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Pilot bioremediation of contaminated soils by hydrocarbons, from an electricity production and distribution site in Ouagadougou, Burkina Faso

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The bioremediation of polluted soils by hydrocarbons is a set of decontamination techniques which exploit the depolluting activity of living organisms in order to reduce the toxicity, mobility or quantity of a contaminant in the environment. This work has enabled the bioremediation of a soil contaminated with hydrocarbons by exploiting endogenous microorganisms of the environment. The depollution method used was the "*off situ*" treatment. Thus, a pilot sample of polluted soil excavated on a site was conveyed to another site accommodating a structure specialized for the biotreatment of the polluted environments. The soil was successively placed in heaps ("heap 1" and "heap 2") on two compartments of a treatment platform and submitted for 08 months to bio-pollution with an improvement in the living conditions of endogenous microorganisms like contribution of substrates, periodic watering and reversal of the medium. Previously, a pre-treatment was carried out on the samples including screening, breaking of large fragments of soil and homogenization. A total oil reduction rate of 62.32% was observed for "heap 1" and 67.92% for "heap 2". The temperature, pH and humidity of the two heaps were varied, respectively between 16 and 39°C, 6.5 and 8 and 35 and 80%. Otherwise, microbiological analysis showed a proliferation of aerobic microflora such as bacteria and molds in the two soil piles.

Key words: Hydrocarbon, polluted soil, bioremediation, microorganisms.

INTRODUCTION

The widespread use of petroleum products in various human activities around the world causes two types of pollution on the environment: one is punctual, localized, brutal and intense, the other diffuse over large areas of land through small amounts of pollutants over a long period (Zein et al., 2006). In both cases, the soils are concerned because of the particles in more or less enormous quantities of hydrocarbons which pour on them.

One of the proven responses to this global

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preoccupation is the remediation of soils, but also of water and air. It includes a variety of decontamination techniques: some are said "*ex-situ*" and aim at the extraction of contaminated material and its treatment in a specific device ("*on situ*" or "*off situ*"), the others are designated as being *in situ* since they allow contaminated material to be treated on site without removing it. The treatments are physical, chemical, thermal or biological (Chu and Kwan, 2003; Van Hamme et al., 2003; Mittal and Singh, 2009). Biological depollution treatment is distinguished by the fact that it exploits the capacity of living organisms (endogenous and/or exogenous), such as microorganisms and plants, to biodegrade or inactivate pollutants such as hydrocarbons under aerobic conditions or anaerobiosis (Peressutti et al., 2003; Vandermeer and Daugulis, 2007). It requires a good knowledge of the environment and its physico-chemical parameters (Tiehm and Schulze, 2003; Aichberger et al., 2005; Vacca et al., 2005; Zein et al., 2006; Zhang et al., 2006; Sawadogo et al., 2015; Okobo et al., 2020).

Several studies have been carried out on the biodegradation of hydrocarbons by microorganisms around the world, but in Burkina Faso, very little research has been carried out (Kaboré-Ouédraogo et al., 2010; Pooja et al., 2014; Sawadogo et al., 2014; Sawadogo et al., 2015; Kaboré-Ouédraogo et al., 2018). However, for the preservation of the health of humans and other living beings with a view to a good ecological balance of the ecosystem, efforts are being made by companies like the National Society of Electricity of Burkina (SONABEL). This state-owned company has designed a major program to clean up parts of land degraded by hydrocarbons used in electric thermal power plants for the production of electricity for several years (SONABEL, 2005). It is in this context that this pilot study was initiated with the general objective of contributing to knowledge in the field of bioremediation of soils contaminated by petroleum hydrocarbons.

The approach of the study consisted, firstly, in monitoring the bio-treatment of soil polluted by total hydrocarbons, physico-chemical parameters during biodegradation (total hydrocarbons, humidity, pH, temperature) and, in a second step, by counting the endogenous microorganisms of the soil samples involved in the biodegradation of hydrocarbons.

