

Full Length Research Paper

Impact of pollution by the hydrocarbons on the biological activity of soils in Ouagadougou, Burkina Faso

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The microbial activity estimated by respirometry test method made it possible to measure the quantity of CO₂ produced per day, from four soils polluted at different degrees by hydrocarbons. These samples have been wet to 2/3 of their maximal capacity of retention and incubated at 30°C for 30 days. The quantity of CO₂ released and measured shows a high value (83.97 mg/100 g of soils against 36.05 mg/100 g of soils) in soils highly polluted by hydrocarbons treated without cow dung. We get higher values (148.98 mg/100 g of polluted soils against 66.52 mg/100 g of not contaminated soils) when the cow dung is used as a ferment. The total aerobic mesophilic flora in the samples of soils has been counted on the nutritive agar. The result is that the total aerobic mesophilic flora varied from 1×10⁴ Colony Forming Unit (CFU) to 50 × 10⁴ CFU before treatment and from 5×10⁴ CFU to 420×10⁴ CFU after 30 days of treatment. During the treatment of the soils, the bacteria and molds microflora increases with time while yeasts disappear progressively.

Key words: Respirometry, microorganisms, contaminated soils, hydrocarbons.

INTRODUCTION

The use of fossil fuels such as hydrocarbons has increased for fifty years. As it is the case in most of the African countries, the main energy sources used in Burkina Faso for the production and distribution of

electricity are hydrocarbons (Ouédraogo and Sodr , 2006; Tetteh, 2015). Their use engender the liquid and gas waste like polycyclic aromatic hydrocarbons, oxide of carbon, oxide of azote, etc., which are sources of

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pollution and greenhouse gas. Therefore, these pollutants have many consequences for the people and their environment (Cerniglia, 1992; Morelli et al., 2005; Ouédraogo and Sodr , 2006; Pinheiro et al., 2013; Jacob and Irshaid, 2015; Sawadogo et al., 2015; Tetteh, 2015; Sawadogo, 2016). The action of pollutants on men is a direct or indirect contact with these hydrocarbons that can sometime be mutagenic or carcinogenic (Pinheiro et al., 2013; Jacob and Irshaid, 2015).

In addition to the pollution of the atmosphere of the water, one of the most worrying forms of pollution is that of the soils on which hydrocarbons are spilled (Colin, 2000; Vandermeer and Daugulis, 2007; Pinheiro et al., 2013). The toxicity of these hydrocarbons varies according to several factors like their nature, composition, concentration, biodegradability, etc (Lecomte, 1998; Vanishree et al., 2014; Wuana et al., 2014). The effect of soils pollution is to question their biological activity which can affect the ecological balance of the ecosystem (Peressutti et al., 2003; Pinheiro et al., 2013; Tetteh, 2015).

This study analyses, the impact of pollution by hydrocarbons on the biological activity of soils. It is specifically about testing the respirometry of microorganisms of four soils from an industrial site, polluted at different degrees by total hydrocarbons. The process passes by a counting of the microorganisms and the introduction of cow dung in the different samples of polluted soils in order to study their biological activity.

MATERIALS AND METHODS

Study site

These studies have been realized in the area of the electric power plant Ouaga 1 of SONABEL (Burkinab  National Electricity Society), in Ouagadougou, capital city of Burkina Faso. For Garmin GPSMAP 62s, the site's altitude is 300.53 m; its position is N12°23.031' and W1°30.927'. In a Soudano-Sahelian climate with a leached tropical ironed soil, Ouagadougou has four big power plants (Ouaga I, Ouaga II, Kossodo, Komsilga), the oldest and most polluted of which is Ouaga I, the site of the present study. Being the first power plant in Burkina Faso, its generating set consume 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) ejects make it impossible to measure exactly the extent of pollution on the site.

Soil sampling and measurements

On a square meter (1 m²) soil, five samples have been collected up to 20 cm of depth, mixed, dried in the shade at open air then sieved at 2 mm of meshing. The samplings are done considering the distance of the power plant's site as well. Apart from the soil « A » that has been sampled at 100 m out of the site, the other three (« B », « C », « D ») have been sampled, respectively at 50, 25 and

5 m from the dump in the site where the power plant is. The physical and chemical characteristics of these soils are shown in Table 1.

