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ORIGINAL ARTICLE

Effect of carbonyl cyanide m-chlorophenylhydrazone (CCCP) on microbial activity and polycyclic aromatic hydrocarbons (PAH) degradation in contaminated river sediments

G. Patricia Johnston[1] · Z. Kalik[1,2] · C. G. Johnston[1]

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Abstract Polycyclic aromatic hydrocarbons (PAHs) are of continued interest because of their carcinogenic nature and persistence in the environment. Among volatilization, sorption, and chemical oxidation, microbial degradation is the main path of PAHs disappearance. The majority of degradation studies have used pure cultures, were spiked with added PAHs, or have been performed using single pollutants. Because contaminated aquatic ecosystems retain mixtures of xenobiotics, experiments using environmental samples that incorporate the role of microbes provide more realistic conditions to study degradation. We tested the inhibitory effects of [carbonyl cyanide m-chlorophenylhydrazone (CCCP)] on microbial respiration, which in turn altered PAH degradation. In this study, 3.5 mM of CCCP (two orders of magnitude higher to what was needed to inhibit growth of *E. coli* in pure culture) was shown to inhibit respiration of indigenous microbes in complex sediment samples by 79 % at 25 °C and 64 % at 37 °C. After 15 days of incubation sediment slurries without CCCP showed higher PAH degradation rates (between 60 and 90 %) compared to sediment slurries with CCCP, which showed much lower degradation rates (<40 %). This study also reported the highest recorded rates of INT reduction (29 nkat/g) due to both short incubation times and the potential to stimulate high microbial activity in the sediments. These data indicated the effectiveness of CCCP as a microbial respiratory inhibitor in sediments, and that indigenous microbes in long-term PAH contaminated anaerobic sediments can be stimulated to degrade PAHs present in their environment.

Keywords Microbial activity · Dehydrogenases · CCCP · PAHs · INT

✉ C. G. Johnston
cgjohnston@ysu.edu

G. Patricia Johnston
gpjohnston@ysu.edu

Z. Kalik
kalik.z@live.sgul.ac.cy

[1] Department of Biological Sciences, Youngstown State University, One University Plaza, Youngstown, OH 44555, USA

[2] St. George's Hospital Medical School, University of London, London SW17 0RE, UK

Introduction

PAHs are toxic and carcinogenic; hence research on their environmental fate and remediation is of current interest. PAHs constitute a large, diverse class of persistent hydrophobic organic compounds that have a high affinity for organic matter and tend to adsorb to sediments (Abbondanzi et al. 2005). The use of microorganisms in mineralization, transformation and/or immobilization of pollutants have shown to be a useful, economic, and advantageous strategy in the bioremediation arena (Lovley 2003; Wackett 2000; Wackett and Bruce 2000; Watanabe 2001).

Microbial degradation is considered the major process involved in PAH disappearance (Chang et al. 2005, 2008; Tian et al. 2008; Kim et al. 2008). Much PAH degradation takes place in the presence of oxygen, yet there is strong evidence of anaerobic degradation of PAHs in sediments (Meckenstock et al. 2004; Ma et al. 2011; Kleemann and Meckenstock 2011). Although most known PAH-degrading bacteria can degrade low molecular weight (LMW)

compounds, a limited number of isolates can grow on high molecular weight PAHs (Johnsen et al. 2005).

The majority of PAH degradation studies have been performed either using pure cultures or spiked sediments with individual PAHs (Chang et al. 2008; Ambrosoli et al. 2005). There are only a handful of studies that showed degradation of mixtures of PAHs (Lei et al. 2005; Wang and Tam 2012). In fact, in most contaminated aquatic environments PAHs interact with sediment, metals, and humics, presenting a much more intricate system than using pure cultures to study degradation (Luu and Ramsay 2003). Thus, experimentation measuring PAH degradation in complex environmental samples with their indigenous microbial communities is needed and could provide fundamental information for potential application in bioremediation.

