isolées de deux sols pollues par les hydrocarbures de Brazzaville, CONGO. *Annales de l'Université Marien NGOUABI Sciences Techniques*, *18*, pp. 23-32. www.annalesumng.org.

[3] Nyolo Lonema, J., Koy Kasongo, R., Mawa Tuzingila, R., & Makaly, E. (2021). Bioremédiation des Boues ex-Réservoirs des Raffineries de Pétrole. *Congo Sciences: Journal En Ligne de l'ACASTI et Du CEDESURK*, *9*(1), pp. 32-44. http://www.congosciences.cd..

[4] Pătrașcu, C., Avram, L., Dobre, L., Panaitescu, C., & Popa, M. (2006). Décontamination microbiologique d'un site historiquement pollué avec le petrole brut. *Scientific Study & Research*, *VII* (3), pp 531-534.

[5] P. W. Kaboré-Ouédraogo,,. P. W. Savadogo, C. A. T. Ouattara, A. Savadogo et A. S. Traoré, «Etude de la Bio-dépollution de Sols contaminés par les Hydrocarbures au Burkina Faso,» *J. Soc. Ouest-Afrique. Chim.,* vol. 30, pp. 19-28, January 2010.

[6] Sawadogo, A., Otoidobiga, H. C., Nitiema, L. W., Traoré, A. S., & Dianou, D. (2016). «Optimization of Hydrocarbons Biodegradation by Bacterial Strains Isolated from Wastewaters in Ouagadougou, Burkina Faso: Case Study of SAE 40 / 50 Used Oils and Diesel,» *Journal of Agricultural Chemistry and Environment,* vol. 5, pp. 1-11.

[7] Ouédraogo, W. P., Otoidobiga, C. H., Tidiane, C. A., Ouattara, A. S., & Traoré, A. S. (2020). «Pilot bioremediation of contaminated soils by hydrocarbons, from an electricity production and distribution site in Ouagadougou, Burkina Faso,» *Scientific Research and Essays,* vol. 14, N°14, pp. 69-77. https://doi.org/10.5897/SRE2020.6688.

[8] USEPA (2003). «Method 8015D: Nonhalogenated Organics Using GC/FID,» *Revision 4 June,* pp. 1-37.

[9] Soltani M. (2004). «Distribution lipidique et voies métaboliques chez quatre bactéries Gram-négatives hydrocarbonoclastes. Variation en fonction de la source de carbone» Paris, 258 P. https://pastel.archives-ouvertes.fr/tel-00009276

[10] Hadjou, O. et Rabhi S. (2015). «Essai de bioremédiation d'un sol pollué aux hydrocarbures par la bioaugmentation et la biostimulation,» Biologie Animale et végétale, Université Mouloud Mammeri de Tizi Ouzou, 52p, Algerie.

[11] A. Rajaona Rafihavanana, «Contribution au traitement des boues et des sols pollués par des hydrocarbures: Optimisation et approche de modélisation du procédé,» Chimie Minérale et de Chimime Physique, faculté de Science d'Antananarivo, 188 P, 2015.

[12] Mansouri A.,2018. «Approche intégrée de suivi d'un procédé de bioremédiation: Application aux sédiments de la lagune de Bizerte,» Génie des Procédés, Université de Nante en co-tutlle avec l'Université de Carthage, 134 P, Nantes.

[13] Hansen, L. D., Nestler, C., Ringelberg, D. et R. Bajpai (2004). «Extended bioremediation of PAH / PCP contaminated soils from the POPILE wood treatment facility,» *Chemosphère,* vol. 54, pp. 1481-1493. https://doi.org/10.1016/j.chemosphere.2003.09.046

[14] Hamdi H., Benzarti S., Manusadžianas, L., Aoyama, I. et Jedidi, N. (2007) «Solid-phase bioassays and soil microbial activities to evaluate PAH-spiked soil ecotoxicity after a long-term bioremediation process simulating landfarming,» *Chemosphere,* vol. 70, N°11, pp. 135-143. https://doi.org/10.1016/j.chemosphere.2007.06.043

[15] Rhbal, H., Souabi, S., Safi, M., Mohamed, T., Arad, M., & Abdelkader, Anouzla Mohamed, H. (2020). «Décontamination des sols pollués par les hydrocarbures,» *Scientific Study & Research: Chemistry & Chemical Engineering, Biotechnology, Food Industry,* vol. 21, N°11, pp. 1-16.