The total hydrocarbons in the soils have been dosed from an analyzer «PetroFLAG» coming from Dexsil Corporation order N° NA, 1996. The method has been the turbidimetry and the results are shown in Table 1.

Organic substrate

The cow dung has been used as a source of nutritive elements and as a source of microorganisms. Its use would be profitable in relation to the chemical products. It contains ten times more organic substances, total carbon and total nitrogen than the studied polluted soils and a good relation of ideal C/N (15). It is a good ferment for the bio-depollution of hydrocarbons (Table 2).

Setting of the microcosm

Follow-up of the microbial activity by the cumulated evolution of the CO₂ released by the soils according to time, the tenor in hydrocarbons and the quantity of the organic substrate

The experimental units composed of 1 L bottles tightly closed. In each bottle are set a 100 g of the soil wet up to two thirds (2/3) of its maximal capacity of retention, a Borel bottle containing 20 ml of soda (NaOH; 0.1N) and another Borel bottle containing 20 ml of distilled water. The first bottle is used to capture the carbonic gas (CO₂) released by the soil's microorganisms (this bottle is replaced every 24 h during the first 15 days and every 48 h during the last 15 days) and the second bottle is used to maintain the soil humidity in the bottle.

The same process has been repeated adding 1% to the samples of soil and 0.1% of the cow dung to stimulate the biological activity of the polluted soils. This experiment is repeated three times for each type of soil. The appliance is incubated at 30°C in a steam room for 30 days (Figure 1).

The CO₂ released during the study and trapped by the soda is precipitated in a sodium carbonate by the barium chloride (3%). The exceeded soda is going to be neutralized by hydrochloric acid (HCl, 0.1 N) with phenolphthalein as a colored indicator. The quantity of the daily CO₂ obtained by the Dommergues formula (1960) is expressed in mg/100 g of dry soil:

$$Q \text{ (mg/100 g of soil)} = [V_{\text{HCl}} \text{ (white)} - V_{\text{HCl}} \text{ (treatment)}] \times 0.6$$

where V_{HCl} = volume of hydrochloric acid (HCl).

The experimental control has been the empty bottle in which the two flasks containing, respectively soda and distilled water have been placed.

Microbiological analysis

Counting of microorganisms in the polluted soils

The counting of total mesophilic aerobic flora, fungus and yeasts has been done, respectively with Plate Count Agar (PCA) and Yeast Glucose Chloramphenicol (YGC) after 48 h and 5 days of incubation.

Table 1. The physical and chemical parameters of the soil (Kaboré-Ouédraogo et al., 2010).

Hydrocarbons content	Type of soil			
	A	B	C	D
Rate of hydrocarbons (g/kg of soil)	9.83±1.21	19.23±0.38	41.97±0.59	136.13±1.39
Maximal capacity of retention	45.0±2.5	34.0±1.0	27.0±2.0	6.0±0.2
Texture (%)				
Clay	17.65	23.53	33.33	11.76
Total silt	25.49	23.53	25.49	21.57
Total sands	56.86	52.94	41.18	66.67
Organic substance (%)				
Total organic substance	2.638	3.569	4.189	4.948
Total carbon	1.53	2.07	2.43	2.87
Total nitrogen	0.172	0.12	0.104	0.074

A: Less polluted, B: moderately polluted, C: much polluted, D: extremely polluted.

Table 2. The physical and chemical parameters of the cow dung (Kaboré-Ouédraogo et al., 2010).

Parameter	Results
Total organic substance (%)	43.871
Total carbon (%)	25.447
Total nitrogen (%)	1.69
C/N	15
Assimilable phosphor (ppm)	0.519

**Figure 1.** Setting of the microcosm in the incubator.

Data analysis

The data have been typed with the Excel software, treated with XLSTAT-Pro 7.5. ANOVA with a probability point $p=5\%$.

