ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY

Impact of nitrogen-polycyclic aromatic hydrocarbons on phenanthrene and benzo[a]pyrene mineralisation in soil

Ihuoma N. Anyanwu[a,b,*], Ojerime I. Clifford[a], Kirk T. Semple[a]

[a] Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, United Kingdom.

[b] Department of Biological Sciences, Federal University Ndufu-Alike Ikwo, P.M.B 1010, Abakaliki, Ebonyi State, Nigeria.

Corresponding author: E-mail: ihuomal@yahoo.com

Abstract

When aromatic hydrocarbons are present in contaminated soils, they often occur in mixtures. The impact of four different (3-ring) nitrogen-containing polycyclic aromatic hydrocarbons (N-PAHs) on $^{12/14}$C-phenanthrene and $^{12/14}$C-benzo[a]pyrene (B[a]P) mineralisation in soil was investigated over a 90 d incubation period. The results revealed that both $^{12/14}$C-phenanthrene and $^{12/14}$C-benzo[a]pyrene showed no significant mineralisation in soils amended with 10 mg kg $^{-1}$ and 100 mg kg $^{-1}$ N-PAHs ($p>0.05$). However, increases in lag-phases and decreases in the rates and extents of mineralisation were observed, over time. Among the N-PAHs, benzo[h]quinoline impacted ^{14}C-phenanthrene mineralisation with extended and diauxic lag phases. Furthermore,$^{12/14}$C-B[a]P and ^{14}C-benzo[a]pyrene–nitrogen-containing polycyclic aromatic hydrocarbons (^{14}C-B[a]P-N-PAHs) amended soils showed extensive lag phases (>21 d); with some ^{14}C-B[a]P-N-PAH mineralisation recording <1% in both concentrations (10 mg kg $^{-1}$ and 100 mg kg $^{-1}$), over time. This study suggests that the presence of N-PAHs in contaminated soil may impact the microbial degradation of polycyclic aromatic hydrocarbons (PAHs) and the impact was most likely the result of limited success in achieving absolute biodegradation of some PAHs in soil.

Keywords: Mineralisation, N-PAHs, benzo[a]pyrene, phenanthrene, bioavailability.

1. Introduction

Exploitation of the biodegradative capabilities of microorganisms for the clean-up of contaminated land has become widely accepted as an alternative to both physical and chemical methods because of its comparatively low cost and environmental sustainability (Sayer and Ripp, 2000; Sutherland et al., 2009; Doley et al., 2017; Menga et al., 2017; Li et al., 2017). Polycyclic aromatic hydrocarbons (PAHs) are among the most abundant organic contaminants with a potential to be degraded. Thus, attention has been paid to the degradation of PAHs and their catabolic pathways are well documented. However, most of these investigations on PAHs mineralisation have focused on single organic contaminants (Grosser et al., 1991; Yoon et al., 2002; Chauhan et al., 2008; Rhodes et al., 2008; Menga et al., 2017; Li et al., 2017). Studies have shown that PAHs degradation processes result in the formation of carbon dioxide (CO_2), water (H_2O) and simple inorganic compounds (maleic acid, fumaric acid, maleanic acid, glutaric acid, acetoacetyl-coenzyme). However, the kinetic efficiency of the pathway, type of reaction and bio-transformed metabolites produced depends on the number of aromatic rings and only limited success has so far been achieved in the biodegradation of some PAHs (Kästner and Mahro, 1996; Juhasz and Naidu, 2000; Lundstedt et al., 2003; Bamforth and Singleton, 2005; Menga et al., 2017; Doley et al., 2017). The reasons for the limited success are not well understood but could be constrained by one or more of the following process: (a) the solubilization of the PAHs, (b) their transport into the cell, (c) the expression of the degradative genes (d) the enzymatic breakdown of the PAHs (e) bioavailability and (f) the presence of other contaminants (Semple et al., 2007; Chauhan et al., 2008; Anyanwu and Semple, 2015b; Menga et al., 2017). Furthermore, interactions between low molecular weight (LMW) and high molecular weight (HMW) PAHs and their metabolites have been reported to play an important role in the induction of the catabolic enzymes, and such interactions can be either synergistic or antagonistic (Demanèche et al., 2004; Mohan et al., 2006; Couling et al., 2010). In the former case (synergistic), the metabolites produced in the degradation of aromatics in one strain may enhance the induction of catabolic enzymes of other aromatics in other strain(s) (cross-induction) (Whitman et al., 1998; Couling et al., 2010; Doley et al., 2017). In the latter case (antagonistic), aromatics or their metabolites may affect degradation due to substrate competition and/or microbial toxicity (Demanèche et al., 2004; Granato et al., 2017). Yet, the impact of more polar and soluble aromatics (such as 3-ring N-PAHs) on LMW and HMW PAHs mineralisation is not well known.

In a similar way, studies on biodegradation of nitrogen-containing polycyclic aromatics in soil have focussed mainly on indole, quinoline, isoquinoline, carbazole and acridine (Pereira et al., 1988; Fetzner, 1998; Millette et al., 1995; Lundstedt et al., 2003; Salam et al., 2017). However, biodegradation of the phenanthrolines (1,10-

phenanthroline, 1,7-phenanthroline, 4,7-phenanthroline and benzo[h]quinoline) have not been recorded in literature.

