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The effect of household waste compost and horse manure on physicochemical parameters and biodegradation of total hydrocarbons in sludge polluted by hydrocarbons discharged from an oil refinery in the Republic of Congo

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ABSTRACT

This study aims at evaluating the effectiveness of organic amendments on the treatment of sludge polluted by petroleum hydrocarbons from a refinery in Congo. The experimental device included 10 mini treatment basins with three randomized replications. The experiment was based on a 365-day landfarming technique combining two organic materials (horse manure and compost) and a mixture of the two, added in proportions of 0.5%, 1% and 1.5%, and a control with no amendment. During treatment, samples were taken and physico-chemical analyses carried out using conventional methods. The results showed that the averages for pH, moisture, electrical conductivity, organic matter and C/N ratio ranged throughout the treatment period from 6.8 to 8.1, 8.5% to 20.3%, 108.46 to 442.88 µs/cm, 8.03% to 9.13% and 36.40 to 61.99 respectively. These physicochemical parameters are in accordance with the hydrocarbon biodegradation standards related to good microbial activity. The total hydrocarbon degradation rates ranged from 34.90 for the control and 74% for the HM+CP 1.5% treatment. Thus, the contribution of organic amendments significantly improved the degradation rate of total hydrocarbons in treated sludge.

Keywords: Organic Amendments, Total Hydrocarbons, Bioremediation, Biodegradation, Pollution, Congo.

INTRODUCTION

The Republic of Congo is the third-largest oil-producing country in the sub-Saharan zone, with production estimated at 339/000 barrels/day in 2019. According to the 2023 Finance Law, the Congolese economy is largely dependent on oil exploitation, which represents 90% of exports and around 60% of state revenues. There are several operating oil companies, the main ones are : Total EP Energies Congo (French), Eni Congo (Italian), SNPC (Congolese), AOGC (Congolese),

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Congorep (Perenco-SNPC joint venture), Mercuria (Swiss), Pelfaco (Nigerian), Perenco (Franco-British) and Wing Wah (Chinese) (MEFSIN, 2021).

However, the country has only one refinery which processes crude oil to extract the marketable fractions: butane gas, premium fuel, kerosene, light diesel and heavy fuel oil. Thus, the refinery guarantees the country's energy security, supplying around 70% of its finished product requirements. It has a processing capacity of 1 million tonnes/year. The refinery is linked to the Djeno oil terminal by a 25 km pipeline. According to the refinery site, the main processing unit is the atmospheric distillation. This unit has a processing capacity of 150 tonnes/hour of crude oil, while other secondary units are used to upgrade gasoline and Jet A1. But, the refining has some consequences. It produces waste such as sludge and oily water, and pollutes the soil with hydrocarbons. Indeed, oil refining waste - effluent and sludge - contains a variety of chemical compounds in varying quantities which are usually hazardous (Rahi et al., 2021).

According to Rahi et al (2021), the refining by products include BTEX and polycyclic aromatic hydrocarbons (PAHs). The US Environmental Protection Agency (US-EPA) categorizes oily sludge as hazardous waste (EPA, 2002). It identified 26 main pollutants to be monitored in refinery effluents (Radelyuk et al., 2021). These sludges are produced by petroleum refining, exploration, transportation and storage activities, as well as the deterioration of storage tanks and pipelines (EPA, 2002; Varjani and Upasani, 2019). Since the hydrocarbon compounds in these sludges are toxic and harmful to the environment and human health (Varjani and Upasani, 2019), it is important to treat these wastes once released into the nature to prevent environmental pollution.

The Republic of Congo passed an environmental law to meet international requirements, that is Law N°. 33-2023 of November 17, 2023, on sustainable environmental management in the Republic of the Congo. That law prohibits the direct or indirect dumping, flowing, discharge or deposit of any solid, gaseous or liquid substance likely to degrade the environment. It also requires operators in the oil sector to repair damage to people, property and the environment.

For example, some operators have set up waste treatment units or outsource the management of their petroleum waste by transforming hydrocarbon sludge into biological agricultural fertilizer using landfarming technology, which is one of the bioremediation techniques (Petrescu et al., 2017; Morabo Okoletimou et al., 2023). This technique involves the application of soil improvers combined with imported inoculum and chemical fertilizers, resulting in additional loads and a fairly limited degradation rate (Kaboré-Ouédraogo et al., 2010; Morabo Okoletimou et al., 2023). Landfarming is a treatment combining biostimulation and bioaugmentation to accelerate the hydrocarbon degradation process as highlighted by several authors (Gkorezis et al., 2016; Lang et al., 2016; Brown et al., 2017; Varjani and Upasani, 2019).

Landfarming is a biotechnology that involves the construction and implementation of onsite or ex site treatment units, regular watering, nutrient input and sludge turning to distribute water/nutrients and aerate the soil, as well as sampling, sludge and leachate analyses (Hansen et al., 2004). Land application of soil amendments is applied to fine soils contaminated with a wide range of other chemical pollutants (Hamdi et al., 2007; Siméon et al., 2008; Brown et al., 2017; Hogan-Itam, 2020;). Also, it is a natural source of micro-organisms capable of degrading petroleum-derived by-products (Hamdi et al., 2007). In addition, this remediation technique involves processes aimed at reducing the bioavailability of target pollutants, preventing their transfer to other media (Wannoussa et al., 2015). However, this process is slow because these pollutants are resistant, not very volatile and hydrophobic. This is the case for aromatic hydrocarbons. Several factors can also influence bioremediation efficiency, including temperature, pH, nutritional status, humidity and the chemical composition of pollutants (Gkorezis et al., 2016; Hogan-Itam, 2020). Also, the use of microbes alone has proven limited due to various abiotic factors and chemical reactions taking place in the treatment medium. Thus, the efficiency of using landfarming bioremediation strategies can be improved by adding soil amendments, inorganic nutrients, biosurfactants, bulking agents, biochar (Hogan-Itam, 2020). These adjustments have recently been examined by Siméon et al. (2008) and Hogan-Itam (2020).