RESULTS

Evolution of the CO₂ production by soil according to time and the tenor in hydrocarbons

During the 30 days of incubation, it was noticed that the CO₂ quantity, for all the types of soil, increases with the pollution degree. Similar results are also obtained in stimulating the process by the cow dung (Figure 2). Moreover, no significant difference of CO₂ production in the less polluted soils (curves (A), (B), (C)) was observed. Yet, curve (D), from the most polluted soil, shows a production twice higher of CO₂ than the other curves.

Thus, at the end of 30 days of treatment, the cumulated amounts of CO₂ produced from the soils « D », « C », « B » and « A » are, respectively:

- (i) 83.97 mg/100 g of soil; 46.37 mg/100 g of soil; 43.35 mg/100 g of soil and 36.05 mg/100 g of soil for the biodegradation of samples not containing the cow dung (Figure 2A);
- (ii) 96.94 mg/100 g of soil; 61.43 mg/100 g of soil; 56.95

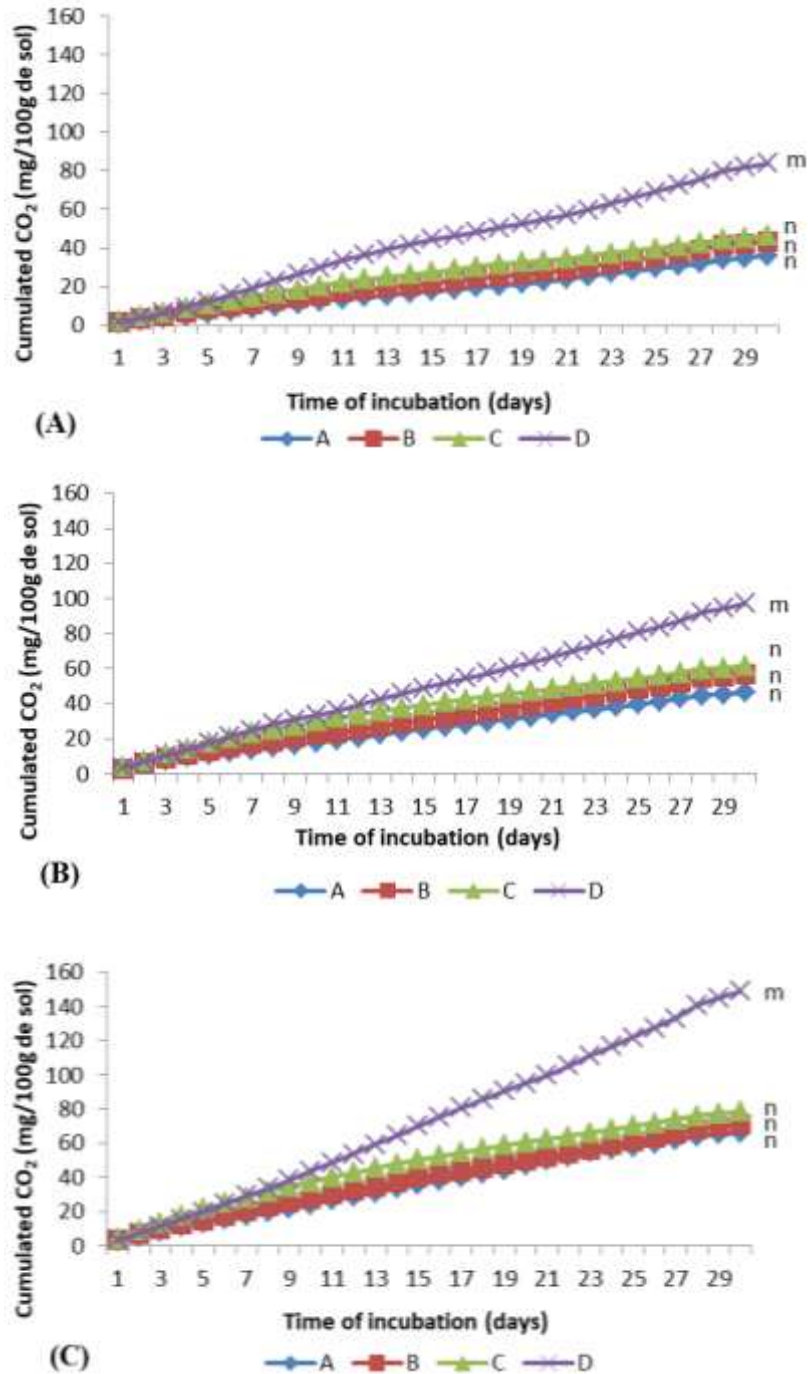


