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Development of Naphthalene Catabolism in Al and Fe contaminated soil

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ABSTRACT: Metals have the potential to affect the biodegradation of polycyclic aromatic hydrocarbons (PAHs) in mixed contaminated soils. This investigation considered the effects of Al and Fe (50, 100, 250 and 500 mg/kg) on ^{14}C-naphthalene biodegradation in soil over 63 d contact time. The presence of Al at low concentrations (50 and 100 mg/kg) stimulated ($p < 0.05$) naphthalene biodegradation in fresh Al amendments; however, naphthalene mineralisation was significantly reduced ($p < 0.05$) in 500 mg/kg aged Al amendment, but this reduction only occurred at 1 and 21 d in 500 mg/kg fresh Al. Naphthalene mineralisation was stimulated ($p < 0.05$) in the highest Fe concentration (500 mg/kg) in both aged and fresh Fe amendments at 42 d. This suggests that Al has a greater impact than Fe on the development of PAH catabolism in soil. Studies on metal-PAH impact in soil facilitates the assessment of risk, hazard and bioremediation potential at sites contaminated with both contaminants. @JASEM

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Highlights, Naphthalene mineralisation was monitored in varying Al or Fe concentrations. Fresh Al amendments stimulated naphthalene mineralisation at low concentrations. Naphthalene mineralisation was stimulated at $t = 42$ d in both aged and fresh Fe amendments at the highest Fe concentration

Naphthalene mineralisation was reduced at the highest aged Al concentration. Naphthalene mineralisation was reduced at the highest fresh Al concentration at $t = 1$ and $t = 21$

Organic and inorganic compounds are released into the environment as a result of human activities which include: fossil fuel and wood combustion (Freeman and Cattell, 1990; Pereira *et al*., 2007; Ibarrolaza *et al*., 2009), industrial activities, use of fungicides, sewage sludge application, mining operations and accidental oil spills (Kim *et al*., 2008; Ibarrolaza *et al*., 2009). PAH contamination in soil is often associated with the presence of high levels of potentially toxic metals (Thavamani *et al*., 2011). Approximately 40% of hazardous wastes sites on the U.S. Environmental protection Agency's National Priority list are co-contaminated with organic and metal pollutants (Sandrin *et al*., 2000; Sandrin and Maier, 2003; Thavamani *et al*., 2011).

PAHs are chemical compounds consisting of atoms of C and H, arranged in the form of two or more aromatic rings (Santos *et al*., 2008; Thavamani *et al*., 2011). PAHs are considered to be ubiquitous environmental pollutants and the concern about their presence, persistence and disposition is increasing since this important class of chemicals have been found to have toxic, mutagenic, and carcinogenic properties (Malakul *et al*., 1998; Maliszewska-Kordybach and Smreczak, 2003; Thavamani *et al*., 2011). Naphthalene is common in petroleum products and is among the 16 PAH compounds designated as "priority pollutants" by United States Environmental Protection Agency (U.S EPA, 2008; Simarro *et al*., 2011), and is one of the best studied PAHs.

Aluminium and iron are the third and fourth most abundant elements in the Earth's crust, respectively (Illmer and Buttinger, 2006; Santos *et al*., 2008). Al is a metal lacking known biological functions and in this respect pertains to the non-essential elements, which is probably due to its very strong toxicity towards all forms of life (Pina and Cervantes, 1996; Illmer and Buttinger, 2006). It is a light metal that make up 7% of the Earth's crust, occurring in the form of harmless oxides and aluminosilicates. However, through acid rain, Al is being released (solubilized) from its natural resevoirs and thereby increasing its concentrations in soil (Pina and Cervantes, 1996). Al is solubilized into toxic forms like aluminium hexaaqua complex ion $[Al\ (H_2O)_6]^{3+}$ which is now present in 40% of the arable lands in the world (Zhang *et al*., 2007).

Iron is an essential element for all forms of life except for members of the genus *Lactobacillus* (Santos *et al*., 2008). It is an important element

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because of its occurrence as a cofactor in various proteins, including Krebs Cycle enzymes and proteins of the respiratory pathway (Dinkla *et al.*, 2001). Aerobic degradation of aromatic compounds by microorganisms proceed via several oxidation steps that are catalysed by oxygenases. Most of these oxygenases contain iron as a cofactor (Dinkla *et al.*, 2001). Thus, aerobic metabolism of hydrocarbons is expected to impose an iron requirement on cells (Dinkla *et al.*, 2001).

Bioremediation of PAHs contaminated sites relies on the metabolic versatility within soil microflora allowing the transformation of PAHs into essentially harmless or at least less hazardous compounds (Ibarrolaza *et al.*, 2009). Microorganisms develop these metabolic capacities by prior exposure to PAHs, and their adaptation to PAHs occur through enzyme induction and increase in the number of degrading organisms (Macleod and Semple, 2002; Lee *et al.*, 2003).

PAH contamination in soil is often accompanied with high levels of potentially toxic metals (Malakul *et al.*, 1998; Amezcua-Allieri *et al.*, 2005; Thavamani *et al.*, 2011). Co-existence of heavy metals with PAHs is a particular challenge for bioremediation of PAHs because co-contamination with metal can delay or even inhibit indigenous microbial activity (Pepper *et al.*, 2002). Metals affect a myriad of microbial activities and this suggests that metals have the potential to affect the biodegradation of PAHs in mixed contaminated soils (Thavamani *et al.*, 2011). We hypothesize reduction of naphthalene mineralisation in freshly added Al or Fe compared to that of aged Al or Fe amendments.

The aims of this study were to assess effects of Al or Fe concentrations on the development of naphthalene catabolism in soil by considering the effects of soil contact time in the presence of increasing concentrations of either freshly added or aged Al or Fe

MATERIALS AND METHODS

Chemicals: Naphthalene and [UL-^{14}C] naphthalene (55 mCi/mmol) were obtained from Sigma Aldrich Co., Ltd, UK. Goldstar multipurpose liquid scintillation fluid was obtained from Meridian, UK. Agar-agar, aluminium sulphate ($Al_2(SO_4)_3.16H_2O$) and ferrous sulphate 7-hydrate ($FeSO_4 \cdot 7H_2O$) were obtained from Fisher Scientific, UK, limited.

Soil: A Dystric Cambisol soil collected from Myerscough Agricultural College (Lancashire, UK) from the upper 10 cm layer, free of any pesticides, nitrogen or phosphate treatment for 20+ years was used for this study. It had a sandy loam texture (pH 6.5; organic matter (OM) content 2.7%; clay content 19.5%; total sand 60.4%; silt 20.0%). The soil was air dried, sieved with 2 mm sieve to remove stones and was kept in the cold room until use.

