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Environmental bioremediation for organometallic compounds: microbial growth and arsenic volatilization from soil and retorted shale

R A Sanford* and D A Klein†
Departments of Microbiology and Environmental Health, Colorado State University, Fort Collins, Colorado 80523, USA

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Nutrient effects on microbial growth and arsenic volatilization from retorted oil shale and soil were evaluated in a laboratory study. Dimethylarsinic acid (DMAA), methanearsonic acid (MAA) and sodium arsenate amendments were used with added nutrients, or with retort process water added to simulate possible co-disposal conditions. In experiments with soil and retorted shale, dimethylarsinic acid showing the highest cumulative arsenic releases, in comparison with added inorganic sodium arsenate (SA). Low but detectable amounts of innate arsenic present in retorted shale could be volatilized with added organic matter. In soil, arsenic volatilization showed a direct relationship to nutrient levels and microbial growth. With shale, in comparison, a threshold response to available nutrients was evident. Distinct increases in fungal community development occurred with nutrients available at a level of 2.5% w/v, which also allowed increased arsenic volatilization. Co-disposal of retort process waters with shale allowed arsenic volatilization without the addition of other nutrients. The presence of retort process water limited arsenic volatilization from the added organometallic compounds DMAA and MAA, but not from SA or innate arsenic. These differences should be useful in the definition of permissive and non-permissive environmental conditions for arsenic volatilization in bioremediation programs.

Keywords: Arsenic, bacteria, bioremediation, energy residuals, fungi, organoarsenic compounds, retorted shale, soil, volatilization

INTRODUCTION

High concentrations of arsenic are found in waste materials from many industrial activities, including coal burning, smelting, mine-dump leaching and oil-shale retorting. Processed oil shale, as a specific waste, may contain arsenic at concentrations ranging from 32 to 65 $\mu g\,g^{-1}$.[1] High-temperature oil-shale retorting increases the mobility of many metalloids, including arsenic, and micro-organisms have the potential to mediate arsenic releases to the environment.[2] Inorganic arsenic in shales is commonly found as different ionic species of valence states As(III) or As(V), and organic species may also be found. The arsenic species cannot be predicted simply by using thermodynamic considerations and solubility relationships.[3,4] Redox, pH, adsorption and especially biological activity influence the types of specific arsenic compounds present and their mobility.

Microbial influences on arsenic transformations have been well documented. Challenger[5] identified trimethylarsine production by *Scopulariopsis brevicaulis* grown on breadcrumbs from arsenic trioxide, methanearsonic acid (MMA), dimethylarsinic acid (DMAA) and arsenite. Thom and Raper[6] similarly described trimethylarsine release from species of *Aspergillus*, *Penicillium* and *Fusarium*. Although these initial studies of arsenic biotransformations involved almost exclusively methylation processes carried out by fungi, aerobic and anaerobic bacteria have been found to methylate and demethylate arsenic.[7–11]

Reductive and methylative pathways for sodium arsenate (SA), MAA and DMAA to volatile forms from soils amended with soybean

*Present address: ECOVA, 3820 159th Avenue NE, Redmond, WA 98052, USA.
†Author to whom correspondence should be addressed.

meal have been documented[12] and volatilization has been observed to increase with increased solubility of the added arsenic compound.[2]

Earlier studies from this laboratory[2,13] have indicated that arsenic volatilization from retorted shales is microbially mediated, and dependent on the availability of a carbon and energy source. In addition, innate arsenic can be volatilized from retorted oil shales, with increased releases being observed under aerobic in comparison with anaerobic conditions.

The objectives of the present study were to evaluate more specific relationships between nutrient availability, microbial growth and aerobic arsenic volatilization from retorted shale and soil treated with SA and DMAA, and to evaluate microbial growth and arsenic volatilization relationships with simulated co-disposal of retort process water with retorted shale.

MATERIALS AND METHODS

Materials

Sodium arsenate (dibasic, 7-hydrate), potassium iodide, ammonium nitrate (NH_4NO_3) and iodine (sublimed, 99% purity) were acquired from the J. T. Baker Chemical Co., Phillipsburg, NJ, USA. Disodium methanearsonic acid (6-hydrate), dimethylarsinic acid (hydroxydimethylarsine-oxide, free acid) and fluorescein isothiocyanate were purchased from the Sigma Chemical Co., St Louis, MO, USA.