Thus, the aim of the present study was to know whether the use of autochthonous organic matter (compost and/or horse manure) can improve the bioremediation of sludge discharged by the oil refinery. This organic matter is supposed to provide microorganisms and nutrients in the degradation medium. The aim is to evaluate the effectiveness of organic amendments or substrates (compost and horse manure) on the depollution of hydrocarbon-polluted sludge.

MATERIALS AND METHODS

Presentation of the study setting

This study was carried out on the premises of the Cité Scientifique in Pointe-Noire, wherein the Chemical Analysis Laboratory of the Institut National de Recherche en Sciences Exactes et Naturelles (IRSEN) Pointe-Noire is located. The exact location of the site is shown in Figure 1.

Polluted sludge and substrates used

The material of this study was a mixture of hydrocarbon-polluted sludge. The polluted sludge was taken from the refinery's sludge collection tank. Organic amendments (household waste compost and horse manure) were used to treat the polluted sludge. Compost was obtained by composting household waste in heaps. The horse manure was collected at the Pointe-Noire horse riding club; it consists of dung and shavings used as bedding.

Sampling of polluted sludge and preparation of experimental device

For the treatment of hydrocarbon-polluted sludge, the biological treatment used in this study was bioremediation via the landfarming technique. Beforehand, 1,000 kg of sludge samples were excavated from the refinery's collection basin (Figure 6), transported to the IRSEN treatment center and subjected to pre-treatment. Pre-treatment was carried out as soon as the sample was received at the treatment site. This first treatment stage consisted in air-drying the sludge for a week and removing any large materials (pebbles, blocks, concrete fragments) to facilitate handling, ensure proper treatment and obtain a homogeneous mixture. After pre-treatment, the polluted sludge was weighed and placed in the mini-tanks at a rate of 20 kg per tank.

Experimental device

The experimental device consisted of 10 mini-basins containing 20 kg of polluted sludge, repeated 3 times (Figure 6). Taking into account the maximum water-holding capacity of the mixture of sludge and hydrocarbon-contaminated soil, 2 L of water was added to all treatments at the start of the experiment. Organic amendments (compost and manure) were introduced 24 hours after the addition of water at different rates of 0.5%, 1% and 1.5% of the dry weight of the sludge to be treated in all treatments except the control. Actually, the various combinations of polluted sludge and different proportions of organic amendments gave rise to the following 10 treatments (Table 1).

The experimental device was a randomized complete block design, comprising 3 replicates and 10 treatments (Figure 7).

The experimental system was monitored for 365 days (from January 2022 to January 2023). Throughout the treatment period, oxygenation by mechanical turning of the sludge in the basins was carried out 3 times a week, and 1L of water was added once a week to maintain a humidity level that allowed microorganisms to develop. On day 150, with the exception of the control, biostimulation was carried out, adding the same quantities of organic substrates (compost and horse manure) to all treatments to stimulate microbial activity, supply nutrients and increase the rate of pollutant biodegradation. On day 210, 800 ml of a solution containing hydrocarbonoclast microorganisms was added to the substrate-containing treatments to increase the hydrocarbon-degrading microflora.

pH and electrical conductivity (EC)

These two (2) parameters were determined using a Backlights 5 in 1 multifunction instrument. The measurement was made in a suspension obtained with 20 g of sample in 50 ml of distilled water. Once the solution had been thoroughly homogenized, it was stirred for 30 minutes on a Bioblock SCHOTT GERATE model 7M 120 electronic magnetic stirrer. After standing for 5 minutes, the instrument probe was introduced into the suspension to measure pH and electrical conductivity (Morabo Okoletimou et al., 2023).

Moisture

Moisture content was determined by oven-drying at 105°C to constant weight. The oven used was a Bioblock Scientific Salvis. The procedure consisted in drying 5 g of the sample in the oven for 24 hours. The sample was then weighed, after drying at temperature in a desiccator, until a constant weight was obtained. The moisture content was calculated according to the following formula (Morabo Okoletimou et al., 2023) :

$H\ (\%) = \frac{W_0 - W_1}{W_0} \times 100$ *Équation 1*

W_0 : Sample weight

W_1 : weight of oven-dried sample

Organic matter (OM)

The organic matter content was obtained using the Loss On Ignition (LOI) method. The oven-dried sludge sample was calcined in a Thermolyne type 48000 furnace at 1100°C for 2 hours. The organic matter content was calculated using the following formula:

$LOI\ (\%) = \frac{m_0 - m_1}{m_0} \times 100$ *Équation 2*

With :

LOI (%): Loss On Ignition corresponding to the content (%) of soil organic matter destroyed by calcination;

m_0 : mass of crucible and oven-dried sample;

m_1: mass of crucible and oven-calcined sample.

The carbon content was determined by the following relationship:

$MO\ (\%) = C(\%)\ x\ 1{,}724$ *Équation 3*

Total nitrogen

The total nitrogen was analyzed using the Kjeldahl method with an autoanalyzer after mineralization of the soil sample. This consisted in mineralizing 2 g of cold soil organic matter for 30 minutes with 10 ml of concentrated sulfuric acid in the presence of a mineralization nitrogen catalyst (mix of 80 g potassium sulfate, 31.25 g copper sulfate and 2 g selenium), then hot for 2 hours on the thermostat. The nitrogen recovered as ammonium was treated with 20 ml distilled water and 30 ml sodium hydroxide titrated to 400 g/L, then distilled as ammonia before determined volumetrically (AOAC, 1990; Morabo Okoletimou et al., 2023).