Nitrogen-containing polycyclic aromatic hydrocarbons (N-PAHs) which are not as much reported can exist, often at high concentrations in soil (Webber, 1994; Švábenský et al., 2009). N-PAH contamination, emanating from petrogenic, pyrogenic and biogenic processes, constitutes major pollution and toxicological problems within the environment (National Toxicology Program, 2001; US Environmental Protection Agency, 2001; IPCS Environmental Health Criteria, 2003; Hazardous Substance Data Bank, 2010; Environment Canada, 2011; IARC, 2012; US Environmental Protection Agency, 2017). Despite the physico-chemical properties of N-PAHs, there is lack of information on the biodegradative impact of N-PAHs on LMW and HMW PAHs in the soil environment, over time.

Biodegradation is a major route of loss for organic contaminants in soil. It is widely known that polluted sites contain mixtures of compounds which vary in their impact to soil microbial community and as well, biodegradation of other aromatics. Irrespective of this, the impact of 3-ring N-heterocyclic aromatics (1,10-phenanthroline, 1,7-phenanthroline, 4,7-phenanthroline and benzo[h]quinoline) on phenanthrene and benzo[a]pyrene mineralisation has not been systematically studied in the soil environment. Conceptually, biodegradation of PAHs in soil by indigenous microbial communities may be enhanced or inhibited by the presence of other chemicals in contaminated sites. Thus, this study investigated if the presence, concentration and contact time of a more polar and soluble heterocyclic aromatics (3-ring N-PAHs) could enhance phenanthrene and B[a]P mineralisation in soil.

2. Materials and Methods

2.1. Chemicals

Phenanthrene (Phen), 1,10-phenanthroline (1,10-Phen), 1,7-phenanthroline (1,7-Phen), 4,7-phenanthroline (4,7-Phen), benzo[h]quinoline (B[h]Q), benzo[a]pyrene (B[a]P) (Table 1) were obtained from Sigma-Aldrich, UK and ^{14}C-phenanthrene, ^{14}C-benzo[a]pyrene was obtained from ARC, UK. Goldstar liquid scintillation cocktail was supplied by Meridian Biotechnologies Ltd, UK.

2.2. Soil preparation

Soil from Myerscough Agricultural College, UK was prepared for this study. The soil was collected from a depth of approximately 5 – 20 cm. The soil texture was sandy-loam (19.5% clay, 60.4% sand, 20.0% silt); organic matter content 2.7% and pH 6.5 (Doick et al., 2003). The soil was air dried at room temperature, sieved through a 2mm mesh and rehydrated back to 45% of the water holding capacity. Soil was amended with PAHs and N-PAH compounds using the method reported in Doick et al. (2003). Soils were placed in bowls: $^{1}/_{3}$ of the soil (100 g, n = 3) was spiked with individual standards of phenanthrene, B[a]P, benzo[h]quinoline, 1,10-phenanthroline, 1,7-phenanthroline or 4,7-phenanthroline dissolved in toluene (2 ml) to give concentrations of 10 mg kg $^{-1}$ and 100 mg kg $^{-1}$ dry soil. The carrier solvent was allowed to volatilise from the soil for 3 – 4 h in a fume hood, after which soils were mixed with the remaining $^{2}/_{3}$ of the soil (200 g). Samples were prepared using soils amended with toluene (only) to serve as a control. The amended soils were then kept in amber glass jars (500 ml) and incubated in the dark at 21 ± 1^{o}C for 1, 30, 60 and 90 d. Soil moisture content was checked regularly and lost water was replenished with deionized water. Recoveries of phenanthrene and its nitrogen-containing analogues from soil over time has been reported (Anyanwu and Semple, 2016a, b; 2015b).

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

The ability of soil indigenous microflora to mineralise ^{14}C-PAHs to $^{14}CO_2$ was assessed at 1, 30, 60 and 90 d soil-contact time. Respirometric assays were carried out in modified 250 ml Schott bottles incorporating a Teflon-lined screw cap containing 1 M NaOH to trap any $^{14}CO_2$, 10 g (n = 3) of soil (wet wt) and 30 ml sterile minimal basal solution (MBS) (Doick and Semple, 2003). Standards prepared in toluene to give a ^{12}C-PAH concentration of 10 mg kg $^{-1}$ and 100 mg kg $^{-1}$ dry weight with an associated ^{14}C-activity of 85 Bq g $^{-1}$ soil dry weight was then added. Analytical blanks consisted of soil with no ^{14}C-amendement. A slurry system with a solid: liquid ratio of 1:3 was used to ensure complete $^{12/14}$C-PAH / N-PAH distribution (Doick and Semple, 2003). Respirometers were shaken at 100 rpm on an orbital shaker (Janke and Kunkel, IKA®-Labortechnik KS 510D) in the dark at 21 ± 1^{o}C and sampled every 24 h for 21 d. Sampling comprised of removal of vials containing trapped $^{14}CO_2$ and replacement, followed by addition of 5 ml goldstar scintillation fluid. The vials were stored in the dark for 24 h before sample quantification were carried out by liquid scintillation counting (LSC) using standard calibration and quench correction techniques (Reid et al., 2001).