To demonstrate PAH degradation by the indigenous microbial communities, we decided to use a non-conventional metabolic inhibitor of microbial respiration, which would thus inhibit microbial biodegradation dependent on respiration. Carbonyl cyanide m-chlorophenylhydrazone (CCCP) is a membrane-based efflux pump (EP) inhibitor that affects all proton motor force-dependent transporters in bacterial cells (Couto et al. 2008), and would therefore inhibit both aerobic and anaerobic respiration (though not fermentation). CCCP has been used in antimicrobial chemotherapy trials specifically as inhibitor of multidrug resistance studies using *Klebsiella pneumonia* (Srinivasan et al. 2014) as well as an inhibitor of mitochondrial respiratory chain in *S. cerevisiae* (Ye et al. 2015). CCCP was also used as an EP inhibitor for *Pseudomonas aeruginosa* in biofilms associated with cystic fibrosis in human lungs (Ikonomidis et al. 2007), for *Mycobacterium smegmatis* (Jin et al. 2010), and for other bacterial pathogens in human and veterinary medicine (Lechner et al. 2008; Rodrigues et al. 2008; Ramón-García et al. 2006). To our knowledge, CCCP has not been used as an inhibitor for microbial respiration in complex environmental samples (i.e., sediments).

Microbial respiration measured by tetrazolium salts [e.g., 2-(p-iodophenyl)-3(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT)], is a valuable tool to estimate rates of biogeochemical cycling and to establish links between the surrounding chemical environment and microbial communities inhabiting sediments (Mermillod-Blondin et al. 2005). It also provides a fast, simple, inexpensive, and repeatable estimation of the metabolic state of microbes (Coello et al. 2010; Dinamarca et al. 2007; Bensaid et al. 2000). Additionally, INT-activity can reflect the potential of bioremediation practices in contaminated sediments. Kaimi et al. (2006) indicated that numbers of aerobic bacteria and soil dehydrogenase activity were higher in the rhizosphere where dissipation rates of diesel oil were also higher. Mosher et al. (2006) and Pratt et al. (2012) reported higher microbial activity in contaminated sediments with high concentrations of PAHs and metals than in relatively uncontaminated sediment, suggesting that perhaps microbes from contaminated sediments had degradative capabilities.

Methods for assessing microbial metabolism and potential capabilities for remediation are needed for effective planning and monitoring. Thus, the first objective of this study was to determine if CCCP could inhibit microbial respiration in complex polluted sediments with PAHs. To do this, conditions were optimized to allow high levels of microbial respiration in sediment slurry incubations (initial aerobic conditions, two relatively high temperatures). The second objective was to determine if PAH degradation was associated with microbial respiration.

Materials and methods

Experimental setup and sampling

Sediments were collected from the riverbanks of the Mahoning River (Northeastern Ohio, USA) at ~1.2 m depth using stainless steel tubes (15 cm long, 5 cm in diameter). Sediments were placed on ice and transferred to the laboratory for analyses. Homogenized sediment was used to make sediment slurries consisting of 1 g of sediment and 5 mL of ultrapure (18.2 MΩ cm) 0.22 µm filtered and autoclaved water. CCCP (Sigma-Aldrich St. Louis, Missouri) was dissolved in acetone using a sonicator at low frequency (Ultrasonics Cleaner, VWR, West Chelsea, Pennsylvania). Previous assays showed that acetone did not interfere with microbial respiration measurements by spectrophotometry (data not shown). 100 µL of acetone containing CCCP was added to the slurries (5.5 mL) to a final concentration of 3.5 mM. Sediment slurries were prepared at room temperature on the bench under normal aerobic conditions (no anaerobic chamber or glove bags). These initial conditions introduced oxygen into the system and mimicked a potential strategy for enhancing both microbial activity and bioremediation. However, 40 mL vials with airtight septa were used to minimize the loss of volatile compounds released from degradation, and also allowed anaerobic conditions to develop over time. Sediment slurries were incubated in the dark at two temperatures (25 °C and 37 °C) with and without CCCP in a shaker at 200 rpm for 15 days (Table 1). Sacrificial 40 mL vials (per triplicate) were set up independently for 1) measurement of microbial activity and 2) PAH extraction at day 0, 7, and 15.

