Bull. Environ. Contam. Toxicol. (1989) 42:395–401

Environmental Contamination and Toxicology

Effects of Two Diesel Fuel Mixtures on Fecal Coliform Bacteria Densities

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One of the major potential environmental impacts from synthetic fuel production plants and conventional petroleum refinement operations is the spillage of the refined product into natural waters. Impacts upon aquatic ecosystems resulting from spills of synthetic fuel would likely be different from those associated with conventional petroleum since products extracted from coal or shale are generally richer in phenolics, aromatic amines and other soluble organic compounds. Also, synfuels have higher water solubilities than equivalent petroleum products giving the potential for higher water concentrations of hydrocarbons.

At the beginning of this decade, about 90% of the energy used by the United States came from oil, coal, and gas with oil furnishing about 50% of that amount (Schriesheim and Kirshenbaum 1981). Synfuels have historically been prohibitively expensive relative to conventional petroleum products. However, a recent report (Lumpkin 1988) indicates that improved technology has reduced the cost of coal liquefaction fuels to an equivalent of $35 per barrel for crude oil. Substantial information exists concerning impacts related to conventional petroleum spills (Evans and Rice 1974; Moore and Dwyer 1974) but there is less information concerning the impacts of synfuels in the aquatic environment (Delistraty 1984; Ghassemi et al. 1981; Giddings et al. 1985). Even less information exists comparing impacts of conventional petroleum to synthetic petroleum products on bacterial densities (Adams and Farrier 1982; London et al. 1984; Segal and Mancinelli 1987).

This study tested the effects of the water soluble fractions (WSFs) of a shale diesel fuel mixture (SDFM) and a petroleum diesel fuel mixture (PDFM) on the growth of fecal coliform bacteria, the group used almost universally as an indicator of bacteriological water quality. The WSF was tested instead of whole oil because acute toxicity results primarily from this fraction (Rice et al. 1977). A wild group of fecal coliform bacteria was used since the objective was to observe effects upon this indicator group encountered in the environment instead of pure laboratory cultures by the routine ambient monitoring and

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measurement technique of membrane filter colony counts as employed by most water quality management agencies.

MATERIALS AND METHODS

Two different assays were performed using the shale and petroleum mixtures. In both assays, the WSFs of each oil were prepared according to the method of Giddings and Washington (1981). For the growth assay, the WSFs from the SDFM and PDFM were combined with portions of domestic wastewater effluent to yield percentage (by volume) WSF mixtures of 40, 20, 10, and 1% (40 WSF, 20 WSF, etc.). The same percent WSFs of the two fuel types were used for the toxicity assay but were formulated using sterile phosphate-buffered water (APHA 1985) instead of effluent as the diluent. Each replicate mixture was inoculated with 1.0 mL of a fecal coliform culture isolated from the same wastewater used in the growth assay. Samples were also included at 100 WSF and 0 WSF in both assays to serve as negative and positive controls, respectively.

The domestic wastewater treatment facility consisted of a mechanically-aerated oxidation lagoon with no effluent disinfection and no industrial waste. During the time shortly prior to and after this study, the discharge from this facility ranged from 0.073 million gallons per day (mgd) to 0.232 mgd. Dissolved oxygen ranged from 3.5 mg/L to 8.0 mg/L while the five-day biochemical oxygen demand typically was 43 mg/L to 97 mg/L. Total suspended solids varied from 53 mg/L to 145 mg/L and pH from 4.5 standard units (su) to 8.7 su. Fecal coliform bacteria densities ranged from 6000/100 mL to 11,000/100 mL.

Triple replicates of 10 mL total volume per replicate were incubated at 25.0 + 0.5 C. The fecal coliform membrane filter technique was used in all tests (APHA 1985). Appropriate aliquots yielding a countable plate from all triplicates were vacuum filtered through 0.45 um pore-size membrane filters (GN-6, Gelman, Ann Arbor, Michigan), and then cultivated on MFC medium (Difco, Detroit, Michigan) for 24 hr in a circulating water bath at 44.5 $\pm$ 0.2 C. Analyses of significant differences in mean densities ($p=0.05$) between control and exposure groups and between SDFM and PDFM exposure groups were made using the Wilcoxon Rank Sum Test and the Kruskal-Wallis H Test (SAS 1985; Wilcoxon and Wilcox 1964). All bacterial densities were transformed from arithmetic values of log10 values.

