

1 **Effects of compost stability and contaminant concentration on the bioremediation**
2 **of PAHs contaminated soil through composting**

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1 **Abstract**

2 The objective of this study was to investigate the effect of two factors: the stability
3 degree ($0.37 - 4.55 \text{ mg O}_2 \text{ g}^{-1} \text{ Organic Matter h}^{-1}$) of different composts derived from the
4 organic fraction of municipal solid wastes and the concentration of a complex mixture
5 of PAHs including Flourene, Phenanthrene, Anthracene, Flouranthene, Pyrene and
6 Benzo(a)anthracene in the bioremediation of soil. The two factors were systematically
7 studied applying central composite design methodology. The obtained results
8 demonstrated that compost stability degree was particularly important during the first
9 stage of the process. Stable composts enhanced the levels of degradation in soil-
10 compost mixture and a degradation rate of 92% was achieved in this period, but only
11 40% was degraded with the least stable compost. The PAHs concentration was also
12 important during the process, since the degradation rates increased with the increase in
13 the PAHs concentration. Moreover, all the individual PAHs demonstrated a notable
14 decrease in their concentrations after the incubation period, but pyrene was degraded to
15 lower levels in some treatments compared to others PAHs.

16

17 **Keywords:** Compost; Polycyclic aromatic hydrocarbons (PAHs); Soil; Stability;
18 Experimental design.

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1 **1. Introduction**

2 The large range of hazardous chemicals with different structures and different
3 toxicity levels that are continuously released from several anthropogenic sources are
4 continuously causing environmental pollution [1]. Polycyclic aromatic hydrocarbons
5 (PAHs) are one of the most encountered pollutants in the ecosystems; as a consequence,
6 soil contamination with these contaminants is a matter of major concern as they can be
7 introduced to the soil by various sources, where these compounds are categorized as
8 toxic for both humans and environment [2]. Indeed, with more concern regarding the
9 ecosystem and more strict regulation like the EU landfill directive (1999/31/EC), which
10 tries to reduce the amount of wastes that can be sent to the landfill, transforming the
11 contaminated soil to such landfills has become limited. Furthermore, the restoration of
12 many contaminated sites is preferable as the available agricultural areas are gradually
13 degraded with time [3]. Accordingly, there is a critical need to develop and implement
14 an effective remediation technology to reduce the threats caused by such contaminants
15 and create a sustainable reuse of soil.

16 Bioremediation can be regarded as an attractive technology that results in the
17 partial or complete biotransformation of many organic contaminants to microbial
18 biomass and stable innocuous end-product. Moreover, this technology is believed to be
19 cost-effective and environmentally accepted [4,5]. The contaminated soil are normally
20 deficient in nutrients that are necessary to support the indigenous microorganisms to
21 develop themselves, or sometimes the microorganisms are only available at low levels
22 that makes the bioremediation process progress at very slow rates [6]. To overcome
23 these conditions, normally the bioremediation of hydrocarbon contaminated soils often
24 rely upon the addition of nutrients or microorganisms (biostimulation and
25 bioaugmentation) [7,8]. Composting as a remediation tool has been considered a

1 suitable technology in the bioremediation of contaminated soils, and it has been used to
2 mitigate these limiting factors, as it also improves the soil properties [9]. On the other
3 hand, applying composting technology provides a sustainable reuse of the organic
4 biodegradable fraction of wastes, which is both microbial and nutrient rich. However,
5 contaminants bioavailability is an important factor affecting microbial degradation rates
6 in soil and sediments as the microorganism are able to attack the target contaminant
7 only when it is dissolved within the materials [10]. Thereby, the selected organic
8 amendment for the bioremediation process should serve to improve and overcome any
9 deficiencies or limitations that influence the process efficiency. One challenge with this
10 type of research is that composting feedstock composition can vary widely from one
11 facility to another; this can affect the chemical and microbial conditions in the
12 amendments [11]. Bioremediation of contaminated soils using composting process
13 depends on a number of physical, chemical, and biological factors that determine the
14 microbial accessibility to the target contaminants [10,12,13], where the amendment
15 properties are of great role in determining the process behaviour. Although various
16 amendments have been applied during the composting of contaminated soil, still much
17 specialized research is needed. Compost stability is one of these factors as this
18 parameter is related to microbial activity within the organic material and it also can be
19 related to the compost composition like humic matter [6]. Until now, no studies were
20 reported that explore this important parameter and its influence on the degradation of
21 PAHs in the soil. For an efficient treatment process, the microbial activity, which is
22 considered the main factor in the bioremediation process, should be maintained at
23 adequate levels [14]. The contaminants concentration is one of the factors that influence
24 the microbial activity as these contaminants may exhibit some toxic or inhibition
25 influence when they exist at high levels. However, low concentration could be below

1 the required level needed to provoke the microbial enrichment to attack this
2 contaminant [15].