2.4. Statistical analysis

Following blank correction, data were statistically analysed using SigmaStat 3.5 version. Effect of exposure time (incubation time), concentration and impact of N-PAHs on the mineralisation $^{12/14}$C-phenanthrene and $^{12/14}$C-benzo[a]pyrene was assessed using analysis of variance (ANOVA) to ascertain the significant differences in lag phases, fastest rates and extents of mineralisation. Differences were found to be statistically significant when $p<0.05$. Data was presented as mean ± standard error (SE) and graphs were plotted with SigmaPlot 10.0 version**.**

3. Results

3.1. Mineralisation $^{12/14}$C-phenanthrene in soil

Following the addition of the ^{14}C-phenanthrene at 1 d, the indigenous microbial communities were able to rapidly mineralise ^{14}C-phenanthrene in Phenanthrene amended soil as indicated by the relatively short lag phases of 2.77 d and 3.14 d in the 10 mg kg $^{-1}$ and 100 mg kg $^{-1}$ amendments over time ($p>0.05$) (Figs 1 and 2). The extent of mineralisation was measured (Tables 2 and 3), and there were significant decreases in the extent to which $^{12/14}$C-phenanthrene was mineralised in soil over time compared with the N-PAH amendments ($p<0.05$). For example, decreases in mineralisation was recorded with increased soil-chemical-contact time (Tables 2 and 3). Furthermore, the rates of mineralisation showed that the mineralisation of $^{12/14}$C-phenanthrene amended soils recorded highest rate of 0.87 ± 0.00% h $^{-1}$ and 0.75 ± 0.30% h $^{-1}$ in the 10 mg kg $^{-1}$ and 100 mg kg $^{-1}$ amendments (1 d), respectively (Tables 2 and 3).

On the other hand, mineralisation of ^{14}C-phenanthrene in the presence of N-PAHs occurred after long lag phases, over time. From the result, soils amended with the nitrogen-containing compounds exhibited extended lag phases of 3.05 d – 5.33 d ($p>0.05$) in the mineralisation of ^{14}C-phenanthrene compared with phenanthrene amended soil over time (Figs 1 and 2). Among the N-containing aromatic chemicals, benzo[h]quinoline (B[h]Q) recorded the longest lag phase of 5.33 d at 1 d in the 100 mg kg $^{-1}$ amendment (Fig. 2). In addition, B[h]Q displayed a diauxic lag phase of 4.55 d after reaching 5% mineralisation in the 100 mg kg $^{-1}$ amendment at 60 d (Fig. 2). The extents of mineralisation were measured, and N-PAHs showed no consistent trend in the mineralisation of ^{14}C-phenanthrene in soil, over time. The extent of mineralisation

recorded high and low values in N-PAH soils over the 90 d incubation. However, low mineralisation values were recorded in both concentrations at 1 d and 60 d; with the exception of B[h]Q amended soils (Tables 2 and 3).

The rates of mineralisation were also measured among the N-PAHs (Tables 2 and 3). N-containing polycyclic aromatics recorded low rates in the mineralisation of ^{14}C-phenanthrene in soil over time. The rates of mineralisation showed that all the compounds recorded their highest mineralisation value at 1 d. For example, B[h]Q recorded the highest rate of 0.90 ± 0.10% h $^{-1}$ (10 mg kg $^{-1}$); while 4,7-Phen recorded the highest value of 0.77 ± 0.10% h $^{-1}$ (100 mg kg $^{-1}$) at 1 d, after extended lag phases of 3.43 d and 3.05 d, respectively (Tables 1 and 2; Figs 1 and 2). 1,10-Phen recorded the lowest rates of 0.17 ± 0.10% h $^{-1}$ in the 10 mg kg $^{-1}$ amendment (60 d) and 0.25 ± 0.10% h $^{-1}$ in the 100 mg kg $^{-1}$ amendment (30 d), after long lag phases of 4.45 d and 4.91 d, respectively (Tables 1 and 2; Figs 1 and 2). From the study, a trend of increased lag phases, diauxic lag phases, slow rates of mineralisation and reduced extents of mineralisation of ^{14}C-phenanthrene were observed in soils amended with N-PAHs in both concentrations, at all the time points. Statistical analysis of data showed significant difference in the lag phases over time ($p<0.05$). However, ANOVA revealed that N-PAHs had no significant positive impact on the mineralisation of ^{14}C-phenanthrene in soil (over the 1 – 90 d soil-contact time) ($p>0.05$), with the exception of 4,7-Phen (100 mg kg $^{-1}$) ($p<0.05$).

