The impact of carbon nanomaterials on the development of phenanthrene catabolism in soil

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Capsule: The presence of high concentrations of MWCNT and fullerene soot affected the development of catabolism

Abstract

This study investigated the impact of different types of carbon nanomaterials (CNMs) namely C_{60}, multi-walled carbon nanotubes (MWCNT) and fullerene soot on the catabolism of ^{14}C-phenanthrene in soil by indigenous microorganisms. Different concentrations (0%, 0.01%, 0.1% and 1%) of the different CNMs were blended with soil spiked with 50 mg kg^{-1} of ^{12}C-phenanthrene, and aged for 1, 25, 50 and 100 d. An increase in concentration of MWCNT- and FS amended to soils showed a significant difference ($P = 0.014$) in the lag phase, maximum rates and overall extents of ^{14}C- phenanthrene mineralisation. Microbial cell numbers did not show an obvious trend, but it was observed that control soils had the highest population of heterotrophic and phenanthrene degrading bacteria at all time points.

Keywords: Catabolism; Carbon nanomaterials; ^{14}C-Phenanthrene; Soil.

1. Introduction

There has been dramatic increase in production and use of nanomaterials in the last decade, which promises to grow in the future; therefore, the release of these materials into the environment is inevitable. Carbon nanomaterials (CNMs) have attracted considerable attention due to their unique physical, electrical and thermal properties. They have been shown to have potential applications in several areas, particularly in hydrogen storage, as semi-conductors, in biomedical applications and environmental remediation [1]. Examples of these carbon nanomaterials are fullerene soot, Buckminster fullerene (C_{60}) and multi-walled carbon nanotubes (MWCNTs). Fullerenes are arranged in a spherical configuration forming a closed graphite ball with only an external surface, while several rolled-up graphite sheets form MWCNT structure, creating interstitial wall spaces inside the inner cavity [2]. Carbon nanotubes have a high surface area to volume ratio, as well as a strong affinity towards organic contaminants like polycyclic aromatic hydrocarbons (PAHs) and other hydrophobic organic contaminants (HOCs) [3, 4]. Fullerenes (C_{60}) are arranged in a spherical configuration forming a closed graphite ball with a single external surface [2]. As CNMs have large reactive surface areas, exhibit strong hydrophobicity and high sorption capacities; they have applications as sorbents of HOCs, such as PAHs, in aquatic and terrestrial environments [5]. Understanding the interactions between organic contaminants and CNMs is therefore essential for evaluating the potential environmental impact of CNMs [6, 7].

Soil is one of the sinks of PAHs and CNMs in the ecosystem and soil microorganisms that interact directly with the soil environment could be significantly affected when exposed to CNMs [8, 9]. Thus, investigating the impact of CNMs on soil microbial activity will provide an insight on how CNMs may affect the fate of organic contaminants in soil. Although, there are a few studies on how CNMs affect soil microorganisms, the results have varied, with some studies finding profound effects of CNMs [4, 9], while others found little or no significant

impact [10, 11]. The varying results may have stemmed from differences in the pre-treatment of fullerenes, which would have altered their physicochemical properties differently [12]. For instance, no significant effect of fullerenes on soil respiration was detected when soils were treated with fullerenes in either 1000 μg g^{-1} soil of granular form or 1 μg g^{-1} soil in aqueous suspension [11]. However, low concentrations of fullerenes repressed the number of fast-growing bacteria immediately after the application of fullerene suspension to soils [12]. Because these materials seem to be extremely resistant to degradation, they might accumulate at specific sites in the geo- and hydrosphere (e.g. soils, groundwater, streams, lakes, sediments, and oceans) or in the biosphere and possibly within specific organisms. The recent rapid development of nanotechnology has driven a considerable number of studies in the use of carbon nanomaterials as soil and ground water remediation materials. The fate of CNMs depends on their size, number, concentration and type of material. It has been reported that CNMs, although engineered, may function similarly to other types of BC in the sequestration of HOCs [4, 13-15]. Therefore, the presence of CNMs in soils and/or sediment may lead to altered bioavailability of HOCs. As a result, understanding the interactions between organic HOCs and CNMs is essential for evaluating the potential environmental impact of CNTs, as well as the potential efficiency as superior sorbent in contaminated soil remediation. Therefore, a clearer understanding on the bioavailability of HOCs in soil in the presence of CNMs is required. To address this, this study investigated the impact of varying concentrations of different CNMs on catabolism of ^{14}C-phenanthrene by indigenous microorganisms in soil.

2. Materials and Methods

2.1. Materials

Non-labelled phenanthrene (> 96%) was obtained from Sigma Aldrich, UK and 9-^{14}C-phenanthrene (radio-chemical purity > 96%, specific activity 55 mCi mmol^{-1}) was obtained from American Radiolabeled Chemical Inc. (ARC). Buckminster fullerene (C_{60}) had a purity of >99.5% and a diameter of 1 nm), multi-walled carbon nanotubes (MWCNTs) had a purity of purity >90%, with a length of 5-9 µm, diameter of 10-15 nm, while fullerene soot (FS) was used "as produced". All CNMs were purchased from Sigma-Aldrich, UK. Chemicals for minimal basal salts (MBS) solution were obtained from BDH Chemicals, UK. Goldstar multipurpose liquid scintillation fluid (LSC) was obtained from Meridian, UK. Sodium hydroxide was obtained from Sigma Aldrich. Plate Count Agar (PCA) was obtained from Oxoid chemicals, UK. General Purpose Agar was obtained from Fisher-Scientific, UK.