Figure 2. Cumulated evolution of CO₂ released by the microorganisms in 100 g of soil treated without cow dung (A), with 0.1% of cow dung (B), with 1% of cow dung (C) as regards to time (days) and the tenor in hydrocarbon [different letters indicate significant differences (p<0.05)]. A: Less polluted soil, B: moderately polluted soil, C: much polluted soil, D: extremely polluted soil.

mg/100 g of soil and 46.35 mg/100 g of soil for the biodegradation of samples containing 0.1% of cow dung

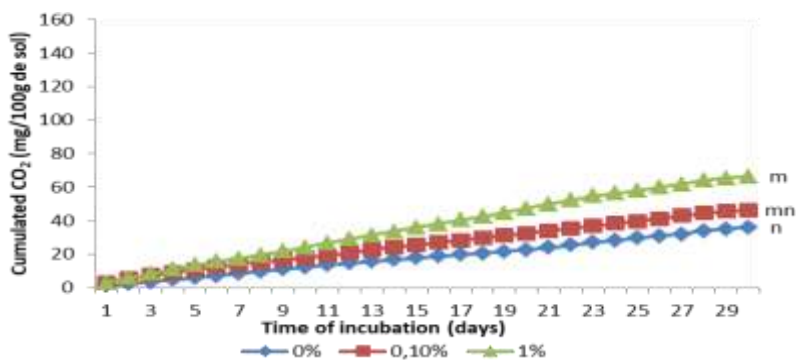


Figure 3. Cumulated evolution of CO₂ released by the microorganisms in 100 g of less polluted soils (A) regarding time (days) and the treatment with cow dung [different letters indicate significant differences (p<0.05)].

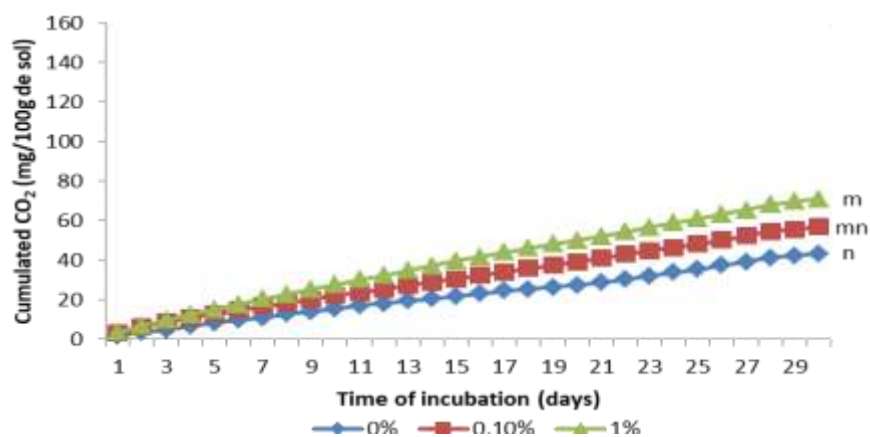


Figure 4. Cumulated evolution of CO₂ released by the microorganisms in 100 g of moderately polluted soil (B) regarding time (days) and the treatment with cow dung [different letters indicate significant differences (p<0.05)].

(Figure 2B);

(iii) 148.98 mg/100 g of soil; 78.81 mg/100 g of soil; 71.13 mg/100 g of soil and 66.52 mg/100 g of soil for the biodegradation of samples containing 1% of cow dung (Figure 2C).

