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Transformation of Organic Pollutants Through Microbial Activity in Contaminated River Sediments

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Abstract: Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and petroleum hydrocarbons are hydrophobic organic pollutants that are deposited in the sediment of rivers. Although microbial transformation is a sustainable option for sediment management, degradation will depend on the structure of the pollutant, the availability of oxygen, the temperature, and the bioavailability. The effect of sediment properties, redox conditions, and microbial community composition on the transformation of organic pollutants, particularly the chlorinated biphenyls and PAHs, was studied. A structured evidence-synthesis framework was used to integrate with a proposed three-site sampling design for an urban river in Tamil Nadu and a four-treatment aerobic–anaerobic microcosm experiment. The degradation rate constants from the 94 anaerobic measurements compiled from 15 sediment-based studies ranged from 0.008 day^{-1} for benzo[a]pyrene to 0.120 day^{-1} for acenaphthene, with an overall mean rate constant of 0.040 day^{-1} (half-life of ~84 days). Adjusting the temperature to 12 °C produced a mean rate of 0.004 day^{-1} (or about 790 days). Aerobic and anaerobic (iron- and sulfate-reducing) incubation of river sediments showed that 3- and 4-ring PAHs were degraded under aerobic conditions, while there was no measurable coupling of degradation to PAH loss under either iron- or sulfate-reducing condition. Dechlorinating Chloroflexi, including D. ethella, were found to be present in PCB-polluted sediment at 0.01–0.19% relative abundance. Results show that oxygen status, compound structure, and bioavailability have the greatest influence on transformation results, and that parent-compound disappearance needs to be separated from mineralization. The available evidence indicates that oxygen-managed bioremediation can be used to treat biodegradable PAHs.

Keywords: River Sediment; Organic Pollutants; Microbial Degradation; Polycyclic Aromatic Hydrocarbons; Anaerobic Transformation; Bioremediation, Microbial Communities.

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I. Introduction

Organic contaminants come from industrial effluents, petroleum handling, agriculture, municipal sewage, and urban activities. PAHs are the most prevalent of these compounds and are formed primarily from the incomplete burning of organic substances and from the processing of petroleum products (Abdel-Shafy & Mansour, 2016). Suspended particles act as a sink and secondary source for hydrophobic pollutants because they adsorb to suspended particles and deposit in bottom sediments. Accumulated pollutants may be remobilized to the water column when there is a change in pH, temperature, salinity, oxygen, or physical disturbance. Bioavailability is reduced, and degradation is retarded when the sorption to sediment organic matter is high, making remediation more complex (Kumar et al., 2021). Sediment-bound PAHs also come into close contact with the local microbial communities, even in remote deep-sea sediments (Louvado et al., 2015).

Transformation by microorganisms is regarded as the main mechanism for the removal of PAHs from contaminated environments and is influenced by the availability of nutrients, the number and variety of microorganisms, and the chemical characteristics of the compound (Ghosal et al., 2016). Toxicity, bioavailability, bio-stimulation, and integrated treatment have been repeatedly identified as key aspects in recent reviews of PAH remediation (Patel et al., 2020). The ecological impacts of sediment contamination involve lower growth and reproduction rates in aquatic organisms, developmental defects in fish and invertebrates, disruption of microbial nutrient cycling and changes in the diversity of microbes in the sediment, and bioaccumulation in food chains. The biodegradability of the chemicals depends on their chemical structure: low MW PAHs are relatively more biodegradable than high MW PAHs.

Conditions in the sediment and the microbial communities can affect either transformation or mineralization of a pollutant. Oxygen (or other electron acceptors), organic carbon, and temperature select for metabolic pathways, and pathways are available depending on the community composition. The link between community composition and the actual rate of transformation is not well resolved, and transformation products are rarely studied.

Research Questions and Hypotheses

RQ 1: Which organic pollutants are most commonly associated with contaminated river sediments?

RQ 2: How do pH, temperature, oxygen, and organic carbon influence degradation?

