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ENVIRONMENTAL FATE AND EFFECTS OF JET FUEL JP-8

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The fate of jet fuel JP-8 was studied in quiescent flask test systems containing water, water/sediment slurries and soil. For each treatment, killed samples were compared to active samples to assess the relative contributions of biodegradation and volatilization in removing JP-8. At appropriate time intervals, flasks were extracted with CS_2 and analyzed by gas chromatography/mass spectrometry. In water and water/sediment slurries, the major removal process was evaporation. No significant differences were noted between active and sterilized flasks, indicating that biodegradation was not a major factor in removal of JP-8 under these test conditions. When removal in water alone was compared to removal in water/sediment slurries, greater losses were observed in water alone, indicating that the presence of sediment sequesters the jet fuel rendering it less susceptible to volatilization. Removal of JP-8 from active soil was not significantly different from removal in sterilized

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19. Abstract (continued)

soil, indicating that in soil as well as water, biodegradation does not play a significant role in removal of jet fuel. Toxicity of JP-8 to microorganisms was assessed by measurement of glucose and hexadecane mineralization. Microbial activity in water was inhibited by JP-8 whereas activity in water/sediment slurries was enhanced by addition of JP-8.

EXECUTIVE SUMMARY

A. OBJECTIVES

The objective of this research was to examine the fate of JP-8 in aqueous and terrestrial environments. Accidental releases of jet fuel are unavoidable consequences of Air Force operations. Surface spills may occur during fueling operations or during transfer of fuel from tankers to storage tanks. Underground release of fuel into the surrounding soil may occur as a result of leakage from underground storage tanks. This type of release may go undetected for considerable periods of time and has the potential to contaminate large quantities of soil and groundwater.

Upon release to the environment, jet fuels are immediately subjected to physical and biological processes which redistribute and/or remove the fuel from the point of release. Aqueous solubility, evaporation, adsorption and biodegradation are the major processes which will affect the fate and transport of jet fuel. Of these, only evaporation and biodegradability will result in loss of the hydrocarbons from the point of release, and only biodegradation will result in the complete destruction of the hydrocarbons. Information on the biodegradability of the jet fuel is therefore essential for an assessment of the environmental fate of spilled fuel.

B. BACKGROUND

Jet fuels are complex mixtures of hydrocarbons. When exposed to the environment, these hydrocarbons are partitioned into environmental compartments according to their physical properties. They may evaporate, they may dissolve in water and be dispersed into the water column, they may absorb onto particles present in sediment or soil and they may be subject to degradative processes.

Evaporation of organic chemicals from surfaces can be related to the compound's vapor pressure and molecular weight. Since this decreases with increasing molecular weight for any homologous series of hydrocarbons, low-molecular-weight hydrocarbons should exhibit relatively rapid evaporative loss. Compounds such as octane, benzene and toluene are lost within hours of a spill, while significant amounts of substituted naphthalenes and alkanes such as hexadecane will persist for as long as 20 days after the spill.

Dissolution of hydrocarbons in water also decreases with increasing molecular weight. Both evaporation and solubility will be greatest for the lower molecular weight hydrocarbons. These two processes will be in competition for the hydrocarbons. Evaporative losses have been calculated to be two orders of magnitude greater than dissolution rates for soluble aromatic hydrocarbons and four orders of magnitude greater for the less readily soluble n-alkanes.

The presence of solid particles, whether as suspended sediments in water or minerals in soil, complicates the above picture because of the process of adsorption, the tendency of a compound to be associated with solid particles. Properties of the organic compound such as water solubility and the tendency to partition into an organic solvent are important in determining adsorption. Equally important are properties of the solid phase including

particle size, organic matter content and mineral fraction.

Those components which persist more than a few hours will become subject to biodegradation. Biodegradation, a process mediated by microorganisms, can convert the hydrocarbons into carbon dioxide and water. The susceptibility of hydrocarbons to biodegradation depends on the hydrocarbon type, straight chain alkanes and simple armatics being more susceptible to biodegradation than branched or cyclic alkanes and complex aromatics. Since biodegradation is microbially mediated, the number of hydrocarbon-degrading bacteria present in a given site will influence biodegradation rate. Sites which have a history of hydrocarbon contamination often demonstrate higher initial biodegradation rates. This phenomenon, termed acclimation, has been related to a larger population of hydrocarbon-degrading bacteria because of prior exposure to the hydrocarbon.

Studies specifically addressing the biodegradation of hydrocarbons in JP-4 have shown that evaporation was the major removal process for the low- molecular-weight, volatile hydrocarbons. Addition of sediment to water samples affected the removal of some hydrocarbon components of JP-4 and the model fuel by reducing the rate of volatilization. For most individual hydrocarbons, biodegradation was not as significant for removal as was evaporation. In some water samples, certain hydrocarbons, such as decane and naphthalene, disappeared at rates significantly greater in the active treatments than in the killed treatments, indicating that biodegradation was occurring in these water samples. However, the extent of biodegradation differed among water samples.

C. SCOPE

The results of previous studies suggested that volatilization was the major process for removal of jet fuels from the environment. Accordingly, the quiescent bottle test was selected as the test method because it would minimize evaporative losses. Since sediment influences the fate of JP-4, treatments containing water alone and water plus sediment were included in the experimental design. In addition to bottles receiving JP-8 as test fuel, bottles treated with JP-4 were included to serve as positive controls. Finally, a series of bottles containing soil were included in this study. Fuel spills and leaks from storage tanks may contaminate the soil as well as the aquatic environment and information on removal from soil would be useful for the assessment of the environmental fate of JP-8. In all studies, samples treated with mercuric chloride were included to compare biological with nonbiological removal processes.

Furthermore, evidence from previous studies indicated that biodegradation was lower in sediment treatments than in water treatments. These results suggested that either toxicity to biodegradative organisms might be occurring or that adsorption to sediment might render the hydrocarbons less available for biodegradation. A toxicity study was therefore included in the study in order to aid in the interpretation of the biodegradation test results.

D. METHODOLOGY

The quiescent-bottle technique was selected as an appropriate technique to monitor the relative rates of evaporation and biodegradation. In this technique, water samples are placed in a square bottle and fuel is added. Bottles are incubated undisturbed on their sides with the caps removed to permit volatilization of the hydrocarbons. At intervals, flasks were extracted with solvent and the extracts were analyzed by gas chromatography-mass spectrometry. This method permits the separation, identification and quantitation of individual hydrocarbon components in jet fuel.

E. TEST DESCRIPTION

For these experiments, water and sediment were collected from a brackish bayou located on Tyndall Air Force Base. JP-8 was incubated under four conditions: (1) water alone; (2) sterile water treated with $HgCl_2$ to kill microorganisms; (3) water supplemented with sediment; (4) water supplemented with sediment as in (3), but sterilized with $HgCl_2$. The tests were conducted in square bottles to which JP-8 (1 percent) was added. After an initial period of shaking to mix the contents of the bottle, they were incubated in the horizontal position in an undisturbed condition. Soil incubations were conducted in a similar fashion. After the appropriate time interval, the remaining fuel was extracted from water, water/sediment slurries or soil by the addition of CS_2 followed by a period of shaking. Extracts were analyzed by high-resolution capillary gas chromatography with mass selective detection.

Toxicity studies were undertaken with JP-8 using water and water supplemented with sediment collected from the same location as for the biodegradation study. The water or water/sediment was dispensed into Erlenmeyer flasks which received either no JP-8, or 0.01 percent, 0.1 percent or 1 percent JP-8. Samples were removed at 0, 1, 2 and 4 days and assayed for toxicity to the general microbial community and effects on the hydrocarbon-degrading portion of the population.

F. RESULTS

Significant loss of jet fuel from water samples occurred over the experimental period. This was due to evaporation as fuel in both active and sterile treatments disappeared at the same rate and to the same extent. Loss of components was related to molecular weight and vapor pressure, with low molecular weight components being removed by day 10 and high molecular weight components persisting to the end of the experimental period.

When sediment was added to the water samples, fuel disappeared at the same rate and to the same extent in active as in sterile treatments, indicating that biodegradation did not play a major role in the removal of JP-8. Statistical analysis indicated that only for 1- and 2-methylnaphthalene was disappearance faster in active treatments
greater than for sterile treatments. Rate of removal of JP-8 from water/sediment slurries was much slower than in the case of water alone. Many of the low molecular weight components which had disappeared by day 10 from water persisted in the water/sediment

slurries. The presence of sediment, therefore, retarded evaporation and inhibited the removal of jet fuel.

The concentration of jet fuel decreased in soil over the experimental period, all but the high molecular weight components disappearing by the end of the experiment. Statistical analysis of the disappearance curves indicated that n-nonane, n-undecane, n-dodecane and 2-methylnaphthalene showed significantly faster disappearance in the active treatments than the sterile treatments. Thus, biodegradation played a limited role in removal of jet fuel components, and was component specific.

Toxicity tests were designed to measure effects of jet fuel on general microbial activity and on hydrocarbon-degrading ability of the microbial population. Addition of JP-8 to test flasks depressed general microbial activity at all concentrations tested, although the lowest concentration demonstrated recovery. JP-8 depressed hydrocarbon degradation in all but the lowest test concentration. Addition of sediment to the flasks reduced the toxicity of JP-8 to the general microbial population, but hydrocarbon-degrading activity was inhibited.

G. CONCLUSIONS

The major removal process of JP-8 in the aquatic environment is evaporation. The more volatile components of the fuel evaporated within the initial 5 days of the experiment; significant removal of all components occurred by the end of the experiment. Some components were still present in significant amounts at the end of the experimental period, particularly n-alkanes such as tetradecane, pentadecane and hexadecane. Addition of sediments to water inhibited the evaporative removal of JP-8, apparently by adsorbing the components of JP-8 thus rendering them unavailable for evaporation.

One possible explanation for the lack of biodegradation of JP-8 in water samples is the toxicity the fuel exerts towards microorganisms. The concentration of fuel used in the quiescent bottle test (1 percent) was inhibitory to microbial heterotrophic activity and hydrocarbon-degrading activity. Thus the persistence of some components of JP-8 until the end of the experimental period may be due to severe inhibition of microbial activity within the test bottles.

As measured by glucose mineralization, JP-8 was not toxic to sediment microorganisms. Nonetheless, hydrocarbon-degrading capabilities of the population were below the detection limit even in the control flasks. One possible explanation may be that sediment-hydrocarbon binding may sequester the hydrocarbon, making it less available for microbial metabolism.

Biodegradation contributed to the removal of JP-8 from the terrestrial environment. Eight of the components disappeared faster in the active treatments than in the sterile treatments. For other components, there was a reduction of the slope in active treatments, but not to a statistically significant extent within the experimental design. This suggests that manipulation of conditions to enhance biodegradation may increase the rate of removal of JP-8 from the terrestrial environment. This agrees well with literature reports on land farming of waste hydrocarbons and the results of a recent study on the biodegradation of JP-4 in a contaminated aquifer. These authors confirmed our findings that biodegradation was compound-specific.

H. RECOMMENDATIONS

Because sorption on sediments reduced evaporation, remedial actions which increase the contact between fuel and sediment should be investigated further.

Biodegradation can contribute to the removal of some components of JP-8 in the soil. Strategies to enhance biodegradation in this environment, such as fertilization and aeration, may be useful in achieving maximum rates of removal of JP-8. Further investigation of enhanced biodegradation seems warranted.

JP-8 showed less of a potential for biodegradation than JP-4. This may be due to increased toxicity of the fuel to microorganisms. Further investigation to determine the toxic components of JP-8 is recommended.

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PREFACE

This research was conducted as part of the 1989 USAF-Universal Energy Systems Summer Faculty Research Program, administered by Universal Energy Systems under Contract No. F49620-88-C-0053.

This report covers work performed between June 1989 and August 1989. The HQ AFESC project officer was Mr. Jim Spain.

This report has been reviewed by the Public Affairs Office (PA) and is releasible to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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SECTION I
INTRODUCTION

A. OBJECTIVES

The objective of this research was to examine the fate of JP-8 in aqueous and terrestrial environments. Accidental releases of jet fuel are unavoidable consequences of Air Force operations. Surface spills may occur during fueling operations or during transfer of fuel from tankers to storage tanks. Underground release of fuel into the surrounding soil may occur as a result of leakage from underground storage tanks. This type of release may go undetected for considerable periods of time and has the potential to contaminate large quantities of soil and groundwater.

Upon release to the environment, jet fuels are immediately subjected to physical and biological processes which redistribute and/or remove the fuel from the point of release. Aqueous solubility, evaporation, adsorption and biodegradation are the major processes which will affect the fate and transport of jet fuel. Of these, only evaporation and biodegradability will result in loss of the hydrocarbons from the point of release, and only biodegradation will result in the complete destruction of the hydrocarbons. Information on the biodegradability of the jet fuel is therefore essential for an assessment of the environmental fate of spilled fuel.

A considerable amount of information is available on the fate and transport of JP-4. Jet fuel JP-8, however, has not been the focus of such investigation. It is currently used in Europe and has been proposed as a substitute for JP-4 in the United States. The current study was undertaken to supply needed information on the environmental fate of JP-8.

B. BACKGROUND

Jet fuels are complex mixtures of hydrocarbons. When exposed to the environment, these hydrocarbons are partitioned into environmental compartments according to their physical properties. They may evaporate, they may dissolve in water and be dispersed into the water column, they may absorb onto particles present in sediment or soil and they may be subject to degradative processes.

Evaporation of organic chemicals from surfaces can be related to the compound's vapor pressure and molecular weight (Tinsley, 1979). Since this decreases with increasing molecular weight for any homologous series of hydrocarbons, low-molecular-weight hydrocarbons should exhibit relatively rapid evaporative loss. This occurs in experimental spill situations. Compounds such as octane, benzene and toluene are lost within hours of a spill, while significant amounts of substituted naphthalenes and alkanes such as hexadecane will persist for as long as 20 days after the spill (Wolfe, 1986). In the natural environment, temperature and wind speed will influence rate of evaporative loss so that the persistence of hydrocarbons will depend on environmental conditions.

Dissolution of hydrocarbons in water also decreases with increasing molecular weight. Both evaporation and solubility will be greatest for the lower molecular weight hydrocarbons. These two processes will be in competition for the hydrocarbons. Evaporative losses have been calculated to be two orders of magnitude greater than dissolution rates for soluble aromatic hydrocarbons and four orders of magnitude greater for the less readily soluble n-alkanes (Harrison et al., 1975). Dissolution cannot simply be related to water solubility, however, since turbulence may create microdroplets which may move away from a slick, depending on water currents.

The presence of solid particles, whether as suspended sediments in water or minerals in soil, complicates the above picture because of the process of adsorption, the tendency of a compound to be associated with solid particles. Properties of the organic compound such as water solubility and the tendency to partition into an organic solvent are important in determining adsorption. Equally important are properties of the solid phase including particle size, organic matter content and mineral fraction. For example, clay minerals demonstrate greater adsorption than other mineral components. For organic compounds that are strongly adsorbed, evaporative losses will be reduced (Tinsley, 1979).

Those components which persist more than a few hours will become subject to biodegradation. Biodegradation, a process mediated by microorganisms, can convert the hydrocarbons into carbon dioxide and water. The susceptibility of hydrocarbons to biodegradation dependson the hydrocarbon type. The following generalizations apply (Atlas, 1981):

1. Straight-chain alkanes are readily utilized by microorganisms, particularly within the size range C_{10} to C_{25}.
2. Alkenes are less readily utilized than are alkanes.
3. Branched-chain alkanes are less readily degraded than straight-chain alkanes. The more extensive the branching, the less readily biodegradable is the hydrocarbon. The presence of a quaternary carbon severely limits biodegradation.
4. Low molecular weight aromatic hydrocarbons are subject to biodegradation at rates which equal or exceed those of straight-chain alkanes. Since these hydrocarbons are toxic to microorganisms, the rate of biodegradation will be concentration dependent.
5. Polycyclic aromatic hydrocarbons are resistant to biodegradation.
6. Cycloalkanes may serve as substrates for microbial attack if alternate growth substrates are available to the microorganisms (cometabolism).

Environmental factors can play determining roles in influencing the rate and extent of hydrocarbon biodegradation. Since biodegradation is microbially mediated, the number of hydrocarbon-degrading bacteria present in a given site will influence biodegradation rate. Sites which have a history of hydrocarbon contamination often demonstrate higher initial biodegradation rates. This phenomenon, termed acclimation, has been related to a larger population of hydrocarbon-degrading bacteria because of prior exposure to the hydrocarbon (Carlson, 1981). Because microorganisms require nutrients such as nitrogen and phosphate for optimal growth, lack of these nutrients may limit hydrocarbon biodegradation. Other factors that may influence biodegradation rates are temperature, pH, salinity and oxygen availability.