MATERIALS AND METHODS

Study site

The studies were carried out at two sites separated by a distance of about 10 km at bird flight. The first site is where the soil was excavated. It is electric power plant Ouaga I of SONABEL (Burkinabé National Electricity Society), always active. The site's altitude is 300.53 m; its position is N12°23.031' and W1°30.927'. Being the first power plant in Ouagadougou, capital of Burkina Faso, its generating set consumes heavy fuel (HFO), some distillated diesel oil (DDO), some oils and some water for them to work (SONABEL, 2005). The oldness of the site (1954), the complexity and the diversity of the industrial effluents (gas, liquid and solid) make it impossible to measure exactly the extent of pollution on the site where the choice of deduction site has been made. However, the sample of the soil of this site was analyzed and showed the effective presence of total hydrocarbons of 33 to 39 g/kg of soil in the first analysis and 9.83 to 136.13 g/kg of soil in the second (Kaboré-Ouédraogo et al., 2010).

The second site is the electric power plant Ouaga III of SONABEL in Kossodo. It is also an active and well-secured site. It hosted the platform built for the pilot bioremediation of contaminated soil and the laboratory for analysis of total hydrocarbons. It is located northeast of the city of Ouagadougou, Position N11° 14.906', W 000° 42.230'; altitude is 254.20 m.

Soil treatment station

The soil treatment station is made up of different structures (Figure 1).

A soil storage platform (before treatment) with a bottom covered with an impermeable polyethylene membrane separating the bedrock from polluted soils. With a capacity of around 170 m^3, it also allows the pre-treatment of contaminated soil (screening, homogenization, organic matter amendment, washing if necessary, etc).

A treatment platform is made up of two compartments of the same size with a border raised by a small bench made of concrete blocks. Each compartment has a length of 5 m, a width of 4 m with a depth of 30 cm. The polluted soil sample was deposited there and bio-treated. To avoid pollution of the bedrock at the host site, a layer of concrete covers the bottom and walls of the treatment tank. On the concrete, a 2 mm thick polyethylene membrane (650 g/m^2) was deposited on it, on which a layer of coarse granite granulated sand (diameter 2-3 mm) was successively installed with a thickness of 5 cm and a layer of crushed granite gravel (diameter 10-20 mm) to a thickness of 10 cm deep. The whole platform was covered by a warning net and by woven plastic mats. The sand and gravel deposited there served as a filter for excess water before it was reused on the sample under treatment. On the whole, the soil sample was deposited in two piles and the whole treatment process was carried out there (Figure 2).

The two compartments, under a shed, were connected to a basin which collected excess treatment water. The shed would limit the influence of external environmental parameters and would avoid the high evaporation of hydrocarbons so that their biodegradation is carried out. It is fitted with a gutter fixed to the posts linked with a PVC pipe leading to a water tank.

A water tank was equipped with a wrapper, recirculation and reuse network for excess treatment and rainwater.

Other devices and accessory materials used included two taps with running water, two buckets, two wheelbarrows, shovels, dabas, an irrigation pump, a flexible hose fitted with a diffuser, a thermometer, a moisture meter, a turbidimeter for metering total hydrocarbons, a lost well, and a concrete block fence to secure the entire treatment site.

Substrate (NPK and Straw)

The NPK substrate (nitrogen, phosphorus, potassium) 15/15/15 is an inorganic fertilizer supplied to the soil as mineral matter and as a regulating element for the balance of the nutritive components of the medium to be decontaminated.

Straw is a vegetable organic matter, coming from grass stems (millet). It was chopped, using a cutter, into pieces about 5 to 10 cm long before being used.