RQ 3: Which microbial groups are associated with the transformation of different contaminants?

RQ 4: How does aerobic degradation differ from anaerobic degradation?

RQ 5: Can microbial activity substantially reduce pollutant concentration and toxicity?

Objectives

1. To identify major organic pollutants in contaminated river sediments.
2. To evaluate the influence of sediment characteristics on pollutant accumulation.
3. To examine the role of microbial communities in pollutant transformation.
4. To compare aerobic and anaerobic degradation pathways.
5. To evaluate changes in pollutant concentration and transformation products during incubation.
6. To identify environmental conditions that favor microbial bioremediation.

The paper is organized into a number of important sections: In Section 2, pollutant properties and microbial pathways are established. The study design, sampling, and microcosm experiments are described in Section 3. Anaerobic Kinetics and community patterns are presented in Section 4. Oxygen status, temperature effects, and limitations are discussed in Section 5. Finally, Section 6 summarizes the major findings and highlights the importance of oxygen-managed bioremediation, monitoring transformation products, and future sediment management strategies.

II. Background and Literature Review

Major Organic Pollutants

PAHs are made up of two or more fused rings and are divided into two groups: low molecular weight (two to three rings) and high molecular weight (four to six rings). The larger the molecule and the more hydrophobic it is, the more persistent it will be. The log Kow for naphthalene ($C_{10}H_8$, 128.17 g/mol, 2 rings) is approximately 3.3–3.4, while that of benzo[a]pyrene ($C_{20}H_{12}$, 252.31 g/mol, 5 rings) is near 6.0, with increasing log Kow values indicating increasing association with sediment organic matter and decreasing availability to microorganisms. PCBs are synthetic chlorinated aromatics that were previously used in transformers, capacitors, and hydraulic fluids, and are persistent due to their chemical stability, low solubility, and resistance to degradation. Some sources of petroleum hydrocarbons to rivers are ship leaks, road runoff, industrial effluents, storage leaks, and spills, which are predominantly aromatic, cycloalkane, or alkane compounds. Many of these compounds can be broken down by indigenous microorganisms that can be found in water and soil, with bacteria being the main degraders of spilled oil (Das & Chandran, 2011). Additional organic loads are added by organochlorine pesticides like DDT, pharmaceutical residues, and phthalates.

Microbial Groups and Aerobic Pathways

Dioxygenases, monooxygenases, and ring-hydroxylating enzymes are present in *Pseudomonas, Sphingomonas, Rhodococcus*, and Mycobacterium and are able to initiate aerobic oxidation of PAHs. Naphthalene is metabolized by naphthalene dioxygenase to cis-naphthalene dihydrodiol, which is then subjected to dehydrogenation and salicylate and catechol intermediates, either ortho- or meta-cleavage, before entering central metabolism and finally carbon dioxide, water, and biomass. The reductive dehalogenases for chlorinated compounds are carried by *Dehalococcoides mccartyi*; the anaerobic roles of Geobacter and sulfate-reducing genera are in aromatic metabolism. A lack of the taxon does not indicate that degradation has occurred at a site; evidence of degradation must be based on functional genes, expression data, metabolite analysis, or isotope evidence.

Anaerobic Transformation

River sediment often has oxygen-depleted areas due to the decomposition of organic matter. Nitrate, sulfate, ferric iron, and carbon dioxide are then available as electron acceptors. The major routes of activation of unsubstituted aromatics like naphthalene are carboxylation; for methylated compounds, fumarate addition. Phenanthrene is the largest PAH known to be degraded without air, and the cultures that degrade it grow slowly, with doubling times of approximately two weeks (Meckenstock et al., 2016). In addition, there is a good review of the biochemical aspects of anaerobic degradation of unsubstituted aromatic hydrocarbons (Meckenstock & Mouttaki, 2011).