3 In this research, we tried to investigate the bioremediation process of soil
4 contaminated by a complex mixture of PAHs using the composting technology, where
5 the mixture of PAHs simulated a real creosote sample. Different composts derived from
6 the organic fraction of municipal solid wastes were suggested as organic amendments
7 considering the effect of their different degrees of stability as an objective to be
8 clarified. We believe that although the development and widespread application of
9 bioremediation process are in a good research position, it still is limited by a lack of full
10 understanding of such important factors (compost stability and pollutant concentration).
11 For this reason, this two factors were studied systematically using central composite
12 design methodology. All the experiments were scheduled and carried out under
13 laboratory-scale level that is representative of real composting conditions.

15 **2. Materials and methods**

16 *2.1. Soil*

17 The soil used in this study was collected from the surface horizon (0-30 cm) in
18 Prades (Tarragona, Spain). The zone where the soil was collected is an agricultural one;
19 hence it is free from any PAHs contamination and preliminary analysis confirmed that
20 there are no PAHs in the used soil or their concentration are below the detection limits.
21 This soil is classified as sandy loam soil and consists of 73% sand, 19% silt and 8%
22 clay. For the experimental purpose, the soil was air-dried, sieved to 2 mm and kept at
23 4°C until use. Some characteristics of the soil were determined and are presented in
24 Table 1.

25

1 2.2. Chemicals

2 Different compounds of PAHs were purchased from (Sigma-Aldrich, Spain).
3 These PAHs include: Flourene, Phenanthrene, Anthracene, Flouranthene, Pyrene and
4 Benzo(a)anthracene with 98-99% purity. According to the US Environmental Protection
5 Agency (USEPA), these PAHs are classified as priority pollutants as they are toxic and
6 carcinogens. As a group of contaminants to be followed during the experiment course,
7 the abovementioned six PAHs were mixed together in a stock solution that was spiked
8 into the mixture soil-compost with a concentration based on total PAHs. The percentage
9 of each individual PAH compound as a part of the total PAHs concentration (Σ PAHs)
10 was 30%, 29%, 9%, 20%, 3.5%, 8.5% respectively. These percentages were determined
11 according to the results of fractionation process of a creosote sample (Creosote lot: 42-
12 13B, Chem Service, SUGELABOR S.A, Spain) in our laboratory using the method
13 3611B of the USEPA, where the volatile part was ignored. A stock solution of the used
14 PAHs was prepared using the previous percentages. Afterwards, they were spiked into
15 the soil to obtain the desired concentrations according to the experimental design matrix
16 (0.1-2 g/kg) as total PAHs. For instance, the low to high concentrations were applied to
17 understand the performance of the process under such conditions considering PAHs
18 concentration as an important factor in a remediation technology. Also the same stock
19 solution was used to calibrate the instrumentation for the PAHs determination.

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21 2.3. Organic co-substrates

22 As an organic co-substrate (amendment) to be added for stimulating the
23 composting process, composts derived from the organic fraction of municipal wastes
24 (OFMSW) were applied during the bioremediation experiments. In this study, five
25 types of OFMSW composts were used during the experimental treatments. The main

1 difference between these composts was the degree of stability “the rate of organic
2 matter decomposition as a result of the microbiological activity” and it is mainly
3 related to the availability of readily degradable substrates. The different levels of
4 stability were determined using the Dynamic Respirometric Index (DRI). Only
5 compost B was obtained from a home composter in the *University Autònoma of*
6 *Barcelona*, where the others were obtained from composting plants located in the
7 Barcelona area (Spain). These composts were selected to be characterized by a
8 different degree of stability ranging from full-stable to unstable compost [16, 17],
9 which would provide the ability to examine their effects on the degradation of the used
10 PAHs and to relate and predict the effect of their major components as a consequence
11 on the bioremediation process. The main characteristics of the used composts are also
12 presented in Table 1.

14 *2.4. Composting reactors and monitoring instruments*

15 The used reactors were Dewar® vessels (4.5-L), which were modified and
16 conditioned to operate in a batch-mode way for the composting experiments. These
17 reactors are thermally isolated, so the process can be kept under the natural composting
18 temperatures, and the influence of ambient temperature can be minimized. Aeration was
19 provided through a pipeline connected to the bottom of the reactor where a plastic mesh
20 is placed to insure a correct distribution of the air through the composting mixture, and
21 the exhausted air exits the reactor through an outlet in the reactor cover. Oxygen
22 concentration was measured by means of an oxygen sensor (Crowcon’s Xgard, United
23 Kingdom), where the inlet of the sensor is connected to the reactor outlet and
24 consequently the oxygen percentage in air was determined. Aeration rate and frequency
25 were adjusted to prevent any limitation or excess in the oxygen percentage in the

1 reactors, consequently, sporadically aeration mode was used and the oxygen
2 concentration was well maintained to insure aerobic conditions (more than 10%).
3 Temperature was monitored by Pt-100 sensors (Sensotran, Spain) connected to a data
4 acquisition system (DAS-8000, Desin, Spain) that was connected to a personal
5 computer. The software used (Proasis®Das-Win 2.1, Desin, Spain) also permits to
6 monitor both the temperature and oxygen content in the reactors. These two parameters
7 (temperature and oxygen concentration) are useful to control and follow the process
8 during the different phases.