Evolution of CO₂ production by the soil regarding time and the treatment with the cow dung

The intensity of the cumulated releases of CO₂ increases with the addition of the cow dung in the same type of soil. In other words, the soil treated without cow dung releases less CO₂ than the one treated with 0.1 or 1% of cow dung; the sample containing 1% of cow dung being the one that releases more CO₂. In Figures 3, 4, 5 and 6, a

significant difference was noticed between the soils treated without the cow dung and those having 1% of the cow dung. The concentration of 0.1% of cow dung has no significant influence on the release of CO₂ in these figures.

The cumulated amounts of CO₂ of the soils containing 0, 0.1 and 1% of cow dung can reach respectively:

- (i) 36.05 mg/100 g of soil; 46.35 mg/100 g of soil; 66.52 mg/100 g of soil in the less polluted soil « A » (Figure 3);
- (ii) 43.35 mg/100 g of soil; 56.95 mg/100 g of soil; 71.13 mg/100 g of soil in the moderately polluted soil « B » (Figure 4);
- (iii) 46.37 mg/100 g of soil; 61.43 mg/100 g of soil; 78.81 mg/100 g of soil in the much polluted soil « C » (Figure 5);

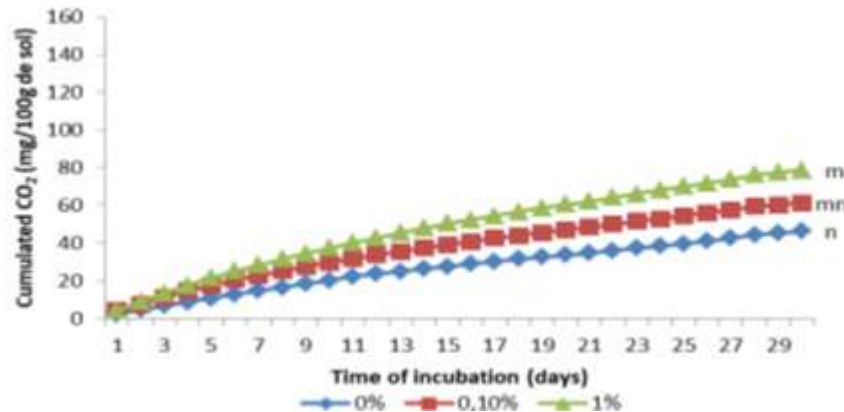


Figure 5. Cumulated evolution of CO₂ released by the microorganisms in 100 g of much polluted soil (C) regarding time (days) and the treatment with cow dung [different letters indicate significant differences (p<0.05)].

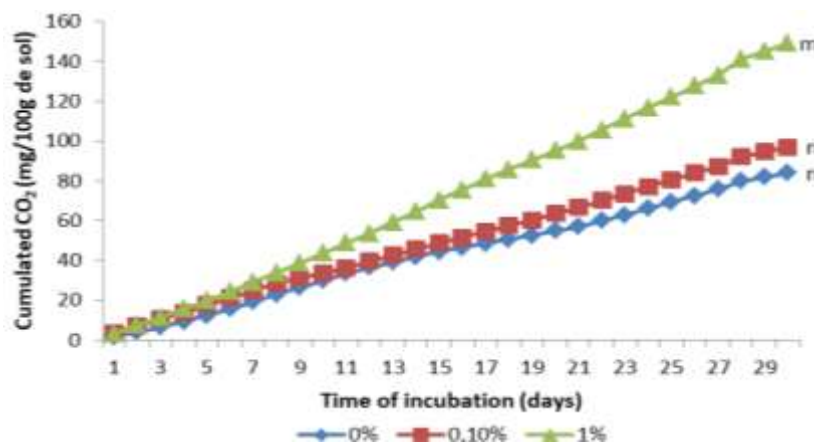


Figure 6. Cumulated evolution of CO₂ released by the microorganisms in 100 g of extremely polluted soil (D) regarding time (days) and the treatment with cow dung [different letters indicate significant differences (p<0.05)].

(iv) 83.87 mg/100 g of soil; 96.94 mg/100 g of soil; 148.98 mg/100 g of soil in the extremely polluted soil « D » (Figure 6).

The slightest pick in each figure results from the soils treated without cow dung (0% of cow dung). The strong release of CO₂ is noticed in the soils treated with 1% of the cow dung.