RESULTS AND DISCUSSION

The PDFM showed significantly lower densities than the SDFM at most culture times in higher WSFs. (Figure 1) The only instance of a significant difference between PDFM and SDFM in the two lower WSFs was at 48 hr in the 10 WSF. The control exhibited typical increases at 12 hr and at 24 hr before a reduction at 48 hr, as expected from a normal growth curve. All negative control samples (100 WSF) exhibited no growth at all culture times. Both fuel

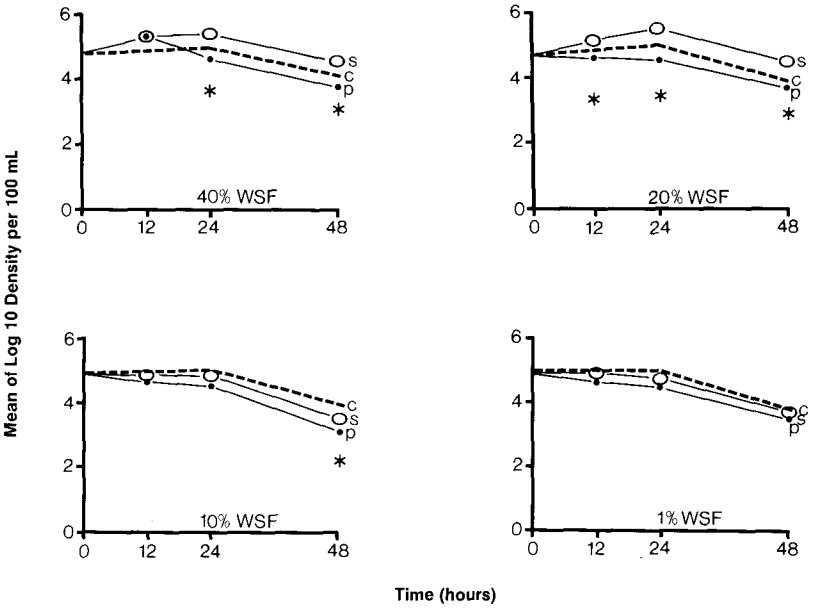

Figure 1. Fecal coliform bacteria densities by percent water soluble fraction (WSF) of shale (s) and petroleum (P) diesel fuel mixtures from growth assay. (C = control; * = significant difference between fuels at $p<0.05$; n=3; both fuels always significantly different from control).

mixtures exhibited significantly less bacteria than the control at all times in the 10 and 1 WSFs. Furthermore, the PDFM showed significantly lower densities than the control at 20 WSF and at 40 WSF, except at 12 hr. The SDFM exhibited significantly higher densities than the control at these two WSFs.

Data from the toxicity assay generally followed the results obtained from the growth assay and suggested that the SDFM supported fecal coliform growth better than the PDFM at the 40 and 20 WSFs, and to some extent, at the 10 WSF (Figure 2). In most cases, both mixtures exhibited densities less than the control. The exceptions were in the 40, 20 and 10 WSFs of the SDFM at 48 hr. As the WSF decreased from 40 to 1%, the difference in densities between the fuels also decreased until both showed the same levels at 1 WSF.

The WSF of the petroleum mixture appeared to provide less growth support from the bacteria than that of the shale mixture. This was evident from both assays as densities from the PDFM were always less than or equal to that of the SDFM. In no instance did the PDFM exhibit higher counts than the SDFM. There was some evidence in the toxicity assay of biostimulation of the bacteria by the SDFM to densities greater than the control as evidenced by

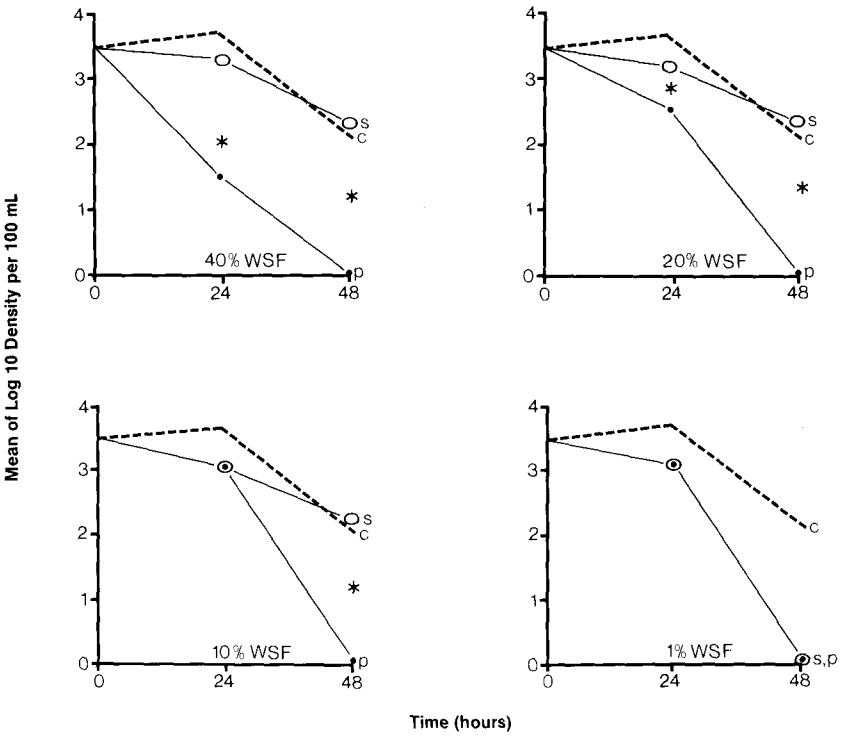

Figure 2. Fecal coliform bacteria densities by percent water soluble fraction (WSF) of shale (s) and petroleum (P) diesel fuel mixtures from toxicity assay. C=control; *=significant difference between fuels at $p < 0.05$; $n=3$; both fuels always significantly different from control).

the levels observed in the 40, 20 and 10 WSFs at 48 hr (Figure 3). This observation was supported by the growth assay as the densities from the SDFM exceeded the control densities at 48 hr in the 40 and 20 WSFs. Likewise, the higher densities obtained from the SDFM over the PDFM are clearly shown in Figure 3. These results generally followed those observed by London et al. (1984) and Gauger and Williams (1987).