Paraho-I-1 retorted shale, with 45.0 $\mu g\,g^{-1}$ As, was collected during construction of successional plots at the Piceance Basin intensive study area (supervised by the Colorado State University Range Science Department).[14] This material was sieved using a 10-mesh (2 mm) screen. The soil used in these experiments was a sandy loam of neutral pH, also acquired from control plots at the intensive study area in the Piceance Basin of northwestern Colorado.[14]

Materials preparation

Leaching of retorted shales, to remove residual salts, was carried out by placing approximately 2 kg of material in a pipet washer over a Whatman No. 1 filter. Approximately 2 dm^3 of deionized water were then passed through the column. The material was removed and dried at room temperature.

Shale and soil were amended with differing levels of nutrients, provided as dextrose and ammonium nitrate, in a modified Bushnell–Haas basal-salts media (0.02 g $MgSO_4$, 1.0 g KH_2PO_4, 0.02 g $CaCl_2.2H_2O$, 1.0 g K_2HPO_4, 0.05 g $FeCl_3.6H_2O$ and 1000 cm^3 deionized H_2O). A C:N ratio of 2.94:1 was used in all amended samples. The following nutrient additions were made to 80 g of soil or shale material: 0.5 g, 1.0 g, 2.0 g, 3.0 g or 4.0 g total glucose plus ammonium nitrate. DMAA (44.8 $\mu g\,g^{-1}$ As) was added to soil and shale materials whilst SA (8.9 $\mu g\,g^{-1}$ As) was only added to shale samples. Soil was moistened with 24.0 cm^3 of basal-salts solution to approximate a water surface tension close to 30 kPa as was used previously.[2] In the co-disposal experiment DMAA, MAA and SA were added to provide a final arsenic concentration of 20 $\mu g\,g^{-1}$. The retort process water used in this study was obtained from Drs R. E. Sievers and M. Conditt of CIRES, the University of Colorado, Boulder, CO, USA. This material was used as a 50% v/v solution where required to provide a final water surface tension of 30 kPa. Soybean meal was used at a 5.7% w/w concentration in this experiment, to duplicate previous studies.[2]

Arsenic volatilization monitoring

The volatile arsenic compound collection apparatus[2,12] consisted of 500 cm^3 Erlenmeyer flasks which contained 80 g samples. This resulted in a retorted shale or soil depth of approximately 1.8 cm. The flasks were connected to 140 mm × 25 mm test tubes by use of glass and polyethylene tubing. Up to ten units were attached in parallel using rubber tubing to supply pre-moistened air at approximately 30 $cm^3\,min^{-1}$. The air flow in each unit was directed over the test material and bubbled through 20 cm^3 of 0.01 $mol\,dm^{-3}$ potassium iodide containing excess iodine in each tube. Neoprene stoppers were used on flasks and tubes, since rubber has been reported to adsorb volatile arsenicals.[15] All samples were incubated at 25°C and sampled at 3, 9 and 15 weeks of incubation.

Chemical analyses

Samples were analyzed for total arsenic at the Environmental Trace Substance Laboratory, University of Colorado, Denver, by Dr R. R. Meglen. A Model 5000 Perkin–Elmer atomic absorption spectrophotometer coupled to a Model 500 Perkin–Elmer graphite furnace and an

AS40 Perkin–Elmer autosampler were used. A nickel matrix modification procedure was utilized for arsenic quantitation.

Measurements of pH were taken at three-week intervals in each experiment. A Fisher Accumet pH-meter was used to analyze slurries of soil or shale and deionized water (1:1.5) with 1.0 g of material analyzed at each sampling.

Biological measurements

Estimates of bacterial numbers and fungal hyphal lengths were carried out microscopically, using 1 g samples removed during volatile-arsenic analyses. Each sample was taken with a sterile microspatula at the center and four additional points across the surface, and blended in 100 cm^3 of bicarbonate buffer (pH 9.6) for 2 min, using a modification of the procedure described by Babiuk and Paul.[16] From the blended solution, separate subsamples were taken for bacterial and fungal analyses. For bacterial enumeration, 10 μl of the solution were spread onto a 1 cm^2 area, using Bellco somatic cell count slides. The slides were dried and stained for 3 min with fluorescein isothiocyanate. Additional 1.0 cm^3 subsamples were placed in individual tubes, stained with 0.5 cm^3 phenolic Aniline Blue and placed as an agar film onto a microscope slide for direct measurement of fungal hyphal lengths.[17,18]

Table 1 Final pH values of shale and soil with dimethylarsinic acid (DMAA) and sodium arsenate (SA), and with nutrients added at five levels, after 15 weeks of incubation