Amendment of soil with ^{12}C-naphthalene, and aluminium or iron: The soil was amended with 50 mg/kg of ^{12}C-naphthalene using the spiking procedure described by Doick *et al.* (2003). Soluble metal salts $Al_2(SO_4)_3.16H_2O$ and $FeSO_4 \cdot 7H_2O$ were used to introduce Al and Fe concentrations of 50, 100, 250 or 500 mg/kg weight of soil, respectively. Soils were each weighed into amber jars and incubated in the dark for 63 d at 20 ± 1 °C (termed Al- or Fe-amended soil).

In a second incubation, soil was amended with ^{12}C-naphthalene only and incubated in amber jars in the dark for 63 d at 20 ± 1 °C. At each time point, 10 ± 0.2 g soils in their various respirometers were amended with 50, 100, 250 or 500 mg/kg Al or Fe (termed freshly Al- or freshly Fe-amended soil). Control soils contained 50 mg/kg of naphthalene only, and analytical blanks were prepared to which no amendment was added to give background values, as well as a no naphthalene control. All soils were sampled in triplicate at 1, 21, 42 and 63 d.

Respirometry : At each time point, soil samples were assessed for naphthalene catabolism using ^{14}C-naphthalene. Sterile minimal basal salts containing (0.3g $NaCl_2$, 0.6g $(NH_4)_2SO_4$, 0.6g KNO_3, 0.25g KH_2PO_4, 0.75g K_2HPO_4, 0.15g $MgSO_4.7H_2O$ in 1 l DI H_2O) (30 ml) was added to 10 ± 0.2 g of the soil in respirometers (n = 3 for each condition). Within each respirometer, a mixture of ^{12}C- and ^{14}C-naphthalene (50 mg/kg and 833 Bq) was added to the soil. Production of $^{14}CO_2$ was assessed in modified Erlenmeyer flasks (250 ml) (Reid *et al.*, 2001). These respirometers incorporated a Teflon lined screw-caped CO_2 trap containing 1 M NaOH (1 ml) within a suspended glass scintillation vial (7 ml). The soils were incubated on a rotary shaker at 100 rpm for 14 d at a constant temperature of 21 °C and 45% relative humidity. The production of $^{14}CO_2$ was assessed daily by the addition of Ultima Gold liquid scintillation fluid to the CO_2 traps and subsequent liquid scintillation counting (LSC, Canberra Packard Tri Carb 2300TR, UK).

Bacterial enumeration: Enumeration of viable bacteria (naphthalene degraders) was achieved by counting the colony forming unit counts/g of soil (CFUs/g soil). This was done at each time point before and after mineralisation using the different conditions. Bacterial enumeration was carried out using 1 g of soil before mineralisation and I ml of soil solution after mineralisation from the different treatments. Soils were mixed with 9 ml of Ringer's solution by whirl-mixing for 30 seconds, sonicated for 1 minute and allowed to stand for 2 minutes. Soil solution (0.1 ml) was serially diluted in 0.9 ml Ringer's solution: aliquots (0.01 ml) of these dilutions were spread on agar-agar plates with naphthalene for isolation of naphthalene degraders. CFUs were counted after 48 h and 7 d.

Statistical analysis: Following blank-correction, Sigma Stat for Windows (version 3.5) was used to statistically analyse the data. Effects of Al or Fe concentrations on lag phase (prior to > 5% mineralisation), ^{14}C-naphthalene mineralisation and bacterial enumeration (naphthalene degraders) were compared statistically with the control. Impact of ageing on lag phase, naphthalene catabolism and naphthalene degraders before mineralisation in the different treatments were also compared statistically with the control using one way analysis of variance and student t-test, at $P < 0.05$ significant level, using three replicates ($n = 3$)

RESULTS

Impact of Al on ^{14}C-naphthalene mineralisation in soil, . Impact of aged Al amendment . Soil was amended with ^{12}C-naphthalene together with increasing concentrations of Al to investigate the impact of the metal on the mineralisation of naphthalene by indigenous microflora. Soils amended with 50, 100 and 250 mg/kg Al had longer ($p < 0.05$; Table 1) lag phases, as compared to the control, but mineralisation was < 5% in 500 mg/kg Al at 1 d. After 21, 42 and 63 d incubation, the lower Al concentrations had no impact on lag phase ($p > 0.05$; Table 1), but lag phases were significantly longer ($p < 0.05$; Table 1) in higher Al concentrations (250 and 500 mg/kg) relative to the control. Lag phases were longest at 1 d, compared to other time points in all amendments.

After 1 d incubation, maximum rates of ^{14}C-naphthalene catabolism were higher ($p < 0.05$) in the control, compared to all other amendments (Table 1). At all other time points however, maximum rates were significantly reduced ($p < 0.05$) in the higher Al concentrations (Table 1), but no impact on maximum rates ($p > 0.05$) were observed in the lower Al concentrations relative to the control. Maximum rates were higher at all other time points, compared to 1 d in all amendments (Table 1).

Extents of ^{14}C-naphthalene catabolism were higher ($p < 0.05$) in the control than in all Al-amended soil at 1 d (Figure 1). After 21 d incubation, however, only soil amended with 500 mg/kg Al had significantly reduced extents of ^{14}C-naphthalene catabolism ($p < 0.05$; Figure 1). Soil amended with 50 mg/kg Al had higher extents of ^{14}C-naphthalene catabolism at 42 d ($p < 0.05$; Figure 1), but these were significantly reduced in higher Al concentrations (250 and 500 mg/kg), compared to the control (Figure 1). Extents of ^{14}C-naphthalene catabolism significantly reduced only in 500 mg/kg Al amendment at 63 d relative to the control (Figure 1). Extents of ^{14}C-naphthalene catabolism were observed to be lowest at 1 d, compared to the other time points in all Al amendments (Table 1).

. *Impact of fresh Al amendment :* Soil was amended with ^{12}C-naphthalene and at each incubation time, increasing concentrations of Al were added to the soil together with ^{14}C-naphthalene. After 1 and 21 d, the Al-amended soil had lag phases that were significantly longer ($p < 0.05$) than the control, except in 50 mg/kg Al-amendment at 21 d (Table 2). After 42 d incubation, however, lag phases were shorter ($p < 0.05$) in the lower Al concentrations, but significantly longer in the higher concentrations ($p < 0.05$) relative to the control (Table 2). Lower Al concentrations had no impact on lag phase at 63 d, but lag phases were significantly lengthened at higher Al concentrations ($p < 0.05$; Table 2). Lag phases were longest at 1 d as compared to other time points in the control and all freshly Al-amendments ($p < 0.05$; Table 2). Lag phase significantly shortened with ageing in 100 mg/kg freshly Al-amendment ($p < 0.05$; Table 2).