Total Hydrocarbons

The analysis of the total hydrocarbon content was carried out in IRSEN's chemistry laboratory. The method used was the EPA 3510C + EPA 8015D 2003. Total hydrocarbons were extracted from polluted sludge using the Soxhlet method, using 10 g of polluted sludge sample and 300 ml of dichlomethane. The extraction lasted at least 8 hours at a temperature of 40°C. At the end of extraction, the residual solvent contained in the hydrocarbon was evaporated under vacuum until total elimination. The mass of total hydrocarbons is equal to the difference between the mass containing the hydrocarbon residue and the mass of the empty flask. The total hydrocarbon content was determined according to the following formula (Varjani and Upasani, 2019) :

$THC = \frac{m_2 - m_0}{m_1} \times 100$ *Équation 4*

With :

- THC : total hydrocarbon content

- m_0 : mass of empty flask;
- m_2 : mass of flask with extract
- m_1 : sample mass

The hydrocarbon degradability rate is expressed in % and calculated from the following formula (Zairi et al., 2002) :

$$Taux\ de\ dégradation\ (\%) = \frac{C_iHCr - C_fHCr}{C_iHCr} \times 100 \qquad Équation\ 5$$

With:

- C_iHC_r : initial residual hydrocarbon concentration
- C_fHC_r : final residual hydrocarbon concentration

Statistical analysis of data

The raw data were entered into Excel (version 2021), which was also used to plot the graphs. The statistical analysis of the data (ANOVA and comparison of means) was performed using OriginPro 2019b 64 bit software. The Bonferroni test was used for comparison of means.

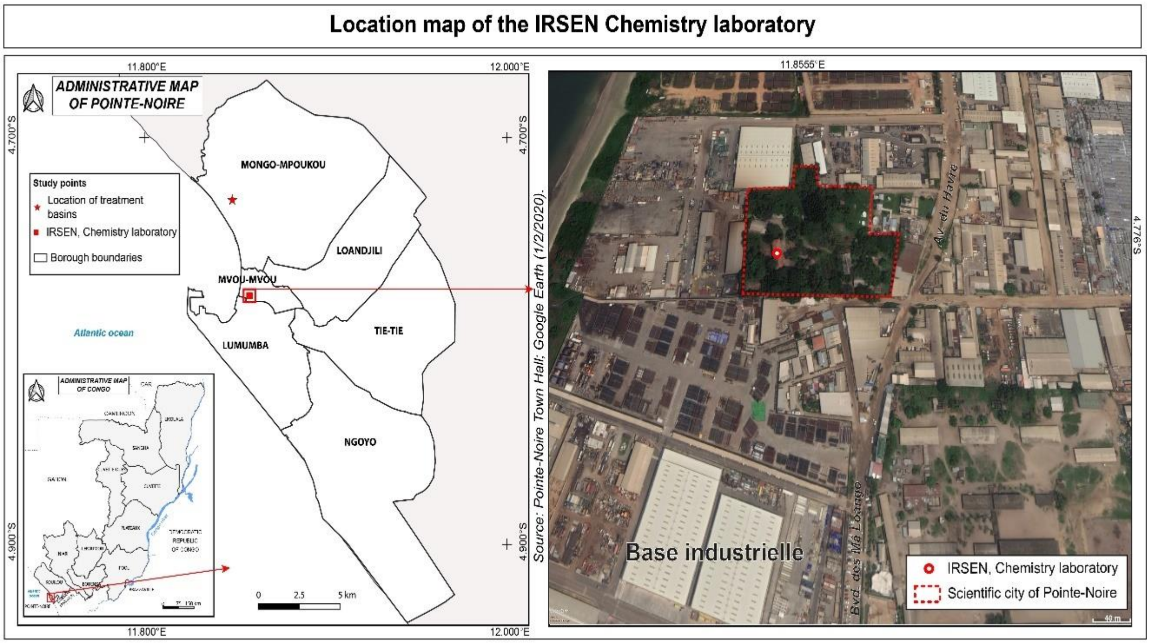


Figure 1 : Location of study site [Sources: Mairie de Pointe-Noire; Google Earth 2020; WGS 84; UTM Zone 32S].

Figure 2 : Polluted sludges.

Figure 3 : Horse manure.

Figure 4 : Compost from household waste.

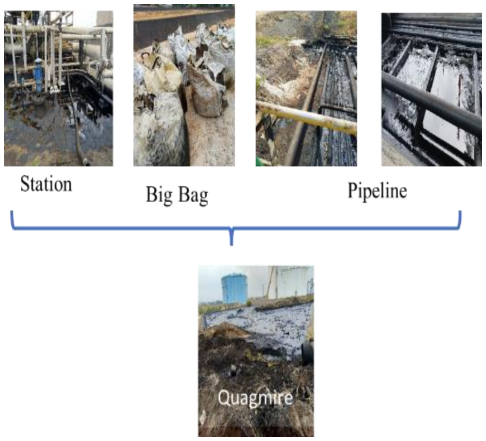


Figure 5 : Sources of polluted sludge stored in the refinery catchment area.

Figure 6 : Landfarming method adopted (pre-treatment of polluted sludge and installation of basins in the bio-center)

BLOCK 1	CP 1.5	CP 0.5	HM+CP 1.5	HM 1.5	HM 0.5	HM+CP 0.5	HM 1.0	CONTROL	CP 1.0	HM+CP 1.0
BLOCK 2	HM+CP 1.5	CP 1.0	CONTROL	CP 0.5	HM 1.0	HM 0.5	CP 1.5	HM+CP 1.0	HM+CP 0.5	HM 1.5
BLOCK 3	HM 0.5	HM+CP 1.0	CP 1.0	HM+CP 1.5	CP 0.5	CP 1.5	CONTROL	HM+CP 0.5	HM 1.5	HM 1.0

Figure 7 : Experimental device (HM : horst manure ; CP : compost).

Table 1: Different treatments tested and their codes.

