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Effect of surface-tailored biocompatible organoclay on the bioavailability and mineralization of polycyclic aromatic hydrocarbons in long-term contaminated soil

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Abstract

A surface-tailored organoclay (palmitic acid-tailored Arquad®-modified bentonite, ABP) was prepared for the selective adsorption of cadmium in the presence of PAH contaminants in a long-term contaminated soil. The efficiency of the organoclay (ABP) and the effect of its parent clays were assessed regarding the microbial viability, metal immobilization and PAHs bioavailability and biodegradation in a long-term (70 days) soil incubation study. The surface-tailored organoclay (ABP) increased the bacterial growth by 5 – 7 fold than the control and parent clay-amended soil. With an increased effect of aging, the ABP immobilized more Cd from the soil solution (2-folds higher than the control soil), and simultaneously increased the bioavailability (1.6 – 1.8 fold) of low molecular weight PAHs related to the control soil and the parent clay-amended soils. The surface-tailored organoclay (ABP) could also increase the mineralization of ^{14}C-labelled phenanthrene by ca. 1.3-fold relative to the control experiment under a 25-days of incubation.

Keywords: Surface-tailored organoclay; Polycyclic aromatic hydrocarbon; Bioavailability; Biodegradation

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1. Introduction

Bioremediation of soils and waters contaminated with polycyclic aromatic hydrocarbons (PAHs) by employing microorganisms can be a serious challenge in the presence of heavy metals because of the potential metal toxicity to the degrading microorganisms (Olaniran et al., 2013; Vig et al., 2003). The metal-PAH mixed contaminated situations are often witnessed in the gasworks sites and motor smelting areas (Brown and Peake, 2006; Thavamani et al., 2012). Material-based adsorption of metals for supporting the viability and facilitating the degradability of microorganisms could help to improve biodegradation, but might not be a suitable option when the adsorbents fix PAHs also in a non-selective manner. The bioavailability of PAHs would be an important issue following their adsorption on the added materials, which would make the removal of PAHs ineffective. However, an adsorbent, which could bind metal selectively without reducing the bioavailability of PAHs, would be efficient to drive the biodegradation in a mixed contaminated situation (Biswas et al., 2016; Malakul et al., 1998; Mandal et al., 2016). The adsorbent should also be compatible with the degrading microorganisms in the soils and sediments to maintain a congenial microenvironment for microorganism, which could deliver an effective bioremediation process.

A surface-tailored organoclay (fatty acid-grafted Arquad®-modified smectite) was previously developed and characterized by the current authors (Biswas et al., 2015, 2016; Mandal et al., 2016). The material showed a selective binding of Cd in a phenanthrene-Cd mixed contaminated situation (Biswas et al., 2016). This clay-based product also showed a higher biocompatibility to bacteria in an aqueous suspension (Mandal et al., 2016) and a field soil (Biswas et al., 2015). However, the biodegradation of PAHs in long-term real contaminated aged soils with the aid of the designer adsorbent was not studied before. It could be more

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challenging to achieve an efficient biodegradation in the aged soil due to (i) the lower bioavailability of PAHs, and (ii) a low microbial activity of native microorganisms (Cébron et al., 2013; Juhasz et al., 2014). The potential application of the newly developed surface-tailored organoclay in the long-term contaminated soil therefore requires assessment in light of microbial viability of the native and inoculated bacteria, immobilization of metals, and the bioavailability and biodegradation of PAHs at a temporal scale. The low molecular weight (LMW, 2-3 benzene rings) PAHs are more susceptible to biodegradation than the high molecular weight ones (HMW, >3 benzene rings) (Semple et al., 2003). However, how these PAHs would behave in terms of their bioavailability over time in the surface-tailored organoclay-amended soils should also be investigated.

The aim of this study therefore was to evaluate the potential of the surface-tailored organoclay to enhance biodegradation of PAHs in a long-term contaminated soil over a long incubation period (70 days) through the assessment of (i) the viability of PAH-degrading bacteria, (ii) metal immobilization, and (iii) bioavailability of total PAHs and biodegradation of LMW PAHs.

2. Materials and methods

2.1 Clay products, soil preparation and microcosm setup

The powder form of raw and modified clay products (B = bentonite, AB = Arquad®-treated bentonite, and ABP = Arquad®-palmitic acid treated bentonite) were obtained from the desiccator-stored stock. The preparation and characterization of these materials have been reported elsewhere (Biswas et al., 2015, 2016; Mandal et al., 2016).

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A long-term PAH-contaminated soil was selected based on its physicochemical characteristics and the total PAH content (Juhasz et al., 2014). The soil was collected from a mine site of South Australia. It had a low clay content (Texture (adjusted to 100% upon organic carbon removal): clay = 4%, silt = 8%, and sand = 88%; organic carbon = 2.1%). The low-clay content soil was chosen because it would likely decipher a more prominent effect of the added clay adsorbents into the soil. The soil physicochemical properties are given in Supplementary Information, SI 1. The soil was incubated in a dark glass jar for the assessment of the bioavailability of contaminants with an aging effect (70 days). Briefly, soil (30 g) was amended with clay adsorbents at the loading rates of (i) 1% and (ii) 5%, and the mixture was conditioned at 30% of water holding capacity (WHC) of the soil upon agitating on an end-over-end shaker for 5 days. Soil without any clay adsorbent served as the control treatment. After conditioning, Cd (150 mg kg^{-1} soil) as $Cd(NO_3)_2$ (> 99% purity, Chem-supply, Australia) was spiked and mixed for another 24 h. At this stage, the moisture level was increased and maintained at 60% of WHC throughout the incubation period (70 days). Soil sub-samples were withdrawn from the microcosms in triplicate at days 7, 35 and 70 for conducting various analyses as discussed in the following sections.