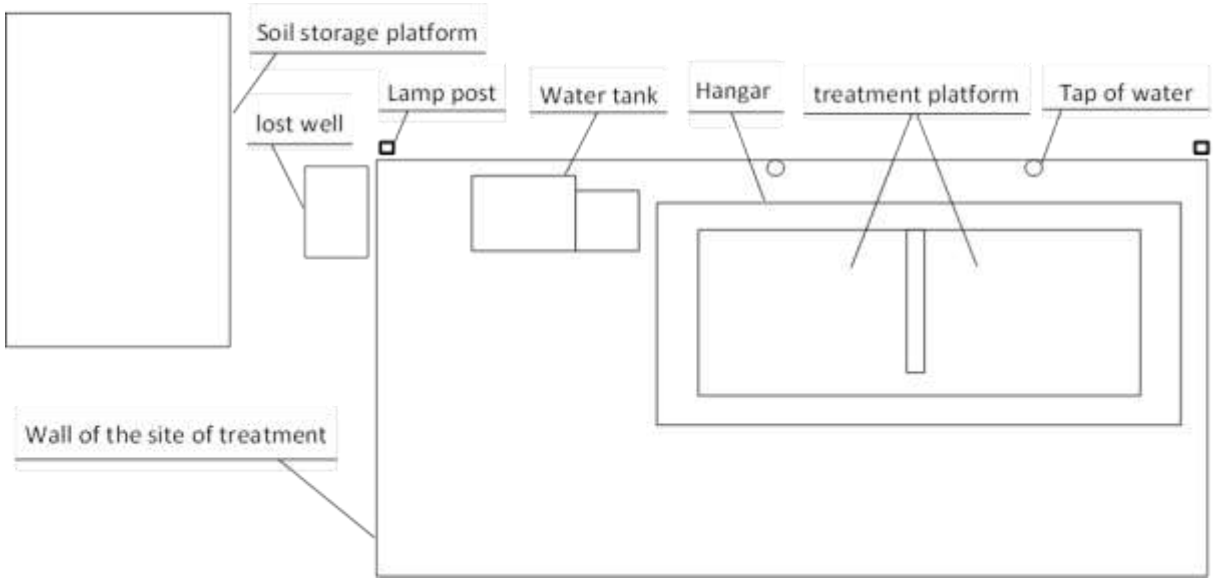

Figure 1. Diagram of the contaminated soil treatment station seen.

Figure 2. Processing platform for the pilot sample of polluted soil and some accessories at the Ouaga III station of SONABEL.

Sampling of polluted soil on the Ouaga I electric thermal power station

Given that the soil treatment was combined with that of the groundwater of the said site, polluted soil came from three wells, each with non-usual dimensions: 1.5 m of diameter and 2 m of deep. It was transported and deposited on the storage platform.

Sampling of polluted soil to be treated

For 18 tons of pretreated soil, 9 kg of NPK and 250 kg of straw are used in each compartment.

Sampling of polluted soil under treatment to be analyzed

Each month, two samples of polluted soil under treatment were taken from the soil piles. Each of them was made up of ten composite samples taken arbitrarily from the entire surface of each pile of polluted soil being treated. A total of eighteen (18) samples of polluted soil being treated were analyzed.

Soil pre-treatment

Pre-treatment involved screening of large stones, breaking large fragments of soil into small pieces (≤ 5 cm in diameter), addition of organic and inorganic substrates, and then homogenization of the soil sample. The mixture to be treated was disposed in heaps in each of the two compartments of the treatment platform. The biodegradation of hydrocarbons was activated by wetting the medium (watering until immersion then draining) at initial rate of 85% humidity.

Bioremediation of soil polluted by hydrocarbons

The mode of bio-depollution of contaminated soil used is the "*off situ*" treatment. The endogenous microorganisms of the contaminated medium were put under adequate conditions in order to optimize their activity for the use of the hydrocarbons of said medium. This technique is used in particular for the bio-degradation of low volatile hydrocarbons like diesel fuel.

Thus, after pretreatment of the medium contaminated with hydrocarbons, the bioremediation was immediately started. It consisted of watering the polluted soil samples 3 times a week, turning them over (for oxygen supply) once a month for 8 months.

