

MICROBIALY MEDIATED ORGANIC MATTER DEGRADATION RELEASES ARSENIC INTO SE ASIAN GROUNDWATER

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Elevated arsenic in groundwaters exploited for irrigation and drinking water is significantly and detrimentally affecting human health in West Bengal, Bangladesh and other regions of SE Asia. A key mechanism for the genesis of arsenic in these waters is microbially mediated reductive dissolution of arsenic-bearing Fe(III) hydrated oxides (Islam et al., 2004). The role of organic carbon, whether from *in situ* organic matter (OM), i.e. OM from within the sediment, or from other sources, is widely recognised. Despite this, there is a paucity of data about the characteristics of OM in these arsenic-rich aquifers and little experimental evidence directly evaluating how OM oxidation is coupled to arsenic release. To investigate this, we characterised OM in several groundwater arsenic “hotspots” in West Bengal and Cambodia. We then conducted microcosm experiments in which OM degradation, iron reduction and co-occurring arsenic release and changes in the bacterial populations were analysed and compared.

In the first stage of this research, we extracted and analysed the lipid biomarkers in seven different sediment horizons (from 0 to 30 m depth) from a West Bengal arsenic “hotspot”. This revealed that some of the OM present is characteristic of the original marginal marine depositional environment. However, in all but the most shallow sample, this was overprinted by abundant hydrocarbons with thermally mature (e.g. petroleum) distributions. These hydrocarbons included *n*-alkanes with no odd-over-even predominance and thermally mature distributions of hopanes and steranes. The presence of this previously unreported source of organic carbon is important as it has the potential to promote microbial activity and associated arsenic release. Indeed, in sandy horizons, the presence of large unresolved complex mixtures and relatively low abundances of *n*-alkanes suggest that indigenous microbes within the aquifer are degrading the petroleum and utilising it as a carbon source. The microbes present in these samples are diverse but include a significant population of *Sulfurospirillum* sp., a known As(V) reducer, providing the first evidence of such organisms mediating arsenic release from West Bengali aquifers.

To determine if this petroleum affects As (III) reduction, we conducted microcosm experiments under reducing conditions and using As-contaminated Cambodian sediments. Arsenic release and iron reduction did not occur in autoclaved sterile microcosms, clearly indicating that these reactions are microbially mediated. In unamended microcosms, arsenic release did occur in a sand-dominated sediment with a relatively low TOC content (0.1%) but containing petroleum-related hydrocarbons (SYII28). However, in a clay-dominated sediment with a relatively higher TOC content (ca 0.6%) but lacking petroleum-related hydrocarbons (SYII9), As release was not observed. Addition of acetate to this sediment caused As to be released at levels comparable to those in the unamended SYII28 sediment microcosm. This demonstrates that (1) the addition of labile OM does stimulate As release, but that (2) with respect to sedimentary OM, it is the type of OM present, not necessarily its total abundance, that governs the magnitude of arsenic release. However, this experiment did not test whether the OM (either petroleum in the unamended sediment or acetate when added as a supplement) is acting as a substrate for the growth of As-reducing microbes or whether it accelerates arsenic release by acting as an electron shuttle.

To determine if petroleum is degraded in these sediments under reducing conditions and co-occurs with As and Fe reduction, we conducted a further set of microcosm-based techniques, using the aforementioned suite of seven West Bengal sediments. As with the previous experiments, arsenic release and Fe(III) reduction was microbially mediated. Moreover, in all seven experiments petroleum *n*-alkane abundances decreased both in total concentration and relative to terrestrially sourced autochthonous organic matter. This strongly suggests that the microbial oxidation of petroleum-related *n*-alkanes could drive Fe(III) reduction and arsenic release.

The implications of this research could be profound. The mechanism by which extensive groundwaters in SE Asia have become contaminated by arsenic is hotly debated but critical to resolving what has been described as a human health catastrophe. Although many questions remain, this work suggests that natural petroleum seepage could be an important control on arsenic release and provide a guide for addressing this challenge.

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REAL-SCALE BIOPILE REMEDIATION OF A KEROSENE-CONTAMINATED SOIL: MICROBIAL DIVERSITY AND GEOCHEMICAL CHARACTERIZATION

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Biopiles technology involves heaping contaminated soils and stimulating aerobic microbial activity through the aeration and/or addition of nutrients, surfactants, moisture and other amendments. Biopiles, like landfarming treatments, have been proven effective in reducing concentrations of the constituents of petroleum products through volatilization and biodegradation. However, general knowledge about biopiles design and efficiency can be increased by detailed studies of microbial processes taking place within the treatment (Chaîneau et al., 2004). Therefore, in this work we selected a soil affected by historical kerosene spills within the last 20 years that had generated an average TPH (Total Petroleum Hydrocarbons) content of 4.000 mg/kg. After a biotreatability study, several biopiles (see below) were on-site disposed for four months to remediate pollution; microbial and geochemical effects of the biodegradation extents were monitored.

Initially, agronomic and geochemical analyses of the soil were performed in order to determine feasibility of the treatment. Results revealed moderate weathering of the hydrocarbons, low initial concentrations of nitrogen and phosphorus, and the presence of appropriate microbial populations. Additional specific microbiological studies were carried out by cultivation of representative soil samples in a synthetic medium for selective enrichment, using kerosene as sole carbon source. The occurrence of several degrading microorganisms, able to attack both aliphatic and aromatic compounds in kerosene, was detected; subsequently, the most abundant of them were isolated, and identified after sequence analysis of 16S bacterial ribosomal DNA. Moreover, confocal laser scanning microscopy of these bacteria, stained with fluorescent indicators, revealed emulsion effects of the hydrocarbons and, interestingly, one of them (*Pseudomonas sp.*) showed the capability of producing a biosurfactant.