Table 1 Experimental conditions used in this study

Experimental conditions	Sediment (g)	Temp. (°C)	CCCP (mM)	No. reps	Days of analyses	No. sacrificial incubations for PAH extraction	No. sacrificial incubations for microbial activity
Sed 25	1	25	–	3	0, 7, 15	9	9
Sed 25-CCCP	1	25	3.5	3	0, 7, 15	9	9
Sed 37	1	37	–	3	0, 7, 15	9	9
Sed 37-CCCP	1	37	3.5	3	0, 7, 15	9	9

Microbial respiration

Microbial respiration was determined by measuring dehydrogenase activity using INT (Sigma-Aldrich St. Louis, Missouri) on day 0, 7, and 15. On each sampling date triplicate subsamples (0.6 mL) from the sediment slurries were pre-incubated for 1 h prior to adding 0.5 mL of 1.08 mM INT. To measure initial respiration (0 min), respiration "killed" controls were prepared by adding 3.0 mL acetonitrile before addition of INT to allow no time for microbial respiration and to account for any background/chemical INT reduction as indicated by Mosher et al. (2003). For measurements at different times (after 3, 6, and 9 min) respiration was terminated by adding 3.0 mL acetonitrile after the addition of INT. It has been reported that reduction of tetrazolium dyes in the absence of cells is possible, but unlikely to contribute to measured absorbances from samples (Wuertz et al. 1998). No-sediment control vials (0.22-μm ultrapure-filtered autoclaved water) were included to check for background absorbances of solvents (none detected). Absorbance of the reduced red-colored formazan product (INTF) was measured at 490 nm (BioMate 5, Thermo Scientific, Waltham, Massachusetts). Rates of respiration were calculated as indicated by Mosher et al. (2003) but were expressed in nkat/g. One kat represents the enzymatic activity necessary to catalyze 1 mol substrate per second to product.

PAH extraction

PAHs were extracted from sacrificial vials at each sampling point per triplicate. There was no subsampling of any kind. The extraction was performed in the incubation vial following a modified extraction method for organic pollutants and microbial lipids based on the Bligh and Dyer method as indicated by Mosher et al. (2006) and Pratt et al. (2012). Dichloromethane (DCM), methanol and 50 mM phosphate buffer (1:2:0.8) were added to slurries along with 50 μL of surrogate and placed at 4 °C for 24 h. After 24 h., DCM and water were added (1:1:0.9) and placed again at 4 °C for 24 h. The next day the organic phase was transferred to a sodium sulfate column. The organic eluate was collected into a graduated glass conical tube. Samples were concentrated to 1 mL under a flow of nitrogen at 37 °C before quantification.

Gas chromatography–mass spectrometry (GC–MS)

To quantify PAHs disappearance over time, GC–MS analyses were performed on a Saturn 2000 (Varian-Agilent, Wilmington, Delaware). The chromatographic column was a 30 m, 0.25 mm inner diameter, 0.25 μm film thickness HP-5MS capillary column (#19091 s-433, Agilent Technologies, Santa Clara, California). Helium was used as the carrier gas at a constant flow rate of 1.2 mL per min. The injector port was held at 275 °C, with an initial oven temperature of 50 °C. The oven was held at 50 °C for 5 min, and then ramped at 10 °C/min to a final temperature of 315 °C, and then held for 2.44 min. The MS was operated in the electron impact mode at 70 eV. The ion source temperature was held at 275 °C. The mass spectra were recorded at a scan mode covering the range of 60–650 mass units for sediment samples. One microliter injection of each sample extract was made in splitless mode using an A200S autosampler (IET, Vernon Hills, Illinois). Quantification was performed using internal standards (as described below). Retention time and the identity of PAHs were confirmed using PAH standards.

Chemical standards used during analyses and PAH extraction

Surrogate standards, 2-fluorobiphenyl, nitrobenzene-d5, and terphenyl-d14, were purchased as a certified high concentration mix (1 mg/mL) from Restek (Bellefonte, Pennsylvania). A certified high concentration mixture (2 mg/mL) of deuterated PAHs (acenaphthene-d10, chrysene-d12, 1,4-dichlorobenzene-d4, naphthalene-d8, perylene-d12, phenanthrene-d10), also purchased from Restek (Bellefonte, Pennsylvania) was used as internal standard. All solvents used for sample extraction and analyses (dichloromethane, acetone, methanol) were Optima grade (Fisher Scientific, Pittsburgh, Pennsylvania).

Sediment properties

Standard methods (APHA 1999) were performed to measure moisture content, organic matter, fixed solids, total solids and volatile solids from sediments used in the slurries. Particle size distribution was determined by using a hydrometer (Fisher Scientific, Pittsburgh, Pennsylvania).