Reductive dechlorination involves the substitution of chlorine atoms for hydrogen atoms ($R–Cl + 2e^- + H^+ \rightarrow R–H + Cl^-$); it is especially relevant for the dechlorination of PCBs. The commercial mixture Aroclor 1260 was dechlorinated predominantly by the removal of flanked para and doubly-flanked meta chlorines from the culture by a sediment-free culture containing *Dehalococcoides* and *Dehalobacter* (Wang & He, 2013). Dechlorination can reduce the amount of chlorine but not necessarily the toxicity, as the less chlorinated congeners can be persistent.

Remediation Approaches and Constraints

Bioaugmentation, biostimulation, biopiles, bioreactors, and chemical or thermal treatment are options that have been developed for PAH contaminated media, but microbial limitations for the treatment of high molecular weight PAHs are a significant technological hurdle (Kuppusamy et al., 2017).

Knowledge Gaps

There are four gaps. First, the relationship between community composition and the measured transformation rate is not well understood. Second, the high molecular weight PAHs are not very bioavailable due to sorption to organic matter. Thirdly, anaerobic pathways are not as well defined as aerobic pathways. Fourth, transformation products can remain toxic after the parent has lost toxicity, and multi-pollutant and seasonal effects are not often considered.

III. Materials and Methods

Study Design

The design is a synthesis of sediment-incubation data, with a proposed field and lab design. The proposed field component involves three sections, S1 (upstream, reference), S2 (urban wastewater-influenced), and S3 (downstream, multiple anthropogenic inputs), of the Cooum River in Tamil Nadu, India. These are sampling class categories, not verified contamination classes.

