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# Influence of Organic Amendments and Moisture Regime on Soil $CO_2$-C Efflux and Polycyclic Aromatic Hydrocarbons (PAHs) Degradation

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**Abstract:** In this study, a 30-day incubation experiment was performed to investigate the interactive effects of soil moisture content and two types of organic manure (animal manure: M and wheat straw: WS) on organic C mineralization and the degradation of PAH compounds. Specifically, washed sandy soil sample free from PAHs was treated with combined standard solution containing six different PAHs; pyrene (Pyr), fluoranthene (Flt), benzo[a]pyrene (BaP), benzo[g,h,i]perylene (BghiP), benzo[k]fluoranthene (BkF), and indeno[123-cd]pyrene (IP). The soil samples treated with PAHs were amended with M or WS and then, the soil samples were incubated and subjected to two levels of moisture content (50% and 100% field capacity, FC). The results indicate that $CO_2$–C rates were the highest at day 1, but they tended to be decreased sharply when incubation time increased. The results showed that the higher rate of $CO_2$-C efflux rate and cumulative were observed in M and WS treatments at 100% FC. Applying organic amendments at 50% FC increased the total cumulative $CO_2$-C from 21.6 mg kg$^{-1}$ to 228 mg kg$^{-1}$ for M and to 216 mg kg$^{-1}$ for WS. Meanwhile, applying organic amendments at 50% FC increased the total cumulative $CO_2$-C from 30 mg kg$^{-1}$ to 381 mg kg$^{-1}$ for M and to 492 mg kg$^{-1}$ for WS. The highest increases at 100% FC could be explained by the optimum water content at field capacity. PAHs concentrations decreased significantly in the presence of organic amendments in relation to enhance $CO_2$-C efflux (soil respiration) and to decrease soil pH. It could be concluded that applying organic amendments might be a useful technique to remediate soil PAHs through mineralization.

**Keywords:** climate change; $CO_2$-efflux; soil respiration; organic manure; PAHs

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## 1. Introduction

The polycyclic aromatic hydrocarbons (PAHs) are one of the most researched classes of organic contaminants found in the environment due to anthropogenic activities [1,2]. These compounds of PAHs have a number of structures and toxicity levels in the environment. Polycyclic aromatic hydrocarbons (PAHs) are hardly biodegradable carcinogenic organic compounds [3]. Therefore, remediation is an urgent tool to remove the PAHs from contaminated sites.

Bioremediation is a commonly used method for treating PAH contaminated environments such as soils, sediment, water bodies and wastewater [4]. Through bioremediation process of PAHs-contaminated soils, soil microorganisms are used for PAHs degradation in situ into less toxic compounds [5]. It has been demonstrated that applying organic waste application to soil has advantage to remove PAHs through degradation processes [6]. The effect of some agricultural wastes on enhancing the degradation of aged PAHs was investigated and they found that the organically agricultural wastes enhanced the degradation of aged PAHs most likely through changing the abundances and community structure of microbial PAH degraders [7]. In a study conducted by Wan et al. [8] to study the effects of organic amendments (including pig manure, sewage sludge and soybean refuse.) on the PAHs degradation, pig manure showed the highest removal efficiency for PAHs than other two organically-amendments of sewage sludge and soybean refuse, mainly due to the decomposition process. Additionally, they investigated the effects of PAHs toxicity on the germination of cress seed and no phytotoxicity was found. Overall, this study suggested that manure is an effective organic amendment for remediation of soil PAHs. It has been previously suggested that the application of composts produced from manure enriched with water-extractable organic matter could be a successful soil amendment in the in-situ PAHs bioremediation [9]. It has been suggested that altering soil properties by the application of organic amendments might control micro-organisms activity, resulting in higher rates of PAHs degradation [10]. The solid organic matrix found in the organic amendment may be favorable for the degradation of PAHs [11]. The influence of organic amendment properties on the degradation of PAHs in polluted soil has been studied by Lukić et al. [12], who reported that the investigation of organic amendments properties could provide a better understanding for a bioremediation process of PAH-polluted soil. Additionally, previous research reported that organic amendments could be promising tools for the remediation of PAHs-polluted soils by stimulating indigenous degraders by providing nutrients and enhancing microbial metabolism, which are responsible for PAHs mineralization [13].

Though there are a number of studies on the influences of organic amendments on soil PAHs degradation, the integrated effects of organic amendments and soil moisture content on soil PAHs degradation in relation to carbon mineralization has not been investigate. Our hypothesis is that amending the PAHs contaminated soils with organic materials would increase the PAHs degradation by enhancing carbon mineralization. Therefore, the aim of this study was to investigate the $CO_2$-C mineralization and the degradation of PAHs in media of sandy soil amended with organic amendments under different moisture regime.