Statistical analyses

One-factor multivariate analysis of variance (MANOVA) was used to determine if there were significant differences among sampling dates, experimental conditions, and the interaction between sampling date and experiment conditions. Pearson correlations were also calculated to establish possible associations between temperature, microbial activity, CCCP and individual PAHs. Statistical analyses were performed using IBM SPSS Statistics 20 for Windows (IBM Corp., Armonk, New York).

Results

Sediments were characterized for organic matter content, moisture content, total solids, fixed solids, and volatiles solids (Table 2). Organic matter content (17 %) was much higher compared to other contaminated sediments elsewhere (Guo et al. 2007), while moisture content (57 %) was similar to previous values reported for these sediments (Mosher et al. 2006; Johnston et al. 2015; Johnston and Leff 2015). Sediments used in the slurry experiment were consistently oily and mostly characterized by silt (56 %) as determined by the particle size distribution analyses (Table 2).

Microbial respiration

Microbial respiration (dehydrogenase activity) was determined by measuring reduction of INT in sediment slurries. Dehydrogenase activity was measured on day 0 after 1 h of initial exposure to temperature, CCCP or both. As shown in Table 3, the highest dehydrogenase activity (29 nkat/g) in samples without CCCP was measured on day 0 when sediments were exposed to 37 °C. At 25 °C microbial activity in samples without CCCP was approximately 50 % of the activity recorded at 37 °C. Furthermore, in the presence of CCCP the lowest initial value of dehydrogenase activity (1.4 nkat/g) was measured in sediments at 25 °C. Surprisingly, dehydrogenase activity in sediments at 37 °C with CCCP was comparable to sediments without CCCP at 25 °C on day 0. After 7 days of incubation, dehydrogenase activity was greatly reduced compared to initial measurements across all experimental conditions (ranging from 0.2 to 0.8 nkat/g). On day 15, dehydrogenase activity was much lowered for most sediments (<0.1 nkat/g), except for sediments without CCCP at 25 °C and 37 °C (~0.1 nkat/g) which remained higher than slurries with CCCP at both temperatures. Multivariate analysis of variance showed that microbial activity was statistically significant among sampling dates (Wilks' Lambda = 0.018, $F = 25.763$, $p = 0.000$), between experimental treatments (CCCP + temperature) (Wilks' Lambda = 0.057, $F = 6.782$, $p = 0.000$) and for the interaction between sampling day and experimental treatments (day × treatment) (Wilks' Lambda = 0.013, $F = 5.324$, $p = 0.000$). In addition, microbial activity highly correlated with individual PAHs (Table 4).

Table 2 Characteristics of sediment used in this study (average and standard deviation in brackets, $n = 4$)

Sediment characteristic	
Total solids (%)	43.6 (8.9)
Volatile solids (%)	16.8 (6.1)
Fixed solids (%)	83.2 (6.1)
Organic matter (%)	16.8 (6.1)
Moisture content (%)	56.4 (8.9)
% Sand	39 (1)
% Silt	56 (2)
% Clay	5 (1)

PAH degradation

To better characterize PAH degradation, PAHs were classified according to the number of benzene rings (BR) as follows: naphthalene, acenaphthylene, acenaphthene, and fluorene (2BR), phenanthrene, anthracene and fluoranthene (3BR), pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene (4BR), benzo[a]pyrene, dibenzo [a,h]anthracene, indeno [1,2,3-cd] pyrene (5-BR) and benzo [ghi]perylene (6BR). For analyses and calculations 5 and 6 BR-PAHs were grouped together. Degradation of PAHs was calculated base on their concentrations remaining in the sediment slurry vials after incubation on day 7 and 15. These concentrations were compared to the original concentrations at day 0.