2.2. Soil and soil spiking

A pasture agricultural soil (Dystric Cambisol) was collected (from the A horizon; depth of 5-20 cm) from Myerscough college, Lancashire, UK. Soil physico-chemical properties are as follows: pH 6.5, organic matter 2.7%, sand 60.4%, silt 20%, and clay 19.5%. The air-dried soil was sieved with a 2 mm sieve to remove roots and stones, and then stored at 4 °C until ready for use. When ready for use, soil was rehydrated with deionised water back to original water holding capacity (WHC). A third of whole soil was first spiked with ^{12}C-phenanthrene prepared in toluene to achieve a concentration of 50 mg kg^{-1}, which was then mixed with a stainless-steel spoon for 3 min followed by a period of venting (1–2 h). Afterwards, the amended soil was mixed with the remaining unspiked soil fraction following the method of Doick et al [16]. Aliquots of soil were then mixed with different concentrations (0%, 0.01%, 0.1% and 1%) of C_{60}, MWCNT or FS. Soil-CNMs aliquots were then sealed in amber glass jars (in triplicate per treatment) and left to age in the dark at 20 ± 2 °C and analysed at 0, 25, 50 and 100 d, respectively. At each time point, fresh ^{12}C/^{14}C-phenanthrene (42 Bq g^{-1} soil)

was spiked to each of the previously aged soils, and respirometry was carried out for 14 d. Blank soils with neither phenanthrene nor CNMs were also prepared.

2.3. Mineralisation of ^{14}C-phenanthrene in soil

^{14}C-Phenanthrenre mineralisation was assessed in modified 250 ml Erlenmeyer flasks and the soils were sampled after 1, 25, 50 and 100 d soil-phenanthrene contact time, as previously described by ~~following the method of~~ Reid et al. [17]. Each respirometer incorporated a Teflon-lined screw cap and a CO_2 trap containing 1 M NaOH (1 ml) within a suspended 7 ml glass scintillation vial. Respirometers were prepared in triplicate, with 10 ± 0.2 g soil (dry weight) and 30 ml sterilised minimal basal salts medium (MBS) to give a soil to liquid ratio of 1:3 [18]. The respirometric flasks were placed securely on an orbital shaker (IKA Labortechnik KS501 digital), incubated at 20 ± 2 °C and shaken at 100 rpm for 14 days to ensure adequate mixing of the slurry over the sampling period. The ^{14}C-activity in the $^{14}CO_2$ trap was assessed after every 24 hours by replacing the NaOH traps and adding liquid scintillation fluid (5 ml) to each spent $^{14}CO_2$ trap. After storage in darkness overnight, trapped ^{14}C-activity was quantified using a Canberra Packard Tri-Carb 2250CA liquid scintillation analyser, using standard protocols for counting and automatic quench correction. An analytical blank (containing no ^{14}C-phenanthrene) determined the level of background activity. We calculated the length of the lag phase (defined as the time taken for mineralisation to reach 5%), the fastest initial rate and cumulative extent of ^{14}C-phenanthrene mineralisation over the 14 days [19].

2.4. Enumeration of bacterial numbers in soil

Colony forming units (CFUs) of culturable heterotrophic and phenanthrene degrading bacteria were determined by plating serial dilutions of soil samples in sterile quarter-strength

Ringer's solution on plate count agar (PCA) using a viable count and General purpose agar amended with ^{12}C-phenanthrene. The density was calculated as colony forming units per gram (CFU g^{-1}) of soil on dry weight basis. The number of bacterial CFUs g^{-1} was counted after 3 and 7 d of incubation at 28 ± 2 ºC [20].

2.4. Statistical Analysis

Following blank correction, statistical analysis of the results from mineralisation assays was done using the Sigma Stat for Windows (Version 3.5, SPSS Inc.). All graphs were presented using SigmaPlot for Windows (Version 10.0, SPSS Inc.). Statistical significance of the addition of the different types of CNM, at different concentrations and soil contact time was determined using analysis of variance (ANOVA) followed by Tukey's test at the 95% confidence level ($P < 0.05$) to assess significant differences.

3. Results

The catabolism of ^{14}C-phenanthrene was monitored for 14 days in soils spiked with various concentrations; 0%, 0.01%, 0.1% and 1% of C_{60}, MWCNT or FS at 1, 25, 50 and 100 d soil-phenanthrene contact time (Figures 1-3).

3.1. Lag phase

The length of the lag phases varied over the course of the experiment and appeared to be dependent upon the concentration of CNMs, the type of CNMs and soil-phenanthrene contact time. Generally, lag phases of greater than 2 days were observed. The shortest lag phases were seen in soils amended with 0%, and the longest in 1% of CNM-amended soils (Tables 1-3). For example, at 1 d, the lag phases for 0% and 1% were 4.24 d and 5.51d, respectively, in C_{60}-amended soils, 7.98 d in MWCNT-amended soils while lag phase was not measurable

for 1% amendment in FS-amended soil. Overall, the length of the lag phases increased (P = 0.03) with an increase in the concentration of amended CNMs. Furthermore, an increase in contact time showed a decline (P = 0.023) in the length of the lag phases, with the shortest was observed after 100 d. Statistical analyses showed that a significant difference (P = 0.038) was observed in the lag phases when 1 d and 100 d were compared, but no difference (P = 0.792) was observed at consecutive time-points (Tables 1-3). A comparison between C_{60}, MWCNT and FS-amended soils, showed that C_{60}-amended soils consistently had shorter lag phases (P = 0.024), in comparison to MWCNT and FS-amended soils, respectively. Additionally, FS-amended soils mineralised <5% at 1 d and 25 d, respectively; therefore, no lag phases were measured. Statistical analysis showed that there were significant differences (P = 0.041), when compared, one against the other. However, this was apparent when only 1% of CNM was analysed, as concentrations <1% showed no difference (P = 0.579).