It is noteworthy that although the counts increased over control levels at certain WSFs, when these increases occurred they were in the SDFM. Also, the SDFM typically exhibited significantly higher densities than the PDFM. This suggests that the SDFM may have been different in chemical nature than the PDFM. Chemical analyses of the total phenol and total organic carbon content of each 100 WSF of the fuel mixtures confirmed this (Perry MJ, MSPH Thesis, Univ. South Carolina, Columbia, SC). Both mixtures had similar phenol concentrations (2.90 mg/L for PDFM; 2.40 mg/L for SDFM) but very different organic carbon content (45.4 mg/L for PDFM; 2432.0 mg/L for SDFM). The phenol and TOC contents of the fuel mixtures in each WSF used in the assays are presented in Table 1. The phenol content as a percent of TOC was 6.4% for the PDFM and 0.1% for the SDFM. This suggests that the differences observed in the densities between the fuel types may have been due

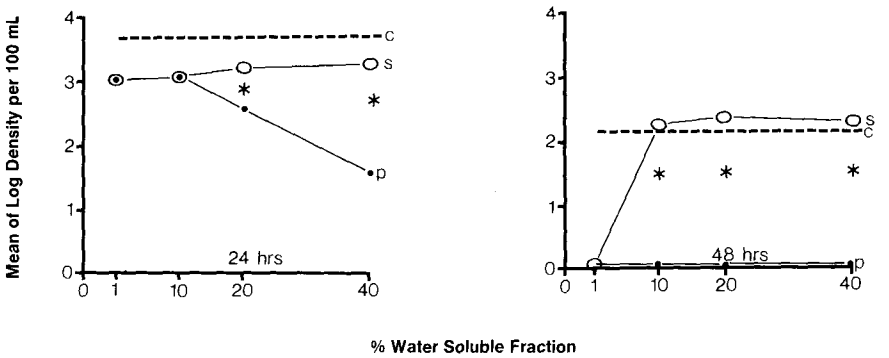

Figure 3. Fecal coliform bacteria densities by culture time from toxicity assay with shale (s) and petroleum (P) diesel fuel mixtures. (C=control; *=significant difference between fuels at p <0.05; n=3; both fuels always significantly different from control).

Table 1. Total organic carbon (TOC) and total phenol (TP) concentrations in the water soluble fraction (WSF) of a petroleum (PDFM) and shale (SDFM) diesel fuel mixture.

Component	Fuel Type	Concentrations in mg/L at % WSF 100	40	20	10	1
TP	PDFM	2.90	1.16	0.58	0.29	0.03
	SDFM	2.40	0.96	0.48	0.24	0.02
TOC	PDFM	45.4	18.16	9.08	4.54	0.45
	SDFM	2432.0	972.8	486.4	243.2	24.3

to phenol toxicity; however, this cannot be strongly supported given the essentially equal absolute concentration of phenol between the two mixtures.

Data from these experiments demonstrated that both fuel mixtures affected the normal densities of fecal coliform bacteria. The PDFM appeared to be toxic to the bacteria while the SDFM appeared to provide some level of biostimulation or mediation against toxicity to yield higher densities than the PDFM and, often, the control culture. While the basic chemical characteristics of the mixtures suggest that differences in phenol content as a percent of TOC may be a factor, the much larger TOC content of the SDFM and its known higher level of aromatics (Ghassemi and Iyer 1981) likely play an important role. Caution must be observed, however, in attribution of growth differences solely to fuel type since London et al. (1984) have shown that the composition of individual fuels is more important than the fuel type.

The release of substances similar to the SDFM into the environment

such as from synfuel conversion plants may stimulate bacterial growth over natural levels giving an erroneous indication of the introduction of fresh fecal material into the environment. Similar effects may result from accidental spills, the normal loss from production areas, losses during transportation and shipping, and runoff via nonpoint sources. As such, careful consideration should be given to the siting of synfuel production plants. In fresh waters, the contamination of a watershed or a portion of a watershed could occur. In estuarine waters, the contamination of shellfish beds would be possible. Where fecal coliform bacteria densities are already elevated, the introduction of a shale DFM may augment those undesirable levels. Finally, these data suggest that discharges of synthetic shale fuels may have a greater impact on natural fecal coliform bacteria densities than their conventional petroleum analogs.

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Received March 14, 1988; accepted July 16, 1988