## 2. Materials and Methods

### 2.1. Solvents

PAHs were extracted and cleaned using residue-analysis grade solvents obtained from Fisher Scientific (Fair Lawn, NJ, USA).

### 2.2. PAHs Standards

Six different PAHs were used in the current study (namely: pyrene (Pyr), fluoranthene (Flt), benzo[a]pyrene (BaP), benzo[g,h,i]perylene (BgP), benzo[k]fluoranthene (BkF), and indeno [123-cd]pyrene (IP)) (AccuStandard, Inc., New Haven, CT, USA). The standard solutions (100 ppb of each PAHs) were prepared for using in the incubation experument. Table 1 shows polycyclic aromatic hydrocarbons (PAHs) priority characterization. Additionally, Figure 1 shows chemical structures of the used PAHs.

**Table 1.** Polycyclic aromatic hydrocarbons (PAHs) priority characterization.

| PAHs | No. of Rings | MW (g/mol) | Solubility in Water at 25 °C (mg $L^{-1}$) | Vapor Pressure (Pa) | Log $K_{ow}$ | Log $K_{oc}$ | LOD (ng/mL) | LQD (ng/mL) | Recovery % |
|---|---|---|---|---|---|---|---|---|---|
| Pyrene (Pyr) | 4 | 202 | $1.3 \times 10^{-1}$ | $3.3 \times 10^{-4}$ | 4.90 | 4.58 | 1.33 | 3.99 | 99.65 ± 2.68 |
| Fluoranthene (Flt) | 4 | 202 | $2.1 \times 10^{-1}$ | $6.7 \times 10^{-4}$ | 4.90 | 4.58 | 1.21 | 3.63 | 97.58 ± 2.66 |
| Benzo[a]pyrene (BaP) | 5 | 252 | $3.8 \times 10^{-3}$ | $7.5 \times 10^{-7}$ | 6.06 | 6.74 | 1.41 | 4.23 | 98.44 ± 2.12 |
| Benzo[k]fluoranthene (BkF) | 5 | 252 | $4.3 \times 10^{-3}$ | $6.7 \times 10^{-5}$ | 6.06 | 5.74 | 1.63 | 4.89 | 98.48 ± 2.39 |
| Benzo[g,h,i]perylene (BghiP) | 6 | 276 | $2.6 \times 10^{-4}$ | $1.4 \times 10^{-8}$ | 6.50 | 6.20 | 2.11 | 6.33 | 99.18 ± 2.09 |
| Indeno[1,2,3-cd]pyrene (IP) | 6 | 276 | $5.3 \times 10^{-4}$ | $1.3 \times 10^{-8}$ | 6.50 | 6.20 | 2.14 | 6.42 | 98.11 ± 2.34 |

PAHs: polycyclic aromatic hydrocarbons; MW: molecular weight; $K_{ow}$: octanol–water partition coefficien; $K_{oc}$: organic carbon partitioning coefficient; LOD: the limit of detection; LOQ: the limit of quantification.