Freshly added Al amendments had significant impact on maximum rates of ^{14}C-naphthalene catabolism. After 1 and 21 d incubation, maximum rates were higher ($p < 0.05$) in the control, as compared to all other freshly Al amendments, except 50 mg/kg amendment at 21 d (Table 2). However, maximum rates were higher ($p < 0.05$) in the lower freshly Al concentrations, but were significantly reduced ($p < 0.05$) in the higher concentrations relative to the control after 42 and 63 d incubation (Table 2). Maximum rates were observed to be higher at all other time points as compared to 1 d in all treatments.

At 1 d, extents of ^{14}C-naphthalene catabolism were significantly reduced ($p < 0.05$; Figure 1) in the 500 mg/kg amendment, compared to the control. After 21, 42 and 63 d incubation, however, lower Al concentrations had significantly higher ($p < 0.05$; Table 2) extents of ^{14}C-naphthalene mineralisation, but these were significantly reduced ($p < 0.05$; Table 2) in higher Al concentrations at 21 d relative to the control. Higher freshly added Al concentrations (250 and 500 mg/kg) had no impact on extents of ^{14}C-naphthalene mineralisation at 42 d and 63 d relative to the control (Figure 1). Extents of ^{14}C-naphthalene catabolism were lowest in most freshly Al amendments at 1 d as compared to other time points (Figure 1).

Impact of Fe on ^{14}C-naphthalene mineralisation in soil: Impact of aged Fe amendment. Soil was amended with ^{12}C-naphthalene together with increasing concentrations of Fe to investigate the impact of the metal on the mineralisation of naphthalene by indigenous microflora. After 1 day, lag phases were significantly longer ($p < 0.05$; Table 3) in all Fe-amendments, with the exception of soil amended with 100 mg/kg Fe, compared to the control. However, after 21 d incubation, lag phase was significantly lengthened in 100 mg/kg Fe ($p < 0.05$; Table 3), but no impact on the lag phase was observed in the other treatments, compared to the control. After 42 and 63 d incubation, higher Fe concentrations had significantly ($p < 0.05$) longer lag phases, but no impact on lag phase was observed at the lower concentrations ($p > 0.05$), compared to the control (Table 3). Lag phases were longest at 1 d ($p < 0.05$) relative to other time points.

Maximum rates of ^{14}C-naphthalene catabolism were significantly reduced ($p < 0.05$) in Fe-amended soil as compared to the control after 1 d incubation, with the exception of soil amended with 100 mg/kg Fe (Table 3). After 21 d incubation, however, only 100 mg/kg Fe amendment had significantly reduced ($p < 0.05$) maximum rates, but other treatments had no impact on maximum rates ($P > 0.05$), compared to the control (Table 3). Maximum rates were significantly reduced ($p < 0.05$) in the higher Fe concentrations, but no impact was observed in the lower concentrations at 42 d ($p > 0.05$; Table 3). Fe-amendments had no effect on maximum rates of ^{14}C-naphthalene catabolism at 63 d ($p > 0.05$; Table 3).

Extents of ^{14}C-naphthalene catabolism were significantly reduced ($p < 0.05$) in Fe-amended soil at 1 d, as compared to the control apart from 100 mg/kg amendment (Figure 2). After 21 d incubation, however, 100 mg/kg Fe showed significant decrease in extents of ^{14}C-naphthalene mineralisation ($p < 0.05$), but no impact was observed in other amendments (Figure 2). Soil amended with 500 mg/kg Fe had significantly higher ^{14}C-naphthalene mineralisation at 42 d relative to the control ($p < 0.05$; Figure 2). Extents of ^{14}C-naphthalene mineralisation were not affected by Fe-amendments at 63 d, and were higher at other time points as compared to 1 d in most treatments.

Impact of fresh Fe amendment: Soil was amended with ^{12}C-naphthalene and at each incubation time, increasing concentrations of Fe were added to the soil together with ^{14}C-naphthalene. Fe amendments had significantly longer ($p < 0.05$) lag phases, compared to the control after 1 d incubation (Table 4). After 21 d incubation, however, 50 mg/kg amendment had significantly shorter ($p < 0.05$) lag phase, but all other Fe-amendments had longer lag phases relative to the control ($p < 0.05$; Table 4). Lag phases were shorter in all freshly Fe-amended soil at 42 d ($p < 0.05$; Table 4), as compared to the control. After 63 d incubation, however, lag phase was significantly longer in 250 mg/kg Fe amendment ($p < 0.05$), as compared to the control (Table 4). Lag phases were longest at 1 d, as compared to other time points ($p < 0.05$; Table 4).

Maximum rates of ^{14}C-naphthalene catabolism were significantly reduced ($p < 0.05$; Table 4) in freshly Fe amendments after 1 d incubation, with the exception of 500 mg/kg amendment. After 21 d incubation, however, 50 mg/kg amendment had significantly higher maximum rates of ^{14}C-naphthalene catabolism ($p < 0.05$), but these were significantly reduced in 250 mg/kg amendment ($P < 0.05$), compared to the control (Table 4). After 42 d incubation, all freshly Fe-amendments had significantly higher maximum rates of ^{14}C-naphthalene catabolism relative to the control ($p < 0.05$; Table 4). Maximum rates were significantly reduced ($p < 0.05$) in 250 mg/kg amendment at 63 d ($p < 0.05$) relative to the control (Table 4). Most treatments were observed to have higher maximum rates of ^{14}C-naphthalene catabolism at all other time points as compared to 1 d (Table 4).

After 1 d incubation, extents of ^{14}C-naphthalene catabolism were significantly reduced ($p < 0.05$) in freshly Fe-amendments with the exception of 500 mg/kg amendment (Figure 2). After 21 d incubation, however, these were significantly reduced ($P < 0.05$) in all amendments with the exception of 50 mg/kg amendment relative to the control (Figure 2). Extents of ^{14}C-naphthalene mineralisation were significantly higher ($p < 0.05$) in all freshly Fe amendments as compared to the control at 42 d (Figure 2). After 63 d incubation, however, 250 mg/kg Fe amendment had significantly reduced ($p < 0.05$) extents of ^{14}C-naphthalene mineralisation, compared to the control (Figure 2). Extents of ^{14}C-naphthalene catabolism were higher ($p < 0.05$) at other time points, compared to 1 d in most treatments (Figure 2).