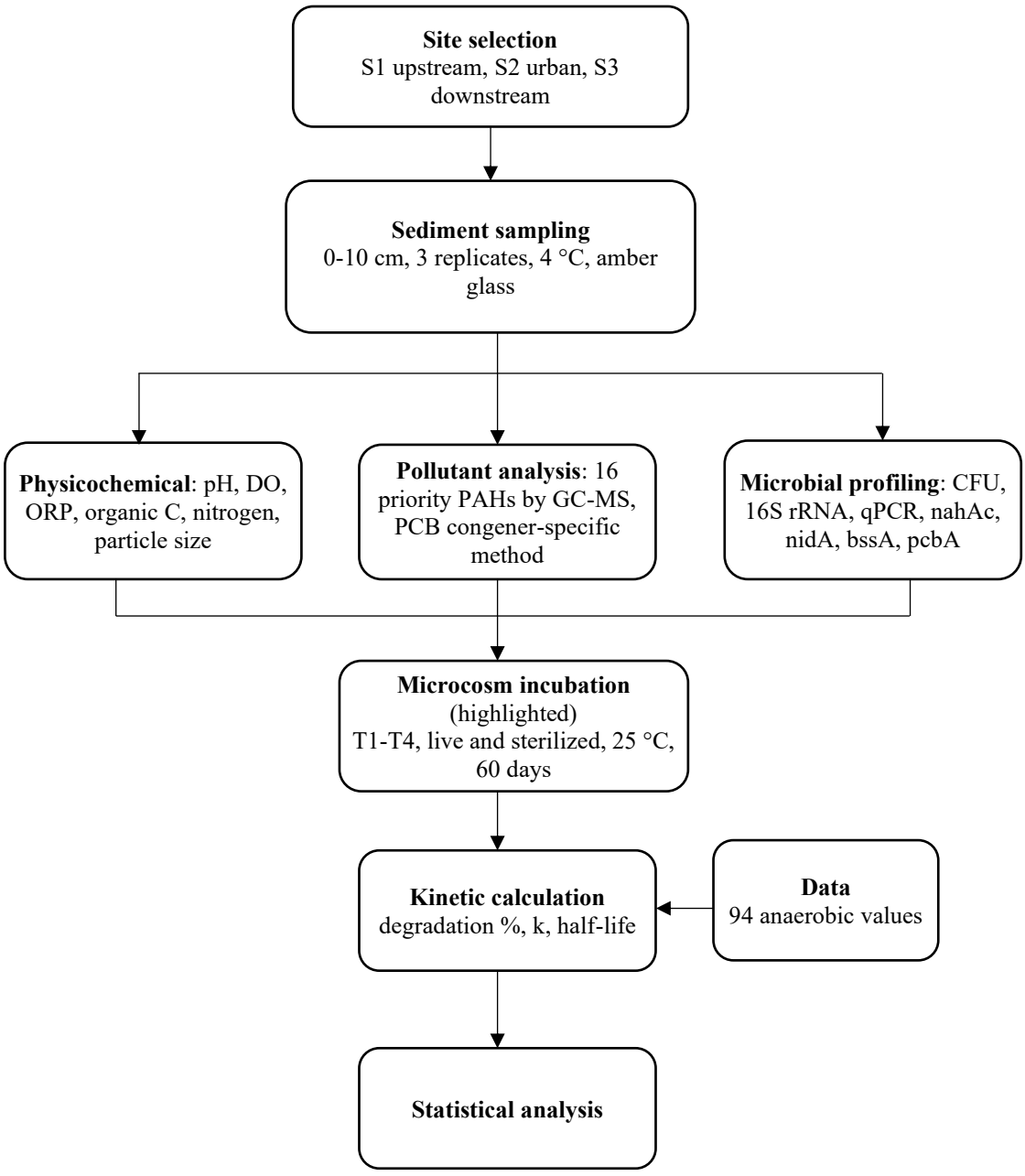


Figure 1: Methodological Workflow of the Study

Figure 1 summarizes the sequence from site selection and sediment sampling through parallel physicochemical, pollutant, and microbial characterization, followed by aerobic and anaerobic microcosm incubation, kinetic calculation, and statistical analysis. Chemical and biological data are from the same aliquots of sediment and are obtained through parallel analytical branches. Sterilized controls remove the biological losses, while the last step involves the transformation of concentration series into rate constants and multivariate relationships.

Sampling and Physicochemical Analysis

Sediment (0- 10 cm depth) collected using a stainless-steel grab sampler and/or corer and stored in pre-cleaned amber glass at approximately 4 °C, protected from oxygen if anaerobic behavior is to be assessed, with at least three independent replicates per site. Parameters measured are pH (calibrated pH

meter), temperature, dissolved oxygen (DO) (probe), oxidation-reduction potential (ORP) (redox electrode; ORP value is given with reference electrode), moisture (gravimetric or moisture meter), organic carbon (Walkley–Black or elemental analysis), total nitrogen (Kjeldahl or elemental analysis), particle size (hydrometer or laser diffraction), nitrate, and sulfate (all parameters are frozen in the sample bottle at −80 °C for molecular analysis).

Pollutant Analysis

Sediment is homogenized, and the 16 US EPA priority PAHs (naphthalene to benzo[g, h, i]perylene) are extracted using either ultrasonic, Soxhlet, or pressurized liquid extraction, cleaned on silica or alumina, and quantified by gas chromatography–mass spectrometry with internal standards and multipoint calibration, according to a validated method from US EPA Method 8270E. Results are given in µg/kg (mg/kg D.W.) and include detection limits, quantification limits, recoveries, blanks and reference-material results. A method is used to analyze PCBs that is congener-specific.

Microbial Characterization

Estimate of culturable abundance is done by serial dilution and plating (CFU/g dry sediment). Amplicon sequencing of 16S rRNA is used to describe community composition. The functional gene copy numbers used are *nahAc* and *nidA* (aerobic PAH dioxygenase genes), *bssA* (fumarate addition activation), and *pcbA*-type reductive dehalogenase genes. Each target is validated using assays because there are differences between the distribution of each gene across strains.

Incubation Experiment and Calculations

The microcosms consist of 50 g of sediment per dry-weight-equivalent, are maintained at 25 ± 2 °C for 60 days and sampled at 0, 7, 14, 30, 45 and 60 days, with at least three microcosm replicates per treatment. Sterilization methods are validated due to possible changes in organic matter and contaminant availability with autoclaving or chemical sterilization.