3.2. Maximum rates of ^{14}C-phenanthrene mineralisation

The maximum rates of mineralisation were measured in all CNM-amended soils, with increasing soil-phenanthrene contact time. The maximum rates of mineralisation ranged from 0.65 to 0.8% h^{-1} for control soils, 0.36 to 0.98% h^{-1}, 0.08 to 0.90 % h^{-1}, and 0.02 to 0.88% h^{-1} in $C_{60,}$ MWCNTs and FS-amended soils, respectively. Overall, control soils (0%) were observed to have the highest values; in contrast, the highest concentration (1%) of CNM-amended soils consistently had the lowest maximum rates of ^{14}C-phenanthrene mineralisation. At 1 d, control had higher values in the maximum rates of ^{14}C-phenanthrene mineralisation, and this was found to be statistically significant (P = 0.021) (Tables 1-3). At other time points, only concentrations >0.1% were found to be significant (P = 0.03) in all amended soils, compared to the control.

Generally, the addition of high concentrations of CNMs significantly (P = 0.032) affected the catabolism of ^{14}C-phenanthrene in all soils (Tables 1-3). Over time, the maximum rates of ^{14}C-phenanthrene mineralisation in control soils (0%) increased after 1 d (P = 0.02), but then reduced slightly; this was not significant (P = 0.764) after 25 d, and at consecutive time-points. For 0.01% and 0.1% CNM-amended soils, contact time was found to have a significant effect (P = 0.012) after 1 d, with the maximum rates of ^{14}C-phenanthrene mineralisation reducing at consecutive time points with an increase in contact time, although this was not significant after 25 d in any of the soils. However, statistical analysis showed that there was a significant reduction (P = 0.019) between 1 and 100 d contact time (Tables 1-3). Interestingly, for C_{60}-amended soils, there was no significant difference (P = 0.212) in the catabolic activity for all treatments. Thus, C_{60} applied at 1% did not show a difference to other concentrations, at all time-points (Table 1). Comparisons between C_{60}-, MWCNT- and FS-amended soils indicated that at concentrations above 0.01%, the maximum rates of mineralisation showed a statistically significant difference (P = 0.009), when C_{60} was compared to MWCNT and FS, respectively. However, MWCNT and FS showed no significant difference (P = 0.1762) when compared to each other (Tables 1-3).

3.3. Total extents of ^{14}C-phenanthrene mineralisation

The extents of ^{14}C-phenanthrene mineralisation declined as the concentration of CNMs increased (Figures 1-3). Generally, 1% CNM-amended soils consistently had the lowest (P < 0.001) extents of ^{14}C-phenanthrene mineralisation compared to that of the control soil (Figures 1-3; Tables 1-3). The total extents of ^{14}C-glucose mineralisation ranged from 36.9% to 47.7% for C_{60}-, 15.2% to 45.4% for MWCNT-, 3.67% to 45.1% for FS-amended soils, respectively. The results showed a concentration-dependent trend in the order: 0% > 0.01% > 0.1% > 1%. The data showed that at 1 d, soils amended with 1% C_{60} and MWCNT only

showed a significant difference ($P = 0.014$) (Figures 1 and 2; Tables 2 and 3), while concentrations >0.01% showed a significant difference ($P < 0.001$) in the FS-amended soils (Figure 3; Table 3). At other time-points, the influence of the addition of C_{60} showed no difference ($P = 0.248$) (Figure 1; Table 1). In contrast, MWCNT- and FS-amended soils showed a significant difference ($P = 0.017$) at 1% and >0.01%, respectively, at 25-100 d (Figures 2 and 3; Tables 2 and 3).

Figure 1 shows that an increase in contact time had no effect ($P = 0.094$) on the extent of ^{14}C-phenanthrene mineralisation in C_{60}-amended soils after 100 d, although there were slight increases in the overall extents of mineralisation. In addition, soils amended with 1% of C_{60}, MWCNT or FS increased as contact time increased, this increase was found to be significant ($P < 0.001$) after 25 d, but not at consecutive time-points afterwards (Figures 1-3, Tables 1-3). The comparison of the total extents of ^{14}C-phenanthrene mineralisation among the three different CNMs showed that C_{60}-amended soils had the greatest values, while FS-amended soils consistently had the lowest values; this was observed in both a concentration-dependent manner and increase in contact time. Although, significant differences (P = 0.001) were observed at 1% and > 0.1% for MWCNTs- and FS-amended soils, respectively, in comparison to C_{60}-amended soils. The trend can be summarised as C_{60} > MWCNTs > FS (Figures 1-3).