[16] USEPA (1998). «Method 9071B, n-hexane extractable material (hem) for sludge, sediment, and solid samples,» *Revision 2,* pp. 1-13.

[17] Goma-tchimbakala, J., Obambi Ngassaï, J. R., Lebonguy, A. A., & Gouolally Tsiba (2020) «Biodégradation des HAP d ' une Huile usée par des Consortiums de Microorganismes Isolés des sols Pollués de Brazzaville, Congo,» *European Journal of Scientific Research,* vol. 155, N°14, pp. 378-387.

[18] Sitou, L. (1994). «Les cirques d'érosion dans la région de Pointe-Noire (Congo): étude géomorphologique». Géographie-physique, Université Louis Pasteur (Strasbourg-I), 240P.

[19] Nizinski, J. J., Galat, G., Galat-Luong, A., Dingkuhn, M. et Fabre, D. (2009). «Evapotranspiration réelle et resistance du couvert d'une savane à Loudetia Arundinacea (Bassin du Kouilou, Congo-Brazzaville)». *Climatologie,* vol. 6, pp. 35-45.

[20] Massengo, A. (1970) «Contribution à l'étude stratigraphique, sédimentologique et mineralogique de serie Plio-pleistocène du bassin côtier du Congo-Brazzaville.,» Université de bordeaux, 159P, 1970.

[21] AOAC (1990), Official Methods of Analysis of the Association of official analytical chemists (AOAC), 15 th éd., vol. 1, H. Kenneth, Éd., 771 P.

[22] D. Baize, Guide des analyse courante en pédologie: Choix-expression-présentation-interprétation, IRA, Éd., Paris, 1988, p. 172.

[23] Piakong, M., & Nur Zaida, Z. (2018). «Effectiveness of Single and Microbial Consortium of Locally Isolated Beneficial Microorganisms (LIBeM) in Bioaugmentation of Oil Sludge Contaminated Soil at Different Concentration Levels: A Laboratory Scale,» *Journal of Bioremediation & Biodegradation,* vol. 9, N°12, pp. 3-9. https://doi.org/10.4172/2155-6199.1000430

[24] Baize, D. et Paquereau, H. (1997). «Teneurs totales en éléments traces dans les sols agricoles de Seine-et-Marne,» *Science du Sol - I. N. R. A. - Orléan,* vol. 4, N°12, pp. 77-94.

[25] Baize, D. (2018). Guide des analyses en pédologie, 3e édition éd., Quæ, Éd., Paris, 323 P.

[26] Benyahia, N. et K. Mahdaoui, K. (2012) «La pollution des sols par les hydrocarbures,» Faculté des Science de laNature et de la vie, Université Abderrahmane Mira de Bejaia, 87P, Algerie, 2012.

[27] Koller, E. (2009). Traitement des déchets industriels: Eau-Air-Déchets-Sols-Boues., 2ème édition éd., Dunod, Éd., Paris, 569 P.

[28] Petard, J. (1993). Les méthode d'analyse de sols, Vols. %1 sur %2Tome 1, (5), ORSTOM, Éd., Paris, France, 196P.

[29] USEPA (2007), «Method 9074: Turbidimetric screening method for total recoverable petroleum Hydrocarbons n soil.,» *Revisio 0, February,* pp. 1-15.