Treatment code	Composition of treatment applied
Control	Sludge (20kg) + water
HM 0.5	Sludge (20kg) + horse manure (rate : 0.5%)
HM 1.0	Sludge (20kg) + horse manure (rate: 1.0%)
HM 1.5	Sludge (20 kg) + horse manure (rate: 1.5%)
CP 0.5	Sludge (20 kg) + compost (rate : 0.5%)
CP 1.0	Sludge (20 kg) + compost (rate : 1.0%)
CP 1.5	Sludge (20 kg) + compost (rate : 1.5%)
HM+CP 0.5	Sludge (20 kg) + horse manure (rate: 0.5%) + compost (rate: 0.5%)
HM+CP 1.0	Sludge (20 kg) + horse manure (rate: 1.0%) + compost (rate: 1.0%)
HM+CP 1.5	Sludge (20 kg) + horse manure (rate: 1.5%) + compost (rate: 1.5%)
HM : **horse manure ; CP : Compost**	

RESULTS

Physico-chemical analysis of the soil improvers used

Table 2 below shows the results of physicochemical analyses of the amendments used in this study at the start of the experiment. Horse manure was richer in organic matter than compost (83% vs. 29%). With nitrogen contents close to 1%, the two substrates had similar nitrogen contents. With pH values above 7.5, and electrical conductivity well above 600 µs/cm, these organic materials were slightly alkaline and very salty.

Humidity trends

Throughout the experiment, a significant variation in moisture content was observed (Figure 8). Indeed, 30 days later, there was an increase in moisture content in all treatments, with no significant difference between treatments at threshold 0.05 . Nevertheless, the highest content (17.17% ± 1.52) was obtained with the control treatment. Moisture content then varied from one period to the next in all treatments, before a sharp drop in moisture content was observed from 300 days for all treatments, with a peak observed at 270 days. Throughout the experiment, from 0 to 365 days, moisture content varied on average from 11.69% ± 6.98 at the start of the experiment to 13.34% ± 2.41 at the end for all treatments. Considering the total duration of the experiment, the average moisture content for all treatments was 14.28% ± 3.0. Despite significant variability in moisture content over the course of the experiment, the Bonferroni test showed no significant differences between treatments (Figure 9) but between periods at threshold 0.05 (Figure 8).

pH evolution

The analysis of variance (ANOVA) test revealed significant differences between dates and treatments ($p < 0.05$). In all treatments, pH increased throughout, from 6.8 to 8.1 (Figure 8). There were 3 phases of pH variation, with significant differences between them and between them and the control treatment. These variations were influenced by biostimulation and bioaugmentation, as pH was around 7 before biostimulation and around 7.5 after biostimulation, while pH values vary between 7.8 and 8.1 after bioaugmentation.

Depending on the treatment, the pH variation showed three distinct groups of pH values which were close to 7.4, 7.5 and 7.6 and showing significant differences (Figure 9): The highest pH values are found in treatments CP 1.5, HM+CP 0.5, HM+CP 1.0 and HM+CP 1.5, while the lowest values are observed in treatments HM 1.5, CP 0.5 and CP 1.0, with Control, HM 0.5 and HM 1.0 having pH values close to 7.5. These differences in pH values could be due to the proportions and types of organic amendments applied.

Evolution of electrical conductivity (EC)

Figure 12 below shows EC fluctuations over the course of the experiment (from 0 to 365 days) for all treatments combined. At 0 day, the mean EC value was 224.40 µs/cm. By day 30, the EC of all treatments declined to 108.46 µs/cm. Thereafter, EC values increased up to 365 days, with a peak of 442.88 µs/cm observed at 360 days.

In addition, figure 13 below shows that, among all the treatments, the control's EC remained the lowest, at 179.82 µS/cm ± 38.72, while HM+CP 1.5, HM 1.5 and HM+CP 1.0 gave higher values, respectively 428.21 µS/cm ± 205.34, 367.52 µS/cm ± 174.29 and 352.55 µS/cm ± 166.23, compared with the remaining treatments. Despite significant variability in EC values over the course of the experiment, the Bonferroni test showed significant differences between treatments and periods at the 0.05 threshold.

Organic matter (OM)

Figure 14 below shows the evolution of organic matter levels during treatment. The overall average OM content over the entire treatment period was 8.72% ± 0.27. According to the Bonferroni test, there were no significant differences between observation dates at the 0.05 threshold. However, there was a slight drop in OM content at the end of treatment (8.94% ± 0.70 at the start of treatment vs. 8.03% ± 0.74 at the end of treatment).

A comparison of MO contents between treatments on all dates showed that there was no significant differences between treatments compared with the control, except treatments HM 0.5, HM 1.0 and CP 0.5, which had significantly higher and different contents (9.15%, 9.03% and 9.09% respectively) from the other treatments at threshold 0.05 (Figure 15).

Evolution of C/N ratio

C/N ratio values ranged from 52 at the start of treatment to 37 at the end, with a peak of 62 at 120 days (Figure 16). The evolution of the C/N ratio over the course of treatment showed a significant increase ($P < 0.05$) on day 120. From day 150, after biostimulation, the C/N ratio decreased until the end of treatment, with no significant differences between dates ($P < 0.05$) . However, a more significant decrease in C/N ratio values was observed from day 300 onwards. Depending on the treatments applied, C/N values ranged from 51 to 39 (Figure 17). Only those treatments that received manure (HM) or a mixture of compost and manure (HM+CP) at doses of 1% and 1.5% showed C/N ratio values that were significantly different from the control and other treatments, on the one hand, and the lowest, on the other one. During the experiment, the Bonferroni test for this parameter showed significant differences between treatments and periods at the 0.05 threshold.

Evolution in total hydrocarbon content (THC)

Throughout the treatment process, there was a significant decrease in TCH content, from 8.00% ± 0.94 at the start of treatment to 3.42% ± 0.92 at the end (Figure 18). This decrease in THC content was accelerated with the addition of soil improvers (biostimulation) at 150 days and with the addition of microorganisms (bioaugmentation) at 210 days. THC levels in the different treatments applied were significantly different according to the dates (Figure 18) and treatments according to the proportions of organic matter applied (Figure 19) at the 0.05 threshold.. All soils treated with manure (HM) had similar THC levels to the control and were not significantly different, whereas treatments with compost alone or mixed with manure showed significant differences with the control and between the two substrate types. The average THC contents of treatments with horse manure (HM), compost (CP) and the HM+CP mixture were 6.65%, 6.20% and 5.71% respectively. These results indicate that the combination of the two amendments was more effective than the amendments alone.