.Impact of Al and Fe amendments on bacterial numbers in soil: Impact of Al and Fe amendments on bacterial enumeration before naphthalene mineralisation. Naphthalene degraders were enumerated in soil amended with ^{12}C-naphthalene together with increasing concentrations of Al and Fe at 1, 21, 42 and 63 d. Al and Fe amendments had significant impact on naphthalene degraders before ^{14}C-naphthalene mineralisation was measured. After 1 d incubation, naphthalene degraders were significantly higher in 50 mg/kg Al amendment, but were lower in other Al concentrations as compared to the control ($p < 0.05$; Table 5). In Fe-amended soil however, naphthalene degraders were higher in all treatments as compared to the control after 1 d

incubation ($p < 0.05$; Table 5). After 21 d incubation, the control had higher ($p < 0.05$) naphthalene degraders as compared to other treatments except in 50 mg/kg Al, 100 and 250 mg/kg Fe (Table 5). Naphthalene degraders were significantly reduced in all Al amendments ($p < 0.05$) except in 50 mg/kg Al amendment, but were significantly higher in 50, 100 and 500 mg/kg Fe, relative to the control at 42 d ($p < 0.05$; Table 5). After 63 d incubation, the control had higher ($p < 0.05$) naphthalene degraders than other treatments. Soil amended with 500 mg/kg Al had naphthalene degraders consistently lower than the control at all-time points, but were below detection limit at 63 d. Naphthalene degraders were observed to be higher ($p < 0.05$) at other time points, as compared to 1 d in most treatments.

Impact of aged Al and Fe amendments on bacterial enumeration after naphthalene mineralisation

After 1 d, ^{14}C-naphthalene mineralisation, naphthalene degraders were significantly higher ($p < 0.05$) in the control as compared to all Al amendments, but were significantly higher in 100, 250 and 500 mg/kg Fe amendments (Table 6). After 21 d mineralisation, however, the control had significantly higher number of degraders than in Al and Fe amended soil (Table 6). Naphthalene degraders were significantly higher ($p < 0.05$) in 50 and 100 mg/kg Al, and 100, 250 and 500 mg/kg Fe after naphthalene mineralisation at 42 d. After 63 d mineralisation, however, naphthalene degraders were higher ($p < 0.05$) in the control as compared to all Al and Fe amendments. Naphthalene degraders were significantly reduced ($p < 0.05$) in 500 mg/kg Al at all time-points relative to the control.

Impact of fresh Al and Fe amendments on bacterial enumeration after naphthalene mineralisation

Fresh Al and Fe amendments had higher naphthalene degraders in 50 mg/kg Al and 500 mg/kg Fe, as compared to the control after mineralisation at 1 d (Table 7). After 21 d and 42 d naphthalene mineralisation, however, all fresh Al and Fe amendments had higher ($p < 0.05$) naphthalene degraders relative to the control, except in 500 mg/kg freshly Al amendment at 42 d. Naphthalene degraders were higher ($p < 0.05$) in the control after 63 d naphthalene mineralisation as compared to freshly Al amendments, except in 100 mg/kg Al amendment (Table 7). However, all fresh Fe amendments had higher ($p < 0.05$) naphthalene degraders than the control at 63 d (Table 7).

DISCUSSION

Indigenous microflora adapted or acclimatized to the presence of naphthalene, resulting in increased catabolic activity over time. This is as a result of reduced lag phases, with general increases in rates and extents of naphthalene mineralisation with increasing contaminant-soil contact time (Tables 1-4). This is in agreement with other authors finding (Macleod and Semple, 2002; Lee *et al*., 2003; Macleod and Semple, 2006; Couling *et al*., 2010; Rhodes *et al*., 2010). Lag phases prior to mineralisation often correspond to the degree of microbial adaptation (Rhodes *et al*., 2010), and has been suggested to be a useful indicator of heavy metal stress to soil microorganisms (Haanstra and Doelman, 1984; Dahlin and Witter, 1998).

Impact of Al on naphthalene mineralisation in soil. Al is a metal lacking known biological functions and in this respect pertains to the non-essential elements (Pina and Cervantes, 1996). Soil microflora adapted to naphthalene degradation more rapidly at low concentrations of Al, compared to that at higher Al concentrations (Tables 1 and 2). Lengthening of lag phases in the higher concentrations of aged and freshly Al could be as a result of reduced or delayed exposure/contact of the indigenous microflora to the contaminant as a result of high metal toxicity, adaptation and selection processes of the community, and it's indicative of increased metal stress (Haanstra and Doelman, 1984; Dahlin and Witter, 1998; Riis *et al*., 2002). This is in agreement with Sandrin *et al*. (2000) who reported delayed onset of exponential growth of *Burkoderia* sp during naphthalene biodegradation in the presence of Cd at 8.9 µm relative to 0 µm and 0.89 µm Cd. Biomass production (microbial growth) was delayed when Al (≤ 400 µm) was added to minimal medium, and only reached the level of Al-free samples about 2-3 days later (Illmer and Buttinger, 2006).

Higher extents of naphthalene catabolism observed in lower concentrations of fresh Al amendments (50 and 100 mg/kg) relative to the control could be as a result of increase in microbial adaptation, microbial numbers and stimulation of the growth of microorganisms by low concentrations of Al (Guzzo *et al*., 1992; Amor *et al*., 2001). This may reflect an "Arndt-Schulz" effect, where non-lethal concentrations of a poison induce, among other changes, an alteration in the permeability that permits a freer flow of nutrients across the plasma membrane (Gogolev and Wilke, 1997). This increase in microbial number and/or activity was reflected further by statistically significant increases ($p < 0.05$) in naphthalene degraders in the control and lower concentrations of Al (CFU/g) after 21 and 42 d relative to 1 d (Table 5). Gogolev and Wilke (1997) reported enhancement of bacterial growth in the presence of flouranthene at low Cd concentration (0.05 mg 1^{-1}). The effectiveness of diesel and black oil degradation were enhanced in the presence of Cu and Mn (Zukauskaite *et al*., 2008). Lin *et al*. (2010) also reported the enhancement of fluorene and phenanthrene removal by *Selenastrum capricornutum* in the presence of metals Cd, Zn, Cu and nickel in a mixture, in a liquid culture. This was thought to be as result of the enhancement of antioxidant enzymes under metal exposure to scavenge reactive oxygen species (ROS) generated under metal stress condition (Lin *et al.*, 2010). Stimulation effect at these lower Al concentrations could also be due to metals reducing competition for nutrients between metal-resistant degraders and metal sensitive non degraders (Sandrin and Maier, 2003).