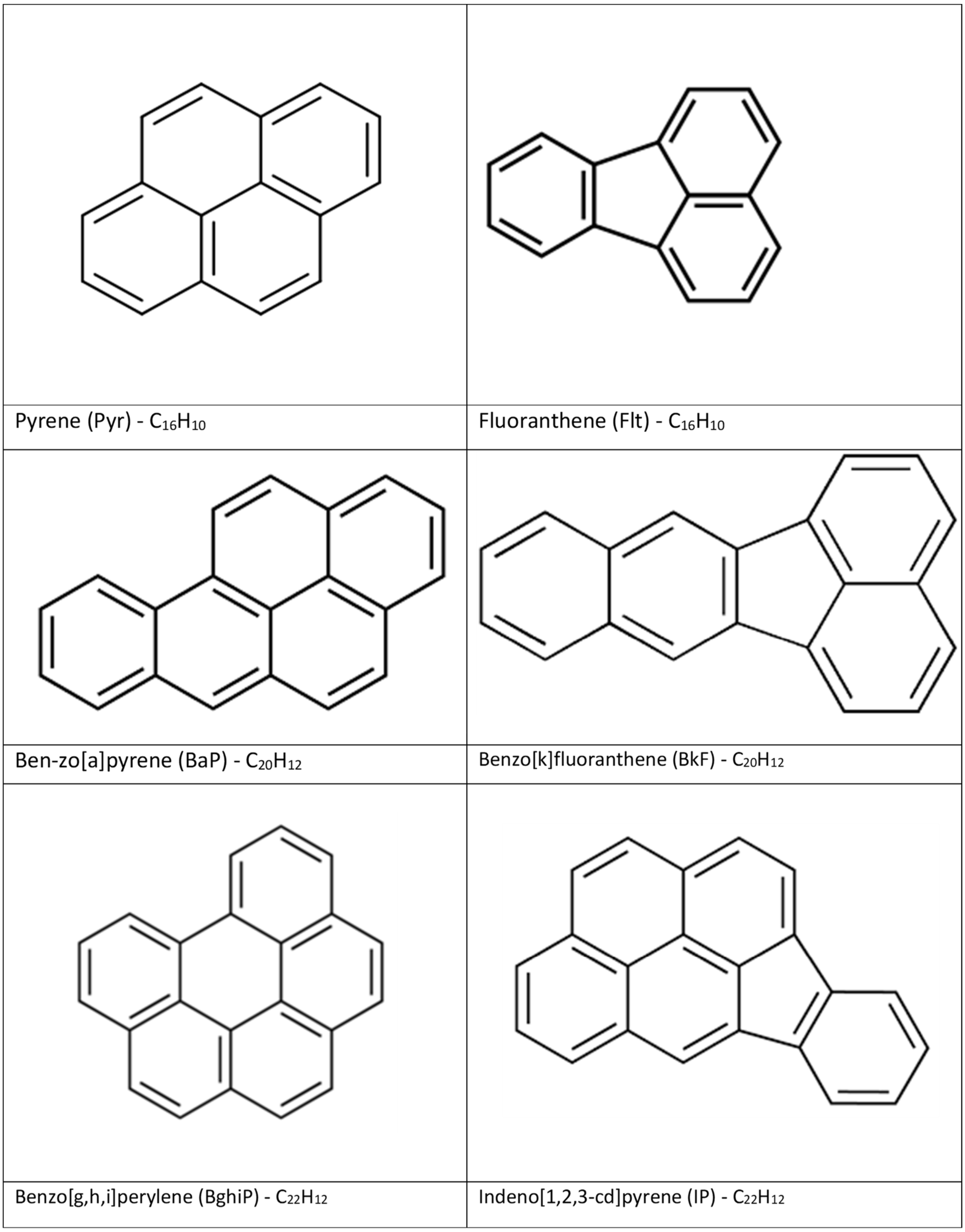

**Figure 1.** Chemical structures of the used PAHs.

### *2.3. Incubation Experiment and Determination of $CO_2$-C Efflux, pH and EC*

The overall goal of this study is to assess the impact of soil moisture regimes and organic amendments of animal manure (M) and wheat straw (WS) as carbon source on biodegradation of the Polycyclic Aromatic Hydrocarbons (PAHs) in soils. A 30-day incubation experiment was performed to investigate the interactive effects of soil moisture content and two types of organic amendments (animal manure and straw wheat) on organic C mineralization and the degradation of PAH compounds in the sandy soil medium. Specifically, washed sandy soil sample free from PAHs, treated with combined standard solution of PAHs (Spiked by 0 and 100 ppb of each PAHs). The animal manure (M) and wheat straw (WS) were added at application rate of 2% (*w*/*w*) to sandy soil. The sand mixtures (25 g) were put in glass vessels (250 mL). Ultra-pure water was added to each soil mixture to bring it to two rates of soil moisture regimes (50% and 100% of field capacity), which were maintained through incubation periods by weighing method. A soil without addition of organic amendments was also incubated as the control (CK) at the two soil moisture regimes (50% and 100% of field capacity). Each treatment was triplicated. In the current study, as a proxy for soil water holding capacity, we used a term of field capacity. The soil moisture content at field capacity is defined as the amount of soil moisture that remains in the soil after all excess water has been drained away [14].

Small vials with 5 mL of 0.5 M NaOH solution were placed in incubation vessels to trap $CO_2$. After the addition of NaOH, the vessels were closed airtight and incubated at 30 °C. The NaOH solution in the vials was changed after 1, 3, 7, 15, and 30 days. The excess NaOH was titrated with 0.1 M HCl after addition of $BaCl_2$ [15]. Total organic C mineralized was calculated as $CO_2$-C rate and cumulative $CO_2$-C evolution [16]. The soil pH and electrical conductivity (EC) were measured at 0, 1, 3, and 30 days of incubation. The soil pH was measured with a pH meter, in a suspension of soil:water ratio of 1:2.5. The soil electrical conductivity (EC) was measured with a EC meter, in extracts of soil:water (1:2.5).