The kinetics of the evolution of the THC degradation rate showed that this rate was low and variable up to 150 days (figure 20). After the addition of organic substrates (biostimulation) at 150 days, the THC degradation rate increased significantly in all treatments, with a peak of 43% in CP 1.5 at 210 days. These degradation rates continued to rise steadily after the addition of hydrocarbonoclast microorganisms (bioaugmentation), reaching maximum values of 67%, 68% and 74% respectively in treatments HM+CP 1.0, CP 1.5 and HM+CP 1.5 at the end of the treatment.

Table 2: Organic substrate results.

Organic substrate	**Nutrients**			**Physico-chemical parameters**	
	N (%)	**C (%)**	**OM (%)**	**pH**	**EC (µs/cm)**
Horse manure	1.03	47.9	82.6	7.53	3 110
Compost	1.09	16.9	29.1	7.84	3 990

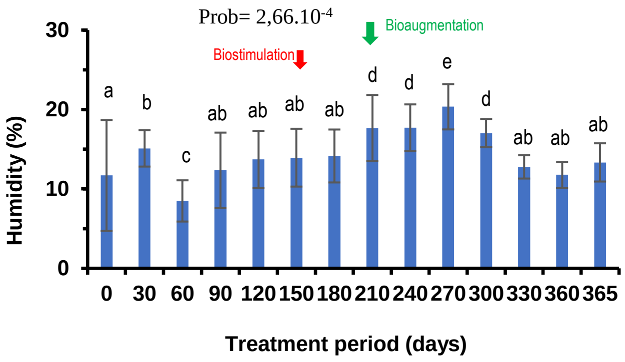


Figure 8 : Moisture evolution by date in all treatments (treatments combined). Levels of significance of comparisons 0.05.

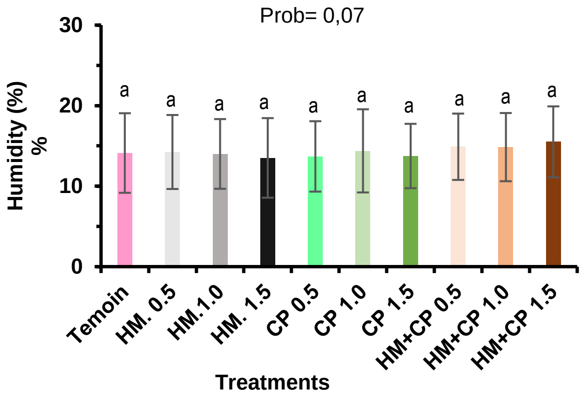


Figure 9 : Average moisture content of treatments over 365 days. Levels of significance of comparisons 0.05.

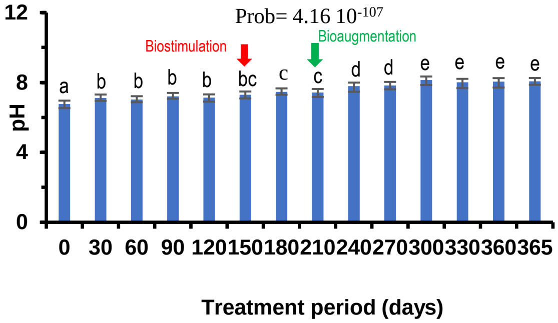


Figure 10 : Evolution of pH by treatment (treatments combined). Levels of significance of comparisons 0.05.

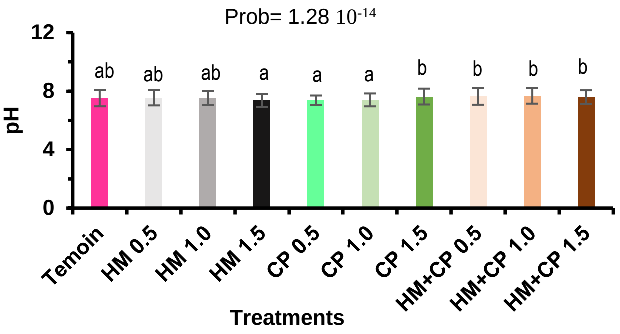


Figure 11 : Evolution of pH in treatments in all treatments over 365 days. Levels of significance of comparisons 0.05.

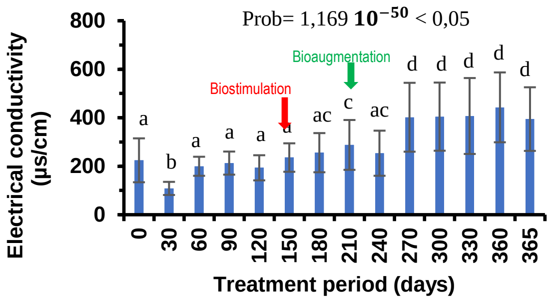


Figure 12 : Evolution in electrical conductivity by treatment (treatments combined). Levels of significance of comparisons 0.05.

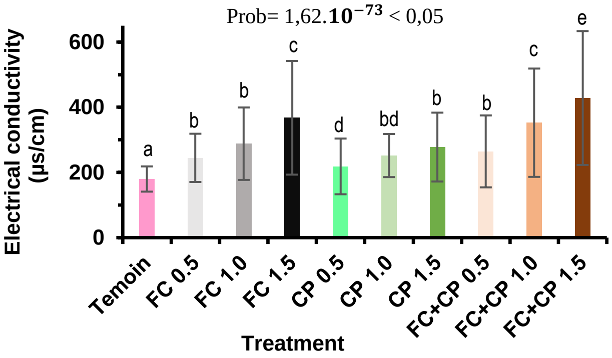


Figure 13 : Evolution of electrical conductivity in treatments as a function of time. Levels of significance of comparisons 0.05.