[30] Ros, M., Rodríguez, I., García, C., & Hernández, T. (2010). «Bioresource Technology Microbial communities involved in the bioremediation of an aged recalcitrant hydrocarbon polluted soil by using organic amendments,» *Bioresource Technology,* vol. 101, N° 118, pp. 6916-6923. https://doi.org/10.1016/j.biortech.2010.03.126

[31] USEPA (2017), «How to evaluate alternative cleanup technologies for Underground Storage Tank Sites: A Guide For Corrective Action Plan Reviewers, landfarming». *Chapter V: Lanfarming,* Vols. 1 sur 2510-B-17-0, Revision of 1994, p. 31. https://www.epa.gov/sites/production/files/2014-03/documents/tum_ch9.pdf

[32] EshghiI Malayeri, B. (1995). «Décontamination des sols contenant des métaux lourds à l'aide de plantes et de microorganismes,» Faculté des Sciences U. F. R. ENSTIB, Université Henri Poincaré, Nancy 1, 116 P.

[33] Marin, J. A., Hernandez, T., & Garcia, C. Ã. (2005). «Bioremediation of oil refinery sludge by landfarming in semiarid conditions: Influence on soil microbial activity,» *Environmental Research 98,* vol. 98, pp. 185-195. https://doi.org/10.1016/j.envres.2004.06.005

[34] Huynh, T. M. D. (2009) «Impacts des métaux lourds sur l'interaction plante/ ver de terre/ microflore tellurique». Université PARIS Est: Ecole Doctorale Sience de la vie et de la santé, 169P.

[35] Desaunay, A. (2011) «Etude et modélisation de la biosorption des métaux par les bactéries. Application au transfert du cadmium et du zinc, seuls ou en mélange par Escherichia coli et Cupriavidus metal- lidurans en colonnes de sable d'Hostun»Sciences de la Terre. Université de Grenoble, 249 P. https://tel.archives-ouvertes.fr/tel-00716409

[36] Belouchrani, A. S., Abdi, N., Lounici, H., Mameri, N. et B. Mouhouche (2013). «Contribution à l'étude de l'effet des micro-organismes dans la bioremediation d ' un sol pollué par le zinc Contribution à l'étude de l'effet des micro-organismes dans la bioremediation d'un sol pollué par le zinc,» chez *The 4th International Congress Water, Waste & Environment (EDE4), December 18-20*, Agadir, Morocco, pp. 74-77.

[37] Bouzabata S. et Djamaa, F. (2015) «Influence des Métaux lourds sur la croissance des souches bactériennes isolées à partir des nodules de Vicia faba et Pisum sativum,» Faculté des Sciences de la Nature et de la Vie, Université des Frères Mentouri Constantine, 85P.

[38] Jacob, C. (2001). «Etude des interactions entre métaux lourds et champignons ectomycorhiziens: mise en évidence de gènes impliqués dans la réponse au cadmium de " Paxillus involutus ",» Sylviculture, foresterie. Université Henri Poincaré - Nancy 1, 253 P.

[39] Hogan-Itam, D. (2020) «The Use of Biochar for Bioremediation of Crude Oil/Hydrocarbon Polluted Soils (Vol 1) – Bioremediation of Polluted Soils, Studies, Results and Possibilities,» *SSRN Electronic Journal,* vol. 1, pp. 1-14. https://doi.org/10.2139/ssrn.3707749

[40] Maynaud, G. (2012). «Adaptation aux métaux lourds de populations de rhizobia impliquées dans la phytostabilisation de déblais miniers:,» Microbiologie, Parasitologie, Université Montpellier II Sciences et Technique du Languedo, 284P.

[41] Abatenh, E., Gizaw, B., Tsegaye, Z., & Wassie, M. (2017). The Role of Microorganisms in Bioremediation-A Review. *Open J Environ Biol*, *2*(1), 38-46. https://doi.org/10.17352/ojeb.000007.