Microbiological analysis

Counting of microorganisms in the polluted soils

The microbiological analysis showed the cultivable

mesophilic aerobic microorganisms on a solid ground. The counting of total microflora, molds and yeasts has been done (Table 3). The total microflora, present in all the samples of soil, varies from 1×10⁴ to 50×10⁴ CFU before treatment and from 5×10⁴ to 420×10⁴ CFU after 30 days of treatment. The molds are absent only in the soil « C » treated without substrate. In the other soils, their number varies from 1.5 to 33 CFU before treatment and from 2 to 200 CFU after 30 days of treatment. The yeasts are in soils « C » and « D » (6.5 to 10 CFU before treatment and 00 CFU after treatment), but absent in the samples « A » and « B » before as well as after treatment of the soil.

The counting of these microorganisms often varies regarding the degree of pollution, the addition of the

Table 3. Counting of microorganisms (total microflora, molds, yeasts) in the polluted soils (CFU/g).

Type of soil	Quantity of cow dung (%)	Before treatment (CFU)			Thirty (30) days after treatment (CFU)		
		Molds	Yeasts	Micro. ($\times 10^4$)	Molds	Yeasts	Micro. ($\times 10^4$)
A	0	10.5 \pm 0.5	-	6 \pm 1	33 \pm 1	-	140 \pm 10
	0.1	23 \pm 2.0	-	8.5 \pm 0.5	43 \pm 1	-	40 \pm 10
	1	33 \pm 1.73	-	9.9 \pm 1.1	23 \pm 1	-	72 \pm 11
B	0	1.5 \pm 0.5	-	1 \pm 1	2 \pm 1	-	5 \pm 5
	0.1	7 \pm 1	-	1.2 \pm 0.1	31 \pm 1	-	12 \pm 0
	1	14.5 \pm 3.5	-	15 \pm 5	35 \pm 2	-	22 \pm 2
C	0	-	10 \pm 0	10 \pm 0	-	-	196 \pm 34
	0.1	2 \pm 1	8 \pm 1	19 \pm 1	-	-	200 \pm 0
	1	4 \pm 0	9.5 \pm 0.5	40 \pm 10	8 \pm 2	-	225 \pm 5
D	0	3.5 \pm 1.5	8 \pm 1	25.8 \pm 4.2	9 \pm 0	-	240 \pm 10
	0.1	5 \pm 0	6.5 \pm 0.5	32 \pm 2	39 \pm 3	-	390 \pm 20
	1	15 \pm 1	7 \pm 2	50 \pm 10	200 \pm 4.36	-	420 \pm 26.46
Dung	-	4000 \pm 1000	-	340 \pm 98.5	-	-	-

Micro.: Total microflora, A: less polluted soil, B: moderately polluted soil, C: much polluted soil, D: extremely polluted soil.

substrate (cow dung) and time. If the addition of the substrate increases the number of the total microflora and the molds, it has no influence on the yeasts. Also, while the biomass of the total microflora and that of the molds increase with time, the yeasts disappear.

DISCUSSION

Evolution of the CO₂ quantity released by the soil regarding time and the tenor in hydrocarbons

One notice that the microbial activity increases with the degree of pollution of soils. The soil « D », containing the highest hydrocarbon rate and therefore having more fermentable organic substances, induces a strong microbial activity in relation to the other three soils. The slightest productions of CO₂ obtained with soil « A » are the result of a microbial activity from less polluted soil, than containing less organic substances.

The release of CO₂ shows a real daily biological activity in each soil. The microbial activity results from the fermentation of the organic substance in the different grounds (Dommergues, 1962). The daily release of CO₂ shows the mineralization of the pollutants. More the quantity of hydrocarbons in a soil is great, more the mineralization's level of its organic substance is high. This results in a stronger CO₂ rejection by the

microorganisms which are in this soil. The easily biodegradable products (light hydrocarbons, microorganisms dead during the desiccation stage, protein substances, sugar, etc.) are the first elements used by the microorganisms (Chaîneau et al., 1995; Aichberger et al., 2005; Zombré, 2006); then the most resistant substances such as the heavy hydrocarbons (aromatic polycyclic hydrocarbons) or lignin are decomposed (Chaîneau et al., 1995; Aichberger et al., 2005).