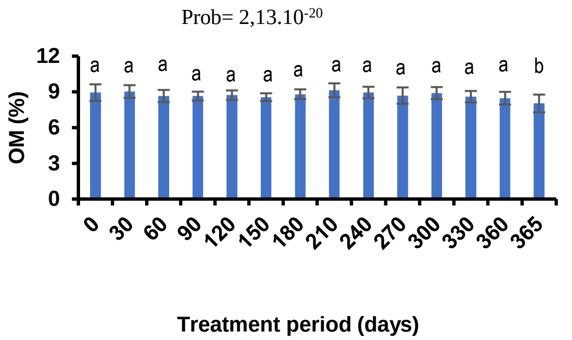


Figure 14 : Evolution of OM according to treatments (treatments combined). Levels of significance of comparisons 0.05.

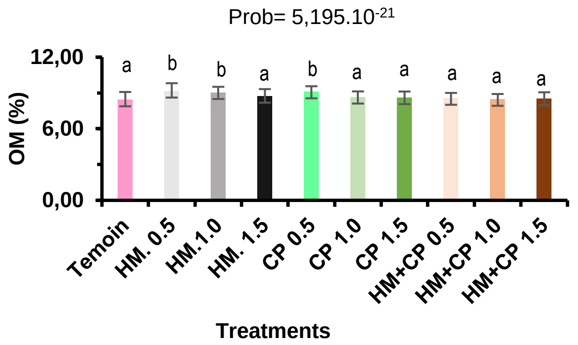


Figure 15 *:* Evolution of OM in treatments as a function of time. Levels of significance of comparisons 0.05.

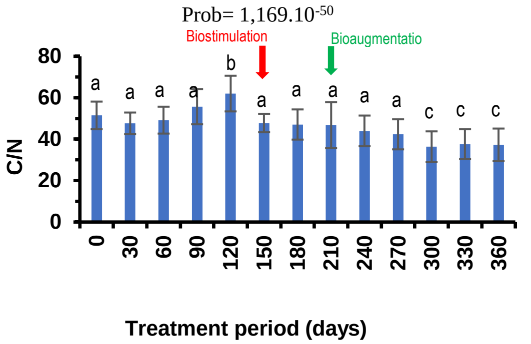


Figure 16 : Evolution du rapport C/N suivant les traitements (traitements confondus). Levels of significance of comparisons 0.05.

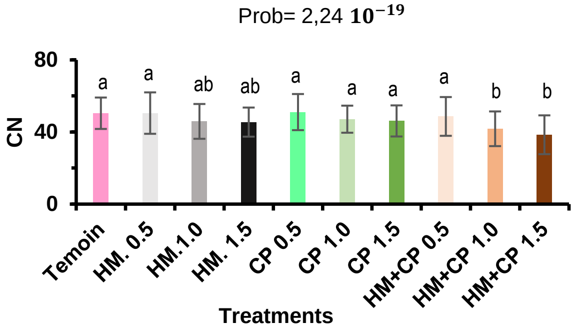
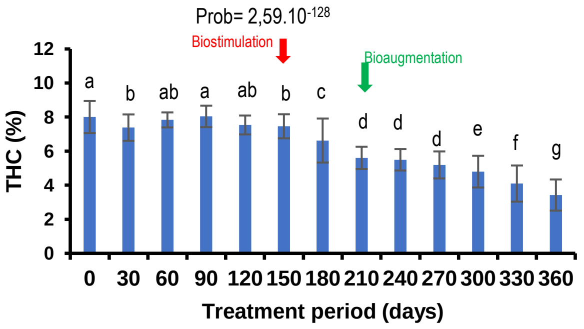


Figure 17 : Evolution du rapport C/N dans les traitements en fonction temps. Levels of significance of comparisons 0.05.



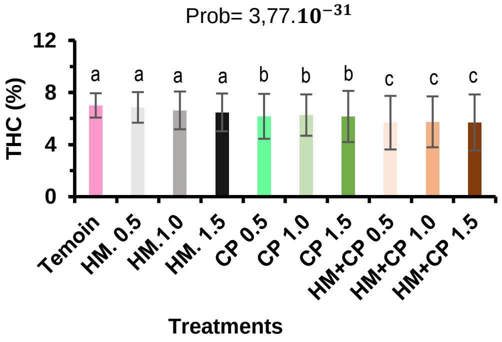


Figure 18 : THC levels by treatment (treatments combined). Levels of significance of comparisons 0.05.

Figure 19 : THC levels in treatments as a function of time. Levels of significance of comparisons 0.05.

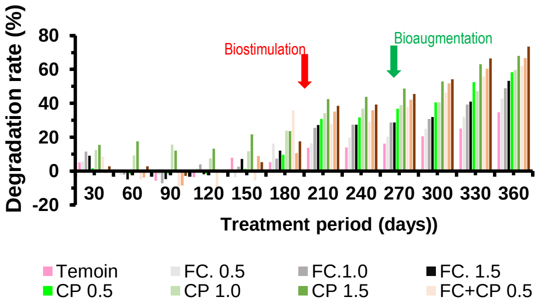


Figure 20 : Degradation rate kinetics during treatment. Levels of significance of comparisons 0.05.

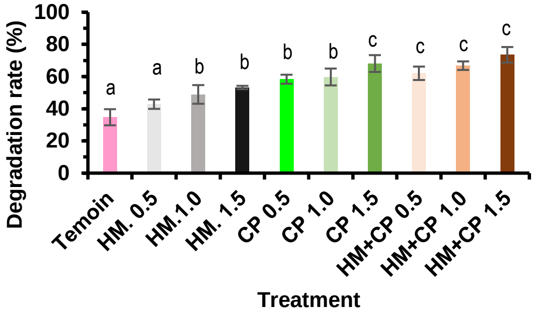


Figure 21 : Degradation rate at 360 days. Levels of significance of comparisons 0.05.