Given that the biotreatability study was successful, four different real-scale biopiles (80 m long x 5 m width x 80 cm depth) were implemented on-site. In short, all the treatments had in common frequency of tilling and watering in order to hold moisture content and oxygenation, while they were differenced in the dosages and types of biostimulants: i) no biostimulant, ii) slow-release fertilizer (SRF) to achieve a C:N:P ratio of 100:10:1 surfactant,

iii) SRF + surfactant, iv) oleophilic fertilizer. Monitoring of microbial diversity and activity were monthly done by direct plate counts and isolation of the predominant degrading microorganisms. These strains are now being compared with those isolated in the previous biotreatability study, with the final objective of understanding the evolution of microbial populations under different biostimulation conditions.

Chemical determinations were performed trying to distinguish between volatilization and biodegradation. In this sense, usually C_{17} /Pristane and C_{18} /Phytane ratios are used to determine the advances of the biodegradation, given the differential biodegradability of linear alkanes with respect to the isoprenoids (Gallego et al., 2006). However, kerosene has negligible contents of C_{16}^+ compounds and, therefore, the use of other isoprenoids such as Farnesane (2,6,10-trimethyl-dodecane) is a feasible alternative. As a result, we have defined a Biotransformation Index for Kerosene (BIK) using $n-C_{13}$ and $n-C_{14}$ abundances, with respect to Farnesane abundance. The use of this index to monitor biodegradation in the biopiles is shown in Figure 1.

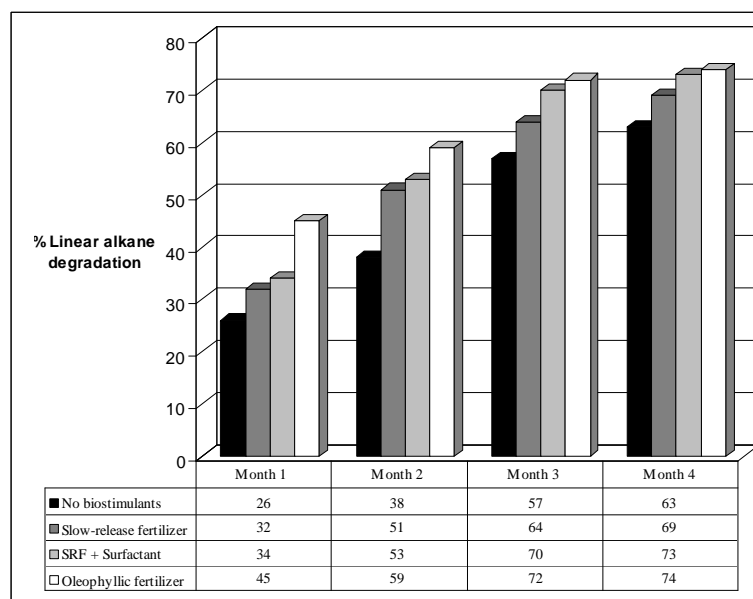


Figure 1. Biotransformation indices of linear alkanes (GC-MS) obtained in monthly sampling after the beginning of the biopile treatments (errors are within 5%). All along the treatment, linear alkane degradation was greater in the biopile where the oleophilic fertilizer was applied, although results in the four biopiles were convergent after four months.

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**BIOREMEDIATION OF 'THE HUMP': ASSESSMENT OF THE
BIODEGRADATION OF A TOXIC AROMATIC HYDROCARBON UNRESOLVED
COMPLEX MIXTURE (UCM) AND MEASUREMENT OF REMAINING
TOXICITY**

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Crude oil is a complex mixture of chemicals, many of which exhibit significant resistance to weathering processes (e.g. biodegradation) when released into the environment. Components of this resistant fraction which are typically unresolved by conventional analytical methods such as gas chromatography (GC) and often referred to as unresolved complex mixtures (UCMs) or 'humps', are toxic (e.g. to marine organisms such as mussels; Donkin *et al.*, 2003). Recent studies have used comprehensive two-dimensional GC time-of-flight mass spectrometry (GCxGC-ToF-MS) to resolve and characterise many thousands of the toxic compounds occurring in UCMs (Booth *et al.*, 2007a). Methods now need to be found to remove or reduce these toxic UCMs in the environment. One approach is use of bacteria to remediate the UCMs by biodegradation.

In the present study, an aromatic hydrocarbon UCM fraction was isolated from an in-reservoir degraded Venezuelan Tia Juana Pesado crude oil. The toxicity of the fraction was determined using a *Vibrio fischeri* Microtox[®] test and invertebrate toxicity investigated using feeding rate assays with mussels (*Mytilus edulis*). The toxic UCM was then subjected to aerobic biodegradation by a natural bacterial consortium enriched from marine sediments collected at Whitley Bay, UK, and previously reported to degrade alkylcyclohexyltetralins proposed as good models for some toxic aromatic UCM compounds (Booth *et al.*, 2007b). The microbial community composition was assessed by PCR-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) of the 16S rRNA gene. The biodegradation of some of the UCM hydrocarbons was determined and quantified using GC-mass spectrometry (GC-MS) and GCxGC-ToF-MS. The latter provided a technique for assessing which aromatic UCM components were susceptible to biodegradation by the consortium and which were still

resistant. The microbial consortium was indeed able to degrade some of the recalcitrant toxic oil fractions and thus seemingly has potential for 'hump' bioremediation.

However the toxicity of the resulting degraded fraction was again determined using the *Vibrio fischeri* Microtox[®] test and mussels. Thus, changes in toxicity resulting from the microbial alteration of the original aromatic UCM composition via biodegradation could be determined and the true potential for bioremediation, rather than bioalteration, assessed.

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