Extents of naphthalene catabolism were significantly reduced at highest Al concentration (500 mg/kg) in aged Al amended soil at all time-points studied, and in freshly Al amended soil at 1 and 21 d relative to the control (Table 2). This could be because of toxic effect of Al on soil microorganisms participating in PAH microbial transformations, which can lead to the decrease of PAH biodegradation rate (Maliszewska-Kordybach and Smreczak, 2003). This is evident in statistically significant decreases in microbial number and/or activity in 500 mg/kg Al before and after ^{14}C-naphthalene mineralisation (Tables 5, 6 and 7), decreases in microbial numbers in the presence of high metal concentrations have been reported in literature (Riis *et al*., 2002; Olivera and Pampulha, 2006; Ibarrolaza *et al*., 2009). Baldrin *et al*. (2000) reported limited PAH degradation by fungus in 500ppm Cd, the authors concluded that this may be due to low manganese peroxidase enzyme activity. The reduction of naphthalene catabolism could also be because of the inhibition of enzymes involved in the degradation of naphthalene at this concentration (Sokhn *et al.,* 2001; Maliszewka-Korybach and Smreczak, 2003; Shen *et al*., 2005). Phenanthrene degradation and dehydrogenase activity were shown to be retarded in soil microcosms, when phenathrene and Cr (V1) (25, 50 and 100 mg/kg) were simultaneously incorporated (Ibarrolaza *et al*., 2009). Irha *et al*. (2003) also reported reduced dehydrogenase activity in four soils contaminated with heavy metals (Cr, Pd, Cu and Cd) and pyrene.

Impact of Fe on naphthalene mineralisation in soil. Fe is an essential element for most organisms and occurs as cofactor in various proteins. In this study, higher extents of naphthalene catabolism were observed in 500 mg/kg aged Fe, and in all concentrations of freshly amended Fe at 42 d relative to the control (Tables 3 and 4). These increases in naphthalene catabolism were accompanied with increases in naphthalene degraders in the control and 500 mg/kg Fe at 42 d (Table 5). Increase in microbial cell numbers in the presence of iron and anthracene, phenanthrene, pyrene, gasoline and diesel oil, as compared to the presence of these hydrocarbons without Fe was reported by Santos *et al*. (2008). These results show that Fe at the highest concentration studied (500 mg/kg) was not toxic to the microbial community and actually enhanced naphthalene catabolism, though this depended on the incubation time. This could be because Fe is an essential element for most microorganisms (Illmer and Buttinger, 2006), and aerobic degradation of aromatic compounds by microorganisms proceed via several oxidation steps that are catalysed by oxygenases which contain iron as a cofactor (Dinkla *et al*., 2001). It is also possible that the higher naphthalene degradation shown by indigenous microflora at 42 d was related to the availability of iron for induction, synthesis and activity of the enzymes involved in naphthalene biodegradation (Santos *et al*., 2008). Dinkla *et al*. (2001) showed increased enzyme activity of the iron-containing toluene monoxygenase and complete removal of toulene in increasing iron concentration, but a reduced enzyme activity and 60 to 70 % removal of toulene under iron-limiting conditions. Santos *et al*. (2008) also reported increases in anthracene degradation (63%) in the presence of Fe (0.1mM), compared to a control (51%), in the absence of Fe from the mean of three pseudomonas isolates. Biodegradation of naphthalene, phenanthrene and anthracene were more effective in iron concentrations of 0.1 mmol l^{-1} as compared to 0.05 (Simarro *et al*., 2011).

Reduced impact at high Fe concentration could be as a result of non-bioavailability of Fe or non-specific binding of Fe to cell surfaces, which may preclude interaction of PAH with bacterial membranes leading to decrease of their toxic effect (Gogolev and Wilke 1997; Maliszewska-Kordybach and Smreczak, 2003; Thavamani *et al*., 2011). Gogolev and Wilke. (1997) reported non enhancement of the deleterious effects of high heavy metal doses by simultaneous addition of flouranthene. Maliszewska-Kordybach and Smreczak. (2003) also gave evidence of smaller enhancement of dehydrogenase activity in PAH and heavy metal contaminated soil than in the case of soil uncontaminated with heavy metals. Non toxicity or enhanced biodegradation at high metal concentrations could also be because high metal concentrations create selective pressure for metal resistant organic compound-degrading microorganisms, which may have reduced competition from metal-sensitive, non-degrading microorganisms, thus increasing biodegradation at higher metal concentrations (Sandrin and Maier, 2003).

*Conclusion:*This study investigated the adaptation of indigenous microbial community in their ability to mineralise ^{14}C-naphthalene in soils amended with aged and fresh Al and Fe. Generally, Fresh Al and Fe amendments seemed to have less inhibitory effect on ^{14}C-naphthalene mineralisation as compared to aged

Al and Fe amendments. This could be because microorganisms are able to accumulate heavy metals from solutions thereby resulting in reduced toxicity. Al showed more toxicity at higher concentrations (as measured by $^{14}CO_2$ evolution and microbial numbers) than Fe. This could be because Al is one of the very few macro-elements without known biological function. However, Fe being an essential element for most microorganisms showed less toxicity. The magnitude and type of combined effects of PAHs and heavy metal depend not only on the type of metal or PAH, but also on the concentrations of mixtures. Biodegradation of naphthalene in soil in the presence of high concentrations of Al might be impeded.

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Table 1: ^{14}C-Naphthalene mineralisation in Al-amended soil

Time (days)	Concentration (mg Al/kg)	Lag phase (h)	Maximum rate (%/h)	Cumulative extent (%)
1 d	Control	26.68 ± 0.28	1.65 ± 0.00	56.48 ± 3.81
	50	62.53 ± 0.04	0.28 ± 0.00	38.36 ± 0.54
	100	54.60 ± 0.57	0.53 ± 0.03	37.76 ± 1.49
	250	102.69 ± 0.89	0.37 ± 0.07	36.88 ± 3.12
	500	LPR	0.01 ± 0.01	1.91 ± 0.20
21 d	Control	3.26 ± 0.19	1.55 ± 0.09	57.32 ± 2.42
	50	4.16 ± 0.32	1.22 ± 0.09	50.94 ± 4.86
	100	3.74 ± 0.18	1.38 ± 0.06	51.56 ± 0.70
	250	19.91 ± 2.23	0.94 ± 0.17	57.86 ± 1.18
	500	134.82 ± 5.43	0.36 ± 0.05	31.60 ± 1.94
42 d	Control	3.82 ± 0.18	1.32 ± 0.06	55.01 ± 2.94
	50	3.69 ± 0.16	1.36 ± 0.06	60.92 ± 0.26
	100	4.51 ± 0.62	1.07 ± 0.13	52.72 ± 3.31
	250	27.56 ± 0.07	0.48 ± 0.01	38.98 ± 1.59
	500	118.28 ± 4.18	0.36 ± 0.05	27.15 ± 0.98
63 d	Control	3.72 ± 0.33	1.37 ± 0.13	55.17 ± 3.07
	50	3.51 ± 0.07	1.43 ± 0.03	57.46 ± 1.22
	100	3.32 ± 0.02	1.28 ± 0.02	50.44 ± 5.61
	250	17.05 ± 1.07	0.56 ± 0.02	48.30 ± 2.42
	500	99.57 ± 1.68	0.43 ± 0.05	35.32 ± 2.63