2.2 Bacterial viability in clay-amended soil

Soil (1 g) was dispersed in a solution (10 mL) of sodium hexametaphosphate (35 g L^{-1}) and sodium carbonate (7 g L^{-1}) by vigorous shaking on an orbital shaker at 300 rpm overnight (Pascaud et al., 2012). The bacterial growth was measured by counting the colony forming units (CFU) on plates containing nutrient agar media after 3-5 days of incubation at 25 °C.

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2.3 Bioavailability of Cd

To measure the bioavailability of Cd in the contaminated soil, a dilute electrolyte (1 mM $Ca(NO_3)_2$) based single extraction procedure was followed (Basta and Gradwohl, 2000; Sarkar et al., 2012). In brief, $Ca(NO_3)_2$ (1 mM prepared in Milli-Q water (resistivity 18.2 MΩ.cm), pH = 6.2) was mixed with the soil (1 g) (soil: extractant = 1:5) in a 10 mL capacity polypropylene centrifuge tube. After gentle shaking on a reciprocal shaker for 24 h at 23 °C, the mixture was centrifuged at 3400 ×g for 20 min, and the clear supernatant was collected into plastic vials. The Cd concentration was measured in the filtered solution (through 0.45 µm nylon membrane, Agilent Australia) using inductively coupled plasma mass spectrometry (ICP-MS) (model: 7500c, Agilent Technologies, USA).

2.4 Concentration of HPCD-extractable PAHs

A non-exhaustive solvent hydroxypropyl-β-cyclodextrin (HPCD) was used as an extractant of the bioavailable fraction of PAHs in the soils. Soil (1 g) was extracted with the following conditions: 1:20 soil: solvent ratio, HPCD (40 mM in Milli-Q water), incubation for 20 h at 25 °C with shaking at 200 rpm (Reid et al., 2000). After centrifugation (3400 ×g for 20 min), the supernatant was decanted completely, and the pellet (soil) was freeze-dried. The residual concentration of PAHs in the pellet following HPCD-extraction (not readily bioavailable) was measured by following an exhaustive extraction procedure using an accelerated solvent extraction system (ASE® 200, Dionex, USA). The ASE® protocol (Application Note 313) was followed (Richter et al., 1994). This met the standard of US EPA method 3545. In brief, soil (1 g) was taken into a stainless-steel ASE® cell and spiked with a surrogate (2,3-benzofluorene) (≥ 98% purity, Sigma-Aldrich, Australia) (1 mg L^{-1} in acetone). Dichloromethane-acetone (1:1 v/v) was used as the extracting solvent. After the automated extraction cycle completed, the extract was collected in a dark vial (20 mL). At this stage, the

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121 extract was stored at 4 °C until all samples were extracted over the entire incubation period.
122 The stored extract was dehydrated with anhydrous Na_2SO_4 and evaporated under a gentle
123 flow of N_2 (solvent evaporator, Dionex SE 500, USA). The residual PAH was then re-
124 dissolved in n-pentane (2 mL) (UniSolv® grade, Merck, Germany) with an internal standard
125 ortho-terphenyl (AccuStandard®, USA) and stored at -20 °C until the gas chromatographic
126 analysis on the next day. Calibrated glass syringes (Agilent Australia) were used in all the
127 handling procedures.

128 A gas chromatography system equipped with flame ionization detector (GC-FID) (Model
129 6890A, Agilent Technologies, USA) was used to measure the PAH concentration. The
130 column (12 m × 0.22 mm ID HT5 0.1 μm) was run in constant flow mode at 2.0 mL min^{-1}.
131 The oven temperature was set at 34 °C for 5 min, raised to 300 °C at ramp 15 °C min^{-1} and
132 held for 5 min. The inlet and detector temperatures were set at 275 °C and 320 °C,
133 respectively. For the detector, hydrogen and airflow were 40 and 450 mL min^{-1}, respectively.
134 Nitrogen (15 mL min^{-1}) and ultrapure helium (60 mL min^{-1}) (13.98 psi) were used as the
135 make-up and carrier gas, respectively. The concentration of PAH was measured against the
136 external standard (16 PAHs, AccuStandard®, USA) with the reference of the internal
137 standard. Using the ASE extraction followed by GC analysis, about 61-91% (mean 77.3%,
138 median 78.2%) recovery of the surrogate was obtained (Supplementary Information, SI 2).
139 The low molecular weight (LMW) PAHs (2-3 benzene rings) and high molecular weight
140 (HMW) PAHs (>3 benzene rings) of 16 PAHs were estimated as the sum of compounds at
141 each category. List of PAHs in each category is provided as Supplementary Information (SI
142 3).

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2.5 Mineralization of LMW PAHs

In a preliminary experiment, the impact of the direct inoculation of a model PAH-degrading bacterium *Mycobacterium gilvum* VF1 on the total microbial respiration was tested (Supplementary Information, SI 4). The growth of *M. gilvum* could be favored by the amendment of clay minerals since this bacterial species could utilize clay fraction more efficiently than sand for their niche building (Uyttebroek et al., 2006). However, in this study, the direct inoculation of *M. gilvum* to the PAH-contaminated aged soil increased the microbial respiration by only 0.8–6% in comparison to the uninoculated control, which was not a significant improvement ($p > 0.05$) (Supplementary Information, SI Figure 3). Therefore, the mineralization of PAHs was tested in the clay-amended soil without any bacterial augmentation. In this case, the native microorganisms played the key biodegradation role unless otherwise any non-biological factor contributed in the process. The possibility of photodegradation was minimized by conducting the experiments in dark conditions.