3.4. Colony forming units (CFUs) of heterotrophic and phenanthrene-degrading bacteria

Table 4 shows the CFUs of heterotrophic and phenanthrene degrading bacteria in soils amended with C_{60}, MWCNTs or FS. Generally, control soils had the highest counts of heterotrophic and phenanthrene-degrading bacteria. The amendment of different concentrations CNMs did not show a clear trend, this was seen in both heterotrophic and phenanthrene-degrading bacterial cell numbers. Over time, the CFUs reduced with an

240 increase in contact time, although there appeared to be more phenanthrene-degrading bacteria
241 than heterotrophs after 50 and 100 d, respectively (Table 4).
242
243
244 **4. Discussion**
245 This study investigated the impact of CNMs on the development of phenanthrene catabolism
246 in soil. In this study, application of high concentrations of CNMs significantly reduced ($P < 0.05$)
247 catabolic activity; the only exception to this was C_{60} which showed no difference across
248 the different concentrations. Generally, this study showed that there were increases in lag
249 phases, and concomitant reductions in the maximum rates and extents of ^{14}C-phenanthrene
250 mineralisation, as concentration of CNMs increased. This decrease may be as a result of
251 enhanced ^{14}C-phenanthrene sorption and a decline in the bioaccessible fraction. This is in
252 agreement to results from previous studies on the impact of black carbon and CNMs on
253 biodegradation [4, 21]. It is plausible that the number of sites available for PAH sorption will
254 increase with increasing CNM concentrations [14, 22]. The strong sorptive properties of CNMs
255 in reducing aqueous concentration and bioavailability of contaminants have been
256 demonstrated by previous authors [4, 14]. Contrary to expectations, this study did not find a
257 significant difference between the extents of ^{14}C-phenanthrene mineralisation when amended
258 with different concentrations of C_{60}; thus, the results suggest that C_{60} had no impact on the
259 biodegradation of the PAH. This is in agreement with a study by Tong, et al. [11], where it was
260 shown that the addition of C_{60} to soil had no effect on microbial activity. With an increase in
261 contact time, there were reductions in the length of the lag phases and maximum rates, but
262 increases in the extents of ^{14}C-phenanthrene mineralisation in CNM-amended soils,
263 suggesting that the indigenous microorganisms were adapting to the presence of the
264 phenanthrene [23, 24]. It is possible that over time, CNMs reduce the bioavailability (rates of

mineralisation), but not the bioaccessibility (overall extents of mineralisation) of the ^{14}C-PAH [25].

Viable counts were used to examine the effects of increasing CNM concentration on the total heterotrophic and phenanthrene-degrading bacteria. As observed, there was a similarity in the amount of heterotrophic and phenanthrene-degrading bacteria in all control soils, but with an increase in amendment of CNMs, there was a reduction in the numbers of culturable bacteria; this suggests that CNMs did influence total culturable cell number [12]. The data obtained from the culturing of indigenous microorganism showed that there was an appreciable number of heterotrophic and phenanthrene degrading bacteria, although the amount of culturable microorganisms seemed to decrease over time [26, 27]. The results showed that there were high numbers of phenanthrene degrading bacteria even at 1% amendment; it can therefore be assumed that the low mineralisation of ^{14}C-phenanthrene at the highest concentration of amendment was not due to the absence of degraders. The higher levels of phenanthrene mineralisation in control soils were also reflected by a significantly large number of phenanthrene degrading bacteria in all CNM amendments. Therefore it can be argued that the fluctuations within microbial communities may be as a result of changes in the respiratory activity of the soil microflora [28]. However, the lower extents of ^{14}C-phenanthrene mineralisation in the 1% amendment of CNMs and at the later stages of aging was not due to the lack of active phenanthrene-utilising microorganisms, but due to sorption effects of the CNMs [4, 12, 29]. It was observed that the low concentrations of C_{60} had reduced CFUs, which is in agreement with results obtained by Johansen, et al. [12]; however, it is not understood how this had no effect on the extent of ^{14}C-phenanthrene mineralisation. It should, however, be noted that this approach only provides relative numbers to be used to compare between samples, as only about 10% of microorganisms from soil samples can be cultured on media in laboratory conditions [30].

290 The type of CNMs was found to have an effect on the development of catabolism in soil, with
291 the trend: C_{60} > MWCNTs > FS. Generally, the extents of ^{14}C-phenanthrene mineralisation
292 were higher in C_{60}-amended than either MWCNTs or FS-amended soils. The data showed
293 that the presence of C_{60} had no effects on the catabolism of ^{14}C-phenanthrene, even at the
294 highest concentration (1%). Significantly less ^{14}C-phenathrene was mineralised in FS-
295 amended soils, in comparison to MWCNT-amended soils. The differences observed in the
296 extents of ^{14}C-phenanthrene mineralisation between MWCNTs and FS-amended soils,
297 especially at >0.1% CNM concentration were more pronounced; this may be due to the
298 different geometries C_{60}, MWCNT and FS [2, 22, 31, 32]. Sorption to C_{60} predominantly occurs on
299 external surfaces because it possesses a spherical structural shape, and C_{60} exists as tightly
300 packed and condensed aggregates [2]. Therefore, ^{14}C-phenanthrene is assumed to be more
301 bioaccessible on C_{60}, in comparison to MWCNT and FS. Hence, the greater extents of ^{14}C-
302 phenanthrene mineralisation in C_{60}-amended soils [32]. Furthermore, the differences obtained
303 in the degree of adsorption between FS and MWCNTs may be attributed to the differences in
304 the aggregation behaviour of FS and MWCNTs, respectively [2, 22, 32]. Previous studies have
305 demonstrated that desorption hysteresis i.e. a rapidly desorbing fraction followed by a slow
306 non-labile desorbing fraction may be responsible for the stronger adsorption of FS, while not
307 generally observed for CNTs [2, 22]. In addition, interstitial spaces and the rearrangement of FS
308 aggregates may cause the entrapment of sorbed ^{14}C-phenanthrene resulting in the rapid
309 desorption of PAH sorbed to external FS surfaces, followed by a slow release of PAH
310 entrapped within aggregates [2, 13] As a result of their cylindrical length, CNTs cannot form
311 closed interstitial spaces, and entrapment within aggregates is not observed [2, 4].
312