LPR Lag phase not reached i.e less than 5% mineralisation

Table 2: ^{14}C-Naphthalene mineralisation in freshly Al-amended soil

Time (days)	Concentration (mg Al/kg)	Lag phase (h)	Maximum rate (%/h)	Cumulative extent (%)
1 d	Control	56.68 ± 0.28	0.75 ± 0.00	40.94 ± 0.88
	50	65.27 ± 0.24	0.37 ± 0.00	32.42 ± 0.83
	100	96.34 ± 0.62	0.59 ± 0.00	38.24 ± 0.85
	250	126.23 ± 3.21	0.38 ± 0.00	37.01 ± 1.25
	500	220.19 ±19.12	0.05 ± 0.01	6.85 ± 0.30
21 d	Control	4.23 ± 0.23	1.19 ± 0.07	52.94 ± 0.62
	50	3.43 ± 0.19	1.47 ± 0.08	55.33 ±3.78
	100	5.70 ± 0.21	0.96 ± 0.04	56.21 ± 2.63
	250	21.22 ± 2.19	0.48 ± 0.02	41.41 ± 0.68
	500	33.63 ± 2.03	0.20 ± 0.03	24.26 ± 1.07
42 d	Control	7.12 ± 0.88	0.72 ± 0.09	30.23 ± 1.90
	50	3.52 ± 0.27	1.43 ± 0.09	47.51 ± 3.12
	100	3.87 ± 0.17	1.30 ± 0.04	53.36 ± 0.53
	250	19.48 ± 1.18	0.45 ± 0.03	33.70 ± 0.45
	500	33.12 ± 2.52	0.33 ± 0.03	32.01 ± 1.50
63 d	Control	4.29 ± 0.41	1.19 ± 0.12	43.54 ± 4.37
	50	3.54 ± 0.28	1.43 ± 0.11	50.52 ± 2.28
	100	3.19 ± 0.13	1.57 ± 0.05	58.77 ± 0.50
	250	15.47 ± 0.48	0.40 ± 0.06	38.54 ± 1.25
	500	30.54 ± 3.87	0.24 ± 0.02	39.31 ± 1.47

Table 3: ^{14}C-Naphthalene mineralisation in Fe-amended soil

Time (days)	Concentration (mg Fe/kg)	Lag phase (h)	Maximum rate (%/h)	Cumulative extent (%)
1 d	Control	26.68 ± 0.28	1.65 ± 0.17	56.28 ± 2.48
	50	47.66 ± 1.17	0.22 ± 0.00	38.48 ± 0.47
	100	28.05 ± 0.10	1.18± 0.09	49.01 ± 2.34
	250	47.96 ± 1.67	0.31± 0.08	34.93 ± 0.62
	500	53.58± 0.21	0.62 ± 0.04	28.46 ± 0.67
21 d	Control	3.26 ± 0.19	1.55 ± 0.09	57.52 ± 2.56
	50	3.73 ± 0.15	1.35 ± 0.05	51.06 ± 2.73
	100	5.27 ± 0.45	0.95 ± 0.06	40.11 ± 2.04
	250	3.93 ± 0.31	1.29 ± 0.10	53.10 ± 1.37
	500	3.79 ± 0.14	1.32 ± 0.03	49.25 ± 1.84
42 d	Control	3.82 ± 0.18	1.32 ± 0.06	53.27 ± 1.44
	50	3.48 ± 0.29	1.46 ± 0.12	57.11 ± 2.92
	100	4.01 ± 0.41	1.38 ± 0.07	49.09 ± 1.73
	250	5.25 ± 0.43	0.97 ± 0.08	52.96 ± 1.37
	500	5.02 ± 0.06	1.00 ± 0.01	61.04 ± 1.00
63 d	Control	3.72 ± 0.33	1.37 ± 0.13	55.17 ± 3.07
	50	3.63 ± 0.38	1.41 ± 0.17	54.84 ± 4.25
	100	4.06 ± 0.57	1.35 ± 0.15	56.23 ± 3.55
	250	5.11 ± 0.82	1.03 ± 0.17	44.02 ± 4.15
	500	5.04 ± 0.87	1.07 ± 0.13	55.04 ± 5.33

Table 4: ^{14}C-Naphthalene mineralisation in freshly Fe-amended soil

Time (days)	Concentration (mg Fe/kg)	Lag phase (h)	Maximum rate (%/h)	Cumulative extent (%)
1 d	Control	56.68 ± 0.28	0.75 ± 0.00	40.94 ± 0.88
	50	60.71 ± 0.33	0.47 ± 0.00	31.36 ± 0.50
	100	77.57 ± 1.11	0.26 ± 0.02	23.33 ± 0.28
	250	73.39 ± 0.82	0.50 ± 0.00	31.78 ± 0.79
	500	74.16 ± 0.02	0.64 ± 0.08	42.94 ± 2.69
21 d	Control	4.23 ± 0.23	1.19 ± 0.07	52.95 ± 0.62
	50	3.41 ± 0.03	1.47 ± 0.01	54.02 ± 0.57
	100	6.26 ± 0.11	0.82 ± 0.02	43.32 ± 0.34
	250	7.25 ± 0.27	0.69 ± 0.03	38.96 ± 0.28
	500	5.10 ± 0.32	0.91 ± 0.09	42.23 ± 1.72
42 d	Control	7.12 ± 0.88	0.72 ± 0.09	30.23 ± 1.90
	50	3.57 ± 0.33	1.42 ± 0.13	52.63 ± 3.36
	100	3.95 ± 0.06	1.39 ± 0.13	50.21 ± 3.31
	250	3.51± 0.11	1.43 ± 0.04	50.91 ± 1.23
	500	4.10 ± 0.39	1.28 ± 0.09	47.89 ± 2.45
63 d	Control	4.29 ± 0.41	1.19± 0.12	43.54 ± 4.37
	50	3.36± 0.23	1.50 ± 0.10	49.62 ± 2.91
	100	4.37 ± 0.38	1.23 ± 0.10	44.83 ± 3.44
	250	9.17 ± 1.02	0.56 ± 0.06	33.59 ± 1.49
	500	3.53 ± 0.33	1.49 ± 0.11	54.90 ± 2.60

Table 5: Indigenous degrader numbers (CFUs/g) in Al and Fe-amended soil at 1, 21, 42 and 63 d before ^{14}C-naphthalene mineralisation