Studies specifically addressing the biodegradation of hydrocarbons in jet fuels have been conducted by Spain and coworkers (1983) and Pritchard and coworkers (1988). In the first study, JP-4, as well as a model fuel made up of known quantities of individual hydrocarbons present in JP-4, was added to water and sediment samples from three aquatic sites, and the disappearance of hydrocarbons was followed over several days. Use of aquatic samples which had been killed by the addition of mercuric chloride allowed comparison of

biotic and abiotic removal processes.

Results indicated that evaporation was the major removal process for the low-molecular-weight, volatile hydrocarbons. Addition of sediment to water samples affected the removal of some hydrocarbon components of JP-4 and the model fuel by reducing the rate of volatilization. For most individual hydrocarbons, biodegradation was not as significant for removal as was evaporation. For those hydrocarbons which were susceptible to biodegradation, such as naphthalene, the extent of biodegradative removal was a function of the presence or absence of sediment in the test and the location from which the sample was taken. The major variable appeared to be the organic matter content of the sediment which differed among the three sampling locations. A high organic matter content of the sediment appeared to reduce the biodegradability of hydrocarbon components by adsorbing the hydrocarbons, thus rendering them unavailable to the microorganisms for biodegradation.

Microbial numbers were monitored during these tests to determine whether toxicity was a factor in biodegradation. For the model fuel, a decline in bacterial numbers was observed during the first 24 hours of the test, followed by an increase in numbers. No such decline was observed for JP-4. Furthermore, microbial numbers and in particular the number of hydrocarbon-degrading bacteria did not vary between the three sites and so differences in biodegradation rates noted from one site to the next could not be related to size of the microbial population.

The second study by Pritchard and coworkers (1988) provided additional data on the fate of jet fuel by comparing shale-derived JP-4 to petroleum-derived fuel using test systems similar to those used in the previous study. Results again indicated that volatilization was the major removal process, particularly for the lower boiling point hydrocarbons. Addition of sediments to the water samples reduced volatility and inhibited biodegradation. In one of the three water samples tested, certain hydrocarbons, such as decane and naphthalene, disappeared at rates significantly greater in the active treatments than in the killed treatments, indicating that biodegradation was occurring in this water sample. The other two sites showed no differences between active and killed water samples. However, $^{14}CO_2$ was released from samples spiked with radiolabeled decane, suggesting that some

biodegradation was occurring in these samples. JP-4 derived from shale was toxic to aquatic microorganisms as evidenced by an inhibition of ^{14}C-toluene mineralization in water samples exposed to the jet fuel.

C. SCOPE/APPROACH

The results of previous studies suggested that volatilization was the major process for removal of jet fuels from the environment. Accordingly, the quiescent bottle test was selected as the test method because it would minimize evaporative losses. Since sediment influences the fate of JP-4, treatments containing water alone and water plus sediment were included in the experimental design. In addition to bottles receiving JP-8 as test fuel, bottles treated with JP-4 were included to serve as positive controls. Finally, a series of bottles containing soil were included in this study. Fuel spills and leaks from storage tanks may contaminate the soil as well as the aquatic environment and information on removal from soil would be useful for the assessment of the environmental fate of JP-8. In all studies, samples treated with mercuric chloride were included to compare biological with nonbiological removal processes.

Furthermore, evidence from previous studies indicated that biodegradation was lower in sediment treatments than in water treatments. These results suggested that either toxicity to biodegradative organisms might be occurring or that adsorption to sediment might render the hydrocarbons less available for biodegradation. A toxicity study was therefore included in the study in order to aid in the interpretation of the biodegradation test results.

SECTION II
MATERIALS AND METHODS

A. FATE OF JP-8

The quiescent-bottle technique of Spain and Somerville (1985) was used to assess the rate of removal of JP-8 from aqueous environmental samples. For these experiments, water and sediment were collected from a brackish bayou located on Tyndall Air Force Base. Water was filtered through a 3.0 μm membrane filter and incubated overnight at room temperature with stirring. The sediment slurry was passed through a 1 mm screen and decanted several times to allow sand particles to settle out. The resulting slurry was incubated overnight with stirring and forced aeration.

JP-8 was incubated under four conditions: (1) water alone; (2) sterile water (containing 0.05 percent $HgCl_2$); (3) water supplemented with sediment to give a concentration of 5,000 mg/L (dry weight basis); (4) water supplemented with sediment as in (3), but sterilized with 0.05 percent $HgCl_2$. The tests were conducted in 150 mL milk dilution bottles containing 25 mL of water or water/sediment slurry. JP-8 (250 μL) was added to each bottle. Bottles were capped, placed horizontally on a shaker and shaken for 15 minutes at 150 rpm to achieve an initial dispersion of the fuel. Following this treatment, caps were removed and bottles were incubated in the horizontal position in an undisturbed condition. For each treatment, 15 bottles were prepared. At 0 time, and 5, 10, 21 and 40 days, triplicate bottles were removed for extraction and analysis. Water and sediment not treated with fuel were used for the enumeration of heterotrophic bacteria by the most probable number (MPN) technique (Koch, 1981).

A soil incubation study was also included. For this study, 25 grams (dry weight equivalent) of soil collected from the campus of Chippola Community College (Chippola, FL), was placed in 150 mL milk dilution bottles. The soil was a sandy loam (76 percent sand, 14 percent silt and 10 percent clay) with a pH of 5.4 and an organic matter content of 5.08 percent (A&L Great Lakes Laboratories, Ft. Wayne, IN). Two sets of bottles were used, one set containing untreated (active) soil and the other receiving soil treated with 2 percent (wt) $HgCl_2$. Each bottle received 250 μL of JP-8. Twelve bottles were prepared per treatment, and triplicate bottles were removed at 0 time, and 10, 21 and 31 days. Three

bottles containing 25 grams of soil (dry weight) were weighed and incubated under identical conditions to the JP-8 bottles. These bottles were weighed weekly to calculate weight loss. The corresponding amount of water was added to the JP-8 bottles to maintain a constant moisture level. The heterotrophic bacterial population in untreated soil was estimated by the MPN method.

B. TOXICITY OF JP-8 TO MICROORGANISMS

Toxicity studies were undertaken with JP-8 using water and water supplemented with sediment (5,000 mg/L). Water was collected from the same location as for the biodegradation study and filtered through a 3 μm diameter membrane filter. It was dispensed into Erlenmeyer flasks which received either no JP-8, or 0.01 percent, 0.1 percent or 1 percent JP-8. All treatments were prepared in triplicate. Flasks were incubated at 30°C on a shaker at 200 rpm. Samples were removed at 0, 1, 2 and 4 days and assayed for glucose mineralization and hexadecane mineralization by the method described below. Incubations with water supplemented with sediment were prepared in a similar fashion except that the 0.01 percent JP-8 treatment was not included. This was done based on the results of a preliminary study which suggested that 0.01 percent JP-8 had negligible effect on glucose mineralization when compared to the control in a water/sediment slurry.

Glucose mineralization was assessed by measuring the production of $^{14}CO_2$ from ^{14}C-labeled glucose added to samples of the water or water/sediment. Five 5-mL samples were removed from each incubation flask and placed in 60 mL serum vials. Two vials received 0.5 ml 2N H_2SO_4 to serve as killed-cell controls. Each vial received uniformly labeled glucose (Pathfinder Laboratories) at a final concentration of 20 μg/L. Flasks were capped with rubber stoppers fitted with center wells containing 0.1 mL of 10N NaOH absorbed onto a filter paper wick. Vials were incubated for 4 hours at 30°C with shaking (200 rpm). At the end of the incubation period, reaction in the active vials was stopped by the addition of 0.5 mL 2N H_2SO_4. Vials were incubated with shaking for an additional hour to ensure complete trapping of CO_2. Wicks were then removed, placed in 10 mL of scintillation cocktail (Ecolume, ICN Biomedicals, Inc., Irvine, CA) and 1 mL methanol, and counted by liquid scintillation counting (Beckman Model LS 9800).

Hexadecane mineralization was assessed by measuring the production of $^{14}CO_2$ when ^{14}C-labeled hexadecane was added to samples of the water or water-sediment incubations. The hexadecane was dissolved in hexane; hexane was selected as solvent because it did not inhibit glucose mineralization when added to water-sediment slurries. Incubations with labeled hexadecane were conducted for 18 hours.

C. EXTRACTION AND ANALYSIS

After the appropriate time interval, the remaining fuel was extracted from water and water/sediment slurries by the addition of 2 mL of CS_2 (containing D_{10}-ethylbenzene as an internal standard). Solvent and sample were shaken for 5 min on a wrist action mechanical shaker, then samples were centrifuged for 10 min at 1200 rpm. A 1 mL sample of the solvent was transferred to an autosampler vial and analysis by gas chromatography/mass spectrometry. Soil samples were extracted similarly except that 15 mL of CS_2 (with internal standard) was used. In order to exclude fine soil particles, the 1 mL samples from soil were drawn from near the top of the solvent layer.

Extracts were analyzed by high-resolution capillary gas chromatography with mass selective detection. The separations were performed using a fused-silica capillary column, 30 meters long, with an internal diameter of 0.24 mm, and coated with 1.0 μm of a bonded and cross-linked stationary phase consisting of 5 percent phenyl-substituted polymethylsilixane (DB-5, J&W Scientific, Inc.). All sample injections were 1 μL in volume. The column temperature was held at 40°C for 4 minutes and then increased to 250°C at a rate of 3°C/minute. The injection port and the gas chromatograph/mass spectrometer interface temperatures were 200°C. Analyses were performed on an HP-5890 gas chromatograph interfaced to an HP-5970B mass selector and equipped with an HP-7673A autosampler (Hewlett Packard Company). An HP-1000F minicomputer was used to control the system, acquire data, and provide gas chromatographic and mass spectral data display and analysis, using vendor supplied software.

1. Water and Water/sediment Samples

The standard contained 27 components, whose names, concentrations and

typical retention times under the conditions given above are listed in Table 1. The components were quantified through their peak areas in total ion chromatograms (TIC) of the injected samples. The peak areas were determined by integrating the TICs. The peak areas and component concentrations in the standard samples were used to calculate response factors for the components, using Equation (1):

$$C = AR \tag{1}$$

where C = component concentration

A = area of the component's chromatographic peak

R = component's response factor.

The concentration of the components in the samples was calculated according to Equation (2):

$$C = ARV_e/V_s \tag{2}$$

where V_e = volume of extract

V_s = volume of the sample before extraction

To improve the precision and accuracy of the analysis, the peak areas were replaced by the ratio between the peak area and the area of the internal standard peak. The internal standard value used was the area of the d_{10}-anthracene peak in the 188 dalton selected ion profile. These internal standard calculations were carried out for all extract and standard solution chromatograms.

Components were recognized in the chromatograms using their Kovat's retention index. These indices are preferable to retention times because they have less variation between chromatograms. They were calculated using the following equation:

$$I = 100n + \frac{t_R(u) - t_R(n)}{t_R(n+1) - t_R(n)} \tag{3}$$

where I = retention index of component u

$t_R(u)$ = retention time of component u

$t_R(n)$ = retention time of the n-alkane component preceding component u

$t_R(n+1)$ = retention time of the n-alkane component following component u

n = number of carbon atoms in the n-alkane component preceding component u

TABLE 1. CONTENTS OF THE STANDARD SOLUTION USED TO CALIBRATE THE WATER AND WATER/SEDIMENT ANALYSES

Compound	Concentration (g/L)	Retention Time (min)
Benzene	0.879	5.54
Cyclohexane	0.779	5.54
Heptane	0.684	7.00
Methylcyclohexane	0.769	8.09
Methylbenzene (toluene)	0.867	10.30
3-Methylheptane	0.706	10.66
1,1-dimethylcyclohexane	0.781	11.38
n-Octane	0.703	12.14
Ethylcyclohexane	0.788	14.12
Ethylbenzene	0.867	15.76
m-Xylene	2.593	16.26
o-Xylene	0.880	17.66
n-Nonane	0.718	18.08
Isopropylbenzene	0.862	19.58
1-Ethyl-3-methylbenzene	0.865	21.84
1,2,4-Trimethylbenzene	3.503	22.23
1,3,5-Trimethylbenzene	0.865	23.74
n-Decane	0.730	24.05
Indan	0.964	26.26
1,4-Dimethyl-2-ethylbenzene	0.877	28.60
n-Undecane	0.740	29.76
n-Dodecane	0.749	35.14
n-Tridecane	0.756	40.18
1-Methylnaphthalene	1.020	41.23
n-Tetradecane	0.763	44.92
n-Pentadecane	0.769	49.38
n-Hexadecane	0.773	53.61

Benzene and cyclohexane coeluted under the chromatographic conditions used. Since the data had been acquired using a mass spectrometer in sequential scanning mode, it was possible to resolve the data into selected ion profiles which could be integrated separately to permit quantitation of the individual species. Benzene was quantified through the mass 78 selected ion profile, and cyclohexane was quantified through the mass 84 selected ion profile. The ion profiles were integrated to obtain the peak area, and Equation 1 was used to calculate the concentrations.

1,3-Dimethylbenzene (m-xylene) and 1,4-dimethylbenzene (p-xylene) also coelute but could not be separately quantified through the selected ion profiling technique, since their mass spectra are identical. They were quantified together, giving a combined figure for the sum of their concentrations. This was considered to be a reasonable procedure since these compounds are closely related structurally and usually have similar response factors.

2. Soil Samples

For soil samples, a splitless injection technique was used, with the injection port being purged 0.33 minutes following each injection. For the soil samples, a slightly different calibration standard was used, as indicated in Table 2. These components were calibrated using their standard chromatograms and Equation 1. The concentrations in the quiescent bottle tests were determined using Equation 2. Note that the response factors for the soil samples had to be determined using the standards injected with a splitless injection, matching the injection technique used for the soil extracts.

D. STATISTICAL ANALYSIS

Two statistical analyses were used on the data sets. For one, the data for each sample type was divided into four groups, one for each sampling day. A Student's t-test was performed to determine if the active set of samples was significantly lower in concentration than the sterile set, using the 95 percent confidence level. A second analysis involved plotting the log of the peak response versus time. The slope and the 95 percent confidence extremes were calculated for each fuel component in the active and in the sterile treatments. The slope of the active sample was considered to be significantly less than that of the sterile sample if the maximum slope of the active sample versus time was less than the minimum slope of the inactive sample versus time.

TABLE 2. CONTENTS OF THE STANDARD SOLUTION USED TO CALIBRATE THE SOIL ANALYSES

Compound	Concentration (g/L)	Retention Time (min)
Benzene	0.439	5.54
Cyclohexane	0.195	5.54
Methylcyclohexane	0.385	8.09
Methylbenzene (toluene)	0.433	10.30
3-Methylheptane	0.353	10.66
1,1-dimethylcyclohexane	0.390	11.38
n-Octane	0.176	12.14
Ethylcyclohexane	0.985	14.12
Ethylbenzene	0.867	15.76
m-Xylene	0.432	16.26
o-Xylene	1.076	17.66
n-Nonane	0.359	18.08
Isopropylbenzene	0.431	19.58
1,2,4-Trimethylbenzene	1.095	22.23
1,3,5-Trimethylbenzene	1.514	23.74
n-Decane	0.365	24.05
sec-Butylbenzene	0.431	24.58
Isobutylbenzene	0.427	24.77
1,2,3-Trimethylbenzene	0.447	25.46
Indan	0.482	26.26
m-Diethylbenzene	1.075	27.01
o-Diethylbenzene	0.660	27.73
1,4-Dimethyl-2-ethylbenzene	0.439	28.60
n-Undecane	0.370	29.76
1,2,3,4-Tetramethylbenzene	0.453	32.86
1,2,3,4-Tetrahydronaphthalene	0.485	33.44
n-Dodecane	0.374	35.14
n-Tridecane	0.378	40.18
1-Methylnaphthalene	0.510	41.23
n-Tetradecane	0.381	44.92
n-Pentadecane	0.384	49.38
n-Hexadecane	0.387	53.61

SECTION III
RESULTS

A. COMPOSITION OF JP-8

The concentrations of the individual hydrocarbon components in JP-8 selected for quantitation are shown in Table 3. Using these values, initial concentrations of these components in the water and water/sediment extracts, assuming 100% recovery, were calculated (Table 4). Initial concentrations in the soil experiments are the same, except that mg/L should be replaced by mg/kg.