9

10 *2.5. Experiments set-up*

11 The PAHs were mixed together according to their percentages to be introduced
12 as the target contaminants during the composting process. These contaminants were
13 spiked into soil to have the initial concentration expressed as total of PAHs according to
14 the values determined by the experimental design technique that were decided to be
15 from 0.1g/kg to 2g/kg (dry matter). After this, the contaminated soil was mixed with the
16 organic amendment at ratio of 1:1 (w:w, dry weight). The mixture was then mixed with
17 bulking agent at a ratio 1:1 (V:V) in an attempt to provide proper porosity to maintain
18 aerobic conditions. All the components of the composting treatments were manually
19 mixed according to the aforementioned ratios, resulting in about 3.5 kg that were used
20 in each reactor. The used bulking agent consisted of wood chips and pruning wastes that
21 were not biodegraded under laboratory composting conditions. Water content of the
22 composting mixtures was adjusted to be within the recommended values (50-60%) by
23 adding tap water before incubation. The composting matrix was left under natural
24 composting temperatures. Aeration flow rate and frequency were monitored and
25 adjusted during the process to avoid any limitation or excess in the oxygen

1 concentration that may affect the process. All the composting mixtures were manually
2 prepared according to the proposed values of the experimental design technique (Table
3 2) and were incubated for 30 days.

4

5 *2.6. Sampling*

6 During the incubation period, the performance of the process was monitored and
7 samples were collected after 10, 20 and 30 days of composting in order to measure the
8 degradation rate in these periods. For sampling, the reactors were opened and the
9 reactor contents were manually well mixed. Then duplicate grab samples (20-30 g) were
10 taken. During each sampling, moisture content was adjusted, if necessary. Thus, the
11 composting mixture was moistened with tap water and remixed well to maintain water
12 content within the optimum values (50-60%).

13

14 *2.7. Analytical procedures*

15 *2.7.1. Co-substrates stability degree*

16 Compost stability was determined using the Dynamic Respirometric Index
17 (DRI), determined according to Barrena et al, [18]. Briefly, this index represents the
18 oxygen consumption by microorganisms to degrade the easily degradable organic
19 matter of a sample within a unit time (h) when it is incubated under optimal and
20 controlled conditions and with a continuous air supply. About 150 g duplicated samples
21 were incubated in 500 ml Erlenmeyer glass flasks provided with a plastic mesh placed
22 in the bottom to support the incubated sample and to ensure equally distributed air. The
23 flasks are perfectly sealed with a rubber pieces where the aeration and exhausted air
24 tubes passing through. The samples are incubated in a thermostatic water bath adjusted
25 at 37°C where the respirometer system is supplied with an oxygen sensor, a control

1 cabinet and air supply system based on mass flow-meters and personal computer unit. A
2 constant air flow was supplied to ensure that the oxygen concentration in the exhausted
3 air is greater than 10%. As a result DRI provides an accurate measure of the biological
4 stability of the organic matter contained in the biomass in form of the maximum
5 respiration activity (Oxygen Uptake Rate) of the samples. The complete details of this
6 analysis can be found elsewhere [19,20].

7

8 *2.7.2 PAHs analysis*

9 The content of the PAHs in the composting mixture was determined after
10 extraction using a Soxhlet extraction process. Duplicated 10 g samples were extracted
11 using acetone/dichloromethane (1:1 v/v) as solvent during two hours. Afterwards, the
12 solvent was left to evaporate and then the remaining residue (extract) was dissolved in
13 10 ml of dichloromethane. A 1- μ l extract of this solution was injected in a gas
14 chromatograph (GC8690N, Agilent, Spain) equipped with flame ionization detector
15 (FID) and a splitless injector. A Zebron ZB-5HT Inferno column (Agilent, Spain) was
16 used. Initial temperature was maintained at 50°C for 1 min, and then it was increased at
17 a rate of 7°C/min until 320°C, then another rate of 20°C/min until 400°C was applied
18 and maintained at this final temperature for 5 min. The concentration of the PAHs was
19 determined after the calibration of the method with standard PAHs samples.

20 To investigate the volatilization of the PAHs during the composting process,
21 samples from the exhausted air were collected using Tedlar bag of known volume [21],
22 and then samples of 1 ml of that air were analyzed using the same GC methodology.
23 However, this test was simply used to check if part of the PAHs decrease is caused by
24 volatilization, but the actual amount of the volatilized PAHs could not be determined as
25 only small amounts of some low molecular weight PAHs were detected.

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2.7.3. Other characteristics

The other characteristics of both soil and organic co-substrates including: moisture content, organic matter content (OM), pH, electrical conductivity, organic carbon, Kjeldahl nitrogen and humic matter fraction were determined on collected samples according to standard methods [22].

2.8. Experimental design methodology

For bioremediation of PAHs-contaminated soil through composting, the influence of PAHs concentration (x_1) and compost stability (x_2) were processed and statistically validated through setting up a Central Composite Design (CCD) technique and a second-order model was produced to correlate the studied factors. A series of experiments were carried out at different points as follow: four experiments using the extreme points ($\alpha=1.414$), four experiments corresponding to a two factors complete factorial design and two experiments at central points. For the validation of the design, two more experiments were conducted at the central points and so 12 experiments as total were carried out and five values for each independent variable were tested.