[42] Mlitan, A. B., Alajtal, A. I., Alsadawy, A. M., & Al, E. T. (2013). Toxicity of Heavy Metals and Microbial Analysis of Soil Samples Collected from the Area around Zliten Cement Factory. *Open Journal of Air Pollution*, *2* pp. 25-28. https://doi.org/http://dx.doi.org/10.4236/ojap.2013.21004

[43] Ledin, M. (2000). «Accumulation of metals by microorganisms — processes and importance for soil systems» *Earth-Science Reviews,* vol. 51, N°11-4, pp. 1-31.

[44] Dommergues, Y. (1977). Biologie des sols, 2ème éd., Paris, 128. P.

[45] Berthelin, J. (1998). «Les micro-organismes dans la transformation des minéraux: incidence sur la formation, le fonctionnement et évolution des sols». In P. Stengel & S. Gelin (Coord), *Sol: interface fragile*, INRA, Paris, pp. 83-91.

[46] Coyne, M. S. (1999). *Soil microbiology: An Exploratory Approach*. ITP (ed.). Delmar, 462P. http:/www.delmar.com.

[47] Chibuike, G. U., & Obiora, C. S. (2014). «Heavy Metal Polluted Soils: Effect on Plants and Bioremediation Methods». *Applied and Environmental Soil Science*, *2014*, 1-12. https://doi.org/10.1155/2014/752708.

[48] Cambier, P., & Mench, M. (1998). «Soil contamination by heavy metals and other trace elements. In S. Stengel, P. Gelin (Coord), *Sol: interface fragile*, INRA, Paris, pp. 161-171.

[49] Bouderhem, A. (2011). «Utlisation des souches bactériennes tellurique autochtones dans la biodétection et la bioremédiationdes sols pollués par les hydrocarbures». Sciences de la Nature et de la Vie, Kasdi Merbah-Ouargla, Faculté des Science et de la vie et Science de la terre et de l'Univers,91P. http://www.indianjournals.com/ijor.aspx?target=ijor:ijmr1&volume=2&issue=4&article=012

[50] Ugboma, C. J., Sampson, T., & Mbonu, N. E. (2021). «Bioremediation of heavy metals from artisanal crude oil refinery (Kpo-Fire) impacted soil using Bacillus flexus and Pseudomonas aeruginosa in Ngie Community, Degema Local Government Area, Rivers State, Nigeria». *Journal of Applied Sciences and Environmental Management*, *24* (12), pp. 2049-2054. https://doi.org/10.4314/jasem.v24i12.6

[51] Rasoanarivony, A. M. (2016). «Bioremédiation des sols pollues l'environnement par les hydrocarbures aromatiques polycycliques (HAP) étude de cas de la région de Tsimiroro,» Chimie, Science et technologie, Université d'Antananarivo, 96 P.

[52] Lukić, B., Panico, A., Huguenot, D., Fabbricino, M., van Hullebusch, E. D., & Esposito, G. (2017). «A review on the efficiency of landfarming integrated with composting as a soil remediation treatment». *Environmental Technology Reviews,* vol. 6, N°11, pp. 94-116. https://doi.org/10.1080/21622515.2017.1310310

[53] Or, D., Smets, B. F., Wraith, J. M., Dechesne, A., & Friedman, S. P. (2007). «Physical constraints affecting bacterial habitats and activity in unsaturated porous media - a review,» *Advances in Water Resources,* vol. 30, N° 16-7, pp. 1505-1527.

[54] Håkansson, I., & Lipiec, J. (2000). «A review of the usefulness of relative bulk density values in studies of soil structure and compaction,» *Soil and Tillage Research,* vol. 53, N° 12, pp. 71-85. https://doi.org/10.1016/S0167-1987(99)00095-1

[55] Lee, S. H., Oh, B. I. et Kim, J. G. (2008). «Effect of various amendments on heavy mineral oil bioremediation and soil microbial activity,» *Bioresource Technology,* vol. 99, n° 17, pp. 2578-2587. https://doi.org/10.1016/j.biortech.2007.04.039

[56] Macaulay B. M. et Rees, D. (2014). «Bioremdiation of oil spills: A reviexof challenges for research advancement». *Annals of Environmental Science,* vol. 8, pp. 9-37.