TABLE 3. CONCENTRATIONS OF STANDARD COMPONENTS IN JP-8

Compound	Concentration (g/L)	Error range*
n-Heptane	0.35	0.01
Methylcyclohexane	0.87	0.04
Methylbenzene (toluene)	2.11	0.07
3-Methylheptane	1.50	0.04
1,1-dimethylcyclohexane	0.27	0.01
n-Octane	6.51	0.08
Ethylcyclohexane	2.26	0.01
Ethylbenzene	2.02	0.002
m-Xylene	10.59	0.05
o-Xylene	4.70	0.08
n-Nonane	20.1	0.10
Isopropylbenzene	4.70	0.01
1-Ethyl-3-methylbenzene	12.3	0.40
1,2,4-Trimethylbenzene	10.9	0.30
1,3,5-Trimethylbenzene	7.31	0.05
n-Decane	30.0	1.00
Indan	3.0	0.90
1,4-Dimethyl-2-ethylbenzene	3.6	0.5
n-Undecane	33.0	1.0
n-Dodecane	25.1	0.9
n-Tridecane	19.9	1.0
1-Methylnaphthalene	1.91	0.05
n-Tetradecane	15.6	0.6
n-Pentadecane	11.0	0.3
n-Hexadecane	4.48	0.09

*Calculated as the absolute value of the difference in concentration estimates from two trials, divided by 2

TABLE 4. CONCENTRATIONS OF STANDARD COMPONENTS IN THE WATER AND WATER/SEDIMENT EXTRACTS

Compound	Concentration (mg/L)
n-Heptane	3.5
Methylcyclohexane	8.7
Methylbenzene (toluene)	21.1
3-Methylheptane	15.0
1,1-dimethylcyclohexane	2.7
n-Octane	65.1
Ethylcyclohexane	22.6
Ethylbenzene	20.2
m-Xylene	105.9
o-Xylene	47.0
n-Nonane	201.0
Isopropylbenzene	47.0
1-Ethyl-3-methylbenzene	123.0
1,2,4-Trimethylbenzene	109.0
1,3,5-Trimethylbenzene	73.1
n-Decane	300.0
Indan	30.0
1,4-Dimethyl-2-ethylbenzene	36.0
n-Undecane	330.0
n-Dodecane	251.0
n-Tridecane	199.0
1-Methylnaphthalene	19.1
n-Tetradecane	156.0
n-Pentadecane	110.0
n-Hexadecane	44.8

B. FATE OF JP-8 IN WATER

Significant loss of jet fuel occurred over the experimental period. This was due to evaporation as shown by the plot of the total chromatogram response versus time (Figure 1), which shows that fuel in both active and sterile treatments disappeared at the same rate and to the same extent. This conclusion is confirmed by statistical analysis of the chromatograms which failed to find any components for which disappearance was significantly faster in the active than in the sterile treatments. Further confirmation is obtained from plots of individual hydrocarbon components of JP-8 (Figures 2-23). Loss of components was related to molecular weight and vapor pressure, with low molecular weight components being removed by day 10 (Figures 2-13) and high molecular weight components persisting (Figures 14-23).

The error bars in the plot represent one standard deviation of the values obtained by triplicate analyses on a given trail day.

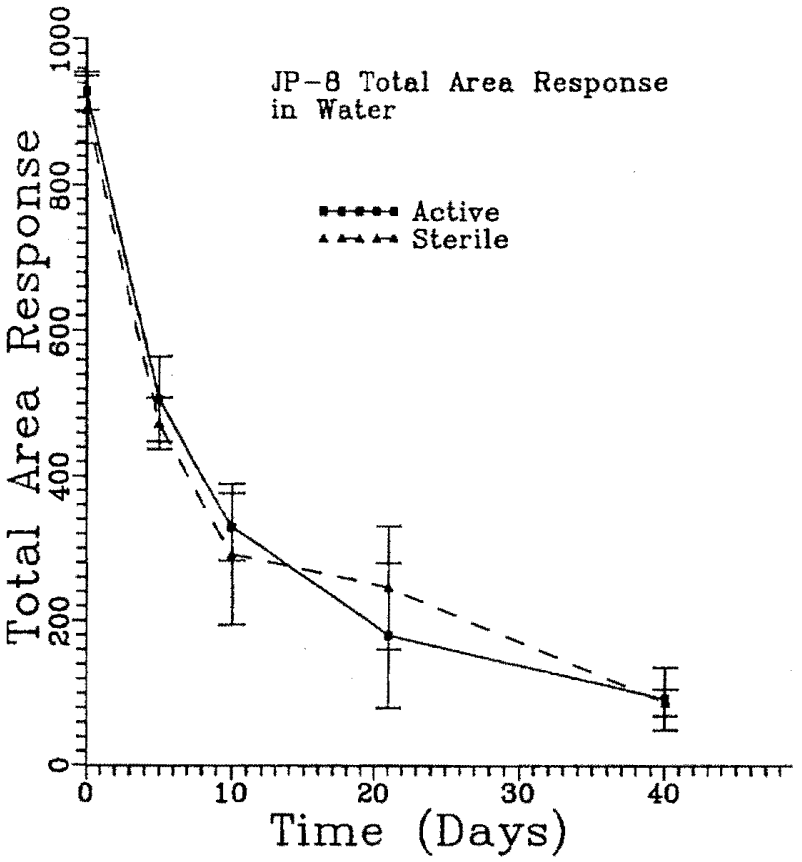

Figure 1. Disappearance of JP-8 from Water as Measured by Total Chromatogram Response

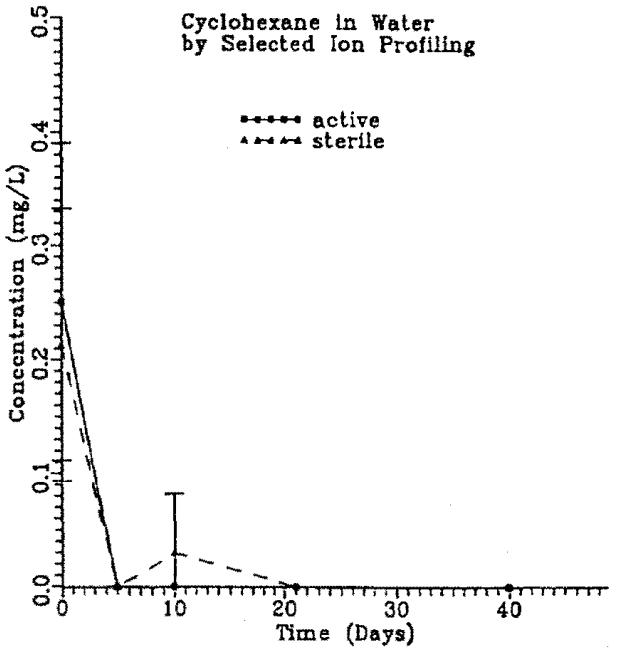

Figure 2. Disappearance of Cyclohexane from Water as Determined by Selected Ion Profile Analysis

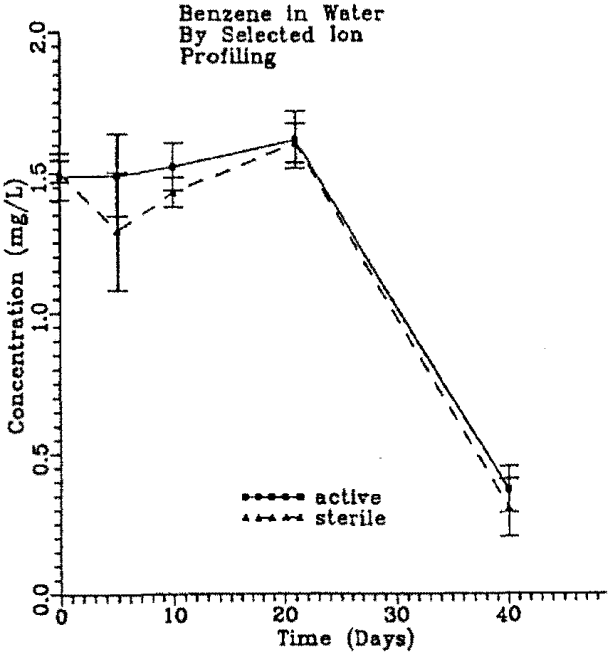

Figure 3. Disappearance of Benzene from Water as Determined by Selected Ion Profile Analysis