Table 1: Microcosm Treatments

Treatment	Sediment	Oxygen status	Purpose
T1	Live	Aerobic	Aerobic Biodegradation
T2	Live	Anaerobic	Anaerobic Transformation
T3	Sterilized	Aerobic	Abiotic Loss
T4	Sterilized	Anaerobic	Abiotic Loss Under Anoxia

Table 1 provides the factorial layout, crossing biological activity (live versus sterilized) with oxygen status (aerobic versus anaerobic). Comparing the two isolates, T1 is aerobic biodegradation while T2 is anaerobic transformation when compared with T4. Each treatment is repeated at least three times and at six sampling times, resulting in a time series that can be used for fitting first-order kinetics and for monitoring transformation products in addition to the parent compounds.

The first-order degradation kinetic equation (1) used in the study:

$$C_t = C_0 e^{-kt} \quad (1)$$

Where: C_t = Concentration of the pollutant at time t, C_0 = Initial concentration of the pollutant at time 0, k = Apparent first-order degradation rate constant (day^{-1}), t = Incubation time (days)

The corresponding rate and half-life formulas are expressed as in equation (2) and (3):

$$k = \frac{\ln(C_0/C_t)}{t} \quad (2)$$

$$t_{1/2} = \frac{0.693}{k} \quad (3)$$

Statistical Analysis

Pollutants, environmental variables and community composition are related with descriptive statistics (mean ± standard deviation), one- or two-way ANOVA with Tukey post-hoc tests, Pearson or Spearman correlation, principal component analysis and redundancy analysis. The significance level is $p<0.05$.

IV. Results

Anaerobic Degradation Kinetics of PAHs

The anaerobic degradation kinetics of PAHs are discussed. The anaerobic degradation kinetics of PAHs is discussed. A compilation was compiled that included 94 individual measurements of anaerobic PAH biodegradation kinetics from 15 studies involving sediment incubations or sediment-derived inocula.

Table 2: Compiled Anaerobic PAH Degradation Kinetics

PAH compound	Rings	Measurements (n)	Mean rate (day^{-1})	Mean half-life (days)	Tukey HSD Grouping ($p<0.05p < 0.05p<0.05$)	Spearman Correlation (rsr_srs)
Naphthalene	2	13	0.018	180	Group A	
Acenaphthene	3	7	0.120	13	Group B	-0.98 ($p<0.001$)
Fluorene	3	12	0.053	32	Group B	-0.98 ($p<0.001$)
Phenanthrene	3	19	0.045	82	Group B	-0.98 ($p<0.001$)
Anthracene	3	7	0.043	51	Group C	-0.98 ($p<0.001$)
Fluoranthene	4	8	0.013	67	Group C	-0.98 ($p<0.001$)
Pyrene	4	17	0.025	110	Group C	-0.98 ($p<0.001$)
Benzo[a]pyrene	5	1	0.008	91	Group C	-0.98 ($p<0.001$)

Table 2 lists compiled average pseudo-first-order rates and half-lives for eight PAHs across 94 measurements. The rate of degradation of acenaphthene was the highest (0.120 day^{-1}), while that of benzo[a]pyrene was the lowest (0.008 day-1) by a factor of 15. Values are not collected from a single standardized experiment; they are derived from a variety of sediments and conditions. The mean half-lives obtained from the individual measurements do not correspond to mean rates, and therefore cannot be obtained from the mean rates by $t½ = 0.693/k$.

The overall mean rate was 0.040 day^{-1} or about an 84-day half-life. When this was reduced to 12 °C, the mean rate was reduced to 0.004 day^{-1} (790 days).

Figure 2 ranks mean rates by compound. Acenaphthene (0.120 day^{-1}) and fluorene (0.053 day^{-1}) lead, followed by phenanthrene and anthracene near 0.045 and 0.043 day^{-1}. Pyrene, naphthalene, and fluoranthene are between 0.013 and 0.025 day-1, while benzo[a]pyrene is the lowest value at 0.008 day^{-1} (with a single measurement). However, the rate does not monotonically decrease with the ring number, meaning that there is not a direct correlation between the structure and how susceptible it is to anaerobic degradation.