313 **Conclusion**

Understanding the effects of CNMs on the catabolic activity of PAHs, such as phenanthrene, have considerable benefits for risk assessment and remediation strategies for contaminated soil. This study investigated the development of catabolism of ^{14}C-phenanthrene in the presence of different carbon nanomaterials. High concentrations of MWCNT and FS reduced the development of catabolic activity of ^{14}C-phenanthrene in soil, whereas the presence of C_{60} had no impact on the development of catabolic activity of ^{14}C-phenanthrene. These results show that the presence of low concentrations of CNMs was not detrimental to the microbial activity, as the soil respiration rates that remained unchanged. Furthermore, the results obtained demonstrated that the application of certain carbon nanomaterials may not affect indigenous microflora, while others may affect them when introduced into the soil at very large quantities. It is advisable that the CNM-containing materials should not be disposed off in large quantities, in the long-term, as it is not particularly understood how this may affect the abundance of pollutant degrading microorganisms.

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399 List of figures

400 Figure 1. Catabolism of ^{14}C-phenanthrene by indigenous microorganisms after addition of
401 C_{60} at contact time: (A) 1 d (B) 25 d (C) 50 d (D) 100 d. Error bars are SEM (n = 3). Legend
402 key: 0% (○), 0.01% (∇), 0.1% (□) and 1% (◊).

403

404 Figure 2. Catabolism of ^{14}C-phenanthrene by indigenous microorganisms after addition of
405 MWCNTs at contact time: (A) 1 d (B) 25 d (C) 50 d (D) 100 d. Error bars are SEM (n = 3).
406 Legend key: 0% (○), 0.01% (∇), 0.1% (□) and 1% (◊).

407

408 Figure 3**.** Catabolism of ^{14}C-phenanthrene by indigenous microorganisms after addition of FS
409 at contact time: (A) 1 d (B) 25 d (C) 50 d (D) 100 d. Error bars are SEM (n = 3).). Legend
410 key: 0% (○), 0.01% (∇), 0.1% (□) and 1% (◊).

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List of Tables

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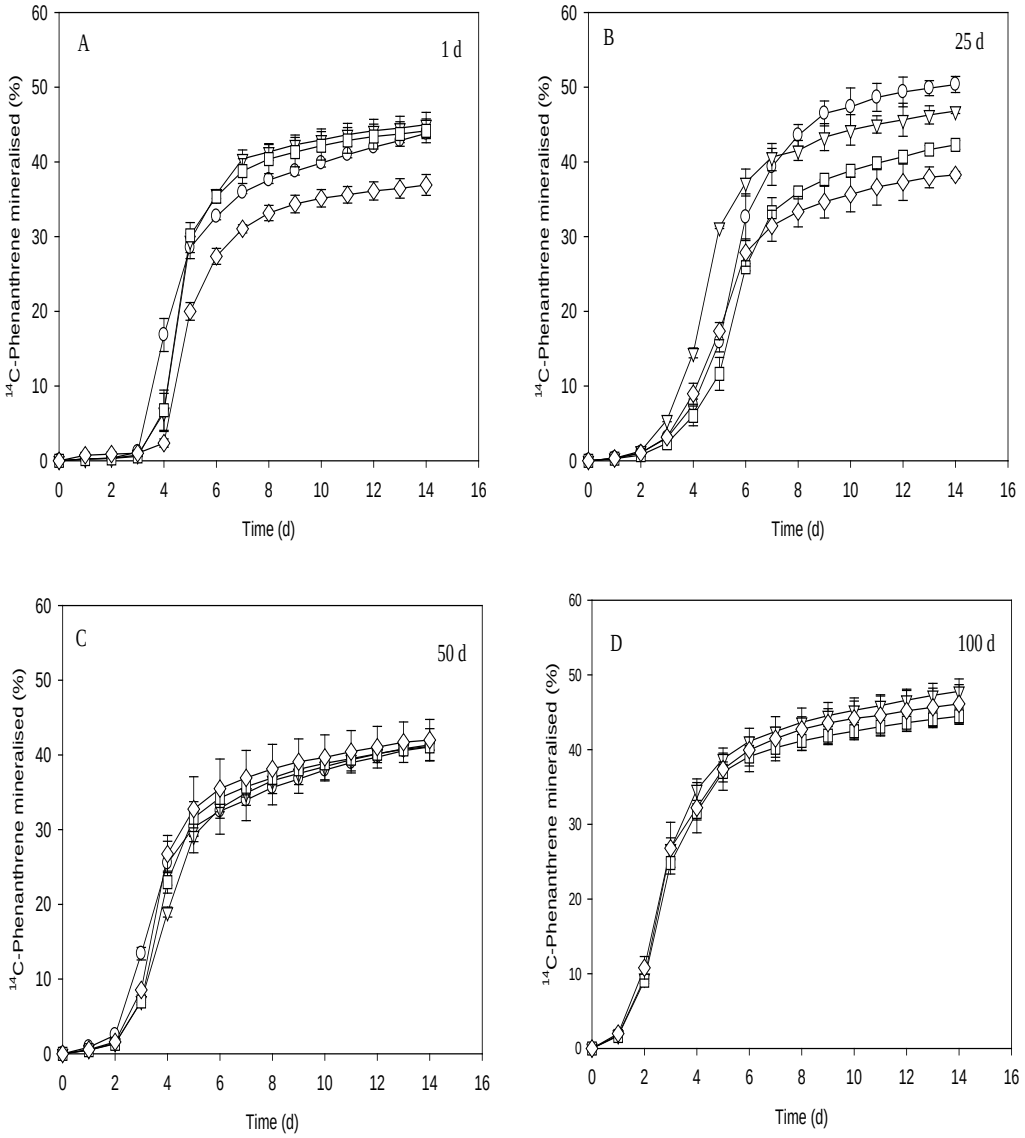
440 Figure 1