To simplify the recording of the conditions and processing of the experimental data, the factor levels were coded with the notations ($-\alpha$, -1 , 0 , $+1$, α). The value of $\alpha=1.414$ was determined according to the number of the studied factors (2). The experiment design technique was carried out as it is explained in the literature [23,24]. Table 2 presents the design matrix, where the coded and the actual values of the studied factors and their combination are described, also the actual response of the process (Y) after 10, 20 and 30 days is also shown.

1 2.9. *Data statistical analysis*

2 Statistical analysis was performed for all variables using the Sigmaplot® 8.0
3 software package (Systat Software Inc, San Jose, USA). The replication of experiments
4 at central point permits the statistical validation of results according to CCD
5 experimental design [23].

6

7 **3. Results and discussion**

8 3.1. *Soil and co-substrates characterizations*

9 In this study several types of OFMSW compost were evaluated as an organic
10 amendment during the composting of PAHs-contaminated soil. Obviously, the available
11 high content of organic matter in these composts in comparison with the available
12 amount in the soil (Table 1) is thought to be needed to support and develop the
13 microbial activity during the bioremediation process. Meanwhile, these composts are
14 suggested to provide valuable populations of microorganisms that presumably can
15 degrade the contaminant [6]. One of the most important characteristics of the applied
16 amendments that play a major role in the remediation process is the humic matter
17 portion [24,25]. Analysis of the composts showed that humic matters are available
18 among them, but their portions are different according to their stability degree. Indeed,
19 humic matter as part of the compost organic matter was found to be increased with
20 stability degree in the sense that, the more stable the compost was the higher humic
21 matter content was observed. However, compost C deviated from this fact to a small
22 extent, which might be attributed to its high content of organic matter (Table 1). The
23 other characteristics are almost considered within the acceptable levels for such process.
24 Generally, the growth factors in the used compost are better than those in the soil;

1 consequently, these organic amendments may have a major role in improving the
2 degradation of the contaminants.

3

4 *3.2. Response surface and statistical analysis*

5 The obtained percentage of PAHs degradation (Y) after 10, 20 and 30 days were
6 used as functions to correlate the studied factors (x_1 and x_2), where second-order
7 polynomial model was used to fit these values as shown in the following equations:

$$8 \quad Y_{10} = 75.2 + 3.36x_2 + 2.16x_1^2 - 2x_2^2 - 1.59x_1x_2 \quad (1)$$

$$9 \quad Y_{20} = 82.33 + 6.57x_1 - 2.3x_1x_2 \quad (2)$$

$$10 \quad Y_{30} = 106.33 - 24.59x_1 - 4.52x_2 + 8.5x_1^2 + 2.4x_1x_2 \quad (3)$$

11 Depending on these equations the regression coefficients (R) of Y_{10} , Y_{20} and Y_{30} were
12 0.83, 0.53 and 0.6 respectively. The obtained regression coefficient after 10 days
13 represents a good regression model, whereas those of 20 and 30 day represent a non
14 perfect regression. In all cases the P values describing the significance levels were not
15 concluding ($P > 0.05$). However, although these statistical values are not within the
16 preferable values to describe the process, the constant variance test indicated that these
17 can be used to predict the degradation rates within $\pm 10\%$.

18

19 *3.3. The composting process*

20 Temperature variations of the composting materials with time in some
21 experimental runs are illustrated in Figure 1. A lag phase was observed even though its
22 duration varied among the treatments, but it was clear in run 9 where about two days
23 were needed to stimulate the microorganisms. As a result of the microbial activity,
24 temperature began to rise to thermophilic ranges that were achieved during the first
25 week in the treatments when less stable composts were used, but it was always in the

1 mesophilic ranges when more stable composts. It is believed that the achieved
2 thermophilic temperatures in the beginning of composting were attributed to the
3 sufficient amounts of easily degradable materials [19,26]. This assumption agreed with
4 results obtained regarding the organic matter degradation (Figure 2) that showed a
5 notable decrease in this stage for the less stable composts. Moreover, high aeration and
6 frequencies were needed during the first stage of the process especially in the less stable
7 composts (data not shown), which implied that the microbial activity was intense.
8 However, as the materials became more stable and the process entered to the cooling
9 phase, less amount of air was needed. The temperature increases as well as the reduction
10 in the organic matter fraction during the whole process are the most important evidences
11 of the process [17,26].

13 *3.4. PAHs degradation*

14 The degradation of the PAHs was assessed during the entire incubation period
15 (30 days). Figure 3 presents the remaining PAHs throughout the different runs. By the
16 end of the incubation period, high rates of degradation (76.11%-96.53% as total PAHs)
17 were achieved among all the experiments except the run 6 where only 45.8% was
18 achieved. For instance, during the first 10 days the highest rate of degradation (92%)
19 was observed in run 2, whereas the lowest rate (18%) was in run 6 during this period,
20 and in the other runs it was within 40%-80%. During the remaining period, a low rate of
21 degradation was observed. The contrast among the different treatments could be clearly
22 visualized during the first 10 days of incubation as different degradation rates were
23 obtained. Thus, the compost stability appeared as an effective factor especially when
24 treatments with the same concentration are to be compared (run 5, 8 and 9). In run 9,
25 which had the most active compost, almost 40% of the PAHs were degraded during that

1 period (10 days). However, almost the double degradation rates were obtained when
2 more stable compost was used. It is worthy to indicate that less stable composts got
3 stabilized with more incubation time. Consequently, degradation rates with these
4 composts improved with time, for instance, degradation rate of 76% after 30 days was
5 obtained in run 9. Nevertheless, this rate of degradation was still less than those
6 obtained under the same conditions when more stable compost was supplied. On the
7 other hand, the rates of degradation were found to be varied under the different
8 concentrations, which imply that the degradation process is also influenced by this
9 factor. In general, the PAHs degradation was influenced by the two factors, where the
10 used amendments were able to enhance the degradation process to a great extent.