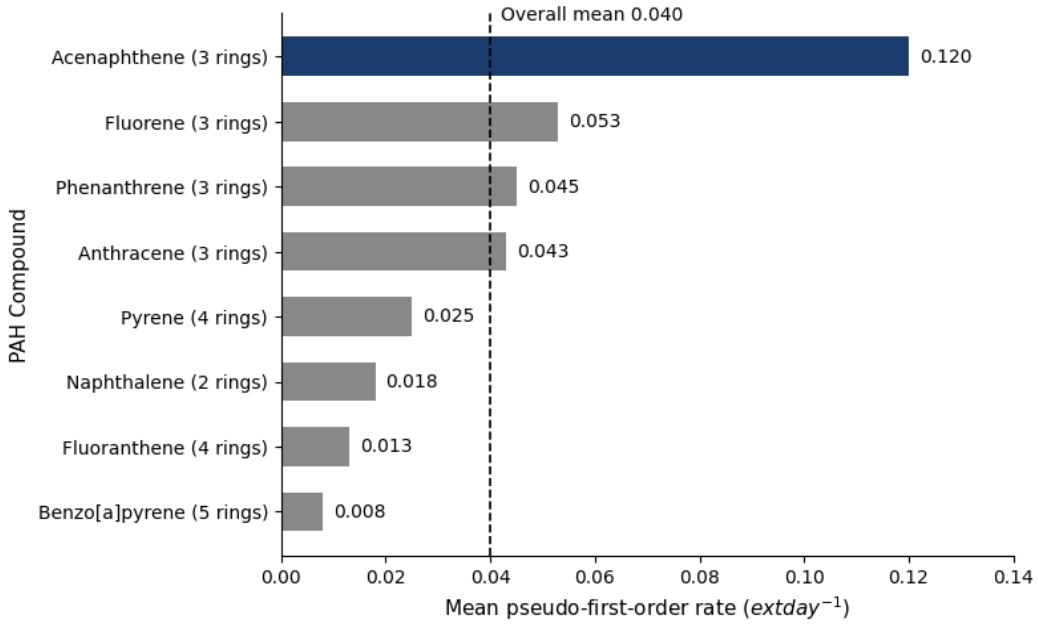


Figure 2: Mean Anaerobic Degradation Rate by PAH Compound

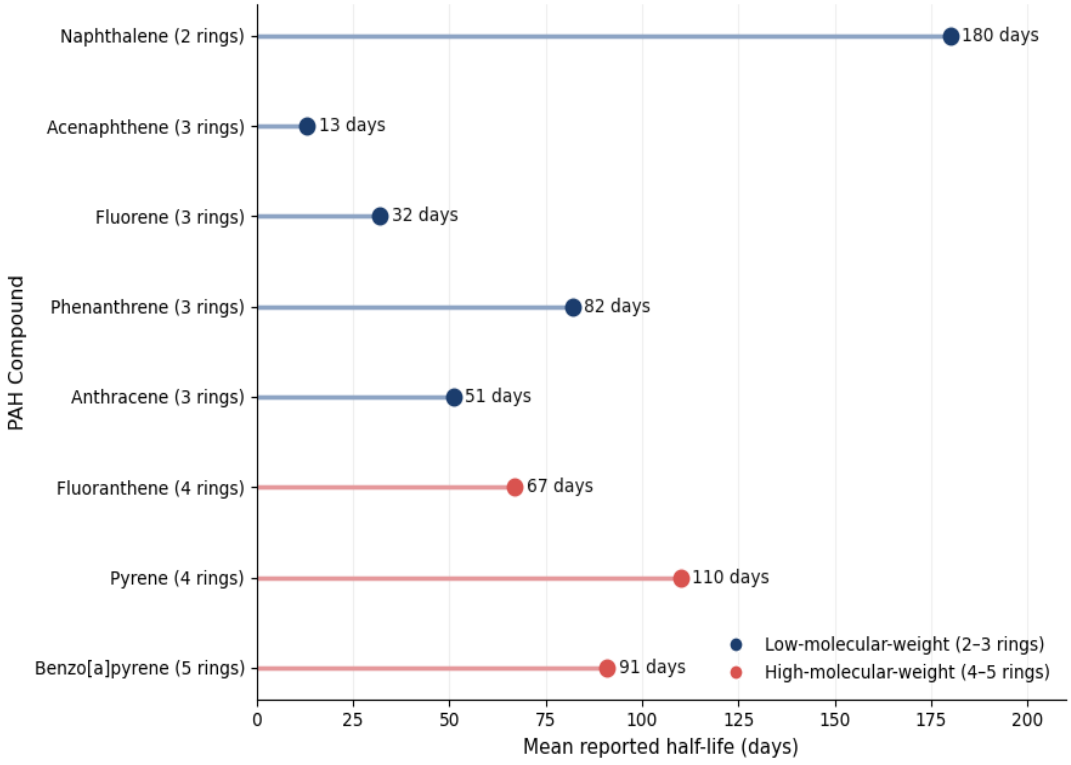


Figure 3: Mean Reported Half-Life by PAH Compound

Figure 3 indicates that acenaphthene is the most rapidly transformed molecule (13 days), while fluorene is 32 days, naphthalene is 180 days, and pyrene is 110 days. Anthracene, fluoranthene, phenanthrene, and benzo[a]pyrene fall in the middle group of 51-91 days. Anaerobic activation of unsubstituted aromatics is a kinetic bottleneck, as evidenced by the persistence of naphthalene under anoxia, despite it being the smallest PAH.

Aerobic Versus Anaerobic Transformation

Indigenous microorganisms in aerated incubations of contaminated river sediment degraded three- and four-ring PAHs. No measurable coupling between PAH degradation and microbial metabolism was found in iron- and sulfate-reducing conditions even for phenanthrene. Microbial respiration is stimulated by cellulose addition, but it did not increase the amount of PAH dissipation, and natural attenuation under anaerobic conditions is considered very slow. Aerobic metabolism is based on the oxygenase-mediated oxidation of *Pseudomonas, Sphingomonas* and *Rhodococcus* species, while anaerobic metabolism is based on activation reactions coupled to nitrate, sulfate or iron reduction or methanogenesis. The two regimes were relatively non-destructive for high-molecular-weight PAHs.

Microbial Community Patterns