Soil analysis methods

Physico-chemical parameters

The total hydrocarbons in the soils were dosed from PetroFLAG® Analyzer System in Soil coming from Dexsil Corporation order N° NA, 1996. The used detection methods have been the turbidimetric development.

The humidity rate was measured humid-meter (Whaterscout TDR 100 – Spectrum), temperature was determined by thermometer (Lee Valley Tools) and pH was obtained with a pH-meter (PCE-PH20S).

Microbiological analysis

The counting of total mesophylic aerobic flora and fungus was done, respectively with Plate Count Agar (PCA) and Sabouraud Agar, after 24 to 48 h of incubation.

Data treatment

The data were analysed using Microsoft Excel and XLSTAT-Pro 7.5. Analysis of variance was ANOVA with a probability point p = 5%. It determined the significant difference among the data obtained.

RESULTS

Degree of pollution of collected samples

At time zero of bio-treatment, the soil samples of "heap 1" and "heap 2", respectively have a total hydrocarbon content of 12.31 ± 0.71 g/kg of dry soil and 12.93 ± 0.82 g/kg dry soil. These values indicate moderately polluted soils.

Reduction of total hydrocarbons

The polluted soil of each pile, submitted to the ambient temperature of the environment, watered three times a week and stirred once a month, showed over time, a significant reduction in total hydrocarbon levels in the polluted soil. Thus, this rate, which was 12.31 ± 0.71 g/kg of dry soil for "heap 1" and 12.93 ± 0.82 g/kg of dry soil for "heap 2" at the start of the treatment decreased, respectively to 4.64 ± 0.16 g/kg of dry soil and 4.15 ± 0.37 g/kg of dry soil in eight (08) months of treatment. A reduction in the total hydrocarbon content of 62.32% in "heap 1" and 67.92% in "heap 2" was observed (Figure 3).

The total oil content of "heap 1" dropped significantly in the first month of treatment until the third month and then in the eighth month. Between the fourth and eighth month, from one month to the next, the difference in the reduction in total hydrocarbons was not significant (Figure 3).

The decrease in total oil in "heap 2" of the polluted soil sample was significant in the first, third, fourth and eighth months of treatment. The biodegradation of total hydrocarbons in the third month was significant compared to the first month. However, it was not significant during the first to second month of treatment, the second to third month, or the fourth to seventh month. The reduction in total oil content in the first three months was quick for the two heaps of polluted soil. Thus, the abatement rate was 51.22% in "heap 1" and 51.03% in "heap 2".

This study, having used the technique of bio-depollution by composting, was able to obtain in 8 months a reduction rate of 62.32% for "heap 1" (with initially 12.31 g of hydrocarbon per kg of soil) and 67.92% for "heap 2" (with 12.93 g of hydrocarbon per kg of soil at the start).

Temperature, humidity and pH of polluted soil during treatment

In addition to the total hydrocarbon content measured, the other physico-chemical parameters (humidity, pH, temperature) of the polluted soil samples were also tested (Table 1).

The humidity of the polluted soil in "heap 1" and "heap 2" varied between 35 and 80% during treatment. The biodegradation medium is watered up to 80% humidity and when the humidity drops to 35% or even 40%, the soil is again wet.

The pH of the bio-treated soil located between 6.8 and 8 in "heap 1" then 6.5 and 8 in "heap 2" was not far from neutrality which is 7. In fact, it was mainly for the majority of the treatment period either neutral or slightly basic. However, it was noted only one month when the pH was slightly acid (5th month for the first pile and 6th month for the second) but without being significantly different from the rest of the pH during the treatment. It did not vary significantly during treatment.