11 Regarding the degradation pattern of the individual PAHs, they demonstrated a
12 notable rate of degradation in the treatments except in the case of pyrene, which had a
13 recalcitrant behaviour especially in the first 10 days where a low rate of degradation was
14 observed in almost all the experiments. However, with more incubation time, its
15 concentrations was decreased but not as the other PAHs. The PAHs degradation order
16 was the same among the experiments and it was, in general: Flourene > phenanthrene >
17 anthracene > flouranthene > benzo(a)anthracene > pyrene. However, in run 6 it was
18 different and was: flouranthene> phenanthrene> flourene> benzo(a)anthracene>
19 anthracene> pyrene. Clearly, neither the solubility nor the octanol-water partition
20 coefficient (K_{ow}) order of these PAHs controlled the degradation. According to [27], the
21 molecular conformation prevails over other parameters in controlling the degradation
22 when the degradation trends for each hydrocarbon are similar under all conditions.

23 In general, the rate of degradation during the first 10 days was faster compared
24 to the rest incubation period where less degradation occurred. In this sense, the organic
25 contaminants could be sequestrated into the matrix of the soil as this process is

1 generally a function of time [28]. Analysis of exhausted air samples indicated that very
2 small amounts of some low molecular weight PAHs were volatilized during the
3 thermophilic stages (especially for temperatures over 50°C), thus a portion of these
4 PAHs reduction is due to volatilization but this amount is so small compared to that
5 resulting from the biodegradation as these elevated temperatures remained for about one
6 week in the reactors with less stable compost. However, the majority of the experiments
7 were in the mesophilic ranges (Figure 1) where no or negligible volatilization occurred.
8 It was also reported that although a portion of the total petroleum hydrocarbons
9 reduction is due to volatilization, abiotic loss has been reported to be generally less than
10 10% at 25°C in the first 30 days [29].

11

12 *3.5. Effect of compost stability on PAHs degradation*

13 The response of the PAHs degradation percentages under different composts
14 stability degrees denoted by DRI (0.37- 4.55 mg O₂ g⁻¹ OM h⁻¹) are illustrated in Figure
15 4. As shown, the process response is changed when different composts were used as
16 well as during the different composting stages indicating that the compost stability is
17 one of the factors that influence the process performance. This effect was significant
18 during the first stage of the composting process (10 days) as the applied composts had
19 to pass different stages according to their composition and dominant microbial
20 enrichment. With compost E (run 9), which is the most active one, only 40.13% of the
21 total PAHs was degraded comparing to the other composts (A and C) under the same
22 conditions (run 5 and 8) where 70.4% and 73.79% of degradation were achieved
23 respectively. The same fact was also observed in run 1 and 3 which demonstrated more
24 degradation as more stable compost was used. Without doubt, these observations are of
25 great interest when the efficiency of the composting technology is to be evaluated with

1 other technologies. However, the highest rate of degradation (92.2%) was observed in
2 run 2 indicating that the PAHs concentration has its influence in this case. For instance,
3 Oleszczuk [30], observed that the influence of the composting process on the
4 contribution of the potentially bioavailable fraction of the PAH depended on the stage
5 of the experiment.

6 Regarding the composting process and as commented before, the thermophilic
7 temperatures are thought to be not suitable for the microorganisms needed to attack the
8 PAHs contaminants as the lowest degradation rate was observed (run 9). These
9 temperatures were reported to inhibit the degradation process [31-33]. Contrarily, the
10 mesophilic temperatures obtained under the same conditions gave higher rates of
11 degradation indicating that these temperatures and the dominant microorganisms under
12 these conditions are preferable for degradation of such compounds. However, other
13 studies are not coincident with these observations [34].

14 The organic matter decrease was proportional with the stability degree, where
15 more reduction occurred in compost E. This reduction shows that these types of
16 composts still have a considerable amount of easily degradable matter which was
17 preferable by the microorganisms rather than other sources of nutrients like PAHs.
18 Accordingly, the mass loss after composting is a suitable evidence indicating the bio-
19 oxidation of the composting matrix.