Statistical analyses showed that there were statistically significant differences among sampling dates for PAHs ($p = 0.000$), but the treatment effect (CCCP + temperature) was only significant for 2BR and 3BR ($p = 0.047$ and $p = 0.003$, respectively). Similarly, the interaction between sampling date and treatment effect (day × treatment) was statistically significant only for 2BR and 3BR ($p = 0.007$ and $p = 0.009$, respectively). Initial total PAH concentrations (132,000 μg/kg) in sediment slurries on day 0 [comparable to measured concentrations from riverbank

Table 3 Microbial activity (nkat/g) measured in sediment slurry incubations over 15-day degradation study under different experimental conditions (average and standard deviation in brackets, $n = 3$)

Experimental conditions	Incubation day 0	Incubation day 7	Incubation day 15
Sed 25	15.49 (3.8)	0.23 (0.0)	0.12 (0.0)
Sed 25-CCCP	1.37 (0.3)	0.19 (0.1)	0.08 (0.0)
Sed 37	28.48 (0.4)	0.77 (0.4)	0.10 (0.2)
Sed 37-CCCP	11.10 (2.9)	0.39 (0.2)	0.05 (0.0)

Table 4 Pearson correlation coefficients between sampling dates (day), microbial activity, and individual PAHs

No of benzene rings	Sampling day	Microbial activity
2 BR		
Napthalene	−0.839**	0.744**
Acenapthylene	−0.187	0.114
Acenapthene	−0.747**	0.676**
Fluorene	−0.800**	0.606**
3 BR		
Phenanthrene	−0.805**	0.676**
Anthracene	−0.620**	0.317
Fluoranthene	−0.734**	0.625**
4 BR		
Pyrene	−0.776**	0.682**
Benzo(a)anthracene	−0.810**	0.632**
Chrysene	−0.688**	0.555**
Benzo(b)fluoranthene	−0.674**	0.454**
Benzo(k)fluoranthene	−0.765**	0.555**
5 BR		
Benzo(a)pyrene	−0.826**	0.680**
Dibenzo(a,h)anthracene	−0.580**	0.511**
Indeno(1,2,3-cd)pyrene	−0.603**	0.514**
6 BR		
Benzo(ghi)perylene	−0.712**	0.617**
Microbial activity	−0.650**	

** $p < 0.01$ show highly significant correlations

sediments described in Johnston and Leff (2015)] decreased substantially by day 15 in slurries at 37 °C and 25 °C without CCCP (67,000 and 44,000 µg/kg, respectively). The highest degradation observed across all treatments was in sediment slurries at 37 °C without CCCP for 2BR- and 3BR-PAHs including acenaphthene, fluorene, phenanthrene, and fluoranthene (ranging from 61 to 89 %). In sediment slurries at 25 °C without CCCP, similar degradation (ranging from 51 to 88 %) was also achieved for the same PAHs. Concurrently, the lowest degradation observed across treatments also occurred in sediment slurries at 37 °C with CCCP, where concentrations of most PAHs remained high (ranging from 69 to 97 %), followed by sediment slurries at 25 °C in which remaining concentrations ranged from 59 to 69 %. In contrast, the combined effect of CCCP and temperature was not statistically significant for 4BR and 5/6BR-PAHs ($p = 0.534$ and $p = 0.180$, respectively) although these PAHs decreased overtime (Fig. 1).

Discussion

PAHs are arguably the most common pollutants in a variety of aquatic environments. As indicated by Johnston and Johnston (2012), PAHs bind to organic matter in sediments and soils and represent a constant impairment to benthic aquatic organisms. Compared to plentiful information on degradation of PAHs (aerobic and anaerobic) using sole carbon sources, there is a scarcity of studies that focus on complex mixtures of pollutants. Additionally, these studies do not represent realistic environmental conditions and certainly do not consider the influence of indigenous microorganisms and their interactions with sediments (Thavamani et al. 2012).

The main aim of this study was to inhibit microbial activity to demonstrate that degradation of PAHs was performed by indigenous microbial communities present in the sediment. This is the first report that measured the inhibitory effect of CCCP on respiration on microbial communities, which in turn decreased PAH degradation. We used sediments slurries containing indigenous microbes under two experimental temperatures to test the efficacy of CCCP. To our knowledge this is also the first time that CCCP, a metabolic inhibitor, which has been only tested in medical microbiological studies (Pournaras et al. 2005; Maniati et al. 2007; Srinivasan et al. 2014), was used in environmental samples. We measured microbial activity by INT reduction since this method provides useful information on the presence of viable microbes and their relationships with surrounding environmental conditions (Margesin et al. 2009). Because microbes respond to environmental stresses, changes in dehydrogenase activity indeed reflect the level of metabolic activity of microorganisms (Bačkor and Fahselt 2005).