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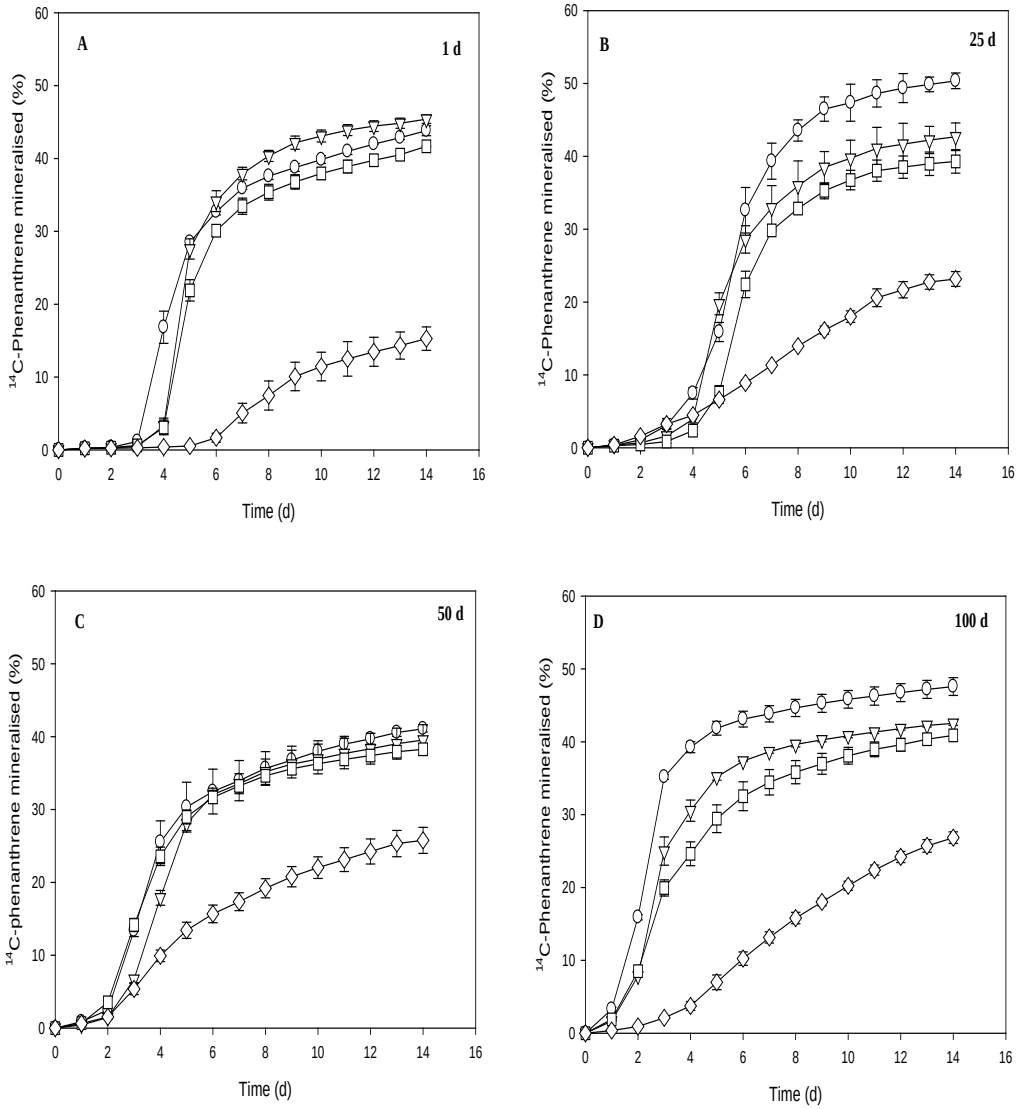
445 Figure 2