The temperature measured was mainly that of the

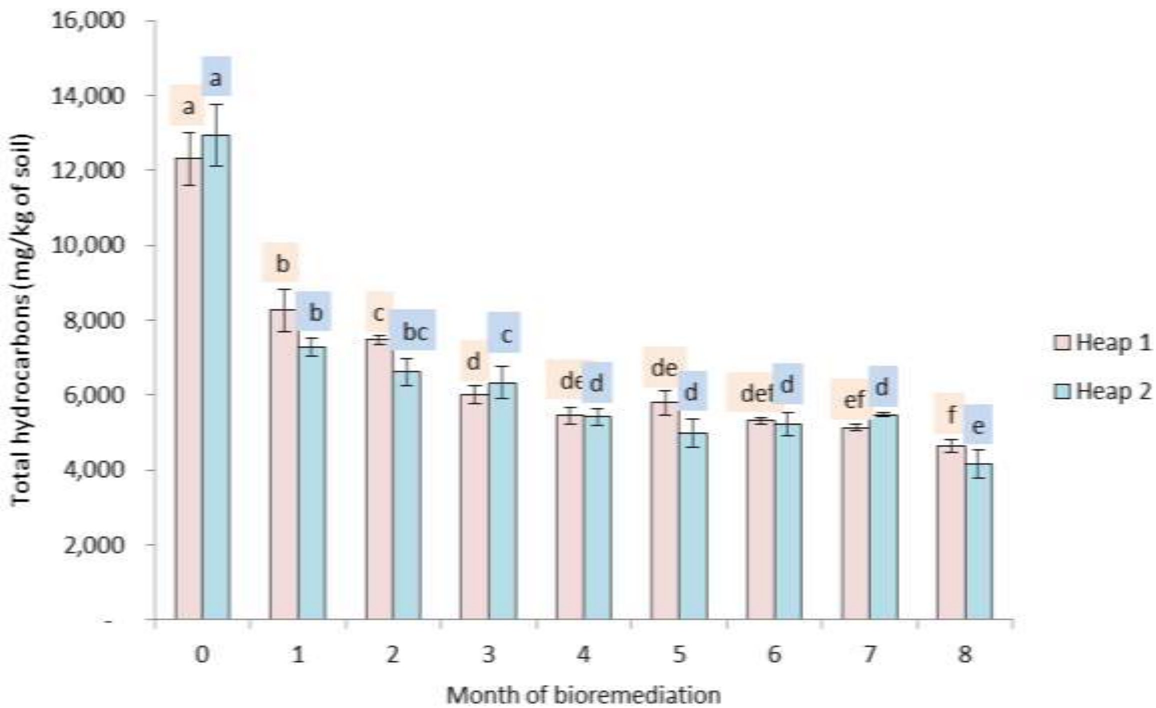

Figure 3. Abatement of total hydrocarbons in polluted soil as a function of biodegradation time. For each heap, different letters indicate significant differences ($p < 0.05$).

Table 1. Temperature, humidity and pH of the soil polluted during treatment.

Month	Temperature (°C) of 2 heaps	Heap 1		Heap 2	
		Humidity (%)	pH	Humidity (%)	pH
0	26-38.4	35-75	7	40-67	7.5
1	22-37	45-75	7.5	45-75	8
2	23,2-33	40-74	7	50-80	7.5
3	24-36.6	40-70	8	40-70	7.3
4	23-37	45-70	7.5	40-70	7
5	19-35	41-71	6.8	35-68	7
6	16-36	40-75	7.2	38-80	6.5
7	18-36	48-75	8	40-78	7.2
8	24-39	40-78	7	40-80	7

ground and the environment under the platform shed. During the eight (08) months of sample processing, the measured temperatures of the day (at 8, 1 and 5:30 h) varied from 16 to 39°C for the two heaps and from 16 to 40°C for the environment under the shed.