	Shale		Soil
Nutrient level (%)	SA	DMAA	DMAA
0.63	8.5	8.4	6.2
1.25	8.2	8.2	6.6
2.5	8.2	8.0	7.0
3.75	7.9	8.0	6.9
5.0	7.9	7.6	6.5

Table 2 Percentage of total arsenic released from retorted shale and soil amended with varied levels of nutrients (glucose and ammonium nitrate) and treated with dimethylarsinic acid (DMAA) or sodium arsenate (SA) after 15 weeks

	Soil		Shale	
Total nutrients[a] (%)	DMAA	SA[b]	DMAA	SA
0.63	0.03	—	0.03	0.04
1.25	0.24	—	0.03	0.04
2.5	0.47	—	0.66	0.27
3.75	1.81	—	1.72	0.56
5.0	2.43	—	1.93	1.23

[a]Percentage based on total mass in grams of nutrient added to 80 g of test material. [b]No data.

RESULTS

The shale and soil pH values after 15 weeks of incubation depended on the nutrient levels added (Table 1). Initial pH values in the shales were approximately equal to 8.6, while the soil had a pH of 7.3. These values tended to change towards neutrality with increased nutrient levels. The values for control samples without nutrients did not change during the experiment. These pH values were not considered of themselves to limit microbial growth or arsenic volatilization processes.

Arsenic volatilization occurred in soil and shale with both DMAA and SA amendments, as shown in Table 2, which provides cumulative volatilization data for the 15-week incubation period. Arsenic treatment, material type and nutrient level had important influences on this process. At higher nutrient amendment levels (2.5–5.0%), DMAA-treated shale samples had higher arsenic releases. Considering all nutrient levels which were used, with soil as the volatilization matrix, a direct relationship between nutrient level and arsenic volatilization was observed whilst, with the shale materials, a distinct threshold nutrient level was required before increased volatilization was observable.

With soil and shale a large increase in cumulative arsenic release occurred between the 2.5% and the 3.75% nutrient levels (Table 2). Most of these volatile arsenicals were released during the first 3-week period of the experiment. In samples amended with water and without added nutrients, less than 0.01% of the added arsenic was volatilized with soils or retorted shale.

The microbiological measurements indicated that the bacteria and fungi showed different responses to arsenic in the two different materials, and in relation to available nutrients. In soil (Fig. 1), progressively higher fungal hyphal length values occurred at 3 weeks with increases in nutrient levels. Especially with the higher nutrient

levels, distinct decreases occurred over the remainder of the experiment, indicating that hyphal degradation had occurred. With shale samples, in contrast (Fig. 2), a nutrient addition level of at least 2.5% was required before distinct fungal hyphal length increases occurred. Also, the responses at a 2.5% nutrient addition level were much more distinct in retorted shale than in soil. At 9 and 15 weeks the hyphal-length values did not decrease as observed with soil. With sodium arsenate-treated shale a similar pattern was observed (Fig. 3), except that the maximum fungal response was delayed until the 9-week reading with the 2.5 and 3.75% amendment levels. Bacterial responses indicated a more gradual increase in relation to the increases in available nutrients. Higher bacterial populations were generally observed over the range of nutrient levels in soils (Fig. 4) than in shale (Figs 5 and 6), possibly due to decreased nutrient availability in soil nutrients or inhibition of bacterial development in this material.

Pearson correlation coefficient analyses, where all fungal hyphal-length and arsenic-volatilization data were compared, indicated an r-value of 0.75 at 3 weeks when all materials and treatments were considered (Table 3). However, this correlation decreased to $r=0.29$ by the 9-week assay, and 0.14 at the end of the experiment. This trend was also shown for the individual material types and arsenic treatments. Pearson correlation coefficients between arsenic volatilized and bacterial numbers were lower than observed with fungal hyphal-length values (data not shown).

Scattergram correlation coefficients of nutrient level with fungal hyphal-length measurements provided additional information on these relationships (Table 4). The strongest correlations, with both DMAA and SA, over the entire experiment occurred with the lower grouped nutrient level. The bacteria, in contrast, showed weaker relationships at 3 weeks, and especially later in the experiment at the lower nutrient level group. This suggests that arsenic releases from DMAA and SA attained between 0.63 and 2.5% total glucose and ammonium nitrate are strongly related to fungal biomass responses.