Time (days)	Concentrations (mg/kg)	Degrader Numbers (CFUs/g)	
		Al-amended soil (A x 10^5 ± B x 10^1)¶	**Fe-amended soil (A x 10^5 ± B x 10^1)¶**
1 d	Control	6.77 ± 1.01	6.77 ± 1.01
	50	8.67 ± 1.53	18. 1 ± 0.23
	100	6.50 ± 0.87	8.50 ± 0.23
	250	2.50 ± 0.00	10.5 ± 4.04
	500	0.50 ± 0.00	7.40 ± 0.55
21 d	Control	21.7 ± 1.47	21.7 ± 1.47
	50	21.4 ± 0.00	10.3 ± 0.00
	100	12.6 ± 0.00	22.0 ± 0.00
	250	3.30 ± 0.00	21.0 ± 0.00
	500	2.40 ± 0.00	15.3 ± 0.00
42 d	Control	23.7 ± 1.47	23.7 ± 1.47
	50	24.2 ± 1.07	24.4 ± 0.92
	100	16. 7 ± 0.30	24.8 ± 1.27
	250	8.00 ± 1.21	22.6 ± 0.81
	500	1.00 ± 0.00	25.0 ± 0.81
63 d	Control	22.5± 0.00	22.5 ± 0.00
	50	17.5 ± 0.00	16.5 ± 0.29
	100	9.60 ± 0.87	16.5 ± 0.29
	250	8.20± 0.00	10.1 ± 1.58
	500	BDL	13.9 ± 0.00

¶ Mean value x 10^5 ± SEM x 10^1, n = 3

Table 6: Indigenous degrader numbers (CFUs/g) in Al and Fe-amended soil at 1, 21, 42 and 63 d after mineralisation

Time (days)	Concentrations (mg/kg)	Degrader Numbers (CFUs/g)	
		Al-amended soil (A x 10^5 ± B 10^1)¶	**Fe-amended soil (A x 10^5 ± B x 10^1)¶**
1 d	Control	20.0 ± 0.00	20.0 ± 0.00
	50	7.90 ± 0.10	19.5 ± 0.29
	100	8.17 ± 1.69	42.0 ± 0.00
	250	4.37 ± 0.43	34.0 ± 0.12
	500	0.40 ± 0.58	40.6 ± 0.55
21 d	Control	21.9 ± 1.67	21.9 ± 1.67
	50	8.17 ± 0.66	16.6 ± 0.35
	100	13.7 ± 1.79	20.4 ± 1.27
	250	3.00 ± 0.00	7.10 ± 1.02
	500	0.30 ± 0.00	21.8 ± 1.79
42 d	Control	22.6 ± 0.00	22.6 ± 0.00
	50	69.0 ± 0.46	21.0 ± 0.00
	100	23.7 ± 1.08	57.0 ± 1.10
	250	14.0 ± 3.33	78.7 ± 0.38
	500	2.05 ± 0.29	66.7 ± 0.78
63 d	Control	98.0 ± 1.27	98.0 ± 1.27
	50	60.7 ± 0.72	23.7 ± 0.23
	100	25.3 ± 0.72	45.0 ± 0.23
	250	6.57 ± 0.38	63.0± 0.23
	500	4.87 ± 0.43	4.50 ± 0.75

¶ Mean value x 10^5 ± SEM x 10^1, n = 3

Table 7: Indigenous degrader numbers (CFUs/g) in freshly Al and Fe-amended soil at 1, 21, 42 and 63 d after mineralisation

Time (days)	**Concentrations (mg/kg)**	**Degrader Numbers (CFUs/g)**	
		Al-amended soil (A x 10^4 ± B 10^1)¶	**Fe-amended soil (A x 10^4 ± B x 10^1)¶**
1 d	Control	13.0 ± 1.15	13.0 ± 1.15
	50	18.8 ± 0.20	10.1 ± 1.21
	100	10.7 ± 1.56	12.6 ± 0.00
	250	10.0 ± 0.00	11.5 ± 1.44
	500	3.20 ± 0.00	15.4 ± 0.78
21 d	Control	2.00 ± 0.00	2.00 ± 0.00
	50	5.00 ±0.57	78.7 ± 1.01
	100	6.07 ± 0.55	65.7 ± 0.00
	250	8.17 ± 0.15	101.0 ± 1.07
	500	3.30 ± 0.38	92.7 ± 1.01
42 d	Control	18.6 ± 0.00	18.6 ± 0.00
	50	30.0 ± 0.00	68.3 ± 0.17
	100	55.0 ± 0.00	23.3 ± 1.70
	250	25.0 ± 0.00	67.7 ± 0.99
	500	4.17 ± 3.76	25.7 ± 0.29
60 d	Control	25.0 ± 0.00	25.0± 0.00
	50	21.4 ± 0.00	84.0 ± 0.00
	100	150.0 ± 5.77	90.0 ± 0.00
	250	6.40 ± 2.31	27.0 ± 0.35
	500	2.17 ± 0.17	96.0 ± 0.35