20 Among the most important properties correlated with the compost stability is the
21 available amount of the humic matter as part of the organic matter. This matter was
22 found to increase with stable compost (Table 1) where more stable composts had more
23 humic matter. Sorption of the organic contaminants with the soil particles usually
24 decreases the degradation rates as these contaminants become less accessible to the
25 microorganism. However, it was found that the humic matter increases the

1 bioavailability of the organic compounds and it can behave as surfactant during the
2 remediation process, which reduces the bond between the soil and PAHs. The more
3 degradation rates with more stable compost confirm this hypothesis and the PAHs were
4 easily desorbed from the soil particles thereby the degradation was stimulated. Plaza et
5 al. [25], demonstrated that during the composting process, the changes underwent by
6 the humic matter are expected to facilitate the microbial accessibility to PAHs. These
7 experiments concurred well and confirm such suggestions. It was clear in the remaining
8 composting period that the degradation process continued among all the experiments
9 and better results had been obtained with more incubation time as the organic matter got
10 more stabilized and the microorganisms were more acclimatized especially with less
11 stable compost. The humic matter found to be more effective to increase the degradation
12 rates than the high temperatures although it is well-known that the produced high
13 temperatures usually increase the kinetics and desorption of such compounds.

14 3.6. *Effect of PAHs concentration*

15 PAHs concentration influenced the degradation rate as observed in the different
16 experiments. Figure 3 presents the percentages of the remaining PAHs after 30 days of
17 incubation, where Figure 4 presents the response of the process under different
18 concentrations. The lowest degradation rate (18%) after 10 days of incubation was
19 observed in the 6th run that has the lowest concentration (0.1g/kg), where the highest rate
20 (92.21%) was obtained in the 2nd run that has a concentration of 1.7g/kg, where
21 degradation rate of 66.5% was achieved with the highest used concentration (2g/kg)
22 during the same incubation period. By the end of the process (30 days), the degradation
23 rate in the 6th run (0.1g/kg) still maintained its order as the lowest achieved rate
24 (45.8%), where it was able to achieve 80.9% with the highest applied concentration
25

1 (2g/kg). However, when comparing the rate obtained with highest concentration, it was
2 less than the other obtained rates where less concentration were used, therefore, the
3 concentration levels are considered crucial when composting process is to be used. For
4 this reason, when low concentrations are present, these concentrations are thought to be
5 below the levels that are assumed to begin the degradation process as the
6 microorganisms start with easily available materials and as these materials depleted
7 quickly before the degradation take place, it will be difficult to keep the required
8 activity. These results agreed with those obtained in [15], where concentration of low
9 PAH did not degrade even when the system was supplanted with additional carbon
10 sources. Furthermore, Jørgensen et al. [35] argued that the degradation of hydrocarbons
11 is governed by first-order kinetics, where the degradation rate of a compound is
12 proportional to its concentration. However, this argument may be validated to some
13 limits as the microbial activity could be affected (retardation or inhibition) when high
14 concentration is available. In this study when the results of high concentrations are
15 compared to other presenting lower concentrations, it is better to assume that retardation
16 conditions were noted.

17

18 **4. Conclusions**

19 During the composting process, it was clear that the potentially available PAHs are
20 influenced by the compost stability and the composting stages as a consequence.

21 Accordingly, the following conclusions were deduced:

- 22 1. The observed different behaviors during the first 10 days of composting
23 demonstrate that less stable compost is not adequate for this type of remediation,
24 but more stable ones can promote the degradation quickly when the process is
25 well controlled.

- 1 2. Humic matter was assumed to facilitate the desorption of the PAHs to be more
2 available for degradation by the microbial activity. Indeed, humic matter was
3 more effective to accelerate the degradation rates than the high temperatures.
- 4 3. By the end of the process, experiments with the less stable compost were able to
5 improve their behavior as the composted materials were more stable, but their
6 results were still less favorable than those obtained with more stable compost.
- 7 4. PAHs concentration was found to influence the process mainly when low
8 concentrations are available, where the lowest degradation rate was obtained.
- 9 5. Both of the studied factors (compost stability and PAHs concentration) had a
10 direct effect on the process behavior; therefore, before carrying out the
11 composting process, initial knowledge about the available conditions may help
12 to have an estimation about the process performance and consequently the
13 expected degradation rates.

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Tables

Table 1

Characteristics of the used composts and soil.

Parameter/Material	Soil	Compost A	Compost B	Compost C	Compost D	Compost E
Moisture content (% , wb) *	6.64±0.01	38.64±0.22	30.1±0.42	53.8±0.25	32.63±0.08	40.55±0.35
Organic matter content (% , db) **	3.68±0.35	44.48±0.39	53.96±0.11	61.63±1.83	44.59±0.35	51.85±1.2
Total Organic Carbon (% , db) **	1.26±0.02	18.52±1.14	24.29±2.79	31.75±0.28	19.43±1.2	20.44±0.28
Total Kjeldahl Nitrogen (% , db) **	0.65±0.14	2.67±0.45	2.62±0.06	4.09±0.12	3.09±0.15	1.94±0.12
pH	6.7±0.02	8.07±0.08	8.63±0.04	8±0.16	8.11±0.01	7.61±0.01
Electrical Conductivity (mS/cm)	0.2±0.01	4.91±0.13	6.46±0.18	5.27±0.14	6.01±0.0	7.13±0.04
Humic Acids (% , db) **	1.5	10.12	11.62	14.6	8.95	4.72
Dynamic Respiration index (mg O ₂ g ⁻¹ OM h ⁻¹)	-	0.37±0.02	0.58±0.4	1.71±0.13	3.07±0.3	4.55±0.1

* wb: wet basis.