The biodegradation of LMW PAHs by the native microbial consortia was assessed by tracing the mineralization of a model ^{14}C-labelled compound - phenanthrene. The carbon tracer (^{14}C) (phenanthrene-9-^{14}C, the structure is provided as Supplementary Information, SI 5) is located at the K-region of phenanthrene, which is the most favorable site for oxidative attack by the degrading bacteria (Hadibarata et al., 2009). Unlabelled phenanthrene or any other carbon source was not supplied to the microcosm soil. Therefore, a direct estimation of ^{14}C as $^{14}CO_2$ would indicate the proportional breakdown of phenanthrene and other readily bioavailable LMW PAHs in the contaminated soil. Each microcosm in duplicate was prepared with the clay-mixed soil (2 g soil with 5% loading of clay products, see section 2.1 for the preparation of soil-clay mixture). A partial soil mixing method was followed for spiking the ^{14}C-phenanthrene tracer (Brinch et al., 2002). In brief, ^{14}C-phenanthrene (100 µL) (stock: 20 kBq

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167 mL^{-1}, dissolved in acetone) was spiked into a portion of soil (0.5 g) in a 20 mL amber glass
168 vial. Following the evaporation of acetone, 1.5 g of soil was mixed with the initial 0.5 g soil,
169 which was the equivalent of 2 kBq radioactivity in 2 g soil (equivalent to approx. 0.09 mg
170 phenanthrene kg^{-1} soil). A preliminary experiment showed that the spiked radioactivity was
171 sufficient to trace $^{14}CO_2$ in a β-counter throughout the incubation period (lower detection
172 limit = 25.6 disintegrations per minute).

173 The vial was then placed in a side-armed biometric flask for measuring the respiration
174 (Supplementary Information, SI 6). The incubation period was counted at this stage as "zero
175 day" and the incubation was kept until the cumulative respiration reached a plateau at ~25
176 days. The side arm of the flask was filled with NaOH (0.5 M, 1 mL) for trapping the
177 evolved CO_2. The alkali was collected periodically into a 20 mL Liquid Scintillation
178 Counting (LSC) vial, and 1 mL alkali was further used to wash the bottom of the side arm.
179 The wash-out was also taken into the LSC vial and mixed with 5 mL LSC cocktail (Ultima
180 Gold, Perkin-Elmer, USA). The activity of the mixture was measured in LSC β-counter (Tri-
181 carb liquid scintillation counter, PerkinElmer, USA) following an 8 h dark incubation for
182 removing any chemiluminescence.

183 **2.6 Kinetics of biodegradation**

184 First-order kinetics often fits with the biodegradation of LMW PAHs in soils (Crampon et al.,
185 2014). However, considering the extended lag-phase (see section 3.5) and the microbial
186 viability relative to the clay amendments, we used a "logistic model" following the principle
187 of the "Lag-phase model" (FOCUS, 2006). The following equation (Eq. 1) was applied:

188 $$C = C_0 \left[\frac{a_{max}}{a_{max} - a_0 + a_0 e^{(rt)}}\right]^{\frac{a_{max}}{r}} \quad \text{.....(1)}$$

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where, C is the total amount of PAH (mg kg^{-1}) present at time *t* (day), C_0 the total amount of PAH (mg kg^{-1}) applied at $t = 0$, a_{max} the maximum value of degradation constant (reflecting microbial activity), a_0 initial value of degradation constant, *r* microbial growth rate.

The biodegradation constant (a_{max}) would also lead to obtain the half-life ($DT_{1/2}$) of biodegradation using Eq. 2:

$$DT_{1/2} = \frac{1}{r} ln \left[1 - \frac{a_{max}}{a_0}\left(1 - 2^{r/a_{max}}\right)\right] \quad \text{.....(2)}$$

2.8 Graphical presentation and statistical analysis

All graphical presentations were produced by using Microsoft® Excel® 2013. The statistical analyses of the effect of treatments on bacterial viability, Cd adsorption, and bioavailability and biodegradation of PAHs were performed using IBM SPSS Statistics 20 software package (IBM Corporation, USA). The following analyses were obtained: Analysis of variance (ANOVA), and posthoc analysis with Duncan's multiple range test at 95% confidence level ($p < 0.05$).

3. Results and discussion

3.1 Bacterial viability in clay amended long-term contaminated soil

The addition of Arquad®-palmitic acid treated bentonite (ABP) significantly enhanced the bacterial viability in the PAH-contaminated soil (Figure 1). Among the three sampling occasions in the unamended control soil, the highest growth of bacteria was found at day 35 ($1.6\times10^6 \pm 4.6\times10^5$ CFU g^{-1}). The growth curve followed a typical 'growth and decline' pattern at the end of 70 days incubation. The addition of 1% of raw bentonite (B) and

Arquad®-treated organoclay (AB) did not significantly change the bacterial growth at day 35 ($1.4\times10^6 \pm 0.5\times10^2$ and $1.9\times10^6 \pm 4.1\times10^5$ CFU g^{-1} in the case of B and AB, respectively). However, a similar loading (1%) of ABP increased the bacterial count by more than two folds ($3.5\times10^6 \pm 2.0\times10^5$ CFU g^{-1}), which was significantly higher than the no clay control (NC) and other clay treatments (B and AB) ($p < 0.05$). A higher loading (5%) of the organobentonite (AB) rather reduced the bacterial growth at day 35, due to the potential toxic effects of the surfactant on bacterial cells (Sarkar et al., 2013; Ugochukwu et al., 2014). However, the surface-tailored organobentonite (ABP) became highly biocompatible and growth-inducer when applied at the higher loading rate (5%). The ABP treatment showed $1.0\times10^7 \pm 3.0\times10^6$ CFU g^{-1}, which was as much as six, five and seven-folds higher than that in the control (NC), B and AB treatments, respectively (Figure 1).