Organic amendments samples (wheat straw and animal manure) applied in the current work were collected from the farm of the Agriculture Faculty, King Saud University, Saudi Arabia. The organic amendments samples were air-dried and ground before using in the incubation experiment. The wheat straw contents of C, N, P, and K amounted to 568, 31.2, 0.41, and 5.16 g $kg^{-1}$, respectively. Meanwhile, the animal (cattle) manure contents of C, N, P, and K accounted for 319, 41.5, 4.1, and 5.3 g $kg^{-1}$, respectively. The detailed analysis of wheat straw is presented in previously published work by Alotaibi et al. [17].

### 2.4. PAHs Extraction, Fractionation and Analysis

In a stainless steel accelerated solvent extractor (ASE) cell (Dionex, Sunnyvale, CA, USA), soil samples were extracted with GC grade acetone/dichloromethane. The detailed procedure for soil samples preparation and PAHs extraction and fractionation as well as their analysis using gas chromatography mass spectrometry (GC-MS) were presented in EL-Saeid et al. [18].

### 2.5. Statistical Analysis

The statistical analysis (ANOVA and Pearson Correlation) for the obtained data was achieved by JASP, a free and open-source program for statistical analysis.

## 3. Results and Discussion

### 3.1. Effect of pH and EC

Table 2 shows the changes in the values of pH following the incorporation of animal manure and wheat straw at day 0, day 1, day 3 and day 30 of incubation. At all incubation times, the pH values of the soils treated with M and WS were lower than the control pH. This decline in the values of soil pH might be due to the acidic organically compounds of the M and WS amendments. Through microbial metabolism and mineralization, the produced organic acids such as hmic acid and amino acid, as well as the released $NH_4^+$ and $CO_2$ may be responsible for lowering pH. Numerous studies have found that the application of organic amendments can result in decreasing soil pH inducing acidic effect [19,20]. At day 0, the M and WS decreased the soil pH from 7.82 to 7.22 and 7.40 at 50% FC, and from 7.83 to 7.18 and 7.33 at 100% FC, respectively. Meanwhile, at day 30, the M and WS decreased the soil pH from 8.21 to 7.40 and 7.44 at 50% FC, and from 8.25 to 7.38 and 7.65 at 100% FC, respectively. It was observed that the pH values tended to increase with time especially at the end of incubation time (day 30). The sensitivity of changes in values of pH following the application of organic materials may be because of the low buffering capacity of the used sand soil.

**Table 2.** Changes in pH and EC values following the application of manure (M) and wheat straw (WS) and at different moisture content.

| **Soil pH** | | | | | | |
|---|---|---|---|---|---|---|
| **Incubation Time, Day** | **50% FC** | | | **100% FC** | | |
| | **CK** | **M** | **WS** | **CK** | **M** | **WS** |
| 0 | 7.82 ± 0.01 | 7.22 ± 0.07 | 7.40 ± 0.02 | 7.83 ± 0.0 | 7.18 ± 0.02 | 7.33 ± 0.01 |
| 1 | 7.85 ± 0.02 | 7.25 ± 0.03 | 7.34 ± 0.0 | 7.91 ± 0.01 | 7.34 ± 0.02 | 7.32 ± 0.06 |
| 3 | 8.01 ± 0.02 | 7.37 ± 0.05 | 7.30 ± 0.02 | 7.93 ± 0.03 | 7.28 ± 0.01 | 7.36 ± 0.02 |
| 30 | 8.21 ± 0.08 | 7.40 ± 0.04 | 7.44 ± 0.01 | 8.25 ± 0.04 | 7.38 ± 0.03 | 7.65 ± 0.12 |
| **Analysis of variance** | | | | | | |
| *F* value | 21.00 | 3.73 | 22.65 | 59.99 | 21.16 | 5.55 |
| *p* value | 0.007 | 0.118 | 0.006 | 0.001 | 0.006 | 0.066 |
| **Soil EC** | | | | | | |
| **Incubation Time, Day** | **50% FC** | | | **100% FC** | | |
| | **CK** | **M** | **WS** | **CK** | **M** | **WS** |
| 0 | 0.47 ± 0.0 | 0.62 ± 0.02 | 0.73 ± 0.01 | 0.47 ± 0.0 | 0.68 ± 0.02 | 0.75 ± 0.0 |
| 1 | 0.45 ± 0.0 | 0.65 ± 0.03 | 0.72 ± 0.01 | 0.51 ± 0.0 | 0.74 ± 0.04 | 0.70 ± 0.0 |
| 3 | 0.46 ± 0.0 | 0.61 ± 0.02 | 0.69 ± 0.0 | 0.48 ± 0.02 | 0.65 ± 0.02 | 0.73 ± 0.01 |
| 30 | 0.44 ± 0.0 | 0.61 ± 0.02 | 0.67 ± 0.01 | 0.48 ± 0.02 | 0.61 ± 0.0 | 0.56 ± 0.02 |
| **Analysis of variance** | | | | | | |
| *F* value | 19.67 | 0.60 | 30.33 | 3.28 | 6.83 | 39.48 |
| *p* value | 0.007 | 0.649 | 0.003 | 0.141 | 0.047 | 0.002 |

CK: Control; M: Animal manure; WS: Wheat straw; ±SD: Standard deviation.