Enumeration of polluted soil microorganisms

The microbiological analysis has permitted to count the microscopic organisms of the environment under bio-treatment. The counting of the microflora in the soil was carried out once a month. The samples taken before treatment in "heap 1" and "heap 2", respectively show that (Figure 4):

(1) The number of total microflora is 4×10^4 and 6×10^4 CFU/g of soil
(2) The number of molds is 24.5×10^2 and 46.5×10^2 CFU/g of soil

According to statistical analyses, the number of total microflora like that of molds in "heap 1" and "heap 2" in the polluted soil sample varied significantly most of the

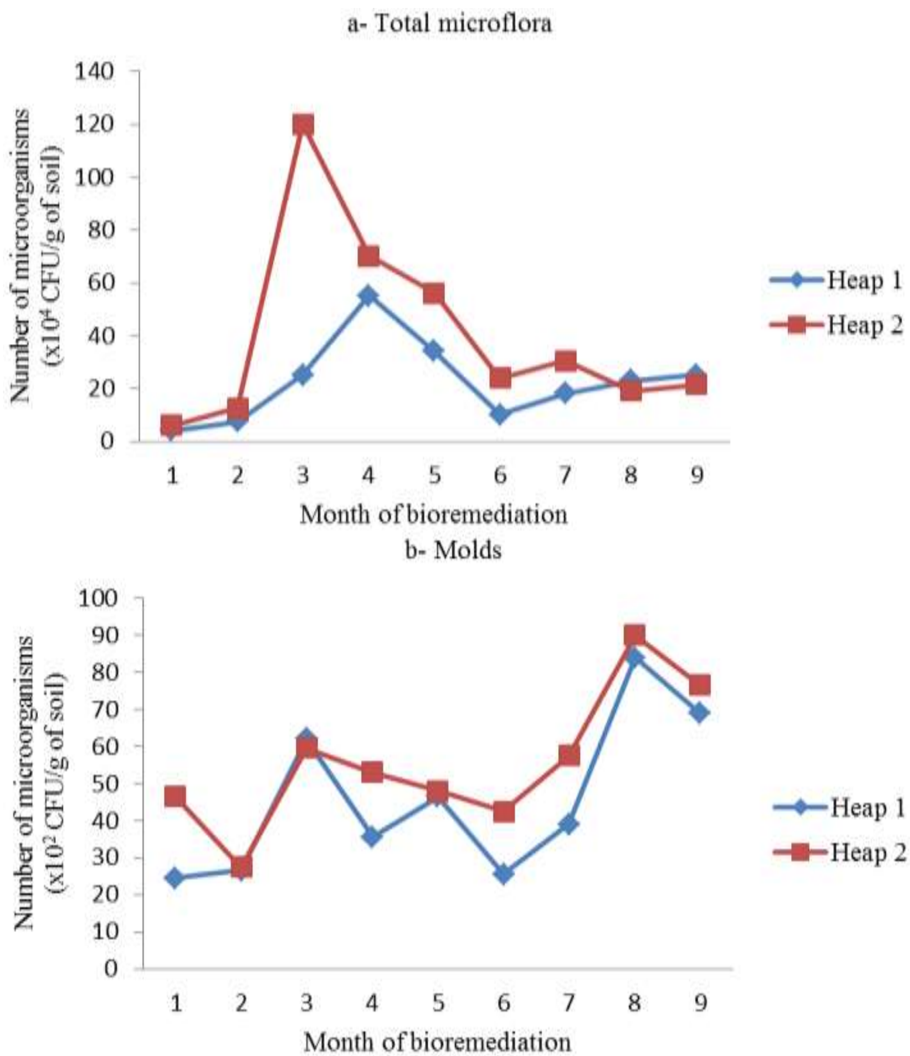

Figure 4. Enumeration of the total microflora (a-) and molds (b-) of the two soil samples "heap 1" and "heap 2".

time of bio-decontamination (Figure 4).

Total microflora

"Heap 1" is the soil sample with fewer microorganisms. Overall, the total microflora varied between 4×10^4 and 55×10^4 CFU/g of polluted soil, with a peak in the third month. During treatment, the number of microorganisms increased significantly until the third month and then decreased significantly until the sixth. From the sixth month, no significant variation was noted, even if the number of microflora increased (Figure 4a).