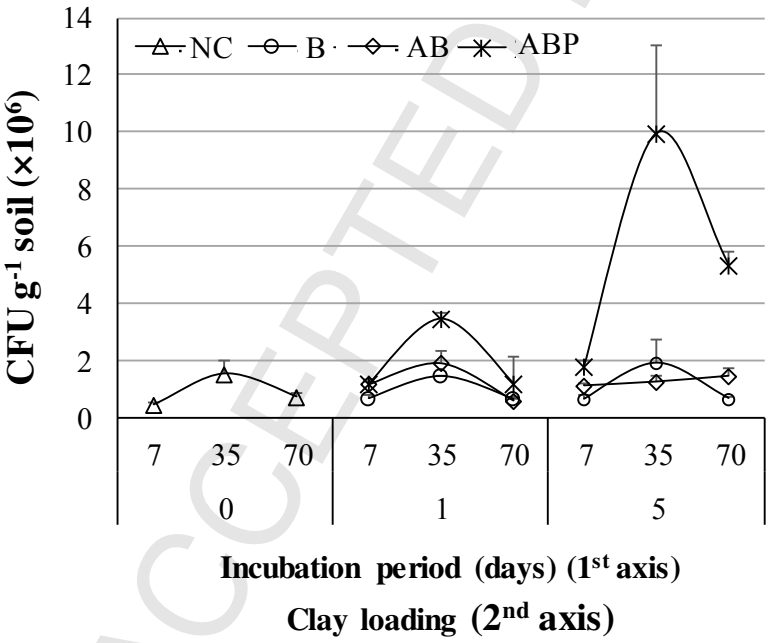

Figure 1. Bacterial growth in clay-amended long-term PAH-contaminated soils. Bar represents the standard deviation of mean; n = 3.

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The clay amendments could increase the microbial growth as reported earlier under other soil conditions such as uncontaminated alkaline soil (Froehner et al., 2009). However, in the current study, the native bacteria in the contaminated soil did not proliferate well over the incubation period when amended with the raw bentonite (Figure 1). Cébron et al. (2015) also reported that the microbial density might not increase significantly due to clay addition in a contaminated aged soil which would have a high toxic load of complex PAHs. It was, however, promising that the surface-tailored organoclay (ABP) provided a congenial microenvironment in the low clay-content experimental soil used in this study, and thus demonstrated a significantly higher bacterial count (Figure 1). Along with the density of potential PAH-degrading bacteria, their community structure is also an important factor that would control the long-term effect of any clay addition to the contaminated soil (Cébron et al., 2015). Therefore, a concurrent microbial diversity study in the clay-amended soils should be conducted in the future. Nevertheless, the higher microbial growth obtained in the current study might lead to an enhanced biodegradation of PAHs in the contaminated soil.

3.3 Bioavailability of Cd in contaminated soil

The bioavailability of Cd in the PAH-contaminated soil was very low (1.0 – 1.6 mg kg^{-1} soil). However, this small bioavailable fraction of Cd in the soil solution could still be harmful to the microorganisms (Olaniran et al., 2013; Vig et al., 2003). It should also be noted that the available chemical extraction-based techniques for the bioavailability assessment of metals in soil microbial toxicity studies might remain largely variable (Giller et al., 1998; Smolders et al., 2009). In the current study, a 1 mM $Ca(NO_3)_2$ solution (pH = 6.2) was used for extracting Cd, which might separate only the soil solution fraction of Cd and thus simulate the Cd that is readily available to the soil microorganisms (Basta and Gradwohl, 2000). With the progress

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of incubation, the bioavailability of Cd slightly increased in the control soil (1.6 ± 0.04 mg kg^{-1} at day 70 against 1.0 ± 0.1 mg kg^{-1} at day 7). Without any clay amendment, a similar trend was reported by Houben et al. (2013) who observed an increase in Cd release in the solution phase of a sandy soil with the progression of incubation time. However, the addition of clay products significantly altered the bioavailability pattern of Cd in the PAH-contaminated soil in the current study (Figure 2). A low loading (1%) of raw bentonite (B) and the surface-tailored organoclay (ABP) reduced the Cd bioavailability significantly ($p < 0.05$). In this condition, at day 35, Cd was detected as low as 1.09 ± 0.02 mg kg^{-1} in bentonite-amended soil and 1.18 ± 0.03 mg kg^{-1} in ABP-amended soil against 1.32 ± 0.01 mg kg^{-1} in the control soil and 1.31 ± 0.03 mg kg^{-1} in the AB-amended soil. This trend prevailed at day 70 also (Figure 2). At day 70, the available fraction of Cd was 0.72 ± 0.11 mg kg^{-1} in bentonite-amended soil and 0.74 ± 0.07 mg kg^{-1} in ABP-amended soil against 1.61 ± 0.04 mg kg^{-1} in control soil and 1.0 ± 0.06 mg kg^{-1} in AB-amended soil, which clearly indicated a greater amount of Cd immobilization by the raw and surface-tailored bentonite. The 5% loading of B and ABP further reduced the Cd concentration in the soil solution over the entire incubation period (0.64 – 1.1 mg kg^{-1} in B-amended soil, and 0.8 – 1.1 mg kg^{-1} in ABP-amended soil). The Arquad®-clay (AB) also showed a strong adsorption affinity to Cd at the 5% loading rate of this material. A small amount of Cd (0.4 – 0.5 mg kg^{-1}) was released from the AB-amended soil over the 70-day incubation period (Figure 2). It has been reported however, that Cd adsorption by AB in aqueous suspension was poor (Biswas et al., 2016). Due to the double alkyl chain of Arquad® and the increased positive charge on AB surface, this organoclay could adsorb anionic contaminants such as oxyanionic form of arsenic in soil (Sarkar et al., 2012). The increased positive charge might repel cationic Cd and reduce adsorption. However, in the current study, Cd was spiked to the PAH-contaminated sandy soil where the speciation of Cd could be affected both by the soil properties and clay