The addition of retort process water resulted in decreases in arsenic volatilization from DMAA and MAA, in comparison with samples without added retort water (Table 5). In contrast, the SA-treated and non-arsenic-amended samples showed increased arsenic volatilization with retort process water present. Retort process water

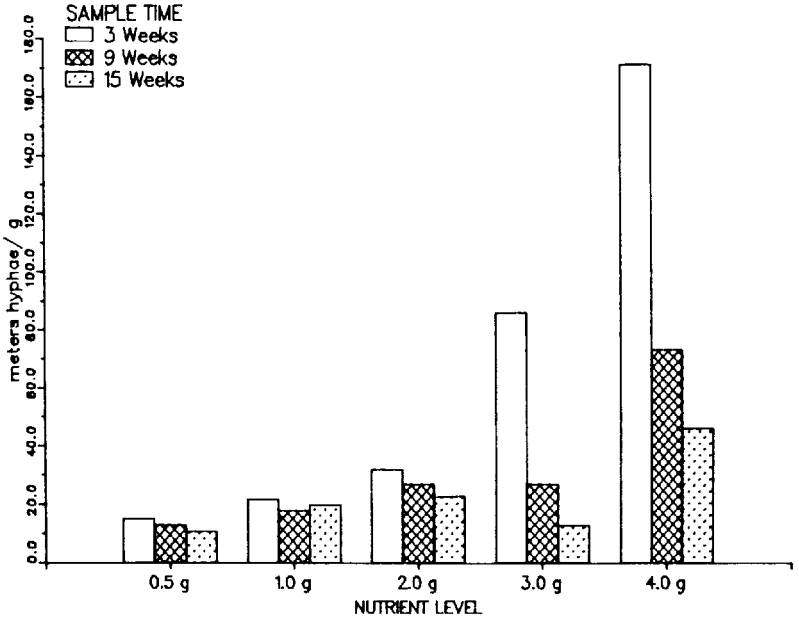

Figure 1 Fungal hyphal lengths at 3, 9 and 15 weeks from soil amended with varied nutrient levels and treated with dimethylarsinic acid (DMAA). Nutrient levels refer to the total mass of nutrients applied to 80 g of shale. On a percentage basis, 0.5 g = 0.63%, 1.0 g = 1.25%, 2.0 g = 2.5%, 3.0 g = 3.75% and 4.0 g = 5.0%.

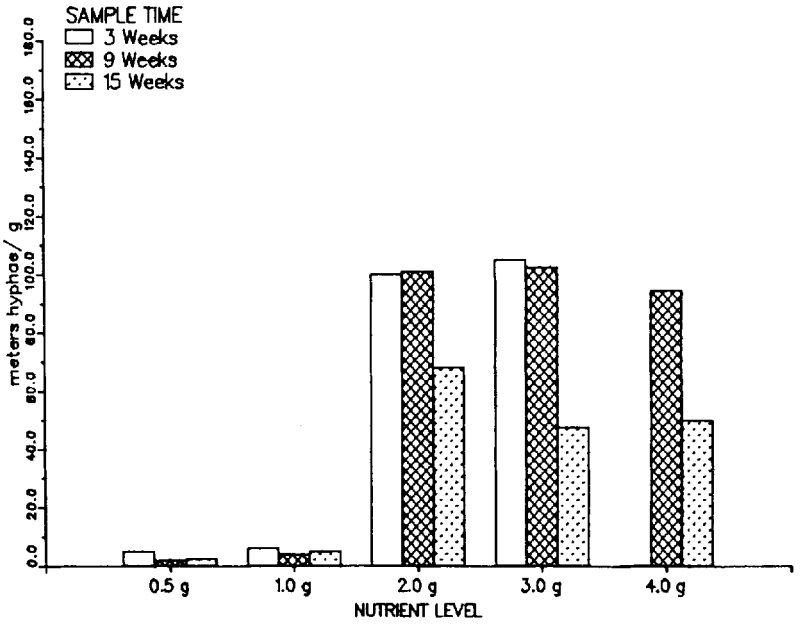

Figure 2 Fungal hyphal lengths at 3, 9 and 15 weeks from retorted shale amended with varied nutrient levels and treated with dimethylarsinic acid (DMAA). Nutrient levels refer to the total mass of nutrients applied to 80 g of shale, as in Fig. 1. The datum for the 3-week sample with 5% material added was not available.