** db: dry basis.

Table 2

Design matrix including factor levels (coded and actual) and their response values for the two factors.

Run	Factor levels				Response*		
	coded		actual		Y ₁₀	Y ₂₀	Y ₃₀
	Concentration (x ₁) (g/kg)	Stability (x ₂) (mg O ₂ g ⁻¹ OM h ⁻¹)	Concentration (g/kg)	Stability (mg O ₂ g ⁻¹ OM h ⁻¹)			
1	-1	-1	0.38	0.58±0.04	81.47	88.26	96.38
2	+1	-1	1.74	0.58±0.04	92.21	96.47	96.53
3	-1	+1	0.38	3.07±0.29	67.55	79.01	89.81
4	+1	+1	1.74	3.07±0.29	75.26	89.09	96.56
5	0	0	1.05	1.71±0.13	73.79	81.82	84.39
6	-α	0	0.1	1.71±0.13	18.61	57.44	45.84
7	+α	0	2.0	1.71±0.13	66.51	78.26	80.98
8	0	-α	1.05	0.37±0.02	70.40	90.79	86.46
9	0	+α	1.05	4.55±0.01	40.13	77.28	76.11
10	0	0	1.05	1.71±0.13	75.61	79.29	78.68
11	0	0	1.05	1.71±0.13	75.07	90.28	85.58
12	0	0	1.05	1.71±0.13	69.35	75.42	85.21

*The response (Y) represents the degradation percentage (%) after 10, 20 and 30 days of composting

Legends to Figures

Figure 1. Temperature profiles of the composting materials over time.

Figure 2. Percentage of organic matter degradation after 30 days of composting.

Figure 3. Percentage of the remaining PAHs after 30 days of composting.

Figure 4. The response of the PAHs degradation (%) under different concentrations and DRI ($\text{mg O}_2 \text{g}^{-1} \text{OM h}^{-1}$) during the incubation period where (A) corresponds to 10 days, B (20) days and (C) 30 days.

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Figure 1: Sayara et al.

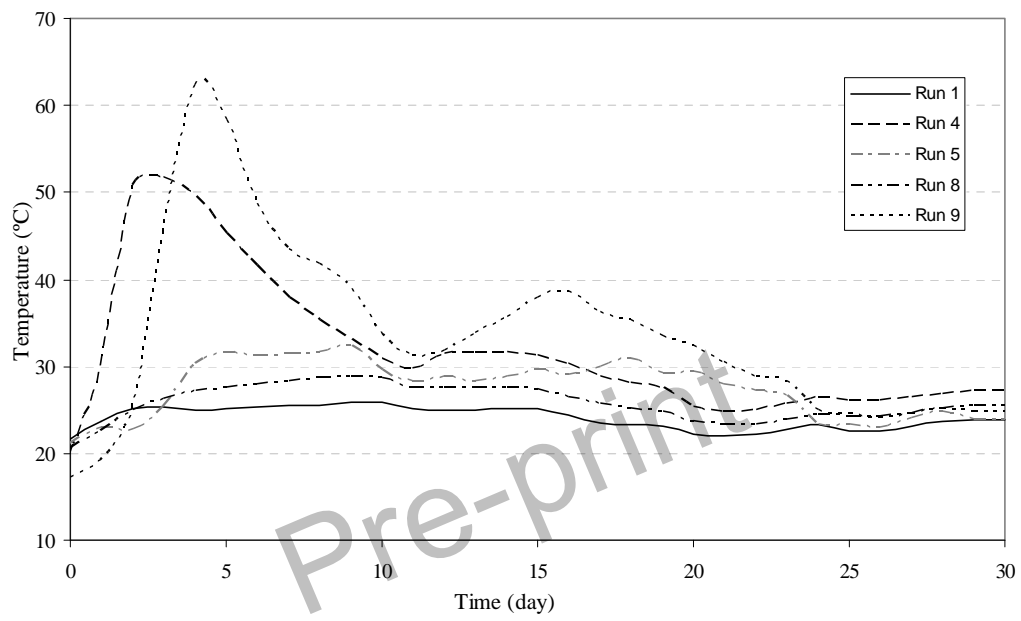


Figure 2: Sayara et al.