amendments (Meers et al., 2005). Only the higher loading rate of AB (5%) immobilized more Cd than B and ABP in the soil. It indicated that the organic surfactant contained in the organoclay might play an important role on Cd adsorption and speciation specially when exposed to PAH-contaminated soil. This seeks a further investigation. However, since the viability of microorganisms is important for enhancing PAH biodegradation, the Arquad-organoclay (AB) showed significant toxic effects (Figure 1), which eventually ruled out the importance of a higher Cd immobilization by this material. In contrast, although the raw bentonite and the surface-tailored organobentonite (ABP) adsorbed similar amounts of Cd over the 70-day aging of the soil (Figure 2), a highly supportive microbial environment was created only in the case of ABP-amended soil (Figure 1) (Biswas et al., 2015; Mandal et al., 2016). The ABP showed specific adsorption of Cd in the PAH-Cd mixed contaminated system, which could maintain a greater fraction of PAHs in bioavailable forms in comparison to the raw bentonite (Biswas et al., 2016).

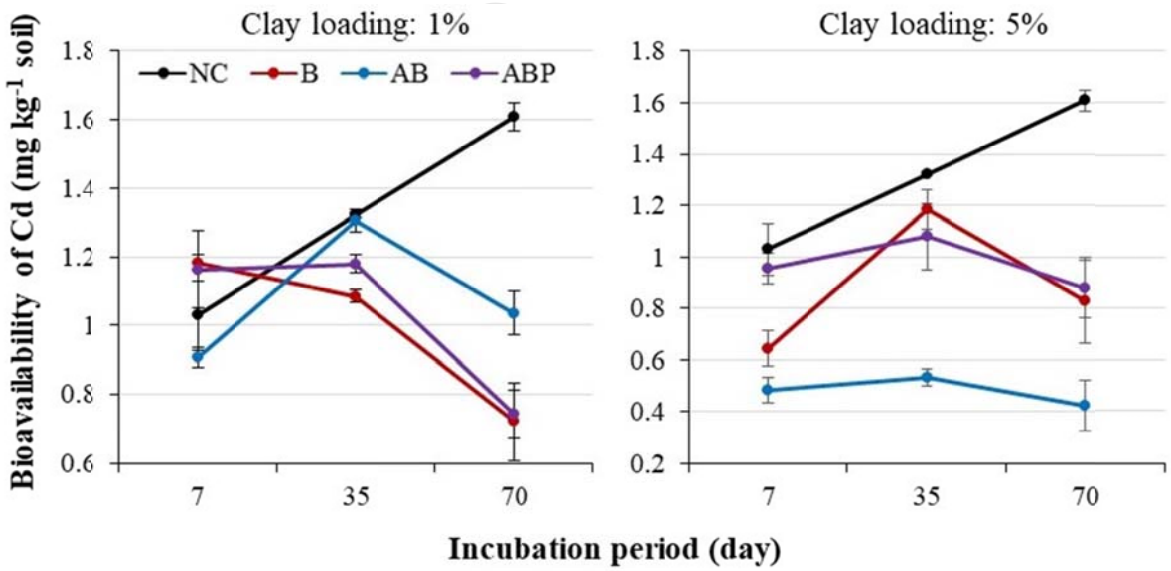

Figure 2. Cd bioavailability in clay-amended PAH-contaminated soils. Bars represent the standard deviation of the mean; n = 3.

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3.4 Concentration of bioavailable PAHs

The biodegradation of PAHs strongly depends on the bioavailability of the compounds in the soil (Crampon et al., 2014; Juhasz et al., 2014). A large fraction of PAHs might remain unavailable to the PAH-degrading microorganisms by entering into the soil pores or by making strong chemical bonds with various soil components. This could potentially constraint the complete removal of PAHs from a contaminated soil. Several methods are available to assess the microbial availability of PAHs in soils, but none of them can fully correlate with the biodegradation potential of the contaminants (Swindell and Reid, 2006). The HPCD extraction is preferentially used for simulating the readily bioavailable fraction of PAHs in soils and sediments (Juhasz et al., 2014; Reid et al., 2000). In this study, the PAH concentration in the pellet (residual fraction) after HPCD extraction was also extracted using exhaustive solvents (section 2.4). The residual fraction of PAHs following the HPCD extraction is not supposed to be available to the functional microorganisms (Reid et al., 2000). However, the residual apparently unavailable fraction could be brought into the bioavailable fraction by amending the soil with the surface-tailored organoclay (Biswas et al., 2016). The organoclay product (ABP) was proven to be a biocompatible material to the native soil microorganisms (Biswas et al., 2015) and a PAH-degrading bacterium *M. gilvum* (Mandal et al., 2016). Also, a higher bioavailability of phenanthrene (an LMW PAH) was observed in the Cd-phenanthrene mixed-contaminated situation (Biswas et al., 2015; Mandal et al., 2016). In the current study also, the ABP showed prominent influences on the amount of readily bioavailable and residual fraction of PAHs in the long-term contaminated soil (Figure 4).