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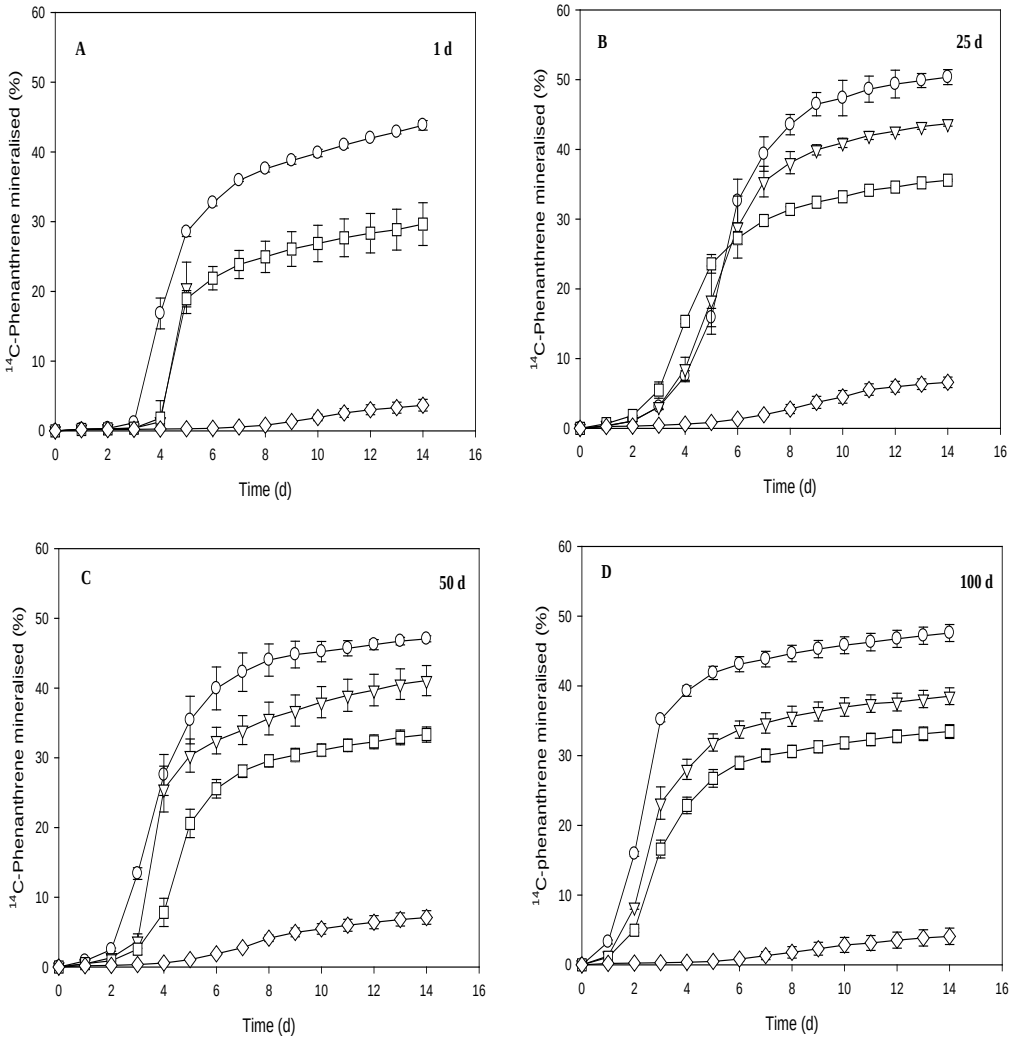
451 Figure 3



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458 Table 1:

Ageing (d)	Conc (%)	Lag time (d)	Max rate (% h^{-1})	Extent (%)
1	0	4.24 ± 0.13	1.10 ± 0.10	47.8 ± 0.68
	0.01	4.74 ± 0.08	0.96 ± 0.01	44.9 ± 1.63
	0.1	4.71 ± 0.01	0.73 ± 0.14	44.2 ± 1.59
	1	5.15 ± 0.01	0.65 ± 0.07	36.9 ± 1.40
25	0	3.44 ± 0.08	0.80 ± 0.01	47.6 ± 1.22
	0.01	3.58 ± 0.07	0.76 ± 0.05	47.7± 1.67
	0.1	3.73 ± 0.08	0.72 ± 0.02	46.5 ± 1.09
	1	3.88 ± 0.09	0.63 ± 0.08	44.1 ± 2.57
50	0	3.23 ± 0.11	0.71 ± 0.06	47.1 ± 0.43
	0.01	3.63 ± 0.03	0.72 ± 0.08	41.3 ± 2.16
	0.1	3.64 ± 0.05	0.67 ± 0.06	41.1 ± 0.50
	1	3.49 ± 0.04	0.67 ± 0.09	42.0 ± 2.75
100	0	2.14 ± 0.09	0.70 ± 0.02	46.6 ± 0.98
	0.01	2.42 ± 0.07	0.70 ± 0.02	46.7 ± 1.24
	0.1	2.45 ± 0.13	0.59 ± 0.03	42.3 ± 1.03
	1	2.29 ± 0.02	0.36 ± 0.09	38.2 ± 1.14

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Table 2:

Ageing (d)	Conc (%)	Lag time (d)	Max rate (% h^{-1})	Extent (%)
1	0	4.24 ± 0.13	1.10 ± 0.10	47.8 ± 0.68
	0.01	5.07 ± 0.03	0.91 ± 0.10	45.4 ± 0.59
	0.1	5.10 ± 0.01	0.65 ± 0.08	41.6 ± 0.06
	1	7.98 ± 0.01	0.15 ± 0.02	15.3 ± 0.34
25	0	3.44 ± 0.08	0.80 ± 0.01	47.6 ± 1.22
	0.01	4.06 ± 0.01	0.71 ± 0.09	42.5 ± 0.30
	0.1	4.51 ± 0.02	0.42 ± 0.06	40.9 ± 0.60
	1	5.39 ± 0.13	0.14 ± 0.02	26.8 ± 0.24
50	0	3.23 ± 0.11	0.71 ± 0.06	47.1 ± 0.43
	0.01	3.67 ± 0.08	0.66 ± 0.08	39.5 ± 2.10
	0.1	3.73 ± 0.11	0.54 ± 0.08	38.3 ± 0.75
	1	4.25 ± 0.04	0.19 ± 0.03	25.8 ± 0.68
100	0	2.14 ± 0.09	0.70 ± 0.02	46.6 ± 0.98
	0.01	2.54 ± 0.11	0.47 ± 0.04	42.7 ± 1.04
	0.1	2.47 ± 0.08	0.44 ± 0.04	39.3 ± 0.14
	1	3.91 ± 0.07	0.08 ± 0.03	23.2 ± 1.09