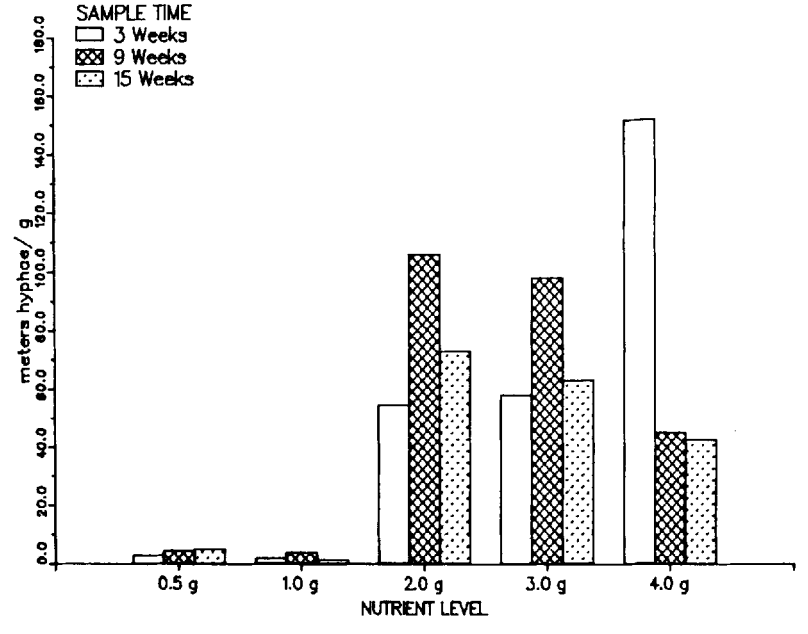

Figure 3 Fungal hyphal lengths at 3, 9 and 15 weeks from retorted shale amended with varied nutrient levels and treated with sodium arsenate (SA). Nutrient levels refer to the total mass of nutrients applied to 80 g of shale, as in Fig. 1.

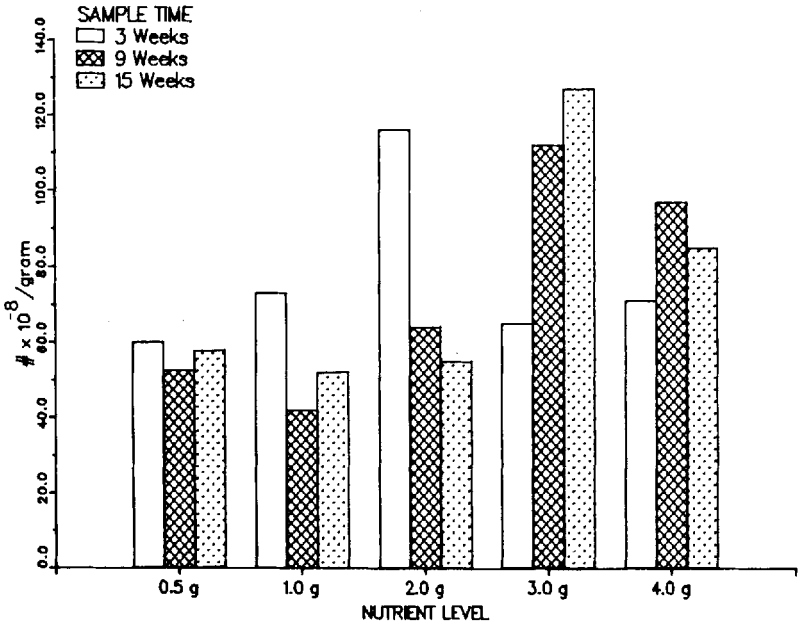

Figure 4 Number of bacteria at 3, 9 and 15 weeks from soil amended with varied nutrient levels and treated with dimethylarsinic acid (DMAA). Nutrient levels refer to the total mass of nutrients applied to 80 g of shale, as in Fig. 1. #, No. of bacteria.