Both the organoclays (AB and ABP at 1% loading) showed a lesser bioavailability of LMW PAHs (HPCD extractable) than the control treatment and raw bentonite (B) at the beginning

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of the incubation (day 7) (Figure 3). However, these differences were not statistically significant ($p > 0.05$) (Figure 3). With the progress of aging (incubation), the organoclays released the LMW PAHs back into the bioavailable phase, and thus resulted in a lesser residual amount (dichloromethane-acetone extractable; ASE method). However, this trend was not observed in the case of control (NC) and bentonite-amended soils (at day 70: ABP 289 ± 53 mg kg^{-1}, compared to AB 313 ± 66 mg kg^{-1}, B 308 ± 31 mg kg^{-1} and NC 350 ± 4 mg kg^{-1} soil). The surface-tailored organoclay (ABP) even significantly increased the bioavailable fraction of LMW PAHs ($p < 0.05$) when applied to the soil at a higher loading rate (5%) (Figure 3). This was indicated by the smaller residual fraction of PAHs following HPCD extraction at the end of 70 days (ABP 218 ± 71 mg kg^{-1}, AB 247 ± 6.0 mg kg^{-1}, and B 404 ± 101 mg kg^{-1} soil). After the 70 days long incubation, the influence of AB on the bioavailability of LMW PAHs was also prominent because the rate of PAH desorption could increase over the aging period (Crocker et al., 1995). However, ABP would be most preferred for a practical bioremediation application because it was able to reduce the Cd toxicity by arresting the metal cations on the fatty acid functional groups which were grafted in the interlayers of bentonite (Biswas et al., 2016). The engineered material simultaneously held the LMW PAHs (e.g., phenanthrene) on the outer surfaces, but due to an weak interaction force the compounds could release back into the soil solution and become available to the microorganisms (Biswas et al., 2016).

In contrast to LMW PAHs, the HMW PAHs showed a different pattern in their residual contents (dichloromethane-acetone extractable) (Figure 3). HMW PAHs could be sequestrated strongly within the soil particles and humin because of their relatively higher aromaticity and lower polarity (Northcott and Jones, 2001). The raw bentonite-amended soil (1% loading) showed a lesser extractability of HMW PAHs by the exhaustive solvents than the control soil (NC), AB and ABP (Supplementary Information, SI Figure 6). Therefore, it

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339 could be an underestimation of HMW PAHs in the residual fraction (loading 1%, Figure 3)
340 due to the poor extractability of these compounds. However, with the increase of loading
341 rates (5%), the extractability improved (Supplementary Information, SI Figure 6). In this
342 case, the modified clay product (ABP) also reduced the amount of HMW PAHs in the
343 residual fraction at the end of 70 days by 28.7% (Figure 3). As expected, the HMW PAHs
344 were more reserved in the soil matrices than LMW PAHs. In biodegradation, the available
345 LMW PAHs are preferentially utilized by the microorganisms (Haritash and Kaushik, 2009).
346 Therefore, a direct tracing of these LMW PAHs at the time of biodegradation could mimic
347 the efficiency of the microbial degradation of the contaminants among different treatments.
348 On the other hand, the fate of HMW PAHs mainly depends on the preferential biodegradation
349 of readily available LMW PAHs (Desai et al., 2008). However, the complexity in the field-
350 contaminated soil may not mimic a direct relation between the degradation kinetics of LMW
351 and HMW PAHs (Desai et al., 2008), which would need a further investigation.

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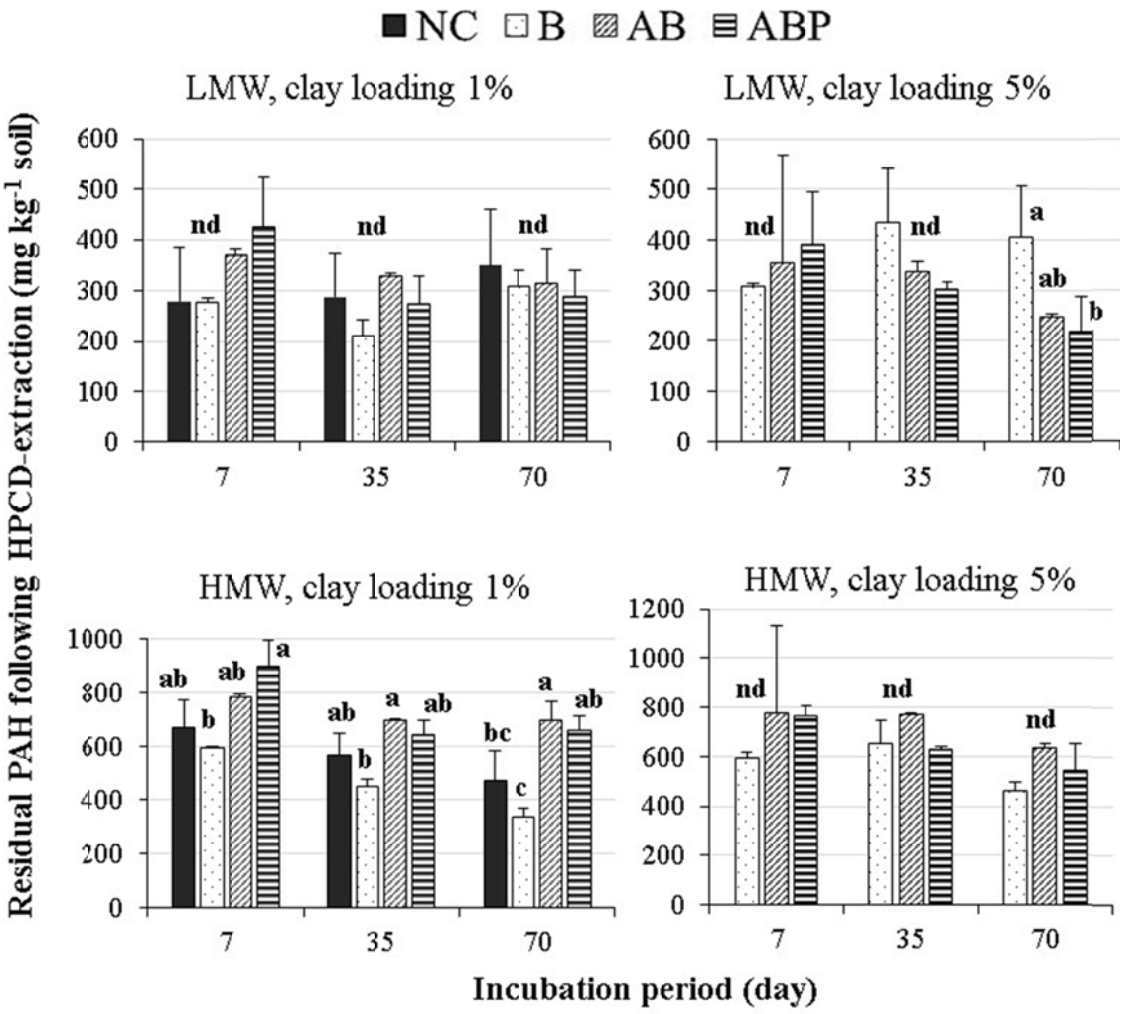