DISCUSSION

The aim of this study was to evaluate the contribution of indigenous organic amendments to the biodegradation of sludge polluted from the refinery, using mesocosm landfarming.

In this study, the results showed that throughout the experiment from 0 to 365 days (figure 8), moisture content varied on average from 11.69% ± 6.98 at the start of the experiment to 13.34% ± 2.41 at the end of the experiment for all treatments, with an average moisture content for all treatments of 14.28% ± 3.0 over the 365 days. These moisture levels can be directly linked to the weekly water supply (Morabo Okoletimou et al., 2023). Indeed, this water supply regulates oxygen diffusion in the treatment basins, prevents their dehydration and also enables the growth of microorganisms (Piakong and Nur Zaida, 2018; Xianagang et al., 2020). According to Xianagang et al. (2020), optimal microbial growth can only take place in the presence of water, because microorganisms need sufficient water to grow.

Indeed, the absence of significant differences between the treatments (Figure 9) could justify the fact that the sludge samples treated came from the same collection basin, so they had the same water retention capacity and the same types of indigenous microorganisms. Several authors had shown in their work that moisture levels differ according to the type of polluted soil, its origin and its degree of pollution as well as its water-holding capacity and the type of microorganisms present in the pollutants (Siméon et al., 2008; Kaboré-Ouédraogo et al., 2010; Morabo Okoletimou et al., 2023). Water retention capacity therefore plays a crucial role in soil humification at the start of treatment. Kaboré-Ouédraogo et al. (2010) assert that for microorganisms to be more active, the soil must be sufficiently moist. For them, it would be preferable for the pollutant (soil/mud) to be wetted to 2/3 of its maximum retention capacity, i.e. 30 ml of water to wet 100 g of soil. In fact, average moisture levels are 14.1% in the control treatment, 13.9% in the horse manure and compost treatments, and 15.1% in the treatments combining horse manure and household waste compost. These water contents are optimal for microbial activity in soils, as the majority of microorganisms function optimally when the medium represents 30 to 85% of the water-holding capacity or between 12 and 30% of its dry weight (Maila et Cloete, 2003; USEPA, 2017). However, in this experiment (figure 8), the increase in moisture was more noticeable on days 30, 210 and 270 respectively after biostimulation (0 and 150 days) and bioaugmentation (210 days). This may be explained by the fact that bioremediation activity is optimal with increasing soil moisture (Maila et Cloete, 2003). In fact, water favors the enzymatic degradation process, as it is essential for the life of microorganisms and improves microorganism/pollutant contact (Ait Oumeraci, 2020). Microbial growth requires the optimal presence of water in the environmental matrix. For optimal growth and proliferation, microorganisms require 12% to 25% humidity (Mukherjee and Das, 2005; Ait Oumeraci, 2020).

The pH changed from neutral to slightly alkaline between 6.8 and 8.1 (Figure 10). This increase in pH could be due to the addition the organic matter with a basic pH that favors the degradation of organic matter, in particular proteins, amino acids and peptides, probably releasing ammonium or volatilizing ammonia (Morabo Okoletimou *et al.*, 2023). Indeed, the treatment with 1.5% compost and all treatments that received the mixture of compost and manure (Figure 11) showed an increase in pH, which would be due to a significant input of nutrients (Namkoong et al., 2002; Ait Oumeraci, 2020; Morabo Okoletimou et al., 2023). These pH variations could also be justified by the microbial activity taking place in the mesocosms, as the values recorded fall within the pH 6 to pH 9 range where bacterial growth is optimal (Maila and Cloete, 2003; USEPA, 2017).

Regarding EC, the analysis of variance (ANOVA) showed that, at the 5% threshold, the means of EC for the factor dates, the treatments and their interaction are significantly different ($p=1.17.10^{-50}$, $p=1.62.10^{-73}$, and $p=1.09.10^{-21}$). With EC values of < 600 µS/cm, these sludges are non-saline in nature (Mehraz and Ouali, 2019). During the landfarming process, the salinity decreased rapidly at day 30; this decrease is thought to be due to the loss of mineral elements entrained by volatilization (Naoual et al., 2015). The increase in EC during this landfarming process would be explained by the increase in mineral elements from the decomposition of organic matter (Naoual et al., 2015). Analysis of this parameter reflects the degree of overall mineralization of the sludge and provides information on the salinity level (El et al., 2011). In addition, EC values are significantly higher and different from values recorded in control samples. This would be due to the added organic substrates which, by degrading the hydrocarbons, release significant quantities of mineral salts into a leachate that is not discharged from the medium (Hamdi et al., 2007; Zhang et al., 2020). In fact, the leachate evaporates and the salts remain in the medium (Naoual *et al.*, 2015). Furthermore, these observed differences (Figure 13) indicate that the quantities of organic matter added for the treatment of polluted sludge have had an effect on EC, as its values increase according to the proportions applied (0.5%; 1.0% and 1.5%). Moreover, the EC values obtained in treatments using horse manure are higher than those obtained in treatments using Compost. Indeed, according to Huber and Schaub (2011), horse manure has less influence on soil organic carbon stock, stimulates soil biomass in quantity and activity, and thus promotes nitrogen mineralization. Compost, on the other hand, stimulates the biomass while further supplying the organic carbon stock by increasing microbial activity and degrading the stable fraction (Huber and Schaub, 2011).

Concerning OM, the absence of significant differences observed between dates and between treatments, with the exception of treatments HM 0.5, HM 1.0 and CP 0.5 (Figure 15), could be due to the low level of THC degradation in the latter treatments. Indeed, the biological activity depends on OM content as it is essential for the growth of microorganisms so high contents could slow down microbial activity in the media; resulting in lower OM degradation rates and low carbon consumption as an energy source (Bouderhem, 2018). The OM concentrations of treated samples are below 10%. They cannot therefore be reused in agriculture, but can be recycled in construction or urban sanitation materials (Petavy et al., 2009). Brady and Weil (2008) point out that soil organic matter generally represents only 1-6% of a soil's dry weight. The high OM contents obtained in this study can be justified by the fact that the presence of hydrocarbons in the sludge increases the organic matter content. Thus, OM is a determining and very important factor because at very high values, mineralization becomes difficult (Kihindo et al., 2023; Sery et al., 2023).