3.2. Mineralisation of $^{12/14}$C-benzo[a]pyrene in soil

Benzo[a]pyrene (B[a]P) amended soils followed similar pattern of increased lag phases (>21 d) in all the amendments and concentrations, at all the time points. The measured rates and extents of mineralisation showed that $^{12/14}$C-B[a]P rates of mineralisation recorded no mineralization in the 10 mg kg $^{-1}$ and 100 mg kg $^{-1}$ (Tables 2 and 3). Maximum extent of mineralisation of 0.78 ± 0.00% was recorded in the 10 mg kg^{-1} $^{12/14}$C-B[a]P amended soil at 30 d (Table 2). In the ^{14}C-B[a]P–N-PAHs amendments, few N-PAHs recorded mineralisation rates of 0.01 ± 0.00% h $^{-1}$ at varying days in the 10 mg kg $^{-1}$ and 100 mg kg $^{-1}$, however, this was not statistically significant ($p>0.05$) (Tables 2 and 3). The extents of mineralisation were also measured and the result showed that most ^{14}C-B[a]P–N-PAHs recorded values <0.1% in the microbial mineralisation of ^{14}C-B[a]P in both concentrations, over time. However, 4,7-Phen (10 mg kg $^{-1}$) and 1,10-Phen (100 mg kg $^{-1}$) recorded mineralisation values >0.80% at 1 d and 30 d, respectively (Tables 2 and 3). Furthermore, the study

190 revealed antagonistic impact of N-PAH amendments in the mineralisation of ^{14}C-B[a]P in soil (see
191 supplementary data).

192

193 **4. Discussion**

194 This study was designed to see if a more polar and soluble aromatic (such as N-PAHs) could stimulate
195 phenanthrene and benzo[a]pyrene mineralisation in soil by measuring the lag phases, rates of mineralisation
196 and extents of mineralisation. However, the results revealed that the presence of N-PAHs negatively affected
197 the rates and extents of ^{14}C-Phen and ^{14}C-B[a]P mineralisation in soil, over time. Inhibition can be seen in any
198 of the following: (a) increases in the length of the lag phase; (b) diauxic lag phases; (c) decreases in the
199 degradation rates, and (d) decreases in the extent of mineralisation.

200 It is clear from the data presented in this study that $^{12/14}$C-phenanthrene was mineralised in the soil
201 following the amendment of 10 mg kg $^{-1}$ and 100 mg kg $^{-1}$ phenanthrene. This is evident from the shorter lag
202 phases, which generally decreased with soil contact time, the rates and extents of mineralisation. This is in
203 agreement with some studies (Reid et al., 2002; Rhodes et al., 2008; Couling et al., 2010; Towell et al., 2010;
204 Menga et al., 2017; Li et al., 2017; Mnif et al., 2017) in which the authors reported observations on the
205 following: molecular mass (Reid et al., 2010), pre-exposure (Rhodes et al., 2008), microbial population and
206 diversity (Towell et al., 2010; Li et al., 2017; Mnif et al., 2017), and the ubiquitous nature of PAHs in the
207 environment at very low concentrations (Couling et al., 2010). Speedy mineralisation of phenanthrene was also
208 recorded by Yoon et al. (2002), who reported that phenanthrene showed faster and stronger catabolic potential.
209 In addition, Anyanwu and Semple (2015b; 2016b) attributed phenanthrene loss in soil to microbial elimination
210 of the original compound (biological degradation), sequestration or removal as a result of soil-contact time
211 (ageing).

212 Furthermore, this present study showed significant decrease in the extents of phenanthrene
213 mineralisation with increased ageing and similar observation was reported by Couling et al. (2010) after 84 d
214 soil contact time. The authors (Couling et al., 2010) attributed the decreases to amount of PAHs remaining in
215 soil and decrease in chemical bioavailability to degrading soil microbial populations. However, sorption of
216 phenanthrene to soil matrix, thereby reducing the bioavailability of the aromatic compound may also be
217 important (Semple et al., 2007; Anyanwu and Semple 2016a, b).

It is widely known that the extent of degradation for different aromatic hydrocarbons are known to differ as a result of physico-chemical parameters such as molecular size, chemical structure, hydrophobicity and solubility (Stroud et al., 2007; Zhu et al., 2017; Doley et al., 2017). Studies have shown that structure and number of aromatic rings in combination with soil physico-chemical variables can affect the overall mineralisation kinetics of a particular compound. Thus, the >21 d lag phase observed with B[a]P in this present study is in agreement with the observations of Grosser et al. (1991) and Zhu et al. (2017). The authors noted that it took extensive lag phase of 3 – 4 weeks and 84 days before the mineralisation of B[a]P in soil and sediment, respectively. Also, studies on PAHs biodegradation in the soil environment have shown that degradation rates are inversely related to the number of aromatic rings (Cerniglia and Heitkamp, 1989; Eriksson et al., 2000). Thus, the <1% B[a]P mineralisation in this study is consistent with the findings of Atlas and Bragg (2015), who noted that contrarily to the low molecular PAHs, high molecular PAHs may not be degraded. This may be attributed to increase in size and angularity of B[a]P which resulted in a concomitant increase in hydrophobicity and electrochemical stability (Harvey, 1997).

The impact of a more polar and soluble heterocyclic aromatics (N-PAHs) was assessed on phenanthrene and benzo[a]pyrene mineralisation in soil. The study revealed that the presence of N-PAHs resulted in relatively increases in the length of lag phases. This confirms the report by Meyer and Steinhart (2000) and Willumsen et al. (2001) in heterocyclic compounds and azaarenes degradation, respectively. In support, Sutton et al. (1996) recorded 15 d lag phase in aerobic degradation of 4-methylquinoline.