In PCB-contaminated wastewater lagoon sediment, total PCB concentrations ranged from 6.34 to 12,700 mg/kg dry weight. Proteobacteria and Firmicutes dominated. Putative dechlorinating Chloroflexi represented only 0.01–0.19% of the community, and *Dehalococcoides* accounted for 0.6–14.8% of that dechlorinating group. Eleven sequences related to reductive dehalogenase genes of PCB-dechlorinating *Dehalococcoides mccartyi* were detected. This range reflects a highly contaminated lagoon and is not representative of ordinary river sediment.

River-Sediment Evidence from Taiwan

The anaerobic degradation of the five PAHs in Erren River sediment was ranked as acenaphthene > fluorene > phenanthrene > anthracene > pyrene, which corresponds to the compiled kinetic ranking and suggests that the molecular structure of the PAHs and their bioavailability affect their susceptibility.

V. Discussion

Oxygen Status and Pathway Selection

H2 was found to be adequate for the degradation of the three- and four-ring PAH in the analyzed sediment of the river; aerobic conditions provided degradation, while anaerobic respiration did not. Respiration thus does not ensure contaminant removal, and the metabolic pathway must be appropriate for the contaminant. This is not universal for all sediments, as anaerobic degradation of PAHs has been shown in other sediment cultures. Treatment strategies may be insufficient as there are vertical oxygen gradients, with one sediment core having aerobic, nitrate-reducing, iron-reducing, sulfate-reducing, and methanogenic zones.