The minimum inhibitory concentration of CCCP (20 µM) used on *E. coli* (Ghoul et al. 1989) as well as other

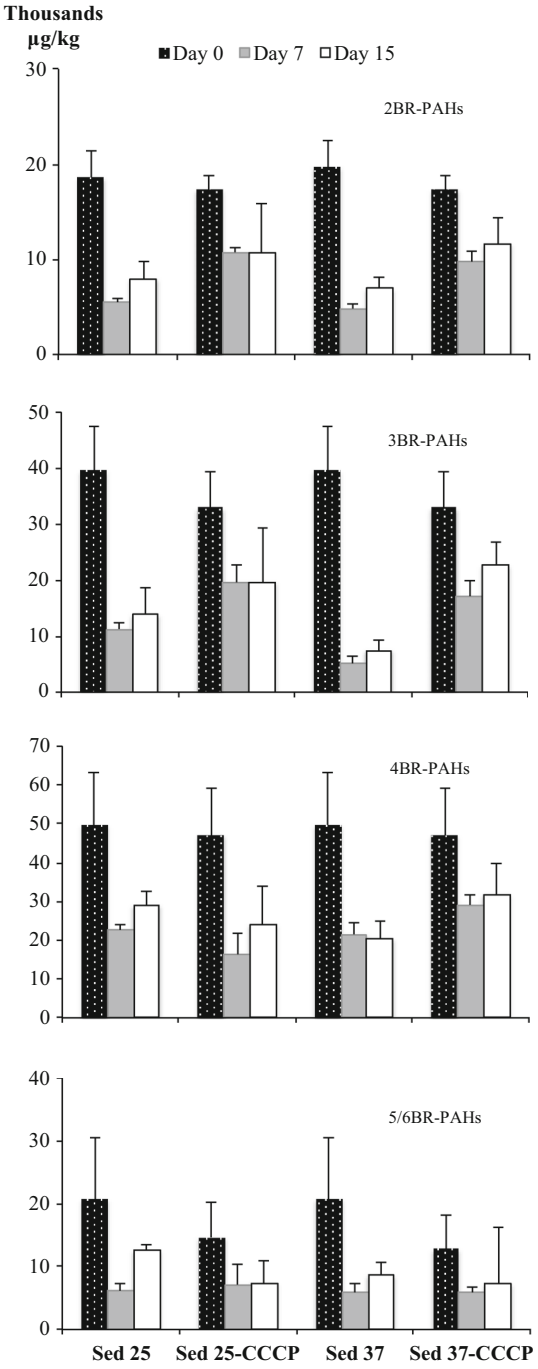

Fig. 1 Measured degradation of PAHs under four experimental conditions (*X*-axis): 25 °C (Sed 25), sediment at 25 °C + CCCP (Sed 25-CCCP), sediment at 37 °C (Sed 37) and sediment at 37 °C + CCCP (Sed 37-CCCP) during a 15 day incubation. In the *Y*-axis PAH concentrations are expressed in thousands of µg/kg. PAHs were classified by number of benzene rings (BR); 2BR: naphthalene, acephthylene, acenaphthene, fluorene; 3BR: phenanthrene, anthracene, fluoranthene; 4BR: pyrene, benzo[a]anthracene, chrysene, benzo[b,k]fluoranthene; 5BR: benzo[a]pyrene, dibenzo[a,h]anthracene, indeno (1,2,3-cd pyrene; 6BR: benzo[ghi]-perylene). Data averaged ($n = 3$) and *error bars* per sampling date are presented. 5 and 6 BR-PAHs were grouped for analyses