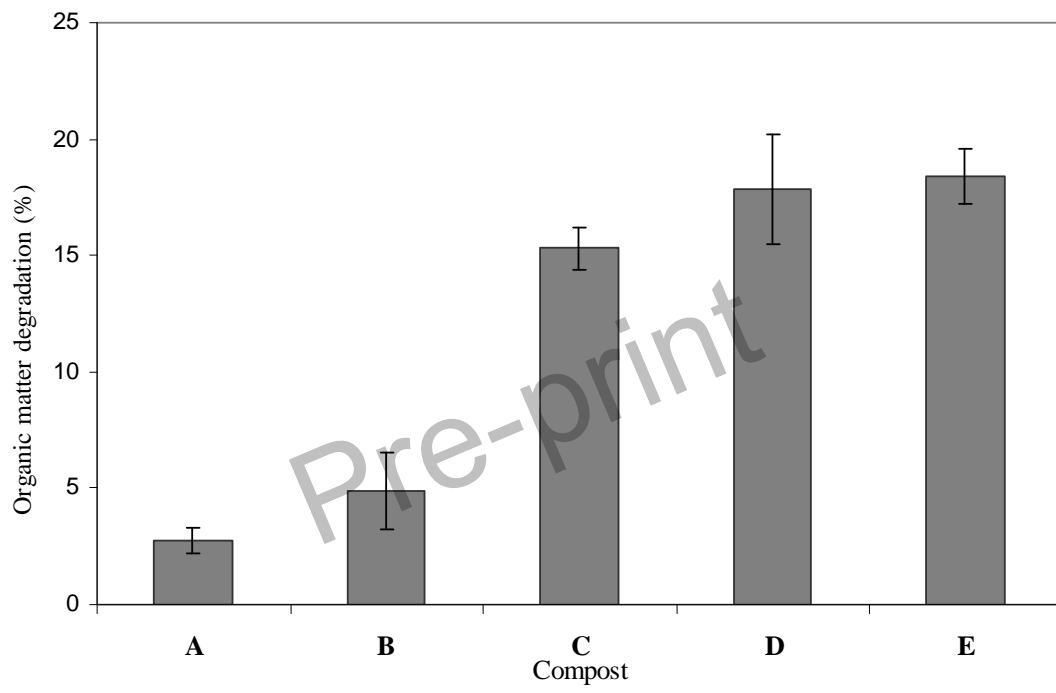


Figure 3: Sayara et al.

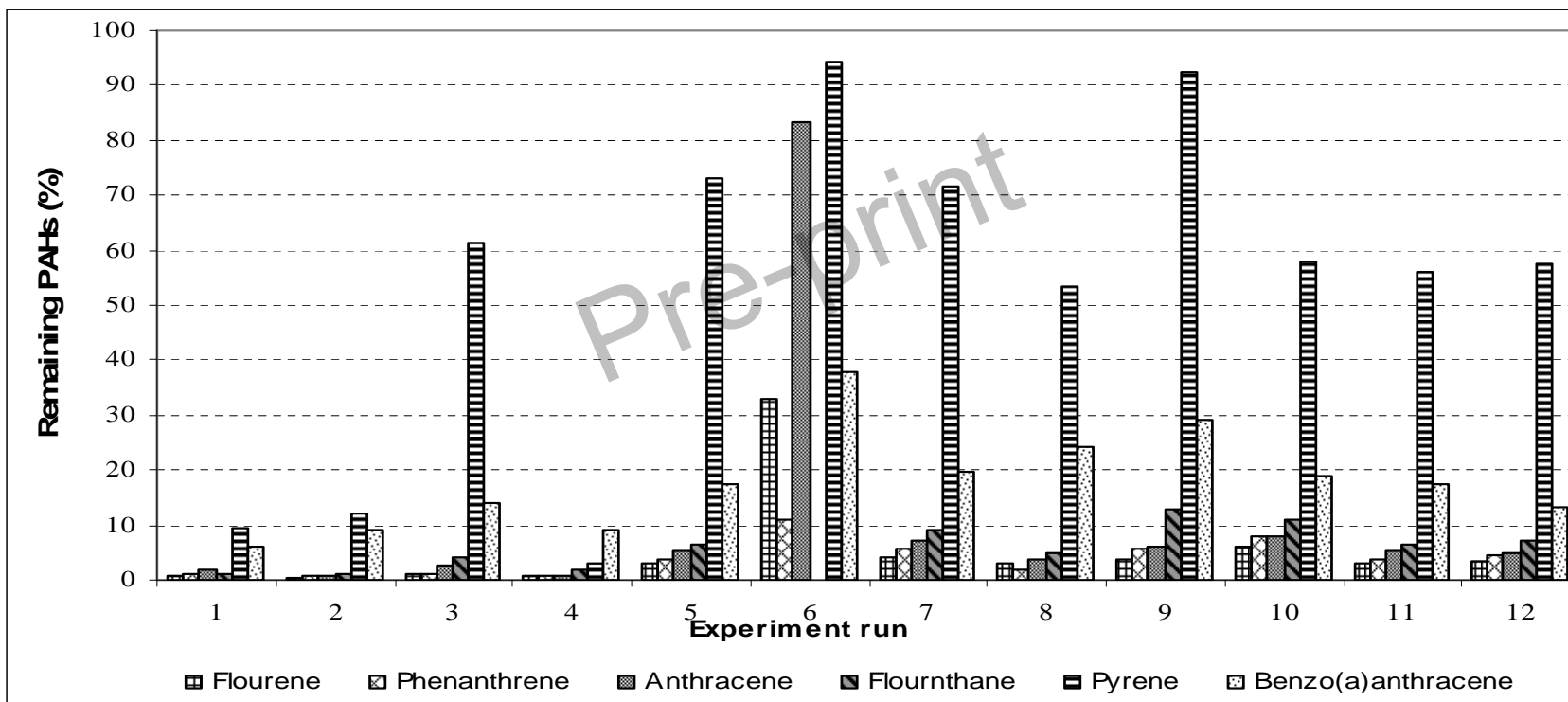


Figure 4: Sayara et al.