Table 2 also shows the changes in the values of electrical conductivity (EC) after the addition of organic amendments of AM and WS. Relative to the control, throughout the incubation period, the investigated of both organic amendments increased the values of EC. For example, at day 0, the M and WS increased the values of EC from 0.47 dS $m^{-1}$ to 0.62 and 0.73 dS $m^{-1}$ at 50% FC, and from 0.47 to 0.68 and 0.75 dS $m^{-1}$ at 100% FC. Meanwhile, at day 30, the M and WS increased the values of EC from 0.44 dS $m^{-1}$ to 0.61 and 0.67 dS $m^{-1}$ at 50% FC, and from 0.48 dS $m^{-1}$ to 0.61 and 0.56 dS $m^{-1}$ at 100% FC.

### 3.2. Treatment Effects on Organic C Mineralization as $CO_2$ Efflux Rate

In the current study, the treatment effects on organic C mineralization were recorded as $CO_2$-C efflux rate (mg C $g^{-1}$ $soil^{-1}day^{-1}$) (Figure 2A) and cumulative $CO_2$-C evolution (mg $kg^{-1}$ soil) (Figure 2B). The results showed that the addition of wheat straw and organic manure enhances carbon dioxide emissions from soils. Applying organic amendments at 50% FC increased the total cumulative $CO_2$-C from 21.6 mg $kg^{-1}$ to 228 mg $kg^{-1}$ for animal organic manure and to 216 mg $kg^{-1}$ for wheat straw (Figure 2B). Meanwhile, applying organic amendments at 100% FC increased the total cumulative $CO_2$-C from 30 mg $kg^{-1}$ to 381 mg $kg^{-1}$ for animal organic manure and to 492 mg $kg^{-1}$ for wheat straw. The amendments of wheat straw and animal manure incorporated into the soils improve $CO_2$-C efflux (soil respiration). Both wheat straw and animal manure could contain easily available C for microorganisms, enhancing $CO_2$-C efflux [21].

The values of $CO_2$-C rates were the highest at day 1, but they tended to be decreased sharply when incubation time increased (Figure 2A). Overall, the lowest rates were recorded at the end period of incubation. This can be attributed to a high portion of the biodegradable organic C. This biodegradable portion is available to soil microorganisms at the beginning but gradually decreased with the time, lowering the microbial activity [22,23]. Several other researchers reported that the $CO_2$ efflux (soil respiration) has two phases: (1) an initial rapid phase having easily available decomposable fraction, (2) followed by a slower phase of decomposition having the more resistant compounds such as such as lignin and other macromolecules [21,24].