352

353 Figure 3. HPCD-extractable fractions of PAHs in long-term PAH-contaminated soil amended
354 with raw and modified clays. LMW = Low molecular weight; HMW = High molecular
355 weight. PAHs represent the sum of either LMW or HMW PAHs. List of PAHs in each
356 category is provided as Supplementary Information (SI 3). The bars represent the standard
357 deviation of mean; n = 3. Different alphabets indicate the significant difference at $p < 0.05$
358 measured by Duncan's test.

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3.5 Mineralization of ^{14}C-labelled phenanthrene

Employing the native soil microbial community, the biodegradation of model LMW PAH (^{14}C-phenanthrene) was enhanced significantly ($p < 0.05$) by the addition of the surface-tailored organoclay (ABP) (Figure 4). At the end of 25 days of incubation, ABP showed 53.4 ± 5.1% mineralization against 40.11 ± 3.2% and 35.1 ± 2.3% in the case of control treatment (NC) and AB amended soil, respectively. The raw bentonite (B) had also shown a similar effect on phenanthrene mineralization (58.7 ± 2.1%), which was not significantly different ($p > 0.05$) from that of the ABP (Figure 4).

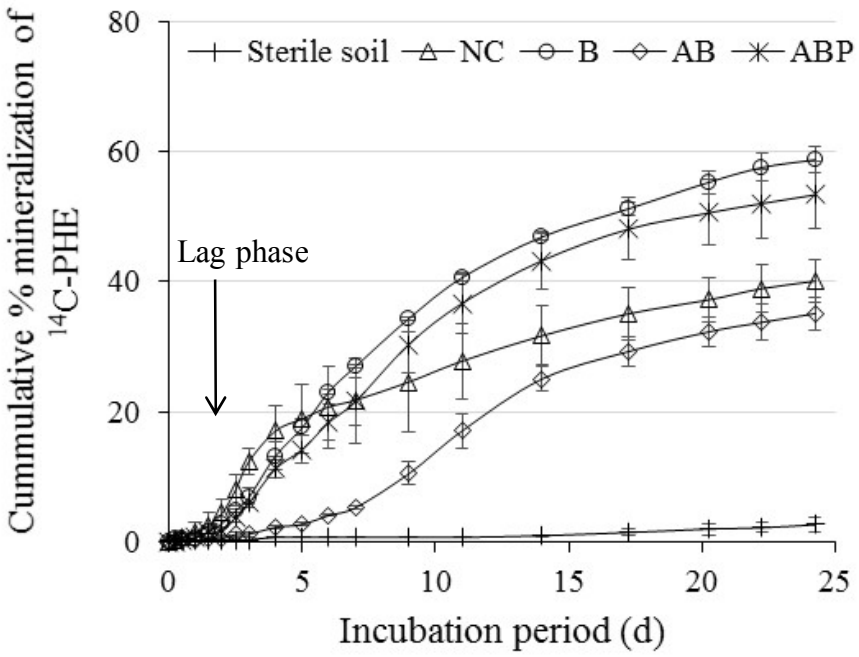

Figure 4. Mineralization of ^{14}C-phenanthrene in the field PAH-contaminated soil amended with raw and modified clays. Bars represent the standard deviation of mean at each sampling point; n = 3.

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373 The 25-day long experiment also showed a kinetically different phenanthrene mineralization
374 patterns among the treatments (Table 1). Both the organoclays (AB and ABP) showed a slow
375 mineralization rate by the native microbial consortia initially (day 4, Figure 4 and 5)
376 compared to the control (NC) and raw bentonite treatment. The heterogeneous surfaces of
377 clays and soil matrices could lead to a temporal disturbance in the utilization of the target
378 compound by the microorganisms (Semenov et al., 2008). As a result, a noticeable oscillation
379 of the microbial activity was observed in the initial stages (day 0–10) (Figure 5). In the
380 overall incubation period, the control soil did not increase the biodegradation rate (very small
381 a_{max} value) likely due to the early death phase of the degrading microorganisms (Figure 5).
382 Similarly, AB too reduced the biodegradation rate as indicated by a small a_{max} value of 0.02 ±
383 0.002 day^{-1}. The toxicity of AB to soil microorganisms persisted for a longer time (Sarkar et
384 al., 2013) (Figure 4 and 5). However, the surface-tailored organoclay (ABP) enhanced the
385 microbial activity by enhancing the respiration rate significantly (Figure 4). The maximum
386 degradation constant (a_{max}) was calculated as 0.04 ± 0.007 day^{-1} for ABP, which was similar
387 to B (0.04 ± 0.002 day^{-1}). The lag-phase model well fitted to the degradation data for all
388 samples with the R^2 value of 0.98-0.99 (Table 1). Although the raw clay (B) and modified
389 organoclay (ABP) were not significantly different in their phenanthrene biodegradation rates,
390 the pattern of mineralization indicated that the biocompatible organoclay (ABP) might have a
391 longer positive influence on the microbial growth with the enhanced bioavailability of PAHs
392 (Figure 1 and Figure 3). The ^{14}C-phenanthrene mineralization results thus supported that the
393 raw bentonite (B) produced a little higher bioavailable fraction of PAHs (see day 7, Figure 3)
394 at the beginning of the incubation. However, with the progress of incubation, ABP released
395 more bioavailable fraction of PAHs.