For the C/N ratio, the increase observed at 120 days could be explained by the pollutant's high carbon content (Figure 16). Thus, the presence of hydrocarbons in the sludge could lead to an imbalance in the C/N ratio as a result of the lower degradation rate of the pollutant (Kaboré-Ouédraogo et al., 2010). On the other hand, the subsequent decrease in the C/N ratio reflects sustained decomposition of the organic matter, which is favored by biostimulation and bioaugmentation: this is the bio-oxidative phase (Naoual et al., 2015). In addition, several studies have shown that nitrogen deficiency or excess can affect the growth of microorganisms and thus slow down the rate of biodegradation (Siméon et al., 2008; Kaboré-Ouédraogo et al., 2010). This decrease is the characteristic of the degradation of organic matter assimilated to its mineralization by microorganisms (Naoual et al., 2015). These results are in line with those of Kaboré et al. (2010). Indeed, in the soil, most of the nitrogen (apart from that from fertilizer) is present in organic matter. This nitrogen becomes available in the soil following the decomposition of organic matter by microbial activity (Kaboré-Ouédraogo et al., 2010; Naoual et al., 2015). This slowdown observed during experimentation is marked by the stabilization phase following mineralization (Naoual et al., 2015). Indeed, the differences in treatments observed on manure (HM) or the compost and manure mixture (HM+CP) at doses of 1% and 1.5% (Figure 17) and the low values obtained compared with the control could be explained by the fact that the percentages of hydrocarbon removal using organic amendments depend on the soil/organic amendment proportions (compost and manure) (Namkoong et al., 2002; Gestel et al., 2003; Ait Oumeraci, 2020). In addition, the C/N ratios of the compost treatments are slightly higher than those of the horse manure treatments, and the latter are higher than those of the compost/manure mixture (CP > HM > HM+CP). This may be explained by the fact that compost contains materials rich in lignin or cellulose (Quimeby, 2022).

With regard to THC, the results obtained could be explained by the addition of organic substrates and hydrocarbonoclasts, which certainly helped to increase microbial activity in contaminated soils and trigger hydrocarbon degradation (Launen et al., 2002; Kaboré-Ouédraogo et al., 2010; Hogan-Itam, 2020; Rhbal et al., 2020). In addition, the low THC degradation rates in some treatments could be explained by the high level of organic matter that is difficult to degrade, reflected in the high values (> 25) of the C/N ratio ((Weber et al., 2001; Quimeby, 2022).

Although THC degradation rates ranged from 35% to 74% in all treatments, THC levels in the various treatments at the end of the experiment (360 days) were still well above the thresholds set between 0.1% and 0.5% by several countries such as Quebec, the Netherlands and the USA (Launen et al., 2002; Petavy et al., 2009 Kaboré-Ouédraogo et al., 2010). In addition, the comparison with the French impact assessment values (IAVs) for the remediation of polluted sites and soils shows that the hydrocarbon content of all treatments is higher than the IAVs for sensitive and non-sensitive soil, which are 0.5% and 2.5% of dry matter respectively (Mossman and Koch-Mathian, 2001). Thus, these treated sludges are not yet suitable for industrial or agricultural use.

The reduction of hydrocarbons in the samples from the landfarming system set up, is very long (low). This seems logical, as the samples tested were taken from the refinery sludge, a landfill site formerly polluted to very high levels. Subsequently, they became highly resistant to the biodegradation process as they were sequestered in the organic fraction of the sludge present in the slough over time (Launen et al., 2002). Indeed, the samples taken have been influenced by natural phenomena such as intrinsic bioremediation and photo-oxidation, so the hydrocarbons in the mesocosms are those that have persisted through these phenomena, their reduction being almost negligible. These results concur with those of Rhbal et al. (2020).

Conclusion

The aim of this work was to evaluate the effectiveness of organic matter on the rehabilitation of sludge polluted by petroleum hydrocarbons from the sludge pit of an oil refinery in Congo-Brazzaville, using the landfarming technique in mesocosms. The results showed that the addition of organic substrates (biostimulation) and microorganisms (hydrocarbonoclasts) accelerated THC biodegradation processes. The results also demonstrated that pH and moisture content were in the optimum range for degradation, and treated sludge was not salty (EC< 600µS/cm) throughout the experiment. The study of the effect of organic amendments on the C/N ratio reveals that it is relevant to add organic substrates (biostimulation) and hydrocarbonoclastic microorganisms (bioaugmentation) to achieve good biodegradation. The monitoring of hydrocarbon concentration at the end of treatment highlighted that the greatest loss of hydrocarbons was obtained with the treatment using the manure and compost mixture at 1.5% (74%) and the treatment with compost at 1.5% (68%). Despite the decrease in THC levels in all treatments at the end of the experiment, THC levels remain high compared with French impact assessment values (IAVs) for the remediation of polluted sites and soils. The results of this work confirm that the objectives set have been achieved. In fact, bioremediation of hydrocarbon-polluted sludge from a refinery with the addition of organic amendments and hydrocarbonoclastic microorganisms via landfarming is technically feasible, as it has physico-chemical properties that favor hydrocarbon degradation. This technique is considered to be an ecological way of eliminating hydrocarbons. The prospects for this work are still numerous, particularly concerning the time and the frequency of nutrient and microorganism input. For an efficient and a rapid biodegradation, it would also be preferable to add biosurfactants to enhance hydrocarbon biodegradation and substantially reduce the treatment time.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

For this study, VVMO and JDDN developed the research methodology and set up the experimental protocol. VVMO carried out all the work involved in monitoring the experiment, from data collection to processing, and writing the article. JDDN, NWN and AAL helped validate the research protocol and improve the manuscript.

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