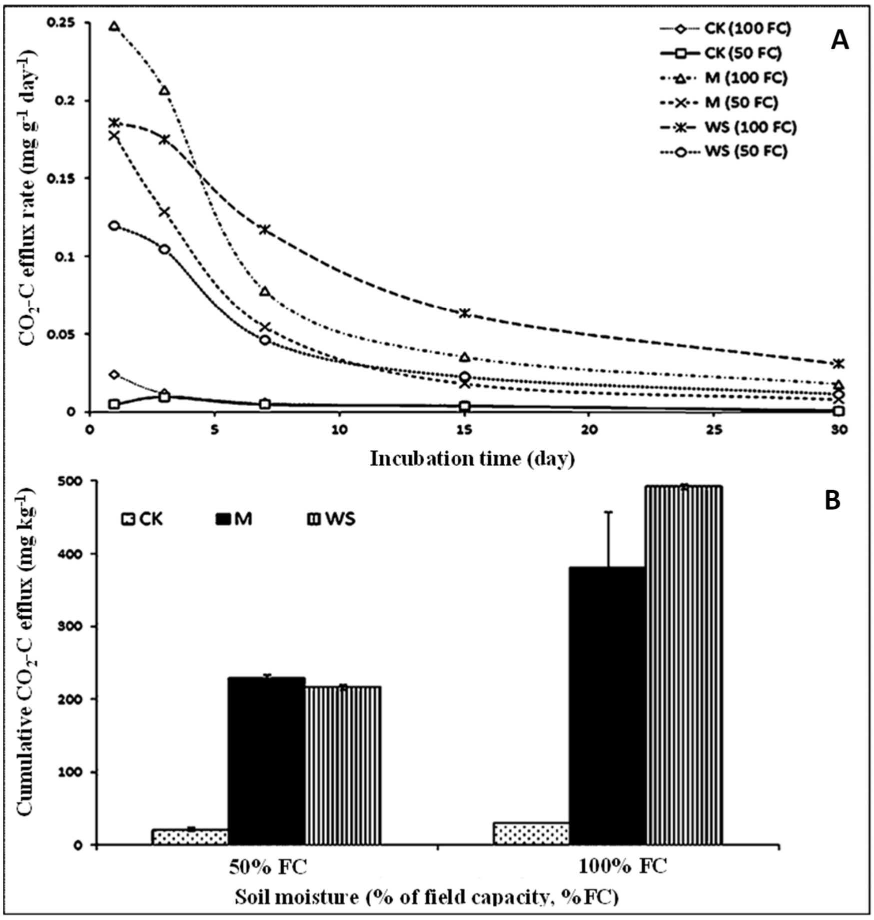

**Figure 2.** Treatment effects on $CO_2$-C efflux rate (**A**) and cumulative $CO_2$-C efflux (**B**).

Overall, the higher rate of $CO_2$-C efflux and cumulative $CO_2$-C were observed in organic manure and wheat straw treatments at 100% FC. The highest increases at 100% FC could be explained by the optimum water content at field capacity. In this context, the dissolved organic carbon of organic amendments at optimum water content can be easily utilized by soil microorganisms as an energy source, resulting in increasing soil microorganism's activity and subsequently increasing soil respiration. Soil moisture is a significant determinant of soil respiration. Soil respiration is thought to enhance when soil moisture increases up to the field moisture capacity [25].

### 3.3. Treatment Effects on PAHs

Table 3 shows treatment effects on PAHs (pyrene (Pyr), fluoranthene (Flt), benzo[a]pyrene (BaP), ben-zo[g,h,i]perylene (BghiP), benzo[k]fluoranthene (BkF), and indeno[1,2,3-cd]pyrene (IP)) concentra-tions (ppb). Additionally, Table 4 shows *p* values among treatments at each incubation time and among incubation time for each treatment. Interestingly to note that PAHs concentrations decreased significantly in the presence of organic amendments (Table 3), especially at days 15 and 30. In this context, the correlation study showed that negative correlation between cumulative $CO_2$-C and the concentrations of all polycyclic aromatic hydrocarbons (r of −0.794, −0.814, −0.776, −0.806, −0.802, −0.812 for Pyr, Flt, BaP, BghiP, BkF, and IP, respectively), as indicated in Table 5. The negative correlation was significantly only for Flt and IP. These negative correlations indicate that the degradation of PAHs increased with increasing $CO_2$-C efflux (soil respiration). Applying organic amendments could provide significant amount of labile carbon resulting in increased soil microorganism activity and subsequent increased soil respiration and PAHs degradation. Włóka et al. [26] found that applying organic fertilizers to soils possess a significant influence on the removal of soil PAHs. Several other studies demonstrated that addition of organic wastes is possible and effective tool for bioremediation of PAHs-contaminated soil [8]. In a study conducted by Zhang et al. [6] to investigate the effect of organic wastes on the plant-microbe remediation for removal of aged PAHs in soils, organic waste application removed soil PAHs to a great extent, depending on the interactional effect of nutrients and dissolved organic matter in organic waste and soil microorganisms. Lukić et al. [12] reported that fresh organic wastes with the high level of soluble organic fraction and protein are suitable for bioremediation of PAH-polluted soil. However, the organic wastes with a lower level of the soluble fraction and protein resulted in a lower potential for PAHs degradation from polluted soil.

**Table 3.** Treatment effects on PAHs (pyrene (Pyr), fluoranthene (Flt), benzo[a]pyrene (BaP), benzo[g,h,i]perylene (BghiP), benzo[k]fluoranthene (BkF), and indeno[1,2,3-cd]pyrene (IP)) concentrations (ppb).