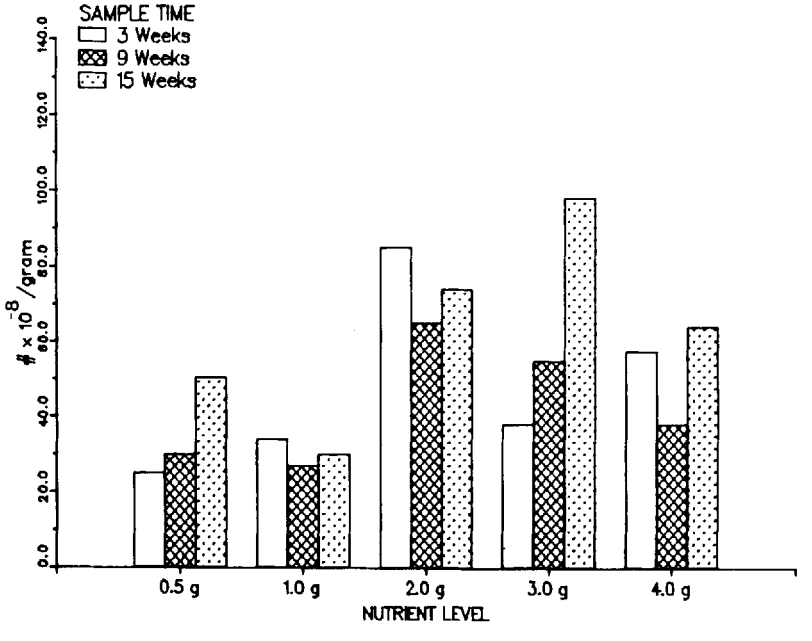

Figure 5 Number of bacteria at 3, 9 and 15 weeks from retorted shale amended with varied nutrient levels and treated with dimethylarsinic acid (DMAA). Nutrient levels refer to the total mass of nutrients applied to 80 g of shale, as in Fig. 1. #, No. of bacteria.

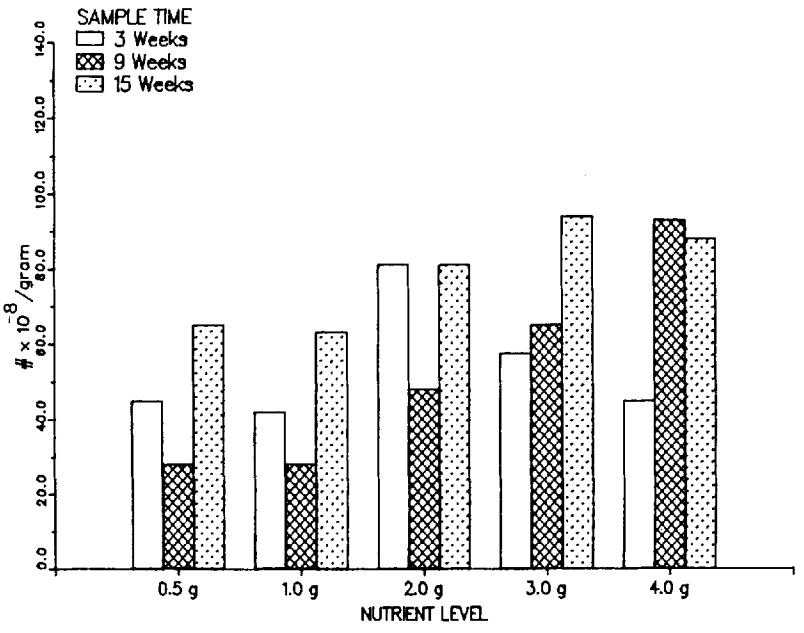

Figure 6 Number of bacteria at 3, 9 and 15 weeks from retorted shale amended with varied nutrient levels and treated with sodium arsenate (SA). Nutrient levels refer to the total mass of nutrients applied to 80 g of shale, as in Fig. 1. #, No. of bacteria.

Table 3 Pearson correlation coefficients between fungal hyphal lengths and cumulative arsenic release in soil and retorted shale amended with varied nutrient levels and treated with sodium arsenate (SA) or dimethylarsinic acid (DMAA)

Time (weeks)	Material type	Arsenic treatments	Pearson correlation coefficient		
			3 weeks	9 weeks	15 weeks
3	All	All	0.75		
	Soil	DMAA	0.93		
	Shale	DMAA	0.66		
	Shale	SA	0.87		
9	All	All		0.29	
	Soil	DMAA		0.70	
	Shale	DMAA		0.57	
	Shale	SA		0.43	
15	All	All			0.14
	Soil	DMAA			0.63
	Shale	DMAA			0.35
	Shale	SA			0.40

also allowed arsenic volatilization, which was not observed in shale materials amended only with water.

Fungal hyphal lengths in retorted process water-treated samples were lower with organo-arsenic amendments than in samples with sodium arsenate and nutrients present (Fig. 7). As a result, nutrient presence or absence did not stimulate arsenic volatilization with DMAA or MAA amendments. This may indicate a direct inhibitory effect of retort process water on fungal growth and DMAA and MAA volatilization.

Table 4 Scattergram r^2 values correlating biological response with cumulative arsenic release from different groupings of percentage nutrient values in grouped soil and shale samples treated with sodium arsenate (SA) or dimethylarsinic acid (DMAA)

Biological response and time	Arsenic treatment	Nutrient level group[a] 0.63–2.5%	3.75–5.0%
Fungal hyphal lengths			
3 weeks	DMAA	0.79	0.10
	SA	0.88	0.10
9 weeks	DMAA	0.60	0.02
	SA	0.91	0.31
15 weeks	DMAA	0.66	0.00
	SA	0.98	0.37
Bacteria			
3 weeks	DMAA	0.58	0.04
	SA	0.51	—
9 weeks	DMAA	0.14	0.06
	SA	0.50	—
15 weeks	DMAA	0.08	0.03
	SA	0.06	—

[a]Percentage based on amount of nutrients added to 80 g of sample.