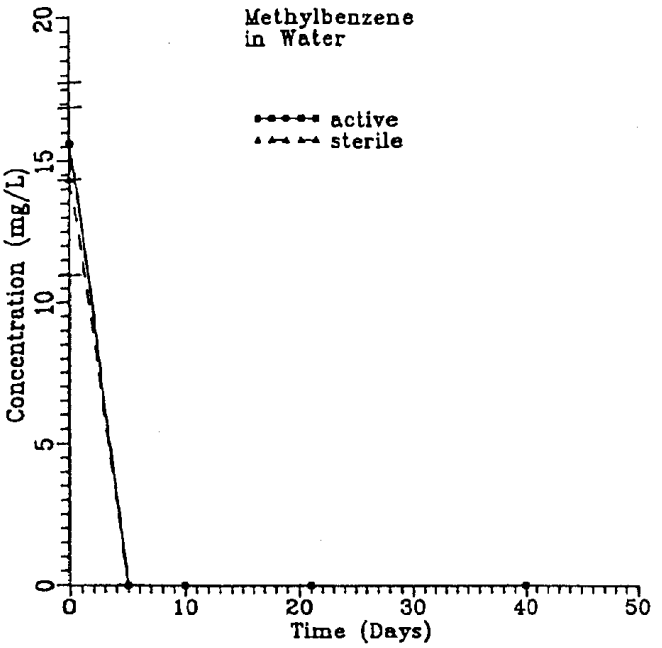

Figure 4. Disappearance of Methylbenzene from Water

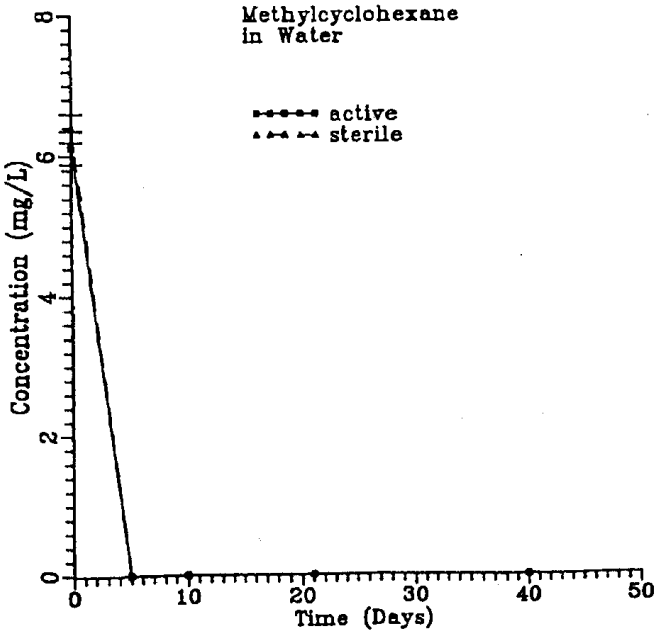

Figure 5. Disappearance of Methylcyclohexane from Water

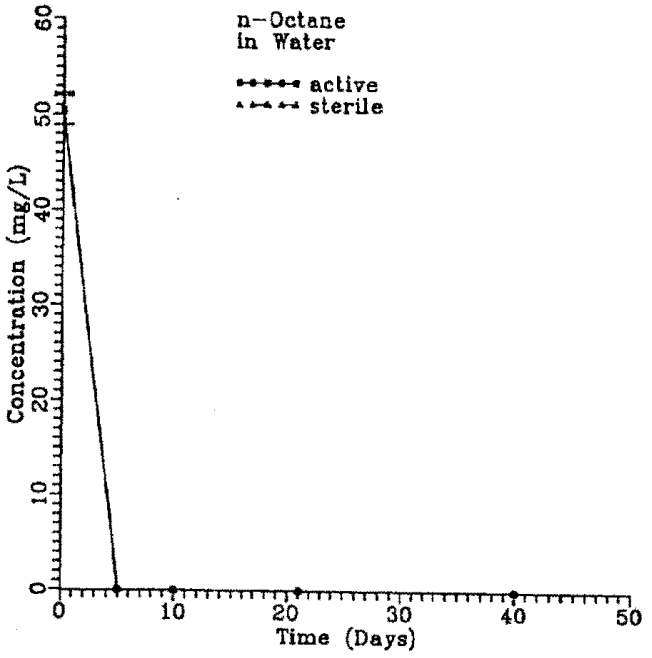

Figure 6. Disappearance of n-Octane from Water

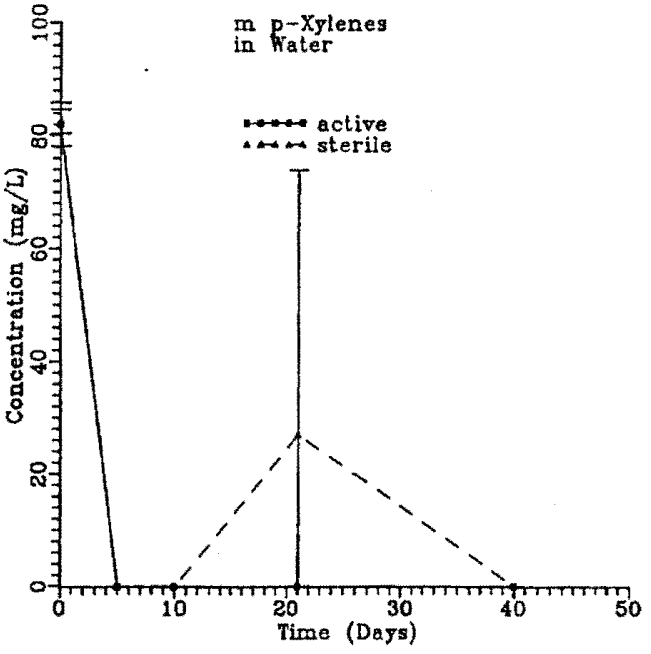

Figure 7. Disappearance of m- and p-Xylene from Water

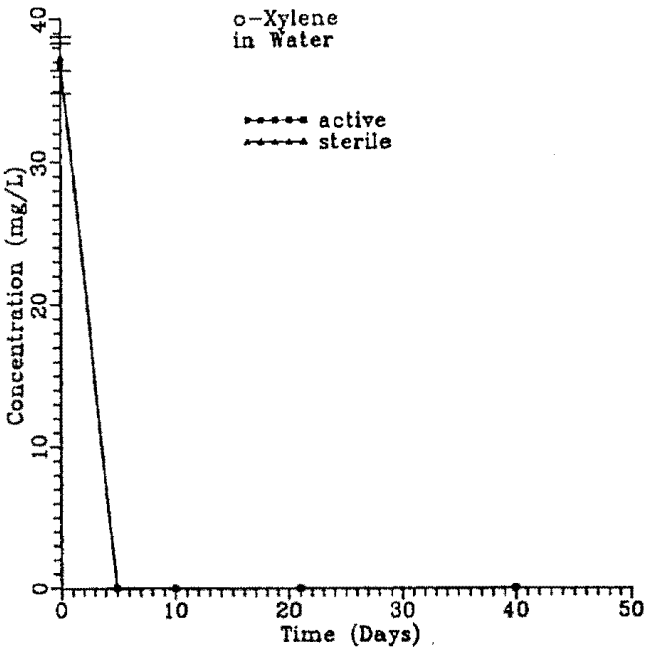

Figure 8. Disappearance of o-Xylene from Water

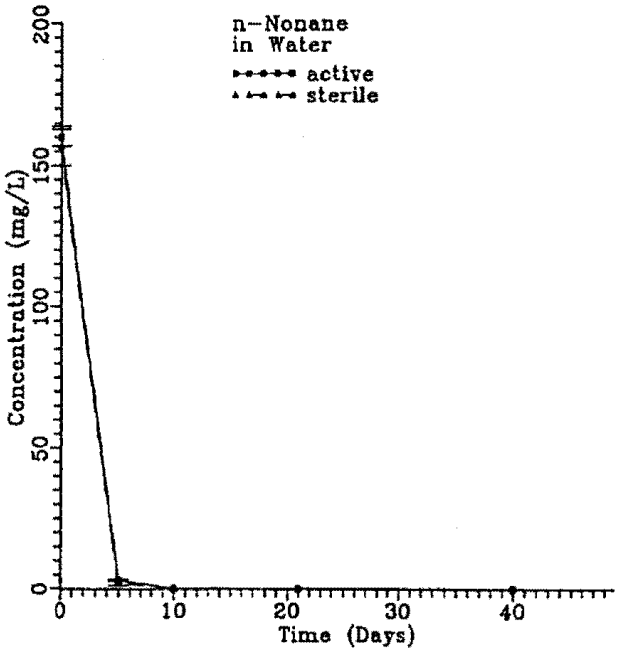

Figure 9. Disappearance of n-Nonane from Water

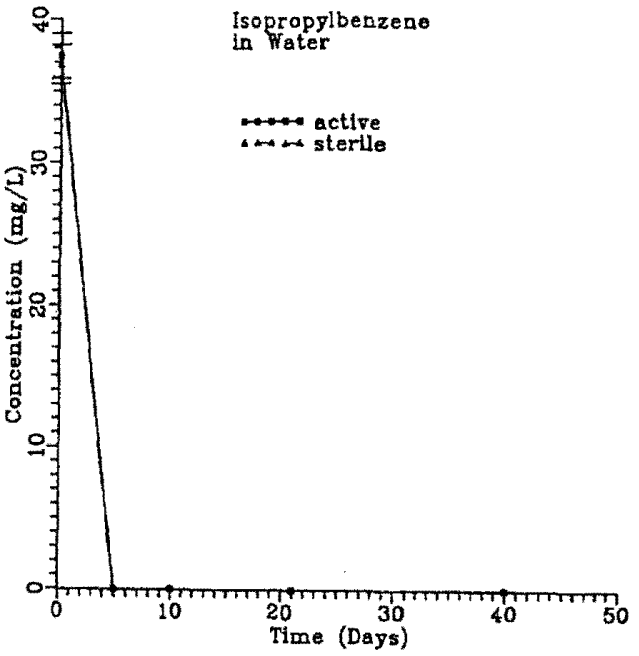

Figure 10. Disappearance of Isopropylbenzene from Water

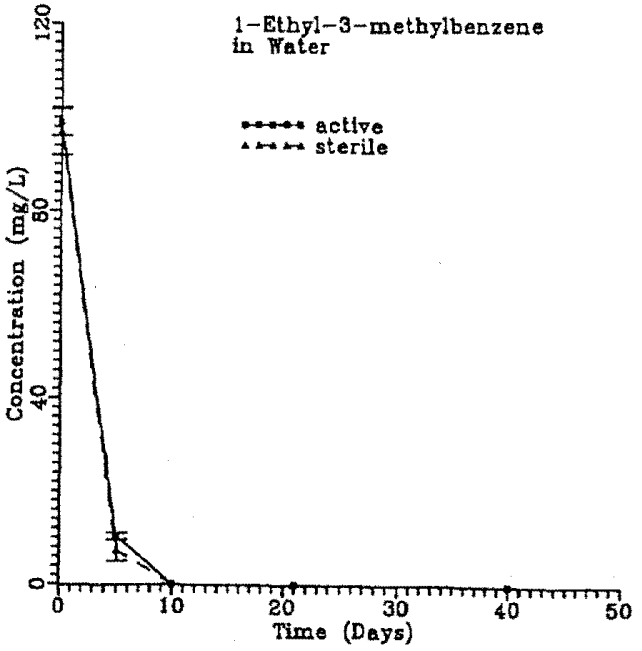

Figure 11. Disappearance of 1-Ethyl-3-methylbenzene from Water

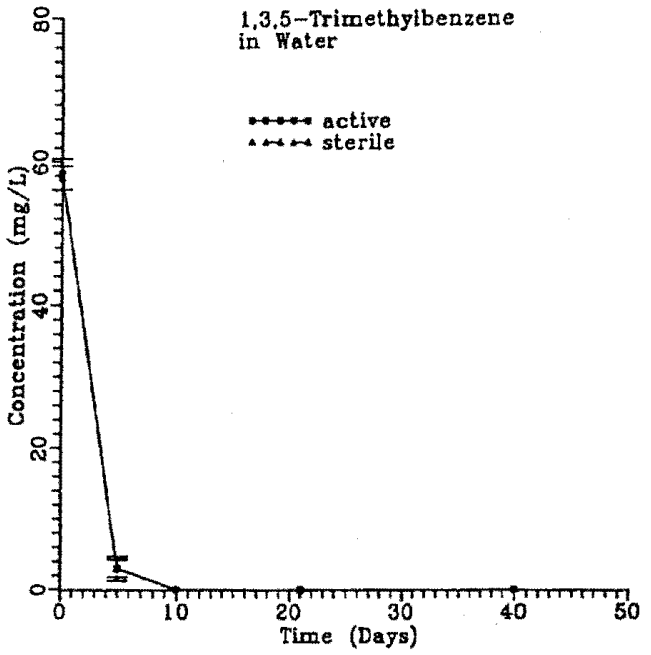

Figure 12. Disappearance of 1,3,5-Trimethylbenzene from Water

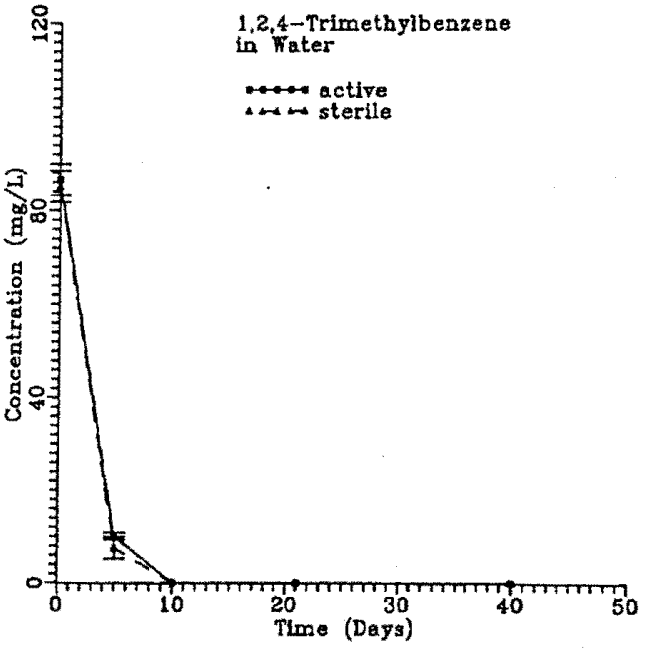

Figure 13. Disappearance of 1,2,4-Trimethylbenzene from Water

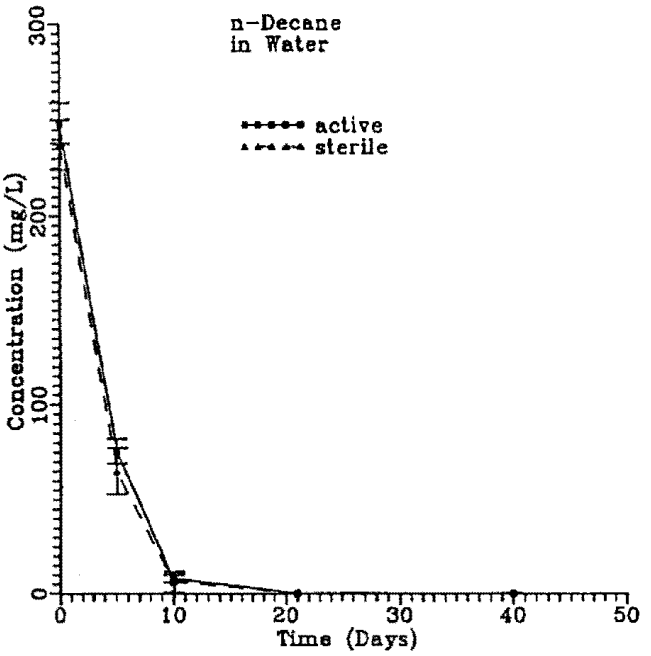

Figure 14. Disappearance of n-Decane from Water

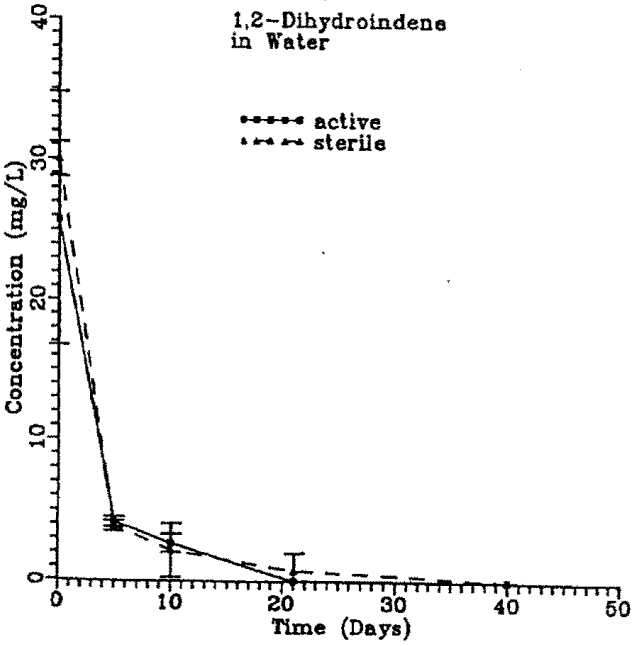

Figure 15. Disappearance of 1,2-Dihydroindene from Water

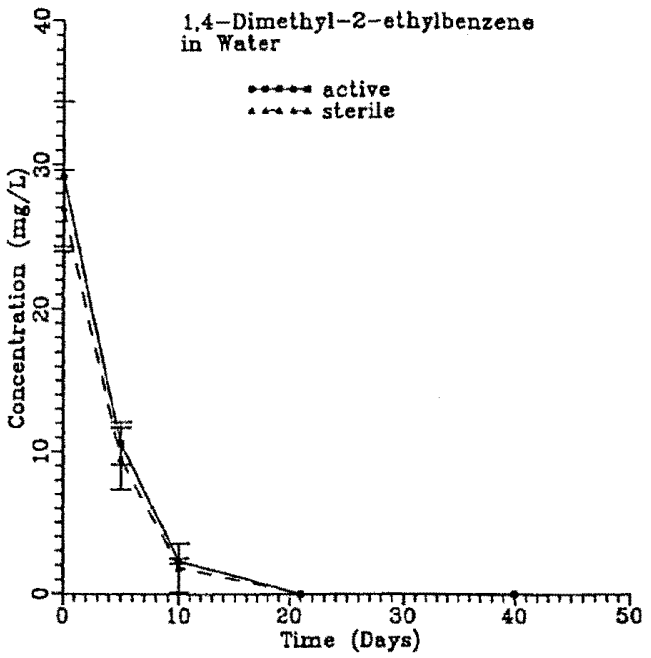

Figure 16. Disappearance of 1,4-Dimethyl-2-ethylbenzene from Water

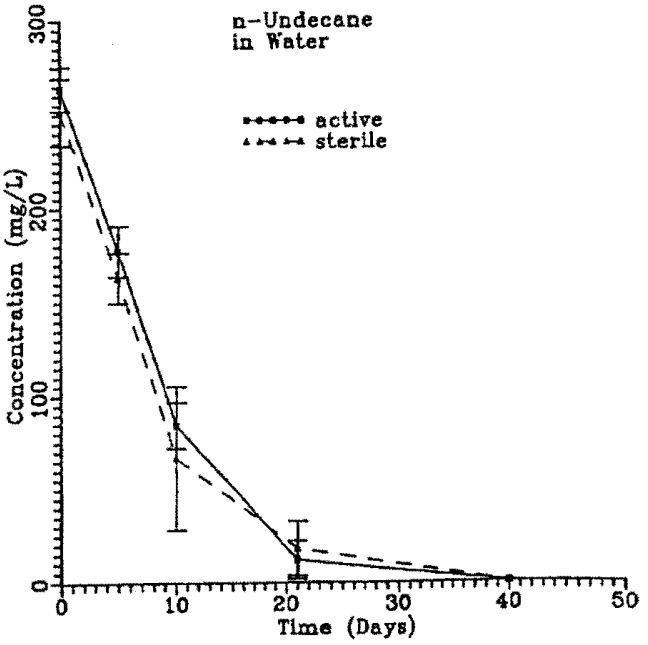

Figure 17. Disappearance of n-Undecane from Water

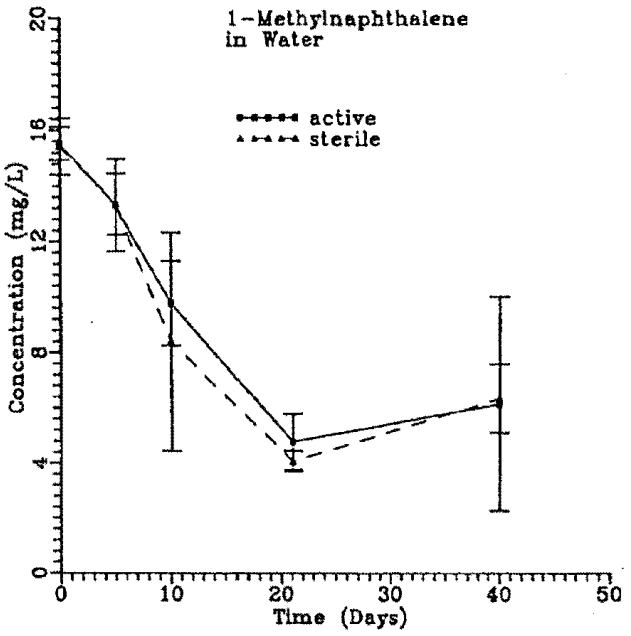

Figure 18. Disappearance of 1-Methylnaphthalene from Water

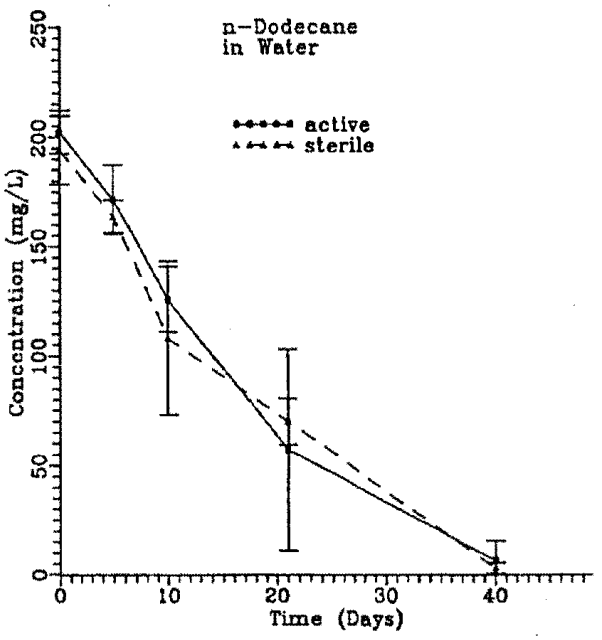

Figure 19. Disappearance of n-Dodecane from Water

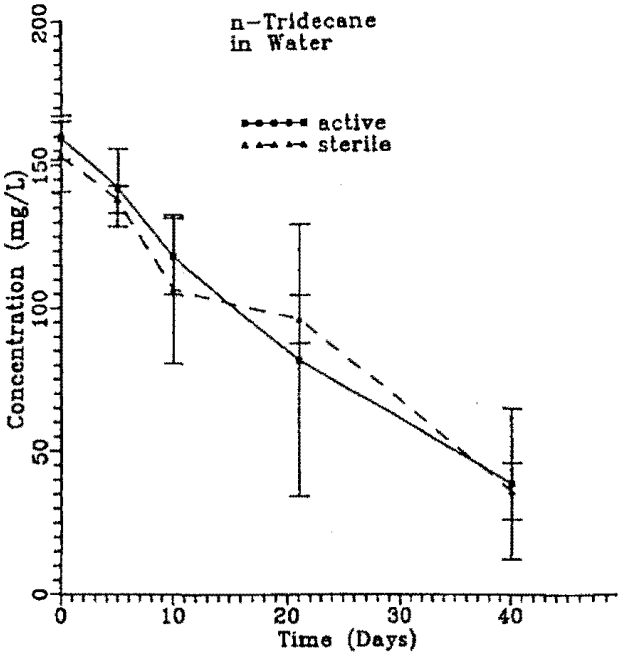

Figure. 20. Disappearance of n-Tridecane from Water

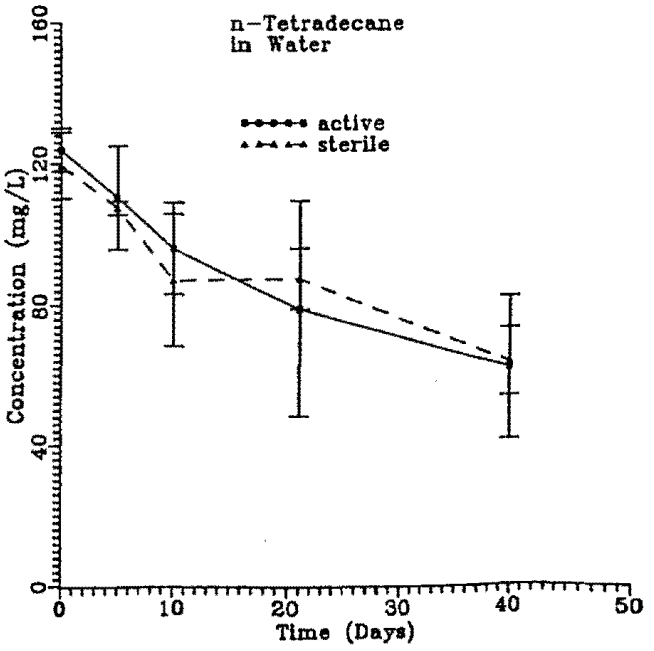

Figure 21. Disappearance of n-Tetradecane from Water

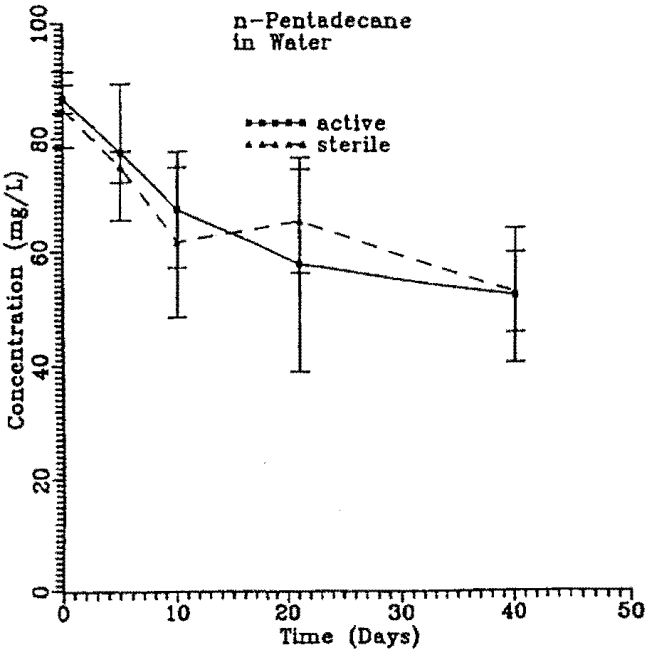