Diauxic lag phase was displayed by B[h]Q despite reaching 5% mineralisation in this current study. This may be associated to the transient build-up of toxic metabolites and/or toxicity. Studies have shown that benzo-quinolines exert greater toxicity when they are bio-transformed to dihydrodiols, hydroxyls and epoxides (Warshawsky et al., 1992). In addition, large differences in N-PAHs physico-chemical properties, such as log K_{ow}, $K_{oc,}$ variations in the type of N-substituents and/or ring position (Table 1) may influence mineralisation rates of the heterocyclic aromatics (Anyanwu and Semple, 2015b, c). However, the induction of catabolic enzymes by microbes to degrade B[h]Q chemical, may be attributed in this study.

When evaluating the environmental fate of organic contaminants, the extent of mineralisation is highly essential. The absence of positive statistically significance difference in the mineralisation of $^{12/14}$C-PAHs in the presence of N-PAHs in this current study, suggests inhibitory effect of N-PAHs on the biodegradation of PAHs in soil. This observation is in agreement with Meyer and Steinhart (2000) and Dyreborg et al. (1996a),

247 who reported inhibitions of hetero-PAHs (N-S-O) in the biodegradation of typical tar oil PAHs in soil and
248 inhibitions of hetero-atoms in toluene degradation, respectively. Although not in the soil environment Lantz et
249 al. (1997) also noted that heterocyclic analogues inhibited PAH degrading bacterium in culture media. In this
250 study, N-PAHs impact could be attributed to their physico-chemical characteristics such as chemical structure
251 (N-atoms), bioavailability, lower K_{ow}, solubility and/or chemical toxicity. This hypothesis is consistent with
252 Millette et al. (1995), who suggested that the order of biodegradability of chemicals in complex mixtures is
253 determined by their polarity, molecular size, structure and bioavailability. In addition, Anyanwu and Semple
254 (2015b; 2016a, b) ascribed N-PAHs inhibitory effects to their recalcitrant fractions, pK_a values and/or N-atom
255 position in the chemical structure.

256 Apart from the physico-chemical properties aforementioned, the impact of N-PAHs on the extent of
257 phenanthrene and B[a]P mineralisation could also be attributed to microbial toxicity and/or inhibition of
258 sensitive microbial populations (Demanèche et al., 2004; Anyanwu and Semple, 2015b; 2016a; Granato et al.,
259 2017), indicating deterioration of ecosystems and the replacement of sensitive specie populations by resistant
260 ones. This can have serious ecological consequences as well as impacts on the efficacy of bioremediation.

261 The significant impact ($p<0.05$) of 4,7-phenanthroline on ^{14}C-phenanthrene mineralisation, may be
262 related to the position of the N-atom on the aromatic structure. Yoon et al. (2002) observed that the rate of
263 carbazole mineralisation significantly increased in phenanthrene-grown cell, linking it to similarities in
264 chemical structure, and suggested the possibility that expression of genes encoding carbazole dioxygenase
265 might be stimulated by phenanthrene. In addition, Lundstedt et al., 2003 reported that the heterocyclic
266 compounds were almost degraded at similar rates to the PAHs with the same number of rings; however, they
267 indicated that some N-heterocycles were degraded more slowly, for example, benzoquinoline.

268 In this current study, N-PAHs impacts were observed with increase in chemical concentration. The
269 observed concentration related effect is in agreement with the findings of Willumsen et al. (2002) who reported
270 inhibitory effect of chemical concentration on azaarene degradation; Sutton et al. (1996), who observed
271 complete inhibition in the degradation of 4-methylquinoline by soil bacterium at 172 mg L^{-1}. In conformity,
272 Anyanwu and Semple, 2015b; 2016a, b) reported concentration-specific impacts of N-heterocyclic aromatics
273 (especially B[h]Q and 1,7-Phen) in soil, over time. Furthermore, the impact of N-PAHs on ^{14}C-B[a]P
274 mineralisation is consistent with the findings Meyer and Steinhart (2000), who reported that B[a]P was not
275 influenced by the presence of hetero-PAHs until 111 d in the PAH / N-S-O soil amendments. However, the

<1% mineralisation of ^{14}C-B[a]P-N-PAH soils over time may be linked to N-PAHs antagonistic interaction and/or microbial toxicity (Demanèche et al., 2004; Anyanwu and Semple, 2015b; 2016a; Granato et al., 2017).

5. Conclusions

The study was designed to see if the presence of 3-ring N-PAHs (a more polar and soluble heterocyclic aromatics) could enhance phenanthrene and B[a]P mineralisation in soil. However, inhibitory impact on ^{14}C-phenanthrene and ^{14}C-B[a]P mineralisation in soil by the presence 3 ring-N-PAHs was observed. The inhibitory impact resulted in increases in the length of lag phases, diauxic lag phases, decreases in the rates and extents of mineralisation. This suggests that losses of some PAH degradation capacity in polluted sites may be due to the presence of N-PAHs. Among the N-PAHs, B[h]Q displayed measurable impact on PAHs mineralisation processes, as shown in the study, indicating the greater toxicity of benzo-quinolines. This an important factor that must be considered in the development of sustainable bioremediation approaches for contaminated soils.