The total microflora of "heap 2", was initially at 6×10^4 CFU/g for the polluted soil sample to be submitted for treatment, increased significantly in the first two months of bio-depollution, decreased significantly in the three months that followed and fluctuated (without a significant difference) for the last three months. In the second month, the highest biomass value (120×10^4 CFU/g) of the medium was noted during the eight months of treatment (Figure 4a).

Molds

The number of molds in "heap 1" was the highest compared to that in "heap 2". During the bio-processing of "heap 1", this number varied significantly up or down from month to month. The number of molds decreased significantly in the first month of treatment ($27.5°10^2$ CFU/g of soil) then oscillated during the treatment time and reached its peak (90×10^2 CFU/g of soil) in the seventh month (Figure 4b).

The number of molds in "heap 2" did not remain constant during bio-remediation. It varied significantly between 24.5×10^2 CFU/g and 84×10^2 CFU/g. There were fewer molds in the first month and more in the seventh month which was marked by a peak, followed by a slight decrease the following month. Like the number of molds in "heap 1", the first three months of soil treatment saw an increase in the number of molds. However, in the last four months, the mold biomass had evolved significantly in a saw tooth fashion (Figure 4b).

DISCUSSION

Bio-depollution of soil polluted by hydrocarbons

The abrupt reduction in the total hydrocarbon content in the first three months and the slight decrease in the last five months of treatment could be explained by several reasons.

(1) A real daily biological activity in each soil linked by the improvement of the living conditions of soil microorganisms. Previously, by following the evolution of the quantity of CO_2 released by polluted soil samples taken on the same study site as a function of time and of the hydrocarbon content, Kaboré-Ouédraogo et al. (2018) confirmed the existence of real daily biological activity.

The increase in number of microorganisms would be the basis for the increase in biological activity which would result from the mineralization of organic matter such as hydrocarbons in the environment hence their considerable drop in the environment (Kaboré-Ouédraogo et al., 2010; Kaboré-Ouédraogo et al., 2018).

Regular watering of the polluted soil samples under treatment helped to maintain the humidity of the environment. This humidity would not only affect the life of microorganisms in the environment but would also facilitate the process of enzymatic degradation and the contact between microorganisms and pollutants. It is the basis of the increase in water activity in the studied polluted soil samples and would have allowed the rapid development of the microorganisms found there. According to Ibrahim et al. (2011) and van Laarhoven et al. (2015), humidity acts on the growth of the mycelium, sporulation and the germination of spores. Thus, in a soil polluted with permanently wet hydrocarbons, it was noted that molds invade it quickly. The importance of this parameter in soil bio-depollution has already been stressed by authors like Naseri et al. (2014).

The overturning (oxygen supply) would have allowed living things in the environment to be in better aerobic conditions to develop and degrade the hydrocarbons found there. Also, an interaction would therefore be made between the surface of the heaps of polluted soil and the atmosphere to allow better aeration of the environment and good aerobic biodegradation by endogenous microorganisms in the environment. Several authors have stressed the importance of oxygen (by increasing or decreasing it in the environment) in the metabolism of microorganisms during the bioremediation of soils polluted by hydrocarbons (El-Kadi, 2001; Yang et al., 2009; Olivera et al., 2015).

The addition of mineral and organic substrates such as NPK fertilizer and straw serves as nutrient supply for soil microorganisms. These nutrients contain chemical elements (nitrogen, phosphate and potassium) capable of stimulating the mineralization of hydrocarbons in the environment (Kaboré-Ouedraogo et al., 2010; Abioye et al., 2012; Olivera et al., 2015). The straw, thanks to its rigidity and spongy and structuring aspect, also increases the porosity of the medium and allows better aeration (which is a main limiting factor of microorganisms like the majority of molds) of the soil sample and good water retention (Qin et al., 2013).