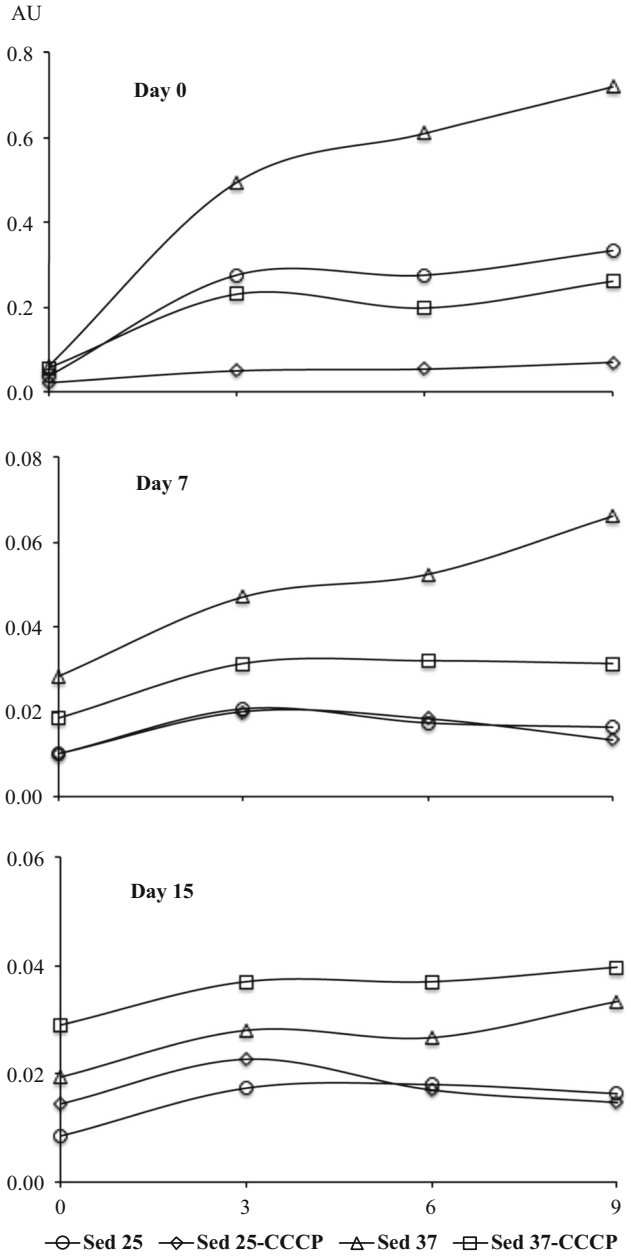

Fig. 2 Microbial respiration measured by INT reduction at 3-min intervals in PAH-contaminated sediment at days 0, 7, and 15 is shown under four experimental incubation conditions: sediment at 25 °C (Sed 25) circles, sediment at 25 °C + CCCP (Sed 25-CCCP) diamonds, sediment at 37 °C (Sed 37) triangles and sediment at 37 °C + CCCP (Sed 37-CCCP) squares. *X*-axis indicates time in minutes since the addition of INT; *Y*-axis indicates absorbance units (AU) at 490 nm

concentrations as indicated by Peachman et al. (2001) and Dror et al. (2008) (5 and 10 µM, respectively) were used as baseline references. By virtue of the intricacy of our sediment samples (petroleum, humics, and metals), a greatly increased (two orders of magnitude) CCCP concentration

(3.5 mM) was chosen. As shown in Fig. 2, the effect of the CCCP as a metabolic inhibitor was most manifested on day 0, where a short pre-incubation time (1 h.) prior to the addition of INT, was sufficient to impair dehydrogenase activity. CCCP inhibited respiration by 79 and 64 % at 25 and 37 °C, respectively after a 9 min incubation period. Previous assays (data not shown) revealed that even higher CCCP concentrations would require more acetone for dissolution, which could have interfered with membrane function and integrity (Lepage et al. 1987).

Sediment slurry incubations were initiated under aerobic conditions to optimize microbial respiration, which did indeed result in high rates of INT reduction on day 0. This result may be explained by the use of short time intervals (rates from the first 3-min after INT addition), which is the shortest incubation time (after INT addition) reported in the literature. In agreement with Mosher et al. (2003), longer incubation times (following INT addition) showed decreased activity (as shown in Fig. 2), perhaps due to substrate limitation. It thus appears that rates of respiration based on longer incubation times most likely underestimate initial rates of respiration, an observation that has not yet been taken into account in the literature for this method.

Microbial respiration was attenuated when measured on days 7 and 15 (Table 3) likely due to the combination of lack of oxygen (anaerobic respiration provides less available free energy) and limitation of metabolically available carbon or nutrients, since no carbon or nutrient supplements were added (the sediment itself was the only source of organic matter, minerals, and nutrients). In fact, microbial activity on day 7 and 15 represented respiration under anaerobic conditions. Although greatly reduced (0.2–0.8 nkat/g) our values were comparable to values reported by Mosher et al. (2003), and demonstrated respiration under anaerobic conditions (Table 5).