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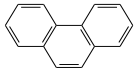
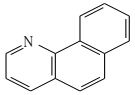
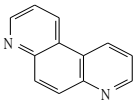
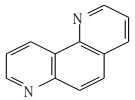
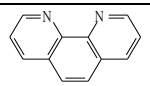
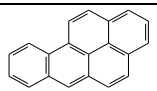
435 **Table 1**

436 Physical and chemical properties of phenanthrene, N-PAH isomers and benzo[a]pyrene

Chemical	Chemical formula	Chemical structure	Molecular mass	Boiling point	Solubility 25°C (mg/L)	Vapour pressure 25°C (mm Hg)	Henry's law (atm-m^3/mole)	Log K_{ow}	Log K_{oc}	Log K_{oa} (m^3/µg)
Phen	$C_{14}H_{10}$		178.2	340.00	1.15	1.21E-04	1.50E-05[a]	4.46	4.32	9.12E-06[b]
B[h]Q	$C_{12}H_9N$		179.2	339.00	78.70	2.08E-05	1.11E-06 [a]	3.43	4.32	0.001[b]
4,7-Phen	$C_{12}H_8N_2$		180.21	361.20	38.04	2.39E-05	1.85E-07 [a]	2.4	4.32	0.022 [b]
1,7-Phen	$C_{12}H_8N_2$		180.21	365.10	30.64	2.39E-05	1.85E-07 [a]	2.51	4.32	0.022 [b]
1,10-Phen	$C_{12}H_8N_2$		180.21	365.10	30.64	2.39E-05	1.85E-07 [a]	2.51	4.32	0.022 [b]
B[a]P	$C_{20}H_{12}$		252.31	377.00	0.17	2.44E-006	4.648E-006	5.99	5.40	7.823

437 a Henrys Law Constant (VP/WSol estimate using EPI values)

438 b Kp Octanol/air model (particle/gas partition coefficient).

439 Source: www.chemspider.com/Chemical-Structure

Table 2

Rates and extents of mineralisation of ^{14}C-phenanthrene and ^{14}C-benzo[a]pyrene in the presence of 10 mg kg $^{-1}$ N-PAHs during the 90 d incubation in soil

Parameters	Time (d)	Phen	1,10-Phen	1,7-Phen	4,7-Phen	B[h]Q	B[a]P	1,10-Phen	1,7-Phen	4,7-Phen	B[h]Q
Rates of mineralisation (% h^{-1})	1	0.87 ± 0.00	0.56 ± 0.10	0.38 ± 0.10	0.65 ± 0.30	0.90 ± 0.10	–	–	–	–	–
	30	0.42 ± 0.00	0.52 ± 0.20	0.74 ± 0.2	0.54 ± 0.00	0.27 ± 0.20	–	–	–	0.01 ± 0.00	–
	60	0.38 ± 0.00	0.17 ± 0.10	0.34 ± 0.10	0.77 ± 0.10	0.40 ± 0.00	–	–	–	–	–
	90	0.52 ± 0.00	0.49 ± 0.00	0.57 ± 0.10	0.62 ± 0.10	0.51 ± 0.00	–	–	–	–	–
Extents of mineralisation (%) (21 d)	1	43.39 ± 0.50*	38.66 ± 1.60	33.67 ± 6.50	41.92 ± 7.90	39.19 ± 1.20	0.05 ± 0.00	0.41 ± 0.10	0.03 ± 0.00	0.82 ± 0.20	–
	30	41.08 ± 0.70*	42.36 ± 5.10	46.46 ± 0.20	49.99 ± 0.20	37.95 ± 5.30	0.78 ± 0.00	0.10 ± 0.10	0.06 ± 0.00	0.64 ± 0.00	0.14 ± 0.00
	60	28.94 ± 0.20*	24.89 ± 0.90	28.04 ± 1.50	33.84 ± 0.10	22.19 ± 1.70	0.11 ± 0.00	0.11 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.01 ± 0.00
	90	29.60 ± 0.10*	27.41 ± 1.70	30.77 ± 0.60	31.76 ± 0.00	31.04 ± 0.20	0.13 ± 0.00	–	0.02 ± 0.00	–	0.12 ± 0.00

Conc = concentration (mg kg $^{-1}$), (–) = no mineralisation, data = amended compounds, * = $p<0.05$.