[57] Lukic, B. (2016). «Composting of organic waste for enhanced bioremediation of PAHs contaminated soils». Materials. Université Paris-Est, 208P. https://tel.archives-ouvertes.fr/tel-01735701v2

[58] Zairi, M., Rouis, M. J.et Shabou, R. «Étude Expérimentale De La Biorestauration Des Sols Souillés Par Les Hydrocarbures,» *Environnement, Ingénierie & Développement,* vol. 3 ème trim, N° 127, pp. 22-29. https://doi.org/10.4267/dechets-sciences-techniques.2384

[59] Roth, E., Fabre, B. et Gunkel, P. (2005). «Environnement, Ingénierie & Développement». vol. 2èm, N°138, pp. 8-14. https://doi.org/10.4267/dechets-sciences-techniques.1895.

[60] Mohamed, N. et Abdelmajid, H. (2016). «Les techniques de dépollution des sols contaminés par les métaux lourds: une revue». *Maghrebian Journal of Pure and Applied Science,* vol. 2, n° 12, pp. 2458-715.

[61] Gautier C. (2007). «Biodégradation des Hydrocarbures en milieu poreux insaturé.,» Physique-Chimie, Université De PAU ET Des PAYS De L'ADOUR: Institut Français Du Pétrole, 270P.

[62] Wannoussa, W., Hiligsmann, S., Tasseroul, L., Lambert, S. D., Heinrichs, B., Masy, T., Weekers, F., Lavigne, B., Delvigne, F., Jacques, P. et Thonart, P. (2015). «Amélioration de la biodégradation du biphényle par Rhodococcus erythropolis t902.1 en présence de Fe_2O_3 et de nanoparticules de fer encapsulées dans un xérogel de silice,» *Déchets Sciences et Techniques,* n° 169, pp. 4-12. https://doi.org/10.4267/dechets-sciences-techniques.3210.

[63] Malika, A. et Roumaissa, A (2018). «Essai de bioremédiation de sol pollué au gasoil par des bactéries hydrocarbonoclastes Essai de bioremédiation de sol pollué au gasoil par des bactéries hydrocarbonoclastes,» Microbiologie, Université des frères mentouri constantine: Faculté des sciences de la nature et de la vie, 82P.

[64] Petavy, F., Ruban, V., Conil, P., Viau, J. Y. et Auriol, J. C. (2009).«Two treatment methods for stormwater sediments – pilot plant and landfarming – and reuse of the treated sediments in civil engineering». *Environmental Technology,* vol. 30, N°18, pp. 37-41, 2009. https://doi.org/10.1080/09593330902990113

[65] Mossman, J. R. en collaboration avec Koch-Mathian J. Y. (2001). «Gestion des sites (potentiellement) pollués et évaluation simplifiée des risques». BRGM/RP-51216-FR, 44 p., 2 ann.

[66] Wang, S., Kuo, Y., Hong, A., Chang, Y., & Kao, C. (2016). «Chemosphere Bioremediation of diesel and lubricant oil-contaminated soils using enhanced landfarming system». *Chemosphere,* vol. 164, pp. 558-567. https://doi.org/10.1016/j.chemosphere.2016.08.128

[67] Couto, M. N. P. F. S., Monteiro, E., & Vasconcelos, M. T. S. D. (2010). «Mesocosm trials of bioremediation of contaminated soil of a petroleum refinery: comparison of natural attenuation, biostimulation and bioaugmentation,» *Environ Sci Pollut Res (2010),* vol. 17, pp. 1339-1346.

[68] Guarino, C., Spada, V. et Sciarrillo, R. (2017). «Assessment of three approaches of bioremediation (Natural Attenuation, Landfarming and Bioagumentation – Assistited Landfarming) for a petroleum hydrocarbons contaminated soil». *Chemosphere,* pp. 1-9. https://doi.org/10.1007/s11356-010-0318-y