Table 5 Percentage total arsenic released from retorted shale samples with and without soybean meal and/or Paraho process water (50% v/v) after 15 weeks: treatments of dimethylarsinic acid (DMAA), methanearsonic acid (MAA) and sodium arsenate (SA) were used, in comparison with non-arsenic-treated samples[a]

Retort water added	Nutrients added	Arsenic treatment DMAA	MAA	SA	None
+	+	0.02	0.03	0.16	0.14
−	+	1.19	0.74	1.13	0.15
+	−	0.07	0.02	0.03	0.06
−	−	<0.01	<0.01	<0.01	0.01

[a]When amended with soybean meal at 5.7% w/w.

Microscopic measurements of bacterial numbers indicated that with nutrients plus retort process water, bacterial development was not inhibited, as occurred with the fungi (Fig. 8). This inhibition of fungal development also was observed with the SA and non-arsenic-amended samples when process water was present.

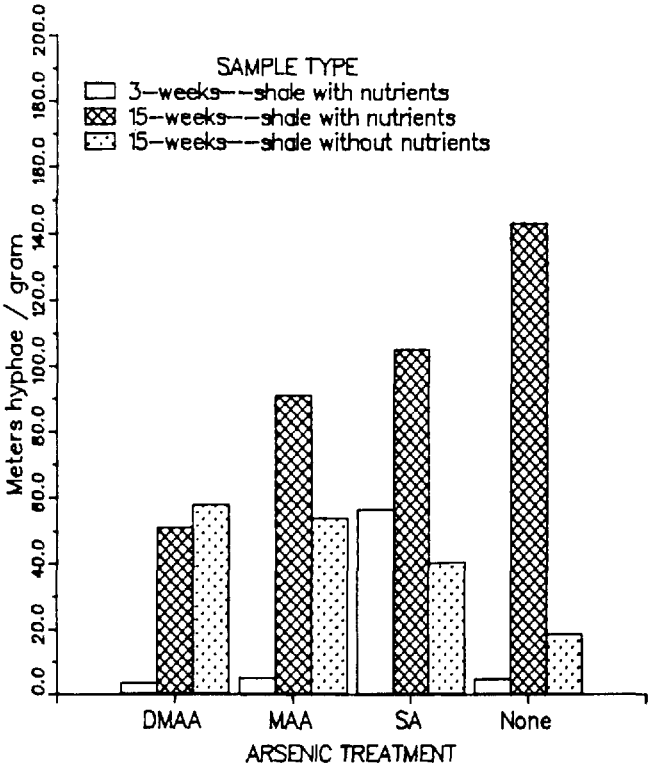

Figure 7 Fungal hyphal lengths at 3 and 15 weeks from retorted shale amended with Paraho process water (50% v/v) and with and without soybean meal (5.7% w/w). Arsenic treatments of dimethylarsinic acid (DMAA), methanearsonic acid (MAA) and sodium arsenate (SA), or samples without arsenic were used.