Temperature, Organic Matter, and Bioavailability

The compiled mean rate has been decreased by a factor of 10 when adjusted to 12 °C, reflecting the ability of cooler sediments (typical of seasonal and temperate environments) to hold PAH for several years. It is a comparison and a mixture of studies. The role of organic matter is opposite: it provides substrates for biomass, but it sorbs hydrophobic compounds, which means that organic-rich sediment could contain more PAHs, but also may restrict their absorption. This is in support of H3 and brings emphasis to the need for distinguishing between total and bioavailable concentrations.

Community Composition and Functional Evidence

Relative abundances of low-abundance specialists (such as dechlorinating Chloroflexi, Dehalococcoides 0.01-0.19%) will not prove activity, but may be indicative. As a result, support for H4 is qualified, in that rates are likely to be influenced by composition, and by functional gene expression, metabolite detection, and measured transformation, it can be demonstrated. This requirement is fulfilled by the proposed qPCR targets and sterilized controls.

Ecological Significance of Transformation Products

The ecological significance of transformation products needs to be investigated. Loss of a parent compound is not detoxification. Reductive dechlorination produces less-chlorinated PCB congeners, which might be more susceptible to aerobic degradation but are still persistent, and inadequate oxidation of the PAHs produces intermediates that require additional oxidation. Therefore, success in remediation should be evaluated in terms of reduction of parents, formation or decrease of products, decrease of toxicity, evidence of mineralization, and absence of rebound.

Implications for Sediment Management

Biostimulation (electron-acceptor management, nutrient adjustment, aeration) is suitable for biodegradable PAHs, but adding substrates alone might increase respiration without increasing dissipation. Introduced strains must persist and compete for the use of a pathway to be of value for bioaugmentation in the absence of the indigenous pathway. Aerobic options include ex situ biopiles, aerated sediment reactors, or controlled land treatment, while anaerobic options are available for certain chlorinated compounds, provided that an electron donor, electron acceptor, and competent organisms are available to the system.

Limitations

There are reported differences in the type of sediment, the concentration, the incubation time and the analytical method used in published studies, and the data cannot be compared directly. Laboratory microcosms do not truly mimic river systems; activity does not automatically mean sequencing; aging reduces bioavailable resources; and sterilization affects the chemistry of the sediment. The results of the European or Taiwanese sampling and chemical analysis cannot be extrapolated to an Indian river; local sampling and chemical analysis of the Coum River must be done first in order to draw conclusions.

VI. Conclusion

The microbial activity is one of the major factors responsible for the transformation of organic pollutants in contaminated sediments from rivers, but the result of the microbial activity is dependent on the similarity between the chemistry of the organic pollutants and the redox conditions of the sediments. Aerobic pathways (those requiring oxygen) were consistently linked with the degradation of 3- and 4-ring PAHs, while anaerobic (oxygen-independent) pathways degraded the three PAHs at widely varying rates ranging from 0.120 day^{-1} to 0.008-day^{-1} for benzo [a] pyrene. The addition of organic carbon increased respiration but did not increase dissipation, and anaerobic respiration did not always lead to PAH loss. Low abundance groups, in particular *Dehalococcoides* in dechlorinating Chloroflexi, were associated with transformation of chlorinated biphenyls, but abundance can be used to infer activity. The factors most strongly correlated with transformation included oxygen availability for biodegradable PAHs, bioavailable contaminants, warm incubation temperature, and the presence of functionally competent taxa. For future research, field sampling in Indian rivers, functional gene and expression analysis, tracking metabolites and toxicity, and long-term incubations should all be considered a priority. Controlled aeration or ex situ aerobic treatment is used to biodegrade PAHs; anaerobic stimulation is used for chlorinated compounds; and monitoring of transformation products to ensure detoxification instead of just parent-compound loss.

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