Figure 22. Disappearance of n-Pentadecane from Water

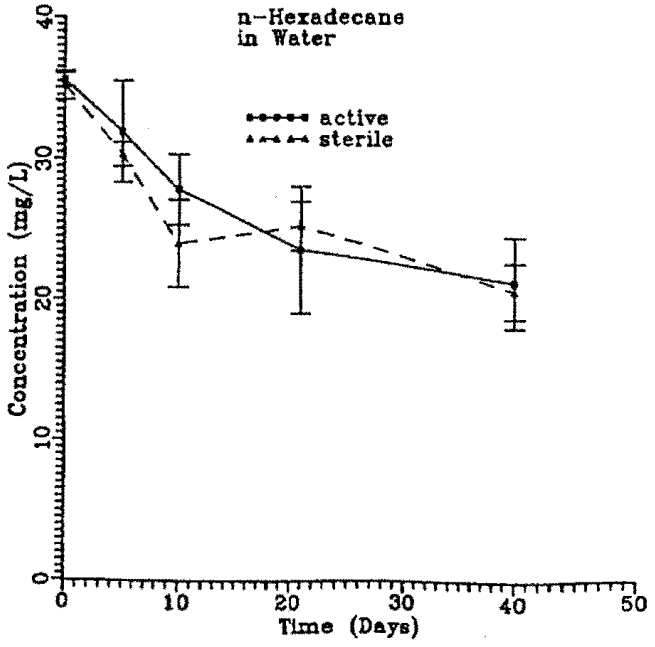

Figure 23. Disappearance of n-Hexadecane from Water

C. FATE OF JP-8 IN WATER/SEDIMENT SLURRIES

As with the water treatments, fuel disappeared at the same rate and to the same extent in active as in sterile treatments (Figure 24), indicating that biodegradation did not play a major role in the removal of JP-8. Statistical analysis indicated that only for 1- and 2-methylnaphthalene was the slope of the disappearance curve in active treatments greater than for sterile treatments. Disappearance curves for individual components of JP-8 are shown in Figures 25-46. Thus, biodegradation played a minor role in the removal of JP-8 from water/sediment slurries.

Rate of removal of JP-8 from water/sediment slurries was much slower than in the case of water alone. This is seen in Figure 24, where fuel decreased only one order of magnitude as opposed to over two orders of magnitude in the case of water alone. In addition, many of the low molecular weight components which had disappeared by day 10 from water persisted in the water/sediment slurries (see Figures 25-36). The presence of sediment, therefore, retarded evaporation and inhibited the removal of jet fuel.

The error bars in the plot represent one standard deviation of the values obtained by triplicate analyses on a given trail day.

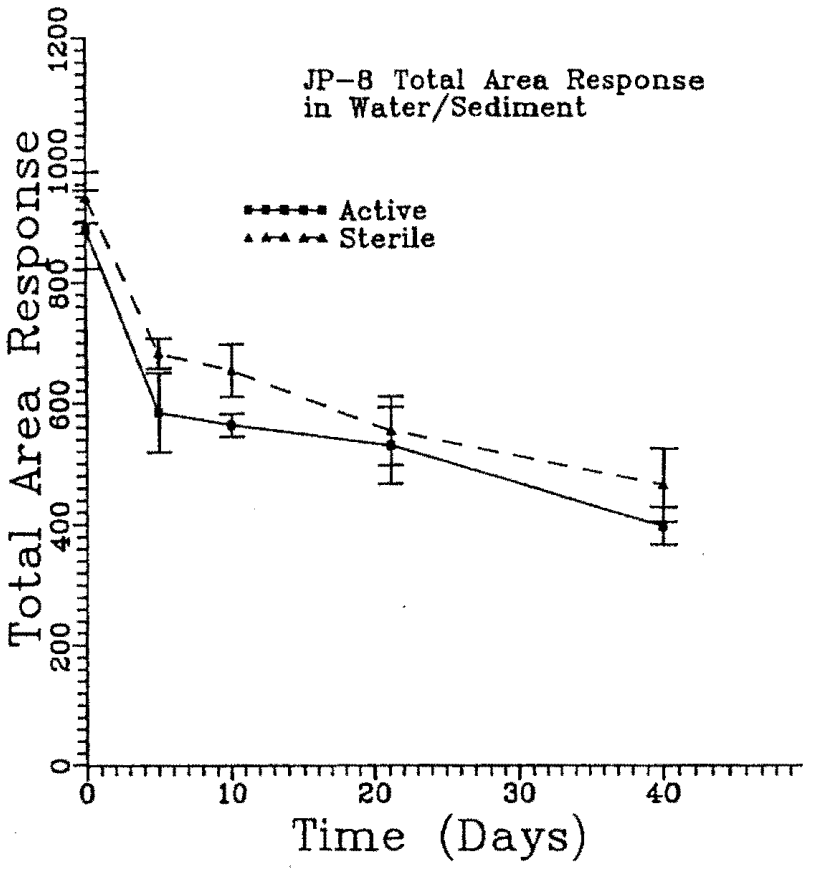

Figure 24. Disappearance of JP-8 from Water/sediment as Measured by Total Chromatogram Response

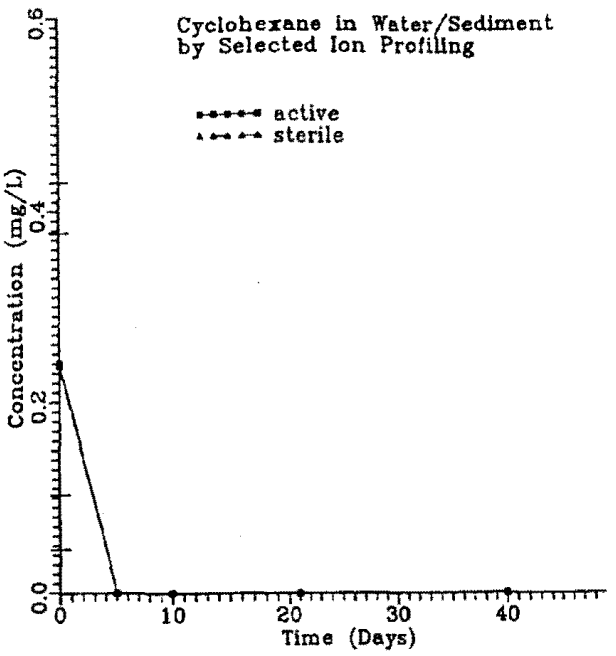

Figure 25. Disappearance of Cyclohexane from Water/sediment as Determined by Selected Ion Profile Analysis

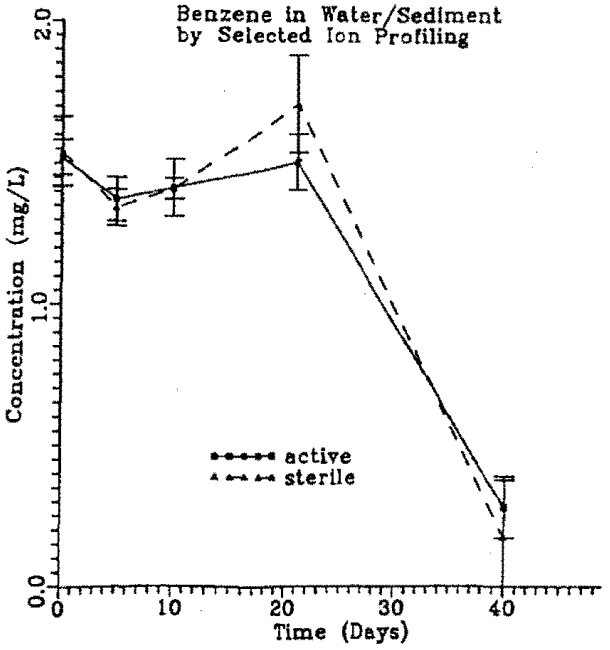

Figure 26. Disappearance of Benzene from Water/sediment as Determined by Selected Ion Profile Analysis