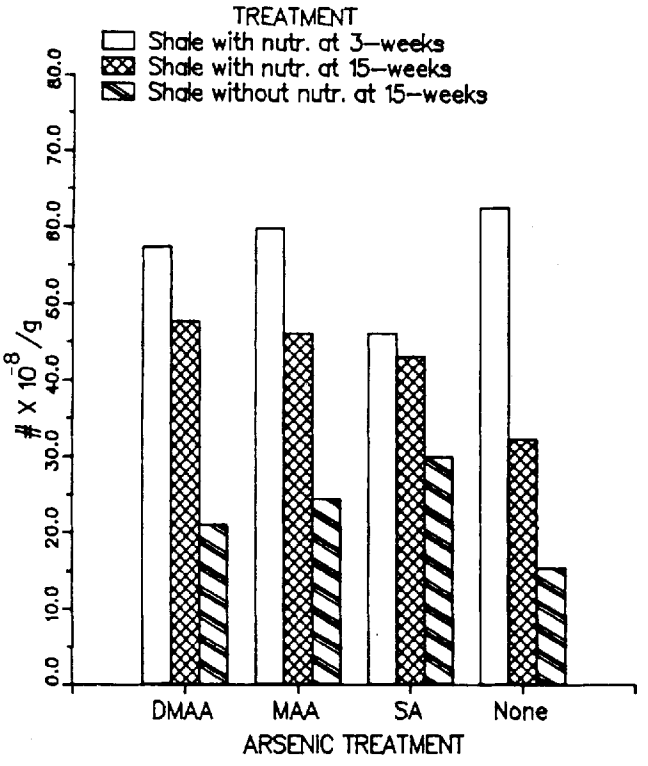

Figure 8 Number of bacteria at 3 and 15 weeks from retorted shale amended with Paraho process water (50% v/v) and with and without soybean meal (5.7% w/v). Arsenic treatments of dimethylarsinic acid (DMAA), methanearsonic acid (MAA) and sodium arsenate (SA), or samples without arsenic were used. #, No. of bacteria.

DISCUSSION

The nutrient level–microbial growth–arsenic volatilization relationships in soil and in retorted shale provided valuable insights into relationships between microbial development and arsenic volatilization in these different materials. In soil the amount of arsenic released was correlated with the nutrient level added; however, this did not appear to be a linear relationship. The volatilization responses in shale were not directly related to the bacterial numbers or the fungal hyphal lengths, as similar populations are observed over the nutrient range 2.5–5.0%, but significant differences in volatile-arsenical production were evident. This would suggest that although essentially maximum populations occurred with 2.5% added nutrient, the development of this population does not allow sufficient excess energy to be available to give maximum volatilization. This maximum, which occurs at 3.75% added nutrients, was followed by decreased volatilization at the highest nutrient addition level in the retorted shale but not in soil. Based on microbial populations, it was not possible to provide an explanation for this inhibition.

In contrast, bacterial numbers did not show these extensive changes or nutrient response thresholds, as a gradual increase in volatilization occurred with higher added nutrient levels. Based on these microbial population–arsenic volatilization relationships in shale, it would appear that fungal growth is predominantly responsible for arsenic volatilization in this material at higher nutrient levels, whilst bacteria would play a more important role under conditions of lower nutrient availability. These differences may reflect the varied threshold responses of bacteria and fungi to nutrients,[19] their differences in substrate exploration strategies[20] and the role of nutrient availability in influencing microbiostasis processes.[21]

This observation supports the hypothesis of a limiting factor being reached, at least in the case of the microbial responses in the shale material.

The differences in arsenic volatilization which occurred when SA- and DMAA-treated responses were compared may be indicative of different energy requirements for the reductive methylation of each compound to occur. Since DMAA is already methylated, the volatilization of this compound may require relatively little energy, whilst SA would require a methylation step prior to volatilization, and a correspondingly greater energy requirement. Also, the bacterial and fungal responses may differ, depending upon the arsenic treatment. For example, SA may be more detrimental to biological activity than DMAA, thus requiring a higher nutrient level to allow similar population responses and arsenic volatilization.

Although it is unlikely that nutrients might be present in natural environments on an all-over level of 2–3%, as used in this study, soil systems or reclaimed areas can contain microenvironments with much higher localized organic matter levels, presumably approaching these concentrations. These organic constituents can include distributed heterogeneously plant fragments,[22] microbial carbon dioxide incorporated into microbial biomass (as can occur with sulfur-oxidizing *Thiobacillus* species)[23] or organic exudates released from plant roots.[24]

The ability of retort process water to stimulate arsenic volatilization from innate arsenic, while inhibiting these processes with added organic matter and especially from DMAA and MAA, has important implications for the management of environmental arsenic volatilization processes.

The problems of arsenic management in surface soil and subsurface environments are emphasized by these results. Although the amounts released and the release rates would be considered as minimal, continuous low-level fluxes of volatile arsenic compounds from waste areas, and especially with co-disposal of retort process waters[25] (in which organoarsenic compounds have been observed[26]), could occur with construction of large surface storage structures.[25] This could lead to continuing low-level releases of arsenic to the environment in soluble and volatile forms. If microbial growth occurs with sufficient moisture and nutrients available, increased arsenic solubilization,[2] as well as volatilization, can occur. Environmental management of arsenic-containing residues will require the clear definition of permissive versus non-permissive environmental conditions for microbial growth. With such information available, it should be possible to manage residual arsenic more effectively, including organoarsenic compounds found in retorted shales[26] in integrated bioremediation programs.

Although strategies for the management of degradation of recalcitrant organic molecules are being developed,[26] the development of similar bioremediation strategies for the management of metals,[27] and particularly for organometallic compounds of environmental interest, remains a continuing need. Hopefully, this and similar studies will assist in the development of criteria for environmental management and bioremediation related to organoarsenic compounds.

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