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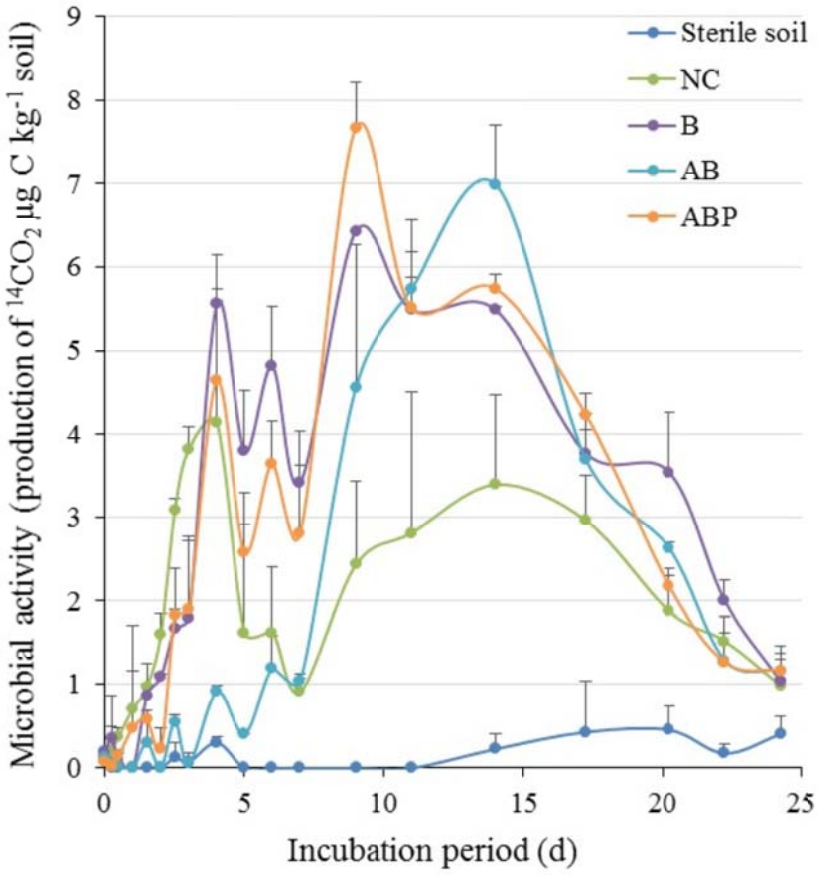

Figure 5. Microbial activity pattern during the mineralization of ^{14}C-phenanthrene in the field PAH-contaminated soil amended with raw and modified clays. Bars represent the standard deviation of mean at each sampling point. For more clarity, see the color figure in the Supplementary Information, SI8.

Table 1. Model fitting parameters for the mineralization of ^{14}C-phenanthrene in the field PAH-contaminated soil amended with raw and modified clays

	a_{max}	$DT_{1/2}$*	R^2	RMSE**
NC	0.00 ± 0.000	41.05 ± 5.49	0.98 ± 0.00	1.77 ± 0.30
B	0.04 ± 0.002	16.64 ± 0.63	0.98 ± 0.01	2.45 ± 0.38
AB	0.02 ± 0.002	33.50 ± 2.91	0.99 ± 0.00	1.38 ± 0.19
ABP	0.04 ± 0.007	19.27 ± 2.95	0.98 ± 0.00	2.30 ± 0.36

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404 * Half-life (Half Dissipation Time); ** Root Mean Square Error

405 **4. Conclusions**

406 In a sandy field soil contaminated with PAHs, the amendment using the surface-tailored
407 organoclay (ABP) increased the bacterial growth significantly more than the parent bentonite
408 and unamended control treatments ($p < 0.05$). A greater fraction of bioavailable PAHs was
409 also maintained in the ABP-amended soil over a 70-day long incubation period. The
410 mineralization of readily available LMW PAHs was significantly higher in the ABP-amended
411 soil than in the control soil. However, the biodegradation efficiency was not as high as the
412 bacterial growth rate between the raw bentonite and modified organoclay-amended soil. This
413 raised a future research question whether microbial functional variability would play a role in
414 the degradation process. Future research is therefore recommended to study (a) the molecular
415 level functional variability of microorganisms in the clay-amended soil, and (b) the
416 improvement of biocompatibility to microorganisms and biodegradation of PAHs by the
417 surface-tailored organoclay under diverse soil environmental conditions.

418

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425 **Supplementary Information**

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426 The supplementary information is available online, which can be obtained via the provided
427 link of this journal.

428 **Conflict of interest**

429 The authors declare that they do not have any conflict of interest.

430 **Ethical approval**

431 This article does not contain any studies with human participants or animals performed by
432 any of the authors.

433

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Supplementary Information

The supplementary information is available online for (i) soil physicochemical properties, (ii) quality control (recovery of PAH during extraction), (iii) ^{14}C-phenanthrene respiration microcosm experimental set-up and microbial growth pattern, and (iv) list of 16 PAHs and the total extractability of them.

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Highlights

- A modified organoclay facilitated native bacterial growth in PAH-contaminated field soil
- It immobilized metals but increased the readily available (bioavailable) fraction of PAHs
- Degradation kinetics with logistic model indicated an extended lag phase in microbial activity
- The ^{14}C-tracer indicated the faster biodegradation of low molecular PAHs from soil

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