Table 3

Rates and extents of mineralisation of ^{14}C-phenanthrene and ^{14}C-benzo[a]pyrene in the presence of 100 mg kg^{-1} N-PAHs during the 90 d incubation in soil

Parameters	Time (d)	Phen	1,10-Phen	1,7-Phen	4,7-Phen	B[h]Q	B[a]P	1,10-Phen	1,7-Phen	4,7-Phen	B[h]Q
Rates of mineralisation (% h^{-1})	1	0.75 ± 0.30	0.49 ± 0.40	0.73 ± 0.10	0.77 ± 0.10	0.48 ± 0.40	–	–	0.01 ± 0.00	–	0.01 ± 0.00
	30	0.67 ± 0.00	0.25 ± 0.10	0.47 ± 0.00	0.26 ± 0.10	0.49 ± 0.00	–	0.01± 0.00	–	–	–
	60	0.57 ± 0.00	o.28 ± 0.10	0.40 ± 0.10	0.36 ± 0.00	0.32 ± 0.20	–	–	–	–	–
	90	0.47 ± 0.00	0.52 ± 0.00	0.62 ± 0.00	0.61 ± 0.10	0.56 ± 0.10	–	–	–	–	–
Extents of mineralisation (%) (21 d)	1	38.36 ± 5.30*	33.73 ± 4.30	43.16 ± 9.20	37.39 ± 0.90*	50.77 ± 4.40	0.09 ± 0.10	–	–	0.02 ± 0.00	0.16 ± 0.00
	30	41.51 ± 3.60*	30.91 ± 2.20	45.33 ± 0.30	42.37 ± 3.30*	45.80 ± 1.90	0.02 ± 0.00	0.88 ± 0.20	0.18 ± 0.00	0.23 ± 0.00	0.20 ± 0.00
	60	25.61 ± 1.10*	20.81 ± 5.20	33.51 ± 0.00	26.38 ± 0.30*	39.43 ± 3.90	0.06 ± 0.00	0.01 ± 0.00	0.19 ± 0.10	0.08 ± 0.00	0.06 ± 0.00
	90	31.40 ± 0.30*	35.03 ± 0.70	36.08 ± 0.80	32.32 ± 1.10*	40.69 ± 0.60	0.12 ± 0.00	0.01 ± 0.00	0.09 ± 0.00	0.15 ± 0.00	0.06 ± 0.00

Conc = concentration (mg kg $^{-1}$), (–) = no mineralisation, data = amended compounds, * = $p<0.05$