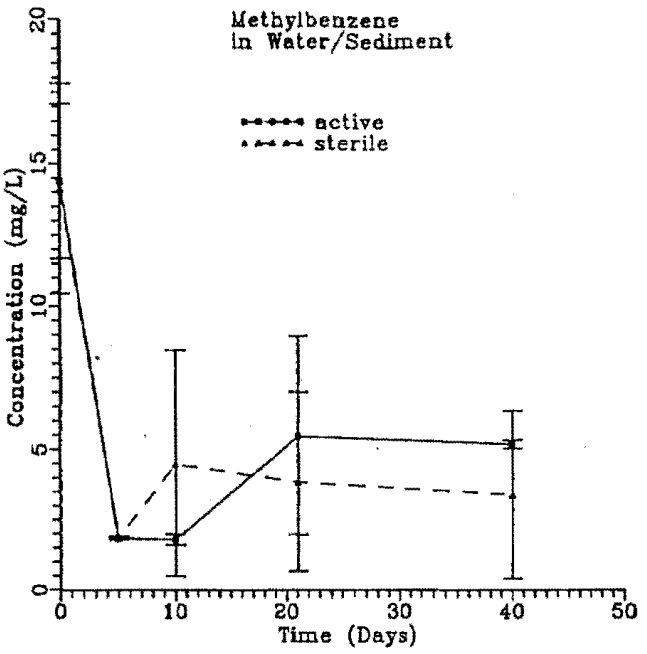

Figure 27. Disappearance of Methylbenzene from Water/sediment

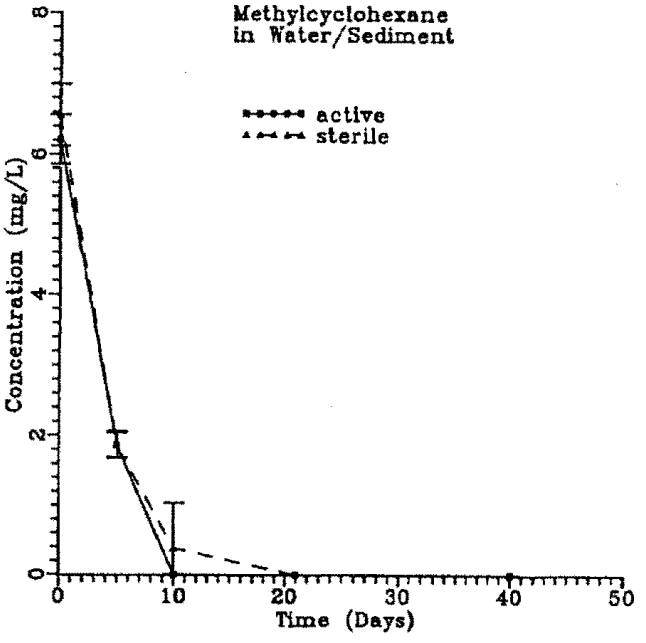

Figure 28. Disappearance of Methylcyclohexane from Water/sediment

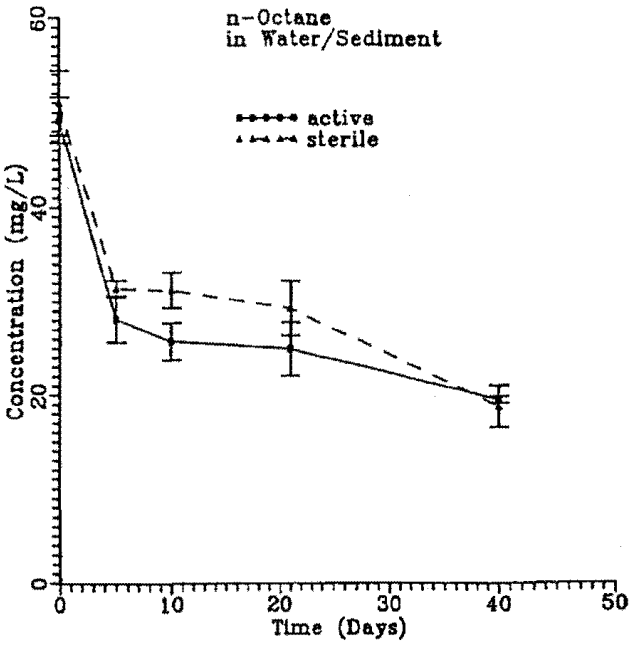

Figure 29. Disappearance of n-Octane from Water/sediment

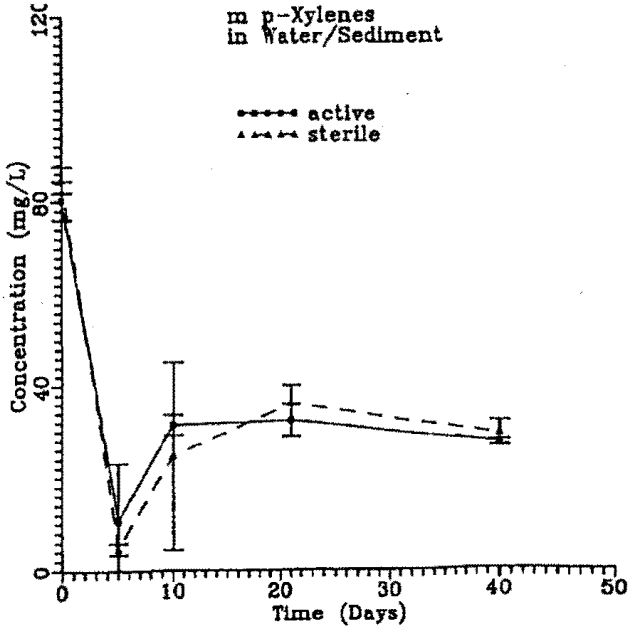

Figure 30. Disappearance of m- and p-Xylene from Water/sediment

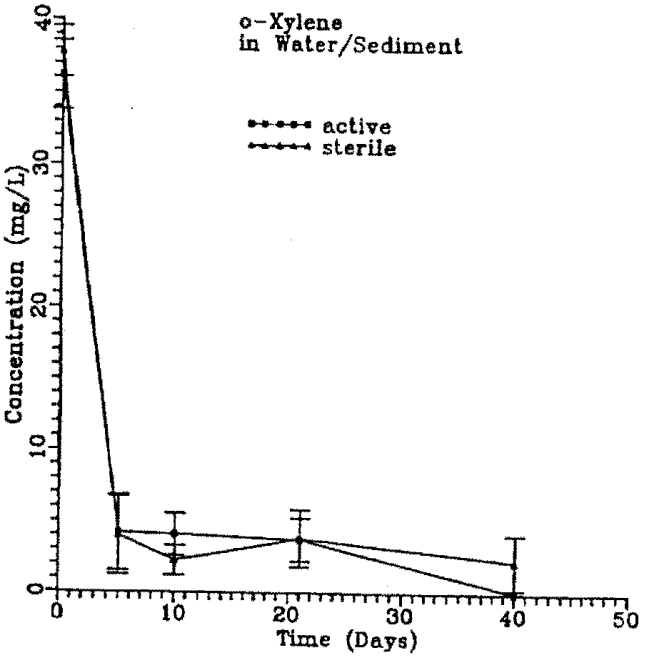

Figure 31. Disappearance of o-Xylene from Water/sediment

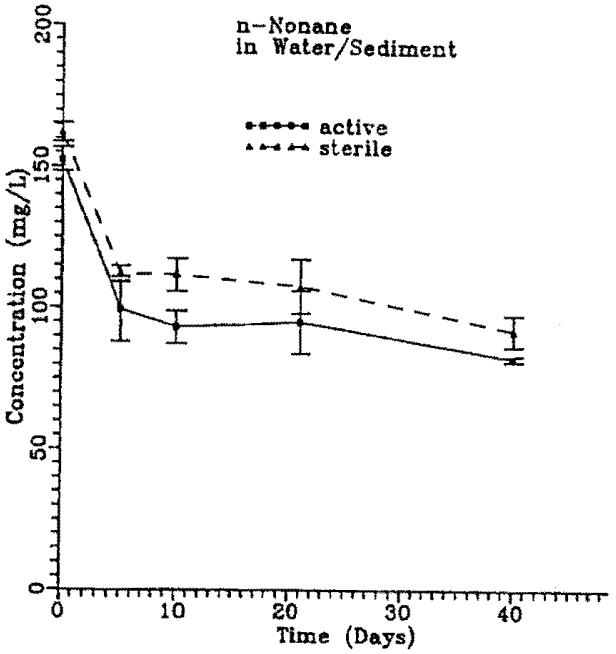

Figure 32. Disappearance of n-Nonane from Water/sediment

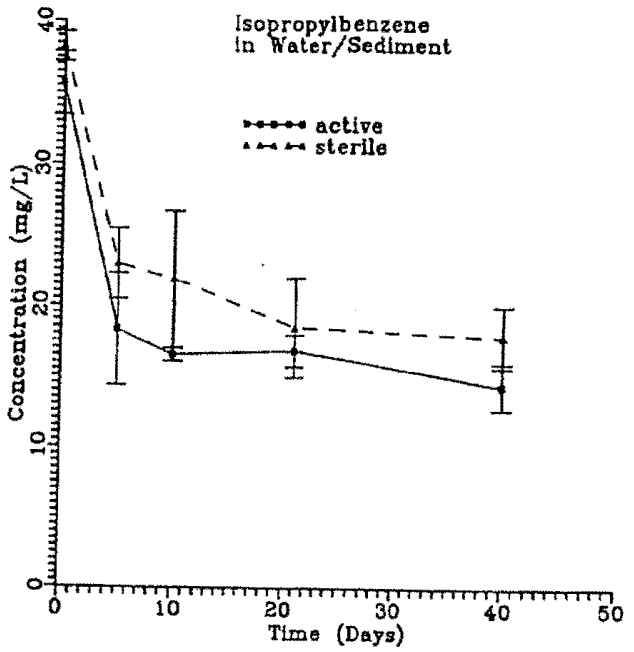

Figure 33. Disappearance of Isopropylbenzene from Water/sediment

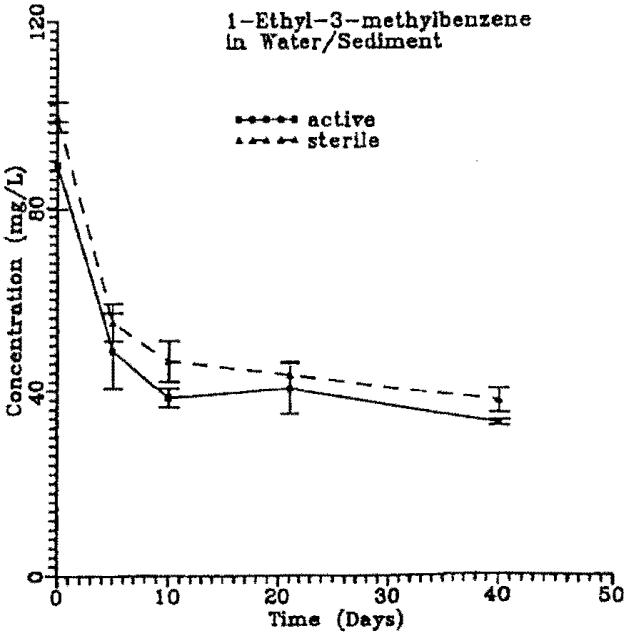

Figure 34. Disappearance of 1-Ethyl-3-methylbenzene from Water/sediment

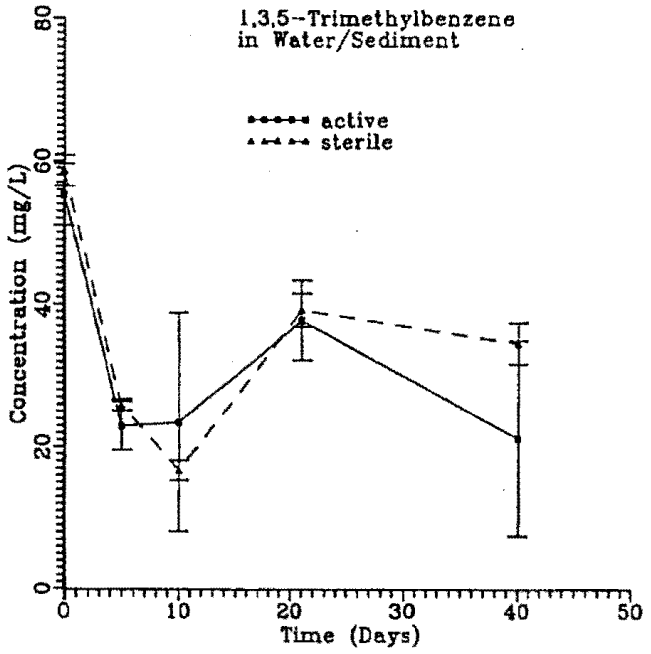

Figure 35. Disappearance of 1,3,5-Trimethylbenzene from Water/sediment

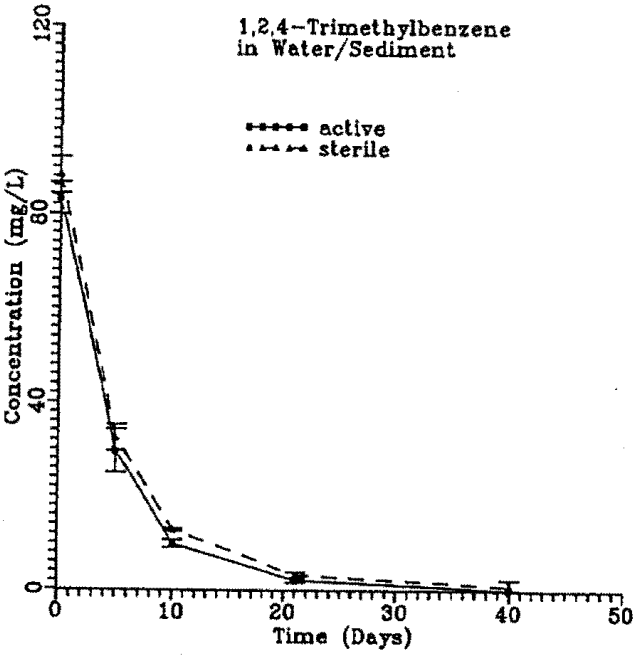

Figure 36. Disappearance of 1,2,4-Trimethylbenzene from Water/sediment

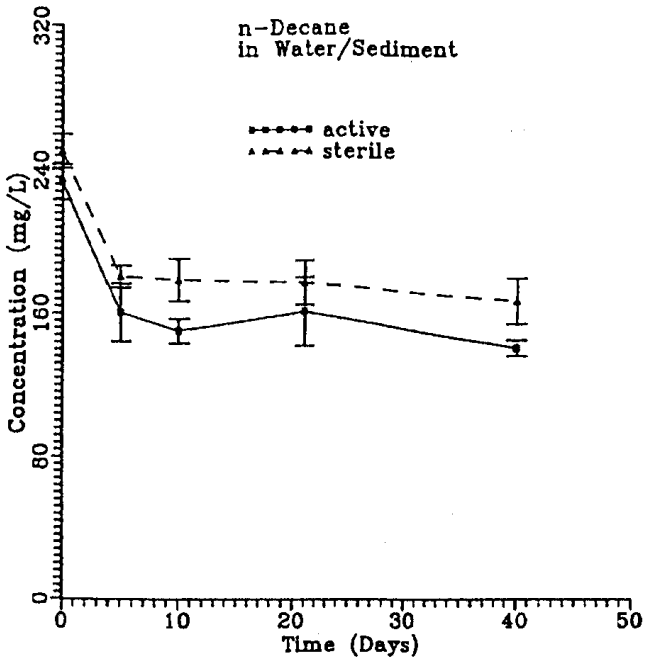

Figure 37. Disappearance of n-Decane from Water/sediment

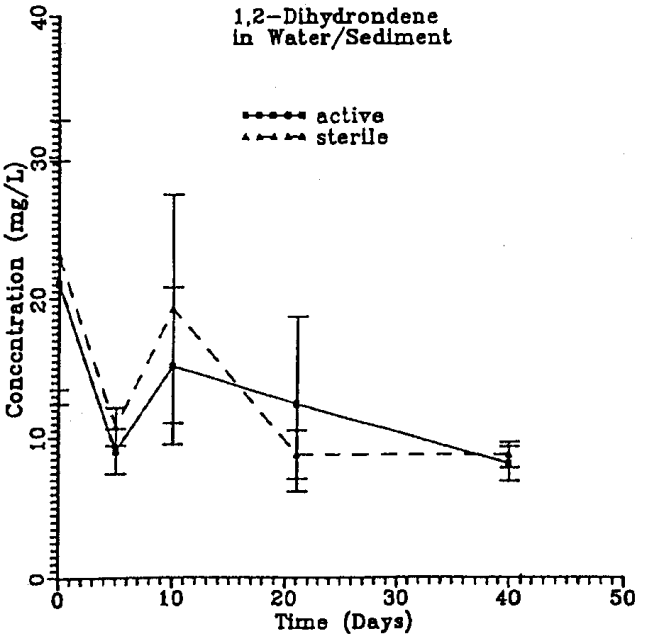

Figure 38. Disappearance of 1,2-Dihydroindene from Water/sediment

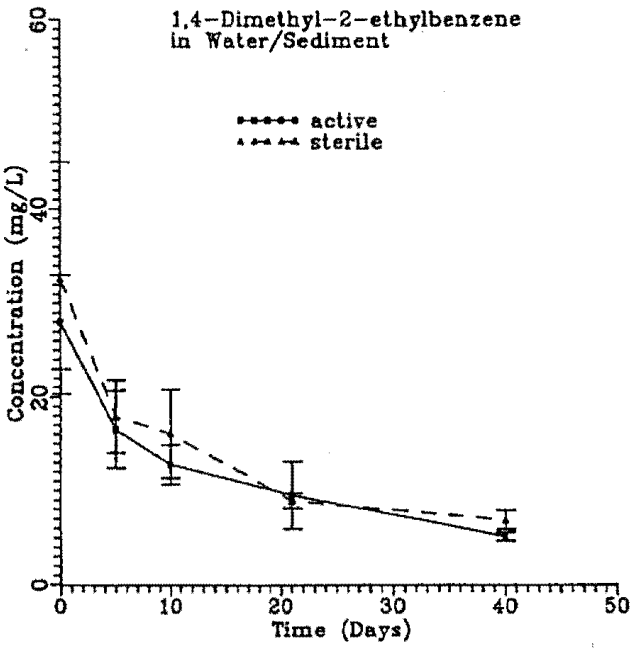

Figure 39. Disappearance of 1,4-Dimethyl-2-ethylbenzene from Water/sediment

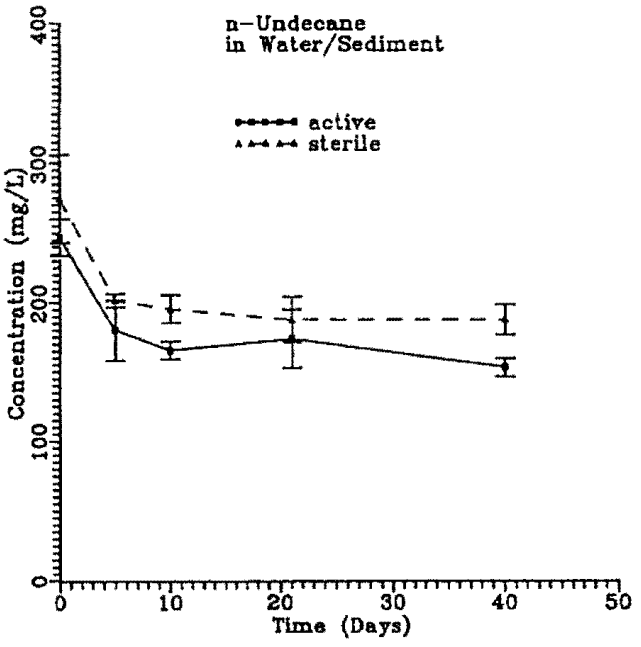

Figure 40. Disappearance of n-Undecane from Water/sediment