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477 Table 3:

Ageing (d)	Conc (%)	Lag time (d)	Max rate (% h^{-1})	Extent (%)
1	0	4.24 ± 0.13	1.10 ± 0.10	47.8 ± 0.68
	0.01	5.19 ± 0.01	0.88 ± 0.18	45.1 ± 0.16
	0.1	5.04 ± 0.02	0.52 ± 0.10	25.8 ± 3.07
	1	>14	0.02 ± 0.01	3.67 ± 0.83
25	0	3.44 ± 0.08	0.80 ± 0.01	47.6 ± 1.22
	0.01	3.34 ± 0.01	0.70 ± 0.07	38.5 ± 1.20
	0.1	3.86 ± 0.08	0.49 ± 0.04	33.4 ± 0.99
	1	>14	0.02 ± 0.01	4.01 ± 1.18
50	0	3.23 ± 0.11	0.71 ± 0.06	47.1 ± 0.43
	0.01	3.05 ± 0.08	0.66 ± 0.08	47.6 ± 2.16
	0.1	3.46 ± 0.01	0.47 ± 0.01	33.3 ± 1.09
	1	10 ± 0.01	0.06 ± 0.01	7.09 ± 0.97
100	0	2.14 ± 0.09	0.70 ± 0.02	46.6 ± 0.98
	0.01	2.53 ± 0.13	0.40 ± 0.04	43.7 ± 2.74
	0.1	3.00 ± 0.09	0.41 ± 0.08	33.3 ± 0.47
	1	10 ± 0.03	0.04 ± 0.01	6.59 ± 0.35

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Table 4:

Ageing (d)	Conc (%)	C_{60}		MWCNT		FS	
		CFU x $10^5 g^{-1}$		CFU x $10^5 g^{-1}$		CFU x $10^5 g^{-1}$	
		Heterotrophs	Phe. Degraders	Heterotrophs	Phe. Degraders	Heterotrophs	Phe. Degraders
1	0	31.6 ± 6.33	54.9 ± 11.3	31.6 ± 6.33	54.9 ± 11.3	31.6 ± 6.33	54.9 ± 11.3
	0.01	1.88 ± 0.88	2.47 ± 1.23	37.0 ± 12.3	0.18 ± 0.07	80.2 ± 13.5	55.6 ± 30.9
	0.1	3.12 ± 0.82	16.5 ± 0.41	3.09 ± 1.85	0.41 ± 0.01	92.6 ± 6.17	93.5 ± 10.8
	1	1.23 ± 0.62	3.29 ± 0.50	24.4 ± 18.5	32.5 ± 20.3	67.9 ± 30.9	48.8 ± 7.04
25	0	12.8 ± 0.61	12.2 ± 0.49	12.8 ± 0.61	12.2 ± 0.49	12.8 ± 0.61	12.2 ± 0.49
	0.01	1.22 ± 0.71	0.24 ± 0.13	0.12 ± 0.06	0.12 ± 0.06	0.30 ± 0.06	0.13 ± 0.02
	0.1	12.2 ± 0.42	1.2 ± 0.03	0.32 ± 0.04	0.24 ± 0.07	0.24 ± 0.12	2.44 ± 0.81
	1	0.55 ± 0.06	0.92 ± 0.07	1.22 ± 0.07	1.40 ± 0.56	4.27 ± 0.61	1.22 ± 0.23
50	0	1.81 ± 0.60	12.2 ± 6.96	1.81 ± 0.60	12.2 ± 6.96	1.81 ± 0.60	12.2 ± 6.96
	0.01	0.96 ± 0.24	2.40 ± 1.06	3.01 ± 0.60	1.61 ± 0.78	0.14 ± 0.09	4.01 ± 0.48
	0.1	0.60 ± 0.45	1.20 ± 0.40	0.13 ± 0.07	0.69 ± 0.40	0.14 ± 0.02	3.60 ± 1.39
	1	0.29 ± 0.21	0.80 ± 0.69	0.42 ± 0.09	0.32 ± 0.20	1.21 ± 0.56	0.80 ± 0.41
100	0	4.81 ± 0.62	4.20 ± 0.96	4.81 ± 0.62	4.20 ± 0.96	4.81 ± 0.62	4.20 ± 0.96
	0.01	5.01 ± 0.02	1.61 ± 0.78	5.01 ± 0.60	0.22 ± 0.11	5.01 ± 0.60	0.12 ± 0.06
	0.1	3.30 ± 0.06	2.20 ± 0.40	3.32 ± 0.06	0.19 ± 0.08	3.32 ± 0.07	0.32 ± 0.04
	1	1.92 ± 0.09	2.00 ± 0.20	1.92 ± 0.09	0.25 ± 0.04	1.92 ± 0.09	0.55 ± 0.06