(2) The pH which did not vary significantly and which remained close to the neutral pH would have enabled good activity of microorganisms (bacteria and microscopic fungi) in polluted soil samples hence its influence on the degradation of the total hydrocarbons of said medium. It has been demonstrated (Lin et al., in 2010; Sawadogo et al., 2014; Nareen, 2018) that the growth of microorganisms is favored by a pH close to neutral or slightly basic conditions. Other authors such as Leahy and Colwell (1990) have attested that under slightly basic conditions, the biodegradation of hydrocarbons is higher even if it depends on certain culture conditions and the types of microorganisms.

(3) The temperature of the two heaps (1 and 2) of soil under treatment ranged between 16 and 39°C. It would have enabled, like the other physico-chemical parameters, a good microbiological activity in the medium and a decrease of the rate of total hydrocarbons during these eight months of soil bio-depollution. Sawadogo et al. (2014) noted that 35 to 38°C was the temperature range that allowed better growth of the two strains of bacteria isolated and cultivated in a medium containing hydrocarbons. Authors such as Leahy and Colwell (1990) have pointed out that the metabolic activity of microorganisms is at a temperature between 30 and 40°C maximum. Thus, Song et al. (1990) and Walworth et al. (2001), during the bioremediation of polluted soil, all concluded that with higher temperatures, the rate of biodegradation of hydrocarbons increased. At the time of this study, during periods when the temperature was below 30°C, the rate of biodegradation of the pollutant would have decreased. However, it did not prevent a significant drop in the level of total hydrocarbons during the eight months of bio-treatment. In short, the improvement in the physico-chemical conditions (humidity control, aeration and monitoring of the variation in pH, etc.) of the environment made it possible to note in the first moments, a good reduction in the rate of hydrocarbons. Physico-chemical parameters play an important role in the reduction of the hydrocarbons in polluted soil samples.

Microbiological analyses of soil polluted by hydrocarbons

In the two soil heaps, the microbiological enumeration of the samples of the polluted soil in bio-treatment has permitted to perceive sometimes an evolution, sometimes a decrease in microorganisms, especially molds in the soil.

The presence of total microflora during the eight months and the increase in their number the first three months for "heap 1" and the first two months for "heap 2" confirm a real biological activity in the soil samples. Peressutti et al. (2003) noted the increase in the number of microorganisms degrading hydrocarbons in the first four months of treatment of polluted soils. The soil, a medium naturally rich in microorganisms, contains a biodiversity of bacterial and fungal strains cultivable under aerobic conditions on solid medium. An interaction, likely to play favorably or not on the bio-decontamination of polluted soil, could be observed between these different microbiological strains (Yamada-Onodera et al., 2002; Zhang et al., 2006; Nikhil et al., 2013; Adekunle and Adeniyi, 2015; Nrior et al., 2017; Williams and Inweregbu, 2019).

The number of microorganisms which varies over time is linked to several parameters such as: (i) the availability of nutrients over time; (ii) the recalcitrance and/or toxicity of certain hydrocarbons such as aromatic polyclinic hydrocarbons; (iii) environmental conditions such as the ambient temperature which varies from one period of the day to another, from one day to another and from one season to another, thus influencing the growth of microorganisms.

Adenipekun and Fasidi (2005), in a study of bioremediation of polluted soil, also showed that the drop in pollutants (total hydrocarbons) would be linked to their mineralization in the environment by microorganisms. They also pointed out that this drop in the studied soil samples would be greater if they were submitted to more treatment time.

Conclusion

This study has permitted to follow, for eight months, the biodegradation of total hydrocarbons in samples of polluted soil. The shutter release for better bio-decontamination of the soil was the improvement of the living conditions of endogenous microorganisms of the environment. Thus, the supply of nutrients, watering, turning and monitoring of certain physico-chemical parameters (pH and temperature) of soil have enabled microorganisms to transform and/or mineralize the environmental pollutant. Based on the present work, it can be concluded and recommended that microorganisms should be used to remediate the soils polluted by total hydrocarbons.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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