¶ Mean value x 10^4 ± SEM x 10^1, n = 3

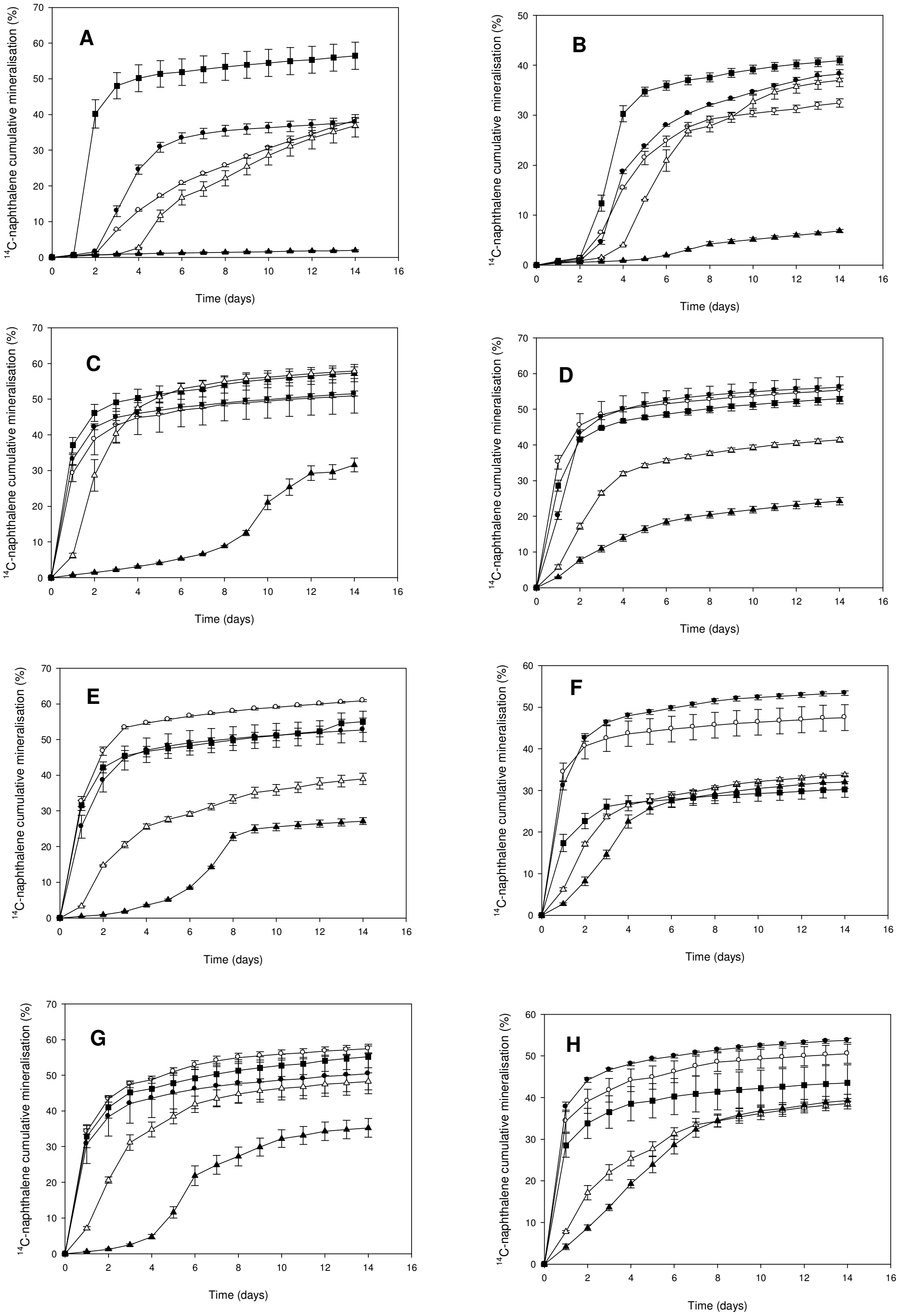

Fig. 1. Development of naphthalene catabolism for Al and freshly Al- amendments at 1 d Al-amendment (A), 1 d freshly Al-amendment (B), 21 d Al-amendment (C), 21 d freshly Al-amendment d (D), 42 d Al-amendment (E), 42 d freshly Al-amendment (F), 63 d Al-amendment (G), 63 d freshly Al-amendment (H), Control (▪), 50 mg/kg Al-amendment (◦), 100 mg/kg Al-amendment (•), 250 mg/kg Al-amendment (Δ), 500 mg/kg Al-amendment ( )

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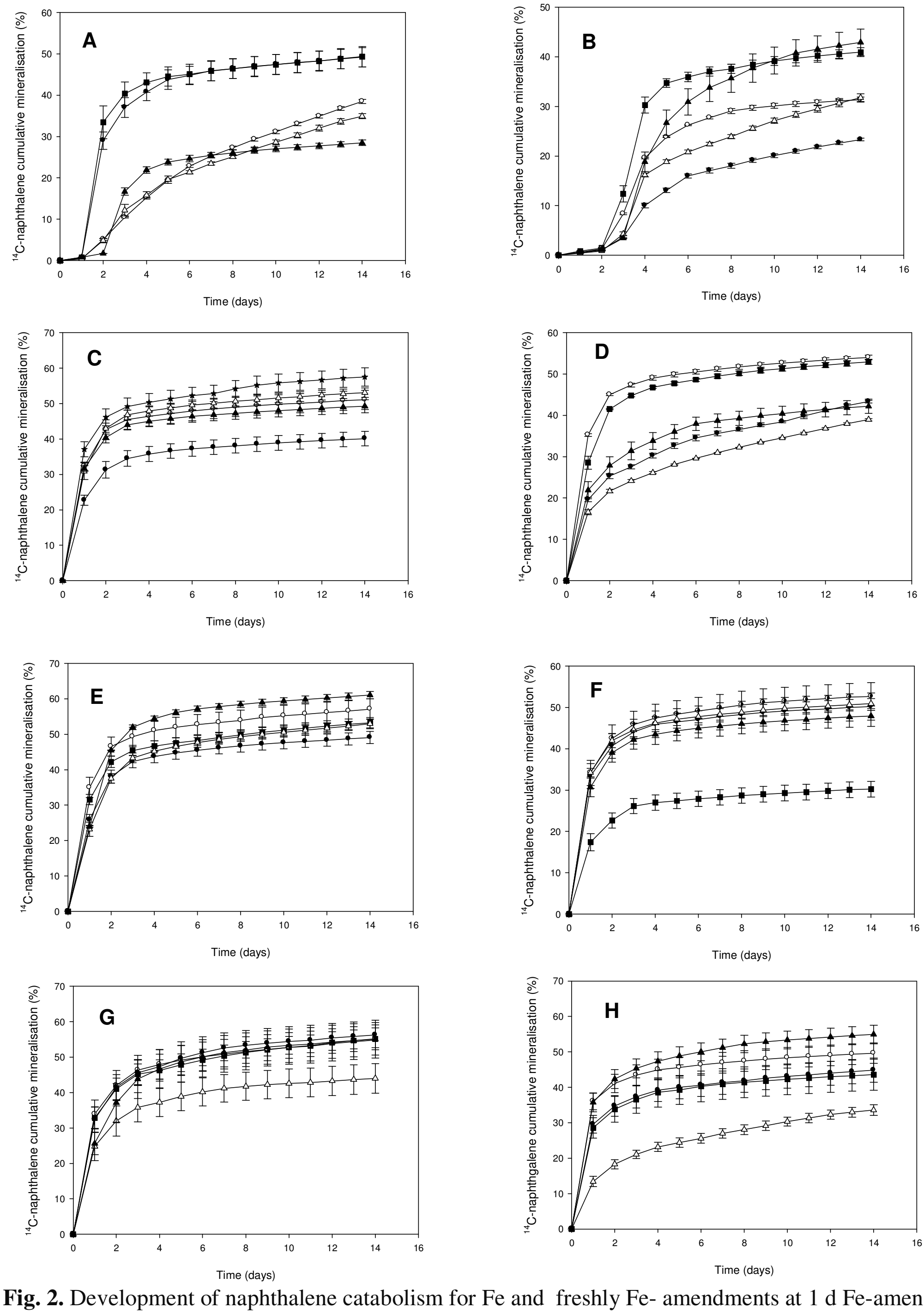

Fig. 2. Development of naphthalene catabolism for Fe and freshly Fe- amendments at 1 d Fe-amendment (A), 1 d freshly Fe-amendment (B), 21 d Fe-amendment (C), 21 d freshly Fe-amendment d (D), 42 d Fe-amendment (E), 42 d freshly Fe-amendment (F), 63 d Fe-amendment (G), 63 d freshly Fe-amendment (H), Control (▪), 50 mg/kg Fe-amendment (◦), 100 mg/kg Fe-amendment (•), 250 mg/kg Fe-amendment (Δ), 500 mg/kg Fe-amendment (   )

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