| **Day 1** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PAHs** | **50% FC** | | | | | | **100% FC** | | | | | |
| | **CK** | **±SD** | **M** | **±SD** | **WS** | **±SD** | **CK** | **±SD** | **M** | **±SD** | **WS** | **±SD** |
| Pyr | 99.5 | 0.10 | 97.1 | 0.01 | 97.2 | 0.08 | 99.4 | 0.05 | 97.7 | 0.16 | 97.1 | 0.01 |
| Flt | 99.6 | 0.06 | 97.3 | 0.05 | 97.3 | 0.05 | 99.6 | 0.06 | 97.3 | 0.05 | 97.3 | 0.05 |
| BaP | 99.6 | 0.04 | 98.5 | 0.02 | 98.3 | 0.01 | 99.6 | 0.04 | 98.5 | 0.02 | 98.3 | 0.01 |
| BgP | 99.7 | 0.20 | 97.2 | 0.01 | 97.2 | 0.01 | 99.7 | 0.20 | 97.2 | 0.01 | 97.2 | 0.01 |
| BkF | 99.6 | 0.09 | 98.3 | 0.11 | 98.3 | 0.11 | 99.5 | 0.02 | 98.3 | 0.11 | 98.3 | 0.11 |
| IP | 99.6 | 0.05 | 97.4 | 0.06 | 97.1 | 0.03 | 99.6 | 0.04 | 97.7 | 0.11 | 97.2 | 0.02 |
| **Day 15** | | | | | | | | | | | | |
| **PAHs** | **50% FC** | | | | | | **100% FC** | | | | | |
| | **CK** | **±SD** | **M** | **±SD** | **WS** | **±SD** | **CK** | **±SD** | **M** | **±SD** | **WS** | **±SD** |
| Pyr | 98.2 | 0.05 | 73.8 | 0.05 | 77.7 | **0.10** | **98.2** | **0.05** | **73.6** | **0.24** | **77.9** | **0.01** |
| Flt | 97.5 | 0.04 | 75.2 | 0.06 | 78.2 | **0.04** | **97.5** | **0.04** | **75.2** | **0.06** | **78.2** | **0.04** |
| BaP | 99.1 | 0.01 | 82.5 | 0.05 | 83.2 | **0.01** | **99.1** | **0.01** | **83.4** | **0.12** | **85.1** | **0.02** |
| BgP | 97.4 | 0.04 | 73.2 | 0.03 | 76.1 | **0.05** | **97.4** | **0.04** | **73.2** | **0.03** | **76.1** | **0.05** |
| BkF | 97.5 | 0.02 | 73.1 | 0.03 | 76.1 | **0.01** | **97.5** | **0.02** | **73.1** | **0.03** | **76.1** | **0.01** |
| IP | 98.9 | 0.54 | 76.3 | 0.01 | 77.3 | **0.01** | **98.9** | **0.54** | **76.6** | **0.27** | **78.4** | **0.01** |
| **Day 30** | | | | | | | | | | | | |
| **PAHs** | **50% FC** | | | | | | **100% FC** | | | | | |
| | **CK** | **±SD** | **M** | **±SD** | **WS** | **±SD** | **CK** | **±SD** | **M** | **±SD** | **WS** | **±SD** |
| Pyr | 97.4 | 0.02 | 50.2 | 0.02 | 51.0 | 0.00 | 97.2 | 0.01 | 52.8 | 0.09 | 55.4 | 0.09 |
| Flt | 95.3 | 0.02 | 50.3 | 0.06 | 52.2 | 0.05 | 95.4 | 0.07 | 51.4 | 0.06 | 54.0 | 0.01 |
| BaP | 97.6 | 0.04 | 60.3 | 0.12 | 63.2 | 0.02 | 97.1 | 0.02 | 62.5 | 0.18 | 67.2 | 0.05 |
| BghiP | 95.6 | 0.05 | 51.7 | 0.01 | 52.2 | 0.08 | 95.4 | 0.04 | 53.8 | 0.04 | 55.2 | 0.11 |
| BkF | 95.1 | 0.02 | 51.3 | 0.11 | 53.2 | 0.09 | 95.5 | 0.02 | 53.3 | 0.02 | 56.2 | 0.15 |
| IP | 96.1 | 0.05 | 52.4 | 0.03 | 54.5 | 0.04 | 96.4 | 0.04 | 54.1 | 0.02 | 56.3 | 0.05 |

CK: Control; M: Animal manure; WS: Wheat straw; ±SD: Standard deviation; Pyrene (Pyr); Fluoranthene (Flt); Benzo[a]pyrene (BaP); Benzo[g,h,i]perylene (BghiP); Benzo[k]fluoranthene (BkF); Indeno[1,2,3-cd]pyrene (IP).