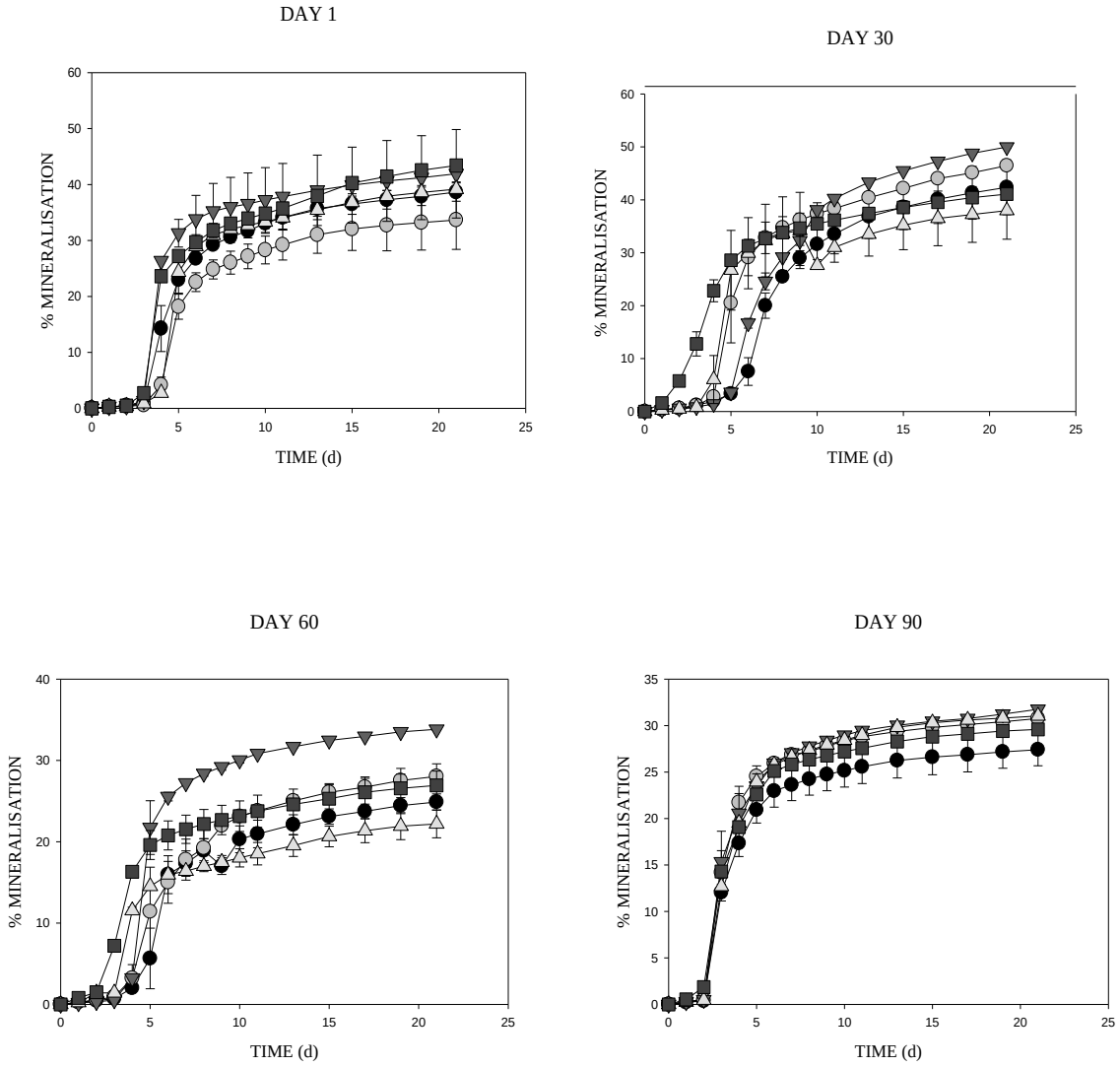
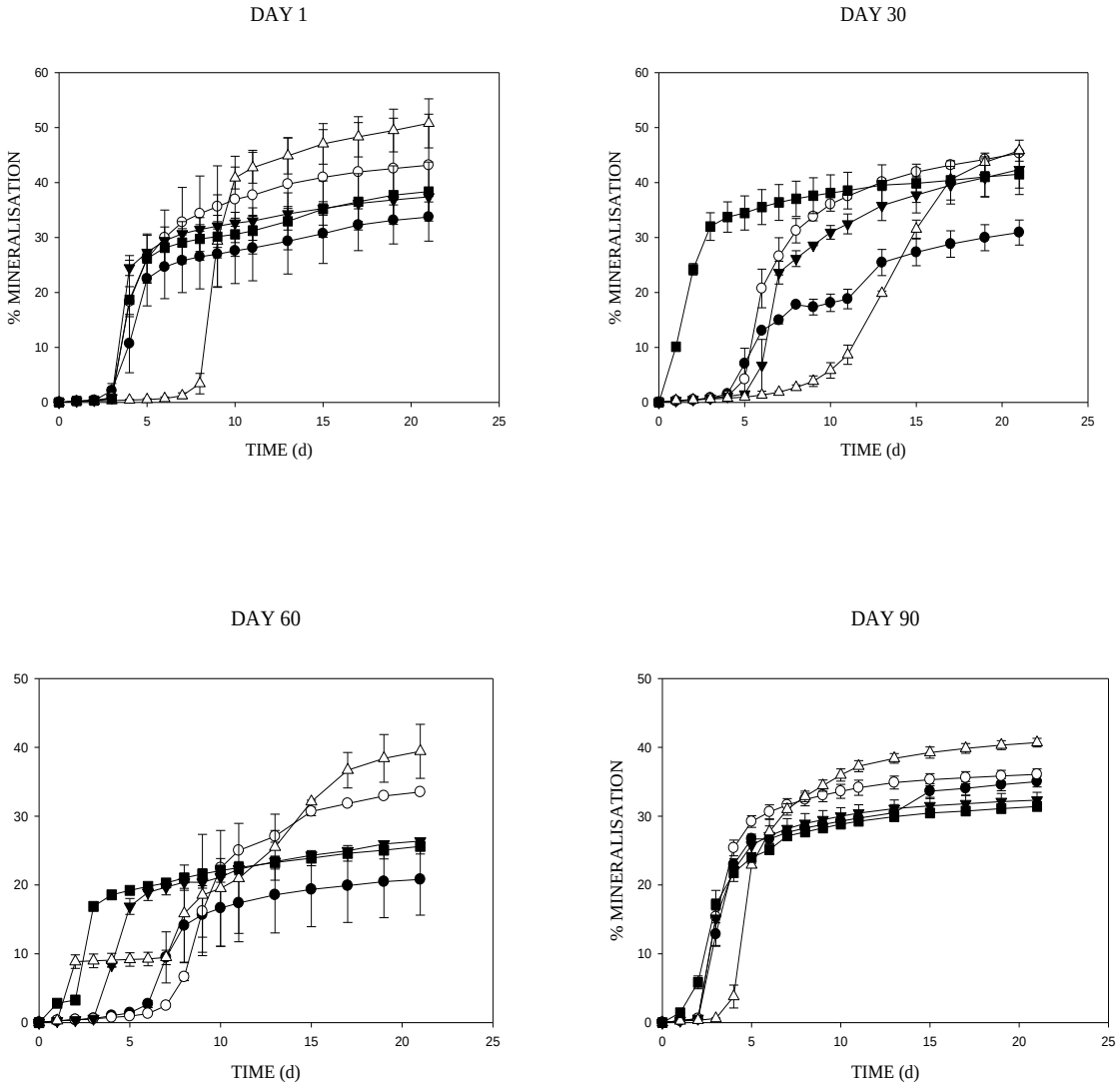


Fig. 1. Mineralisation of $^{12/14}$C-phenanthrene in the presence of 10 mg kg $^{-1}$ N-PAH amended soils during the 90 d incubation. The 1 – 90 d graph shows: 1,10-Phen (●), 1,7-Phen (○), 4,7-Phen (▼), B[h]Q (Δ) and Phen (■).



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467 **Fig. 2.** Mineralisation of $^{12/14}$C-phenanthrene in the presence of 100 mg kg $^{-1}$ N-PAH amended soils during the

468 90 d incubation. The 1 – 90 d graph shows: 1,10-Phen (●), 1,7-Phen (○), 4,7-Phen (▼), B[h]Q (Δ) and Phen

469 (■).