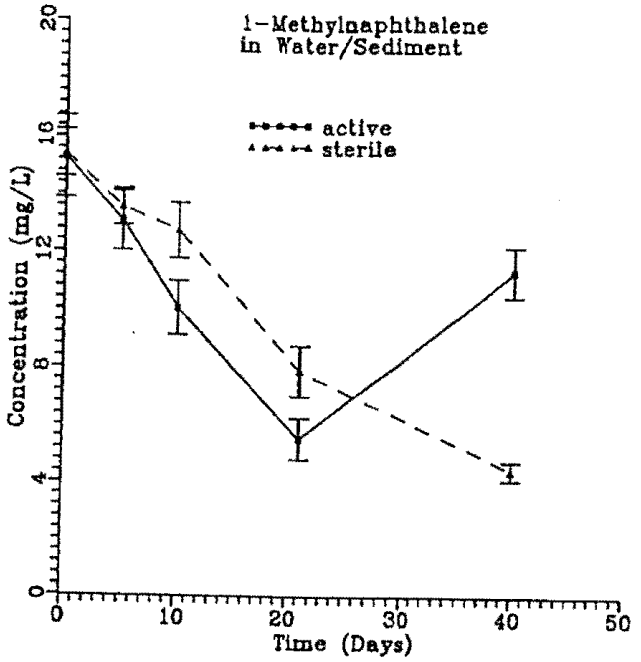

Figure 41. Disappearance of 1-Methylnaphthalene from Water/sediment

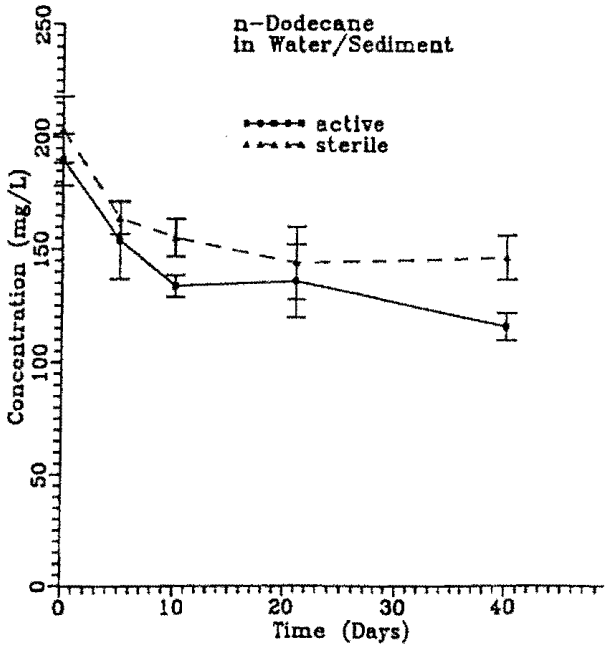

Figure 42. Disappearance of n-Dodecane from Water/sediment

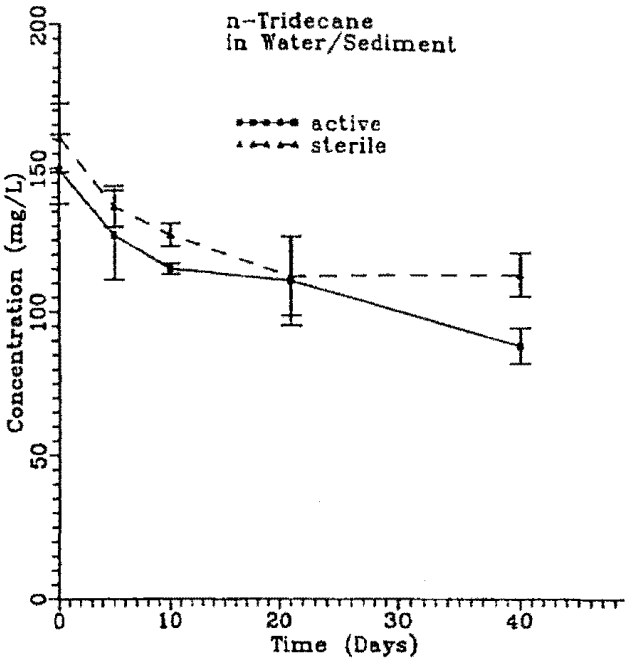

Figure 43. Disappearance of n-Tridecane from Water/sediment

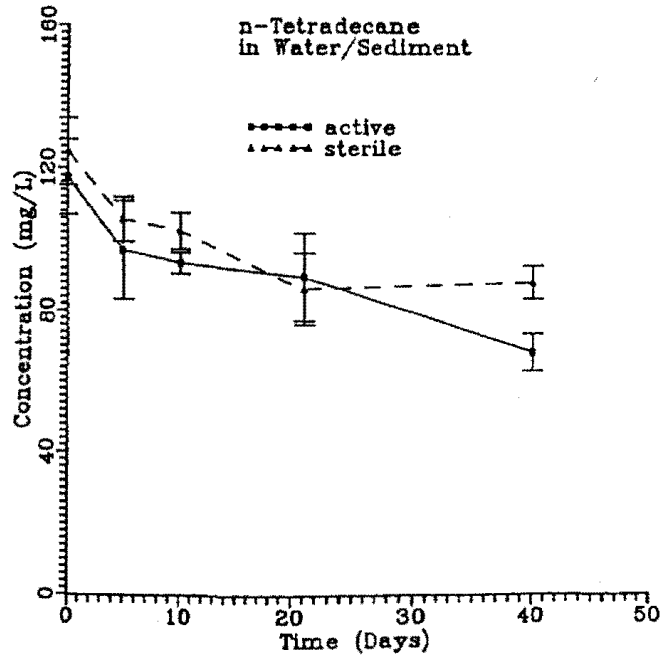

Figure 44. Disappearance of n-Tetradecane from Water/sediment

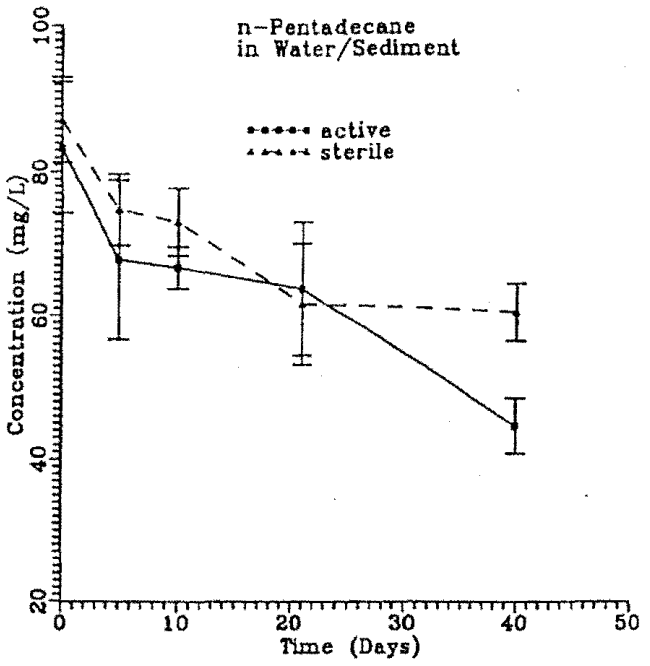

Figure 45. Disappearance of n-Pentadecane from Water/sediment

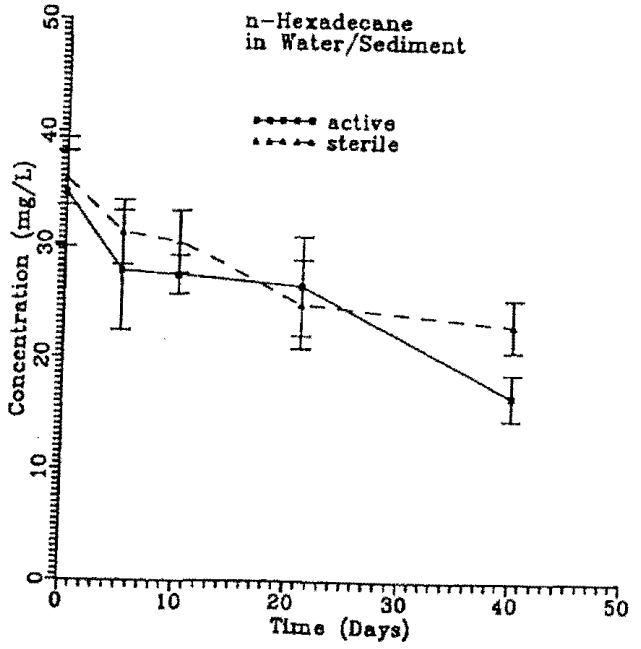

Figure 46. Disappearance of n-Hexadecane from Water/sediment

D. FATE OF JP-8 IN SOIL

The concentration of jet fuel decreased in soil over the experimental period as evidenced by the total chromatogram response (Figure 47) and plots of individual fuel components (Figure 48-65), where all but the high molecular weight components had disappeared by the end of the experiment. Statistical analysis of the disappearance curves indicated that n-nonane, n-undecane, n-dodecane and 2-methylnaphthalene showed significantly greater slopes in the active treatments than the sterile treatments. Thus, biodegradation played a limited role in removal of jet fuel components, and was component specific.

The error bars in the plot represent one standard deviation of the values obtained by triplicate analyses on a given trail day.

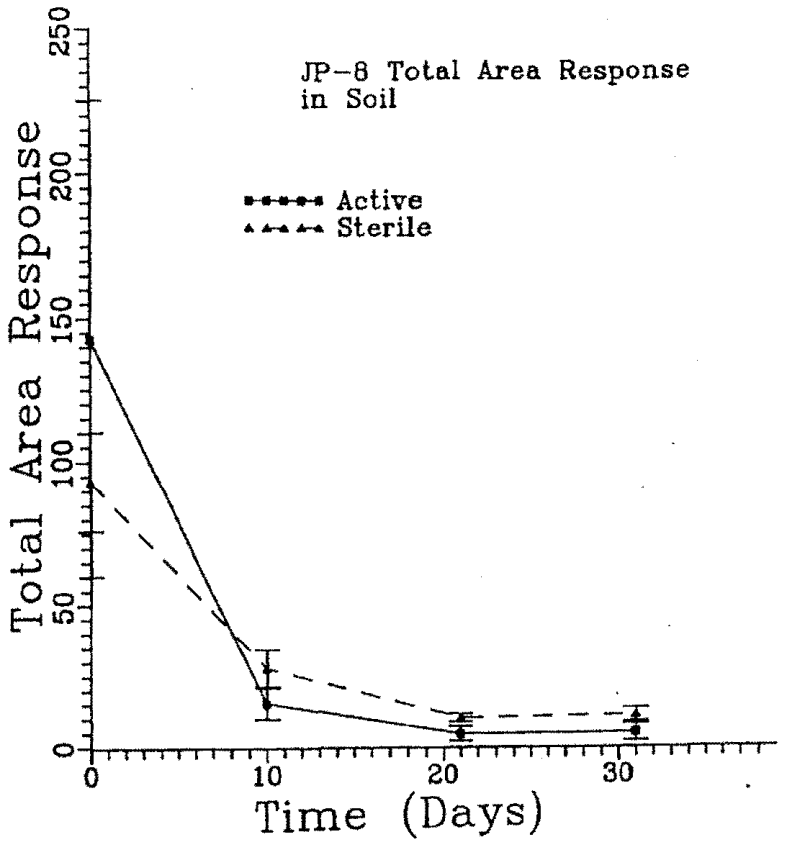

Figure 47. Disappearance of JP-8 from soil as measured by total chromatogram response