**Table 4.** ANOVA table showing $p$ values among treatments at each incubation time and among incubation time at each treatment.

| **$p$ Values among Treatments at Each Incubation Time** | | | | | | |
|---|---|---|---|---|---|---|
| | **Pyr** | **Flt** | **BaP** | **BghiP** | **BkF** | **IP** |
| | | | 50% FC | | | |
| 1 | 0.000 | 0.000 | <0.0001 | 0.001 | 0.005 | <0.0001 |
| 15 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| 30 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| | | | 100% FC | | | |
| 1 | 0.001 | 0.000 | <0.0001 | 0.001 | 0.004 | 0.000 |
| 15 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| 30 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| **$p$ Values among Incubation Time at Each Treatment** | | | | | | |
| | **Pyr** | **Flt** | **BaP** | **BghiP** | **BkF** | **IP** |
| | | | 50% FC | | | |
| CK | <0.0001 | <0.0001 | <0.0001 | 0.000 | <0.0001 | 0.009 |
| M | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| WS | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| | | | 100% FC | | | |
| CK | <0.0001 | <0.0001 | <0.0001 | 0.000 | <0.0001 | 0.011 |
| M | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| WS | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

CK: Control; M: Animal manure; WS: Wheat straw; ±SD: Standard deviation; Pyrene (Pyr); Fluoranthene (Flt); Benzo[a]pyrene (BaP); Benzo[g,h,i]perylene (BghiP); Benzo[k]fluoranthene (BkF); Indeno[1,2,3-cd]pyrene (IP).

**Table 5.** Pearson correlation coefficient (r) among various parameters (the concentrations of PAHS, pH or cumulative $CO_2$-C).

| **Variables** | **pH** | **Cum. $CO_2$-C** | **Pyr** | **Flt** | **BaP** | **BghiP** | **BkF** | **IP** |
|---|---|---|---|---|---|---|---|---|
| pH | — | | | | | | | |
| Cum. $CO_2$-C | −0.722 | — | | | | | | |
| Pyr | 0.983 *** | −0.794 | — | | | | | |
| Flt | 0.981 *** | −0.814 * | 0.999 *** | — | | | | |
| BaP | 0.99 *** | −0.776 | 0.998 *** | 0.997 *** | — | | | |
| BghiP | 0.979 *** | −0.806 | 1 *** | 0.999 *** | 0.997 *** | — | | |
| BkF | 0.984 *** | −0.802 | 1 *** | 1 *** | 0.998 *** | 1 *** | — | |
| IP | 0.981 *** | −0.812 * | 0.999 *** | 1 *** | 0.998 *** | 1 *** | 1 *** | — |

* $p < 0.05$, *** $p < 0.001$. Pyrene (Pyr); Fluoranthene (Flt); Benzo[a]pyrene (BaP); Benzo[g,h,i]perylene (BghiP); Benzo[k]fluoranthene (BkF); Indeno[1,2,3-cd]pyrene (IP).

Additionally, the correlation study showed that highly significant positive correlation between pH and the concentrations of all polycyclic aromatic hydrocarbons (r of 0.983, 0.981, 0.99, 0.979, 0.984, 0.981 for Pyr, Flt, BaP, BghiP, BkF, and IP, respectively) (Table 5). This suggests that the degradation of PAHs increased with decreasing the values of pH. In a study conducted by Yu [27] to investigate the influence of pH on the degradation of PAHs in sludge during biological fermentation, the pH of 7.7 showed the best effect on PAHs degradation; however, the pH of 8.2 was the worst. Kästner et al. [28] reported that soil neutralization is favorable for the degradation of PAHs. Similarly, Pawar [10] found that altering pH is the most effective tool to remediate PAHs polluted soil and it has been found that soil pH of 7.5 is highly suitable for the degradation of PAHs, mainly due to enhance microbial populations, and microbial biomass. In the current study, at day 30, the application of M and WS decreased the soil pH from 8.21 to 7.40 and 7.44 at 50% FC, and from 8.25 to 7.38 and 7.65 at 100% FC. Therefore, our results suggest that the altering pH values (to be in the range of 7.38–7.65) by the addition of animal manure or wheat straw might be responsible for enhancing the degradation of PAHs as indicated by highly significant correlation between pH values and the concentrations of PAHs.

## 4. Conclusions

The results indicated that the higher rate of $CO_2$-efflux rate and cumulative $CO_2$-C were observed in organic manure and wheat straw treatments at 100% FC. The correlation study showed that negative correlation between cumulative $CO_2$-C and the concentrations of PAHs, suggesting enhancing PAHs degradation with increasing C mineralization. On the contrary, highly significant positive correlation were found between pH and the concentrations of PAHs. Based on the obtained findings, the application of organic amendment to sandy soil may create good biodegradation conditions by increasing soil microbial activity and decreasing soil pH, resulting in increasing soil respiration and PAHs degradation. Further research, therefore, still needed to investigate the effects of applied organic amendments under field conditions on bioremediation of PAHs polluted soils.

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