Cumulated evolution of CO₂ released by the soil regarding time and the treatment with the cow dung

The slightest quantities of CO₂ got came from the soils treated without cow dung (0% of cow dung). The highest quantities are obtained in the soils treated with 1% of the cow dung. The cow dung has therefore a stimulating effect on the biological activity in the treated samples. It brings to the soil not only an additional ferment, but also some chemical elements like carbon, nitrogen and phosphor. The ferment degrades the organic substances of the soils and the chemical elements stimulate the metabolism of the microorganisms and therefore their growth (Chaîneau et al., 1995; Aichberger et al., 2005; Adekunle and Adeniyi, 2015; Jacob and Irshaid, 2015). The cow dung has, at a certain concentration (1%), a positive influence on the biological activity like the

mineralization of the pollutants in all the soils after 30 days of treatment.

Microbiological analysis

Counting of microorganisms in the polluted soils

Bacteria are present in all the studied samples of soils. Their number increases regarding the pollution of the ground (their tenor in hydrocarbons) and the quantity of cow dung. During the treatment of different soils, their number increases progressively from simple to double in all samples. They would be able to use the hydrocarbons in their growths (Dash et al., 2013; Sawadogo et al., 2014; Vanishree et al., 2014; Adekunle and Adeniyi, 2015; Jacob and Irshaid, 2015; Sawadogo et al., 2015; Sawadogo, 2016).

The number of molds also increased with the addition of the cow dung but is not influenced by the tenor of the soil in total hydrocarbons. They would actively participate in the biodegradation of hydrocarbons in the soil (Okerentugba and Ezronye, 2003; Zhang et al., 2006; Damisa et al., 2013). Many researches confirm that fungi have been capable of biodegrading the hydrocarbons (Vanishree et al., 2014; Adekunle and Adeniyi, 2015).

The yeasts, only present in the soils « C » and « D » disappear after the 30 days of incubation. The high concentration of hydrocarbons would inhibit their biodegradation and would limit the use of oxygen (Adenipekun and Fasidi, 2005; Vanishree et al., 2014). However, much research has shown that they can degrade hydrocarbons (Zhang et al., 2006; Vanishree et al., 2014).

Generally, for their metabolism and reproduction, all living being previously seen use hydrocarbons as source of carbon. Various microorganisms have the capacity to use hydrocarbons as their source of carbon and energy (Adekunle and Adeniyi, 2015). They also need nitrogen and phosphor that also could be found in the cow dung.

These microorganisms would be able to degrade hydrocarbons in the soil (Lecomte, 1998; Okerentugba and Ezronye, 2003; Zhang et al., 2006; Adekunle and Adeniyi, 2015). They would probably make it possible to bio-decontaminate the soil of pollutants such as petroleum hydrocarbons; hence, their decrease (at least 10.64% of abatement after 30 days) more or less considerable at the end of the study (Kaboré-Ouédraogo et al., 2010).

Conclusions

This study made it possible to see the impact of total hydrocarbons on the microbial activity in four soils

polluted at different degrees. Thus, the biological activity of the soils has been measured, thanks to the CO₂ released daily by the microorganisms. It is shown that released CO₂ is very important in soils with a high tenor in hydrocarbons, therefore a high quantity of organic substances. It is once more important after an addition of cow dung to the soils. The measured CO₂ results essentially from a mineralization of the organic substances by the microorganisms.

The presence of hydrocarbons in the soils in this study shows that these fossilized organic substances play an important role in the microbial activity of the ground. Their use by the microorganisms of the soil would involve their biodegradability that could make a depollution of contaminated grounds by the organic substances.

The microbiological analysis made it possible to have a high microbiological diversity in the samples of soil. It made it possible to note an increase of the microbial biomass regarding the increase of the tenor in hydrocarbon, the bringing of cow dung and the time. This microbial biomass plays a role more or less important in the degradation of hydrocarbons. A molecular isolation and characterization of efficient microorganisms in the biodegradation of the hydrocarbons would be performed in subsequent studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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