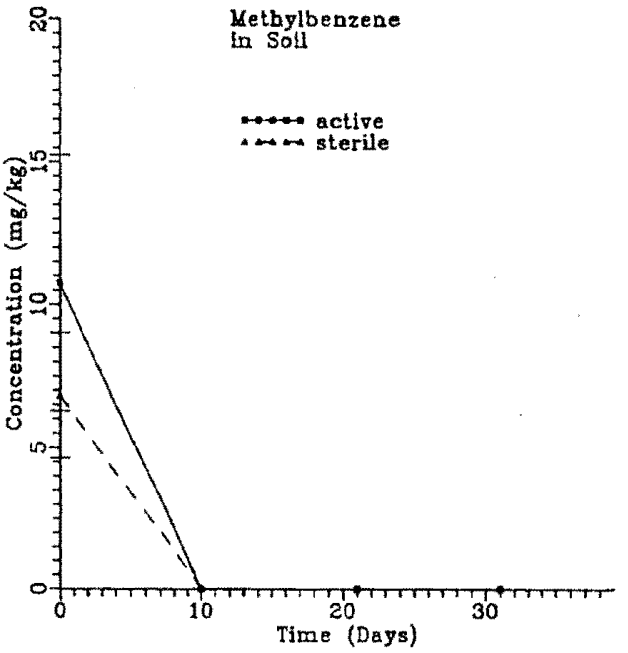

Figure 48. Disappearance of Methylbenzene from Soil

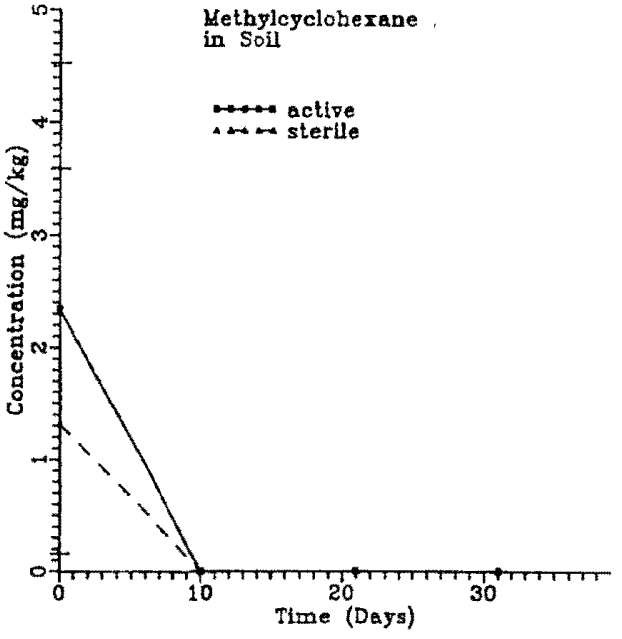

Figure 49. Disappearance of Methylcyclohexane from Soil

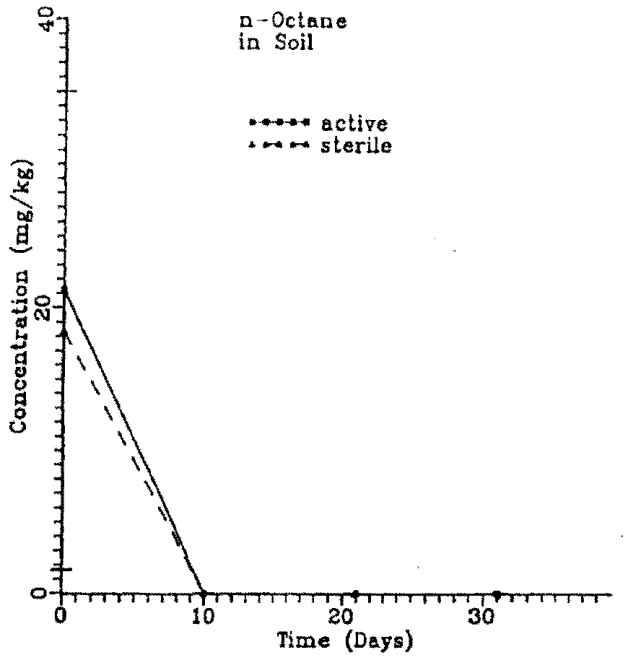

Figure 50. Disappearance of n-Octane from Soil

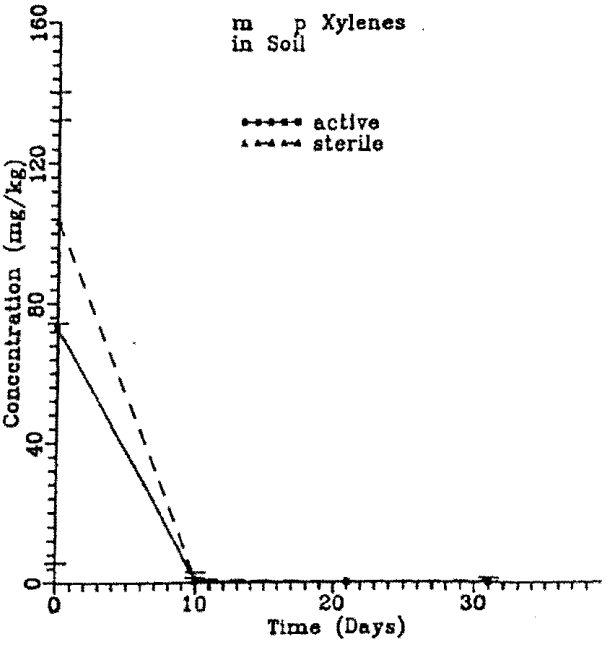

Figure 51. Disappearance of m- and p-Xylene from Soil

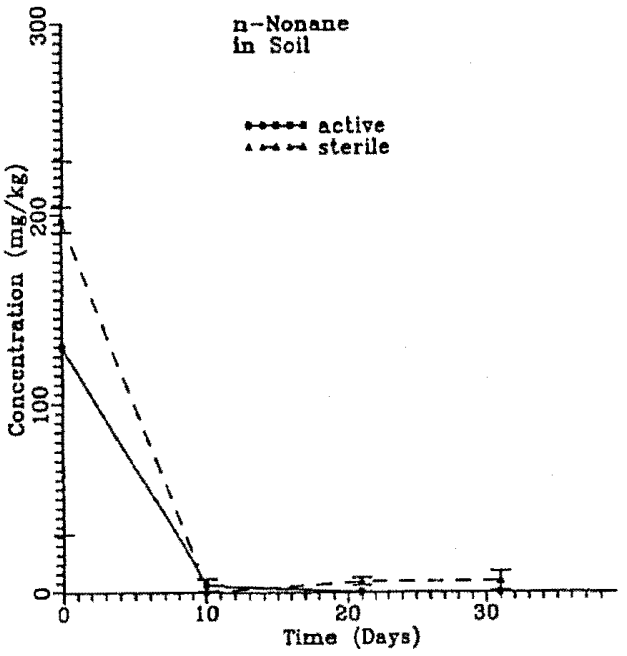

Figure 52. Disappearance of n-Nonane from Soil

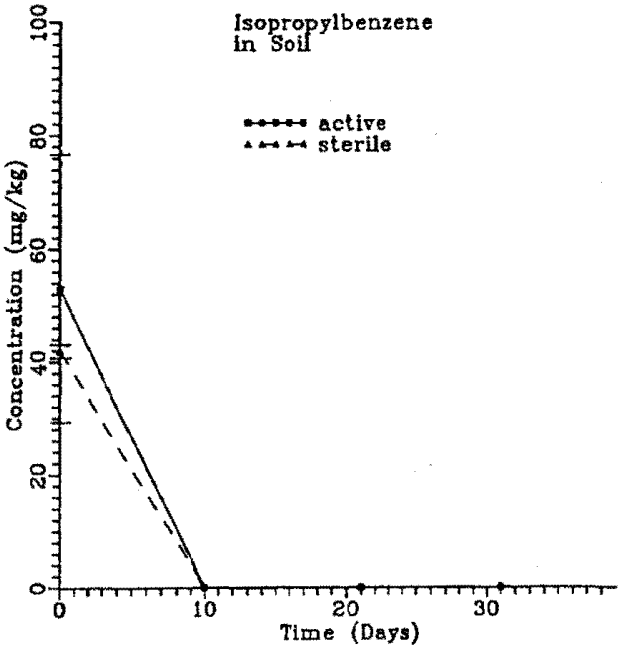

Figure 53. Disappearance of Isopropylbenzene from Soil

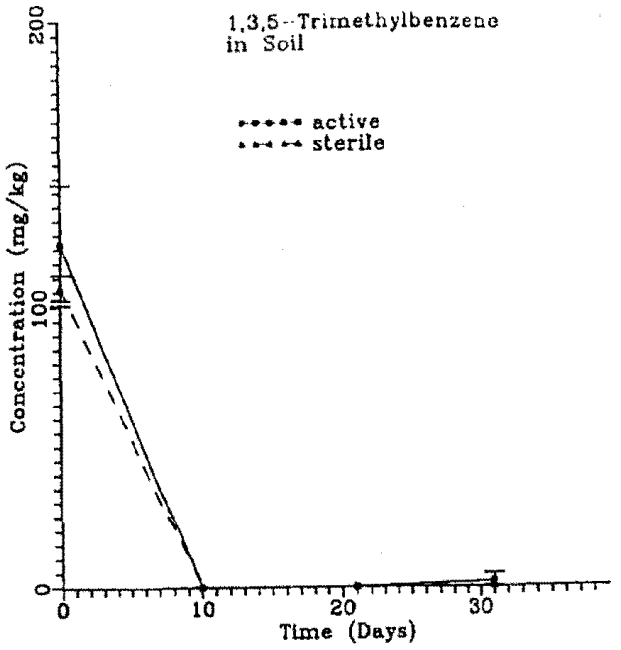

Figure 54. Disappearance of 1,3,5-Trimethylbenzene from Soil

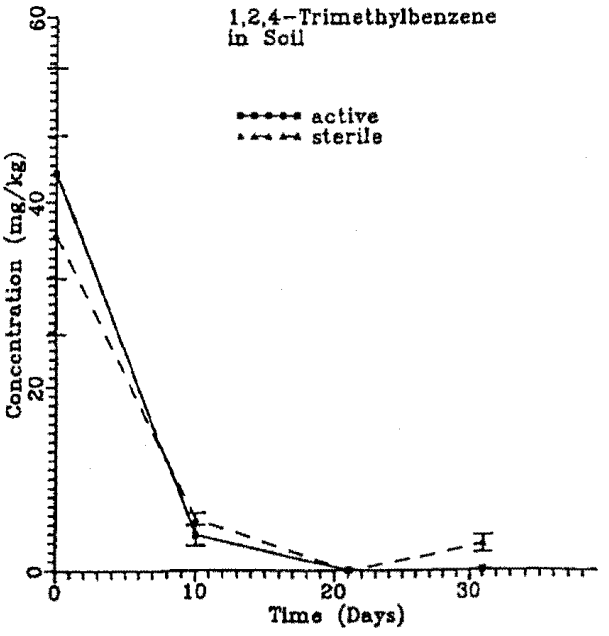

Figure 55. Disappearance of 1,2,4-Trimethylbenzene from Soil

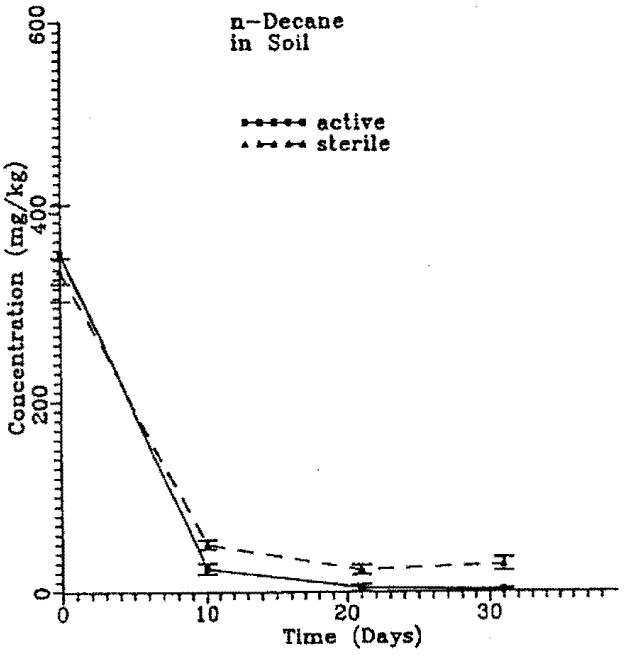

Figure 56. Disappearance of n-Decane from Soil

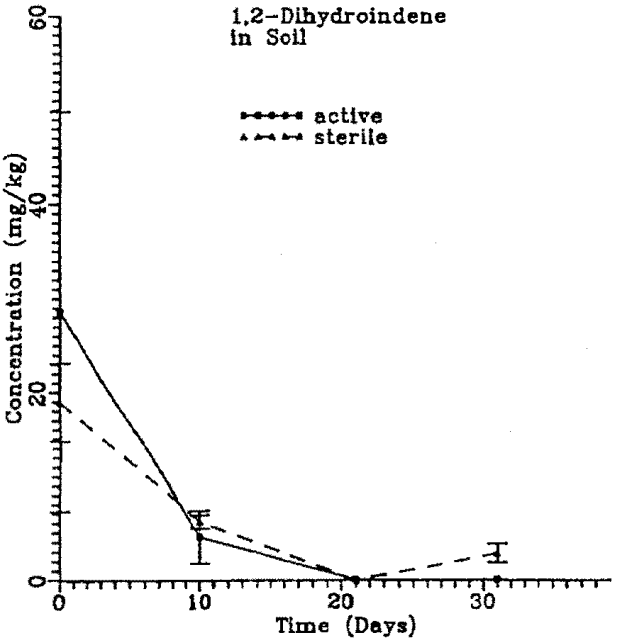

Figure 57. Disappearance of 1,2-Dihydroindene from Soil

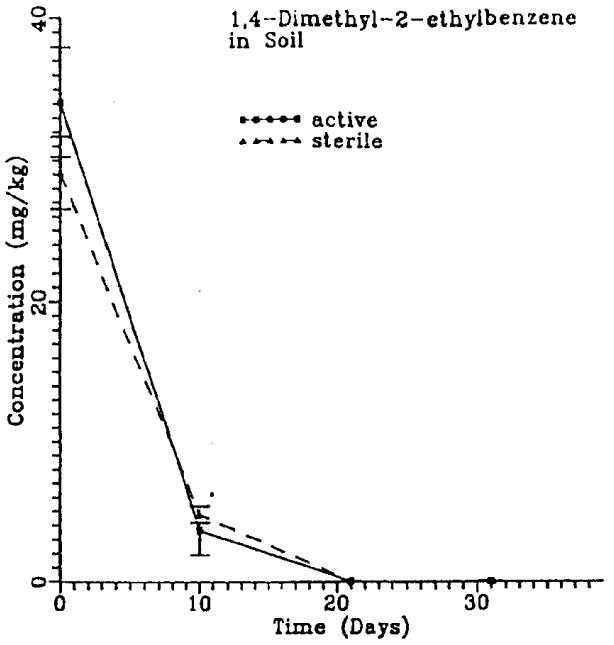

Figure 58. Disappearance of 1,4-Dimethyl-2-ethylbenzene from Soil

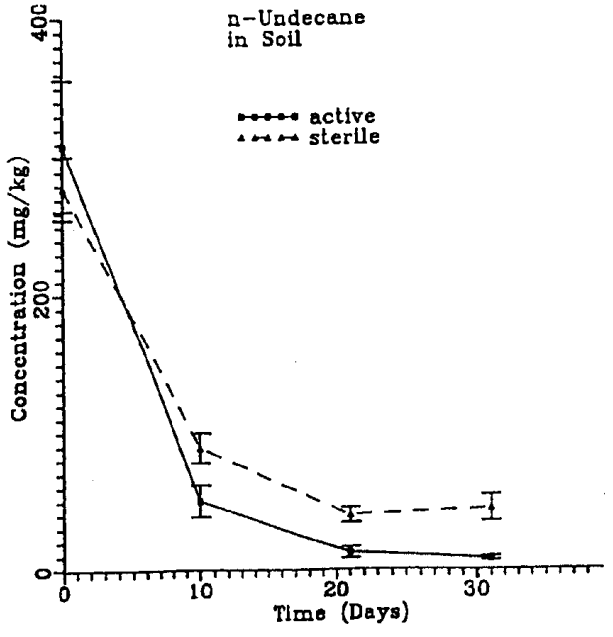

Figure 59. Disappearance of n-Undecane from Soil

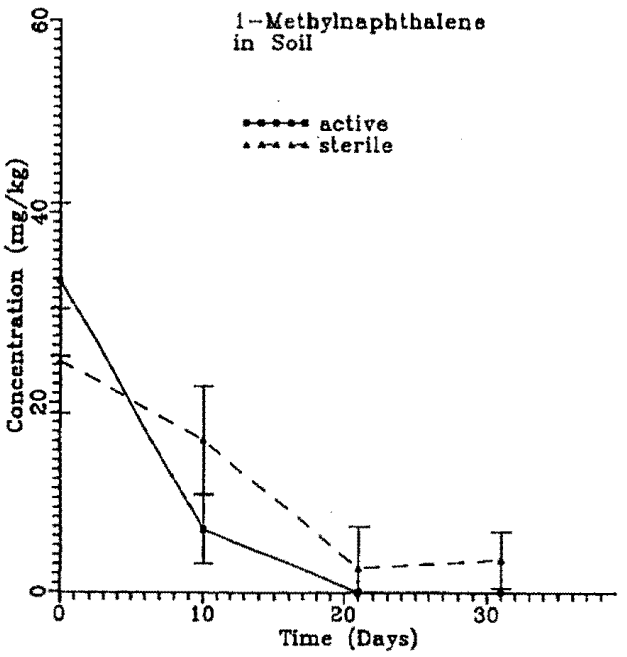

Figure 60. Disappearance of 1-Methylnaphthalene from Soil

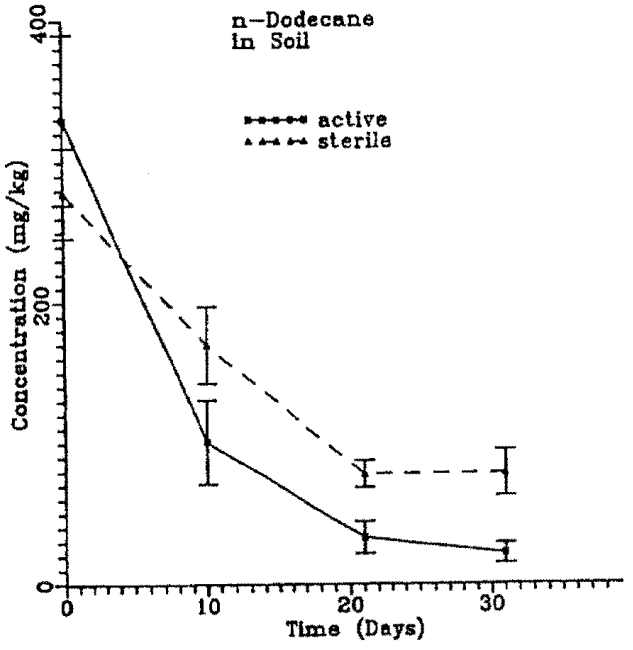

Figure 61. Disappearance of n-Dodecane from Soil

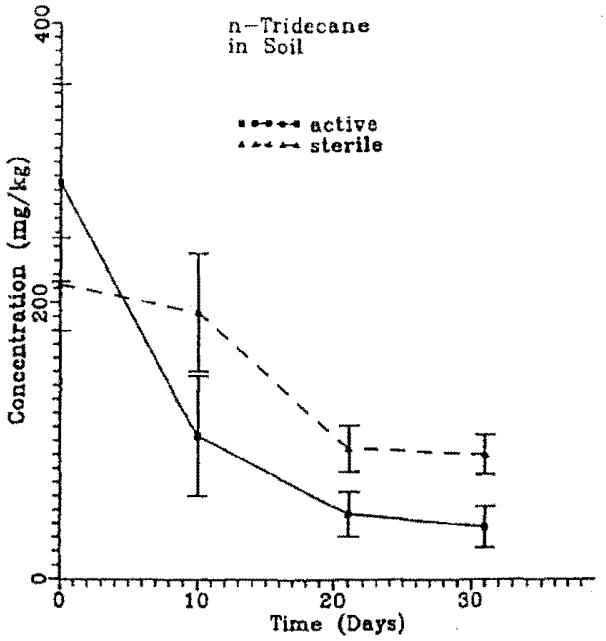

Figure 62. Disappearance of n-Tridecane from Soil

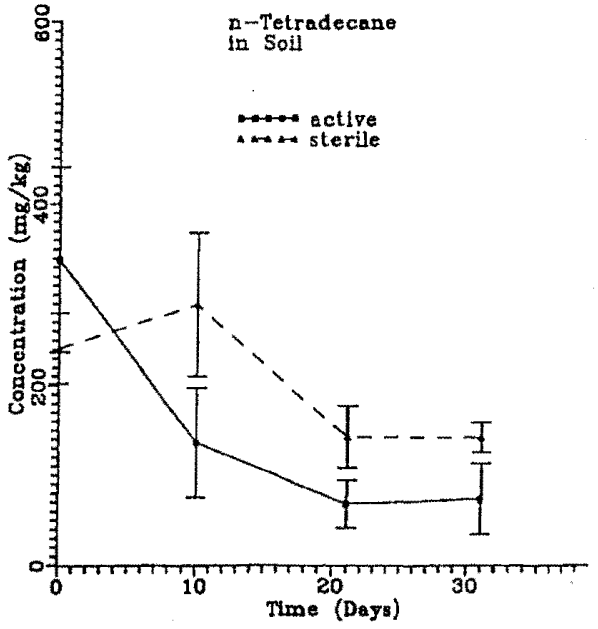

Figure 63. Disappearance of n-Tetradecane from Soil

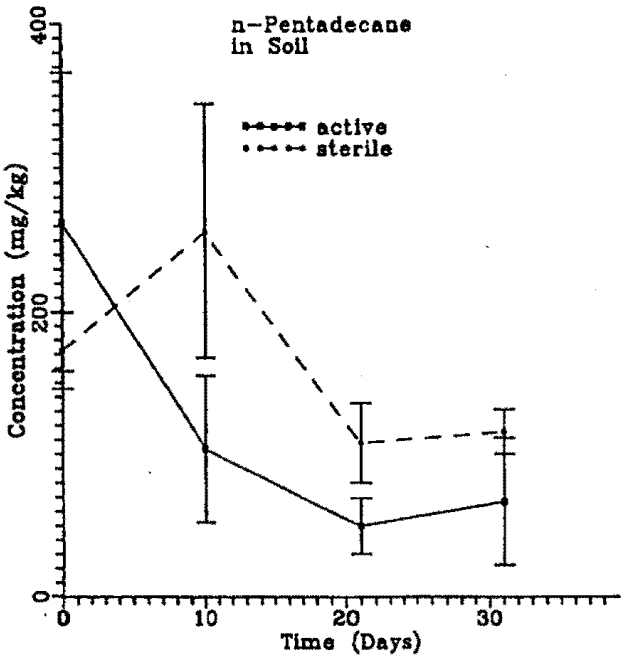

Figure 64. Disappearance of n-Pentadecane from Soil

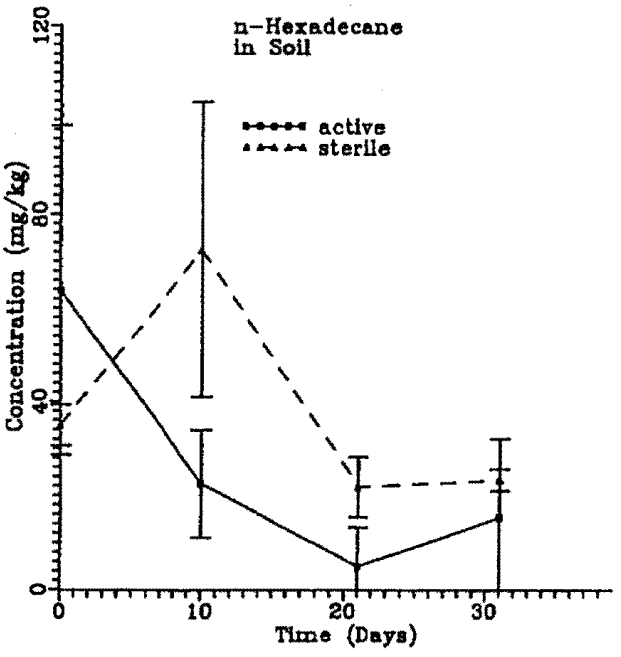

Figure 65. Disappearance of n-Hexadecane from Soil

E. TOXICITY OF JP-8 TO MICROORGANISMS

Microbial activity in water was inhibited by all concentrations of JP-8 as indicated by a depression of glucose mineralization in comparison to the control (Figure 66). Over the time period of the experiment, microbial activity in the water treated with 0.01% JP-8 increased to the control level. Microbial activity in the other treatments remained low. Hexadecane mineralization (Figure 67) was higher in the water treated with 0.01% JP-8 than it was in the control, but by day 4 had decreased to the control level. Hexadecane mineralization in 0.1% JP-8 treated water was negligible at day 1, and was low but measurable on day 2 (fraction utilized 0.006) and 4 (fraction utilized 0.003). Hexadecane mineralization in the 1.0% JP-8 treatment was negligible at all sampling times.

Results of the toxicity assays with water/sediment slurries are shown in Figure 68. Addition of JP-8 at concentrations of 0.1% and 1.0% enhanced microbial activity as indicated by an increase in glucose mineralization when compared to the control. This increase in microbial heterotrophic activity did not extend to hydrocarbon degradation. Mineralization of hexadecane was negligible in all water/sediment treatments at all time periods.

The error bars in the plot represent one standard deviation of the values obtained by triplicate analyses on a given trail day.