Because microbial metabolism and respiration are usually increased under higher temperatures, sediments incubated at 37 °C showed higher dehydrogenase microbial activity than the 25 °C incubation on day 0 and throughout the length of the experiment. In addition, our results indicated much higher microbial respiration throughout the experiment compared to those previously reported (Table 5) including for other similar contaminated environments (Margesin et al. 2008; Kaimi et al. 2006; Robidoux et al. 2004).

PAH microbial degradation

Our data indicated that microbial communities in anaerobic riverbank sediments appeared to have a great potential for degradation of PAHs when exposed briefly to oxygen. Experimental conditions were carried out (a) under tightly sealed vials to minimize PAH loss from the system, and (b) under dark conditions to minimize photo-oxidation to mimic natural conditions in the sediments (Quantin et al. 2005). Yet, it is not undeniable that other mechanisms of PAHs disappearance might still have occurred during the 15-day incubation (Haritash and Kaushik 2009). Nevertheless, the inhibitory effect of CCCP on microbial activity clearly carried over as inhibited PAH degradation. PAH degradation included a biotic component and perhaps facultative anaerobic bacteria were involved in the high initial PAH degradation when oxygen was present in the vials (Lei et al. 2014). By day 7, the vials were likely anoxic, had reduced respiration, and consequently PAH degradation slowed down or ceased entirely (Fig. 1). This was most evident in sediment slurries with no CCCP at 37 °C for the majority of 2 and 3 BR-PAHs, and exceptionally for few 4 and 5BR-PAHs. In agreement with Lei et al. (2005), indigenous microbes were likely responsible for the degradation of most 2, 3 and some 4 ringed PAHs in sediment slurries with no CCCP addition. To a lesser extent, the same pattern was observed for slurries at 25 °C without addition of CCCP where concentrations of 2, 3, and some 4 ringed PAHs decreased over time. Verrhiest et al. (2002) also reported degradation of a 3 and 4 ringed PAH mixture (phenanthrene, fluoranthene, and benzo[k]fluoranthene) by unidentified indigenous microbes from natural freshwater sediments, and demonstrated that the degree of degradation depended on the physicochemical properties and bioavailability of PAHs in the sediments. Xia et al. (2006) corroborated this finding, indicating that biodegradation of PAHs in rivers depended on the sediment content in water, reporting that rates of degradation by PAH-degrading bacteria (identified by culturing techniques) increased with sediment content.

Table 5 Comparison of dehydrogenase respiration by INT reduction found in this study and previously reported

Type of soil/sediment	Nkat/g	Incubation time	Reference
PAH-contaminated sediment	0.90	30 min	Mosher et al. (2003)
Alpine soils	0.11	1 h	Margesin et al. (2009)
Diesel contaminated soils	0.05	6 h	Kaimi et al. (2006)
Explosive-contaminated soils	0.01	24 h	Robidoux et al. (2004)
PAH-contaminated sediment	0.33	10 min	Pratt et al. (2012)
PAH-sediments without CCCP	16–29	3 min	This study

The long history of PAH contamination in the Mahoning River (Johnston et al. 2015) suggests that microbial communities may have adapted to use these organic compounds as carbon sources. The role of indigenous microbial communities in degradation studies of complex mixtures of PAHs and other pollutants is still poorly understood and has not been well documented for river sediments. More studies on natural systems and focused on communities adapted to contamination are needed to address problems of PAH recalcitrance.

This study demonstrated that CCCP, a potent metabolic inhibitor for respiration in pure cultures, can also be applied in more complex environmental systems such as sediment contaminated a priori with PAHs, metals, and petroleum as evidenced by decreased dehydrogenase activity. However, CCCP concentrations needed to be two orders of magnitude higher to have a strong inhibitory effect on respiration in this environmental matrix. In addition to inhibiting respiration, CCCP inhibited degradation of lower molecular weight PAHs, linking their degradation to microbial metabolism. Our findings provide an alternative tool for environmental scientists to include, along with respiration, as a variable to study in complex systems. This approach also elucidates the degradation potential of extant indigenous microbial communities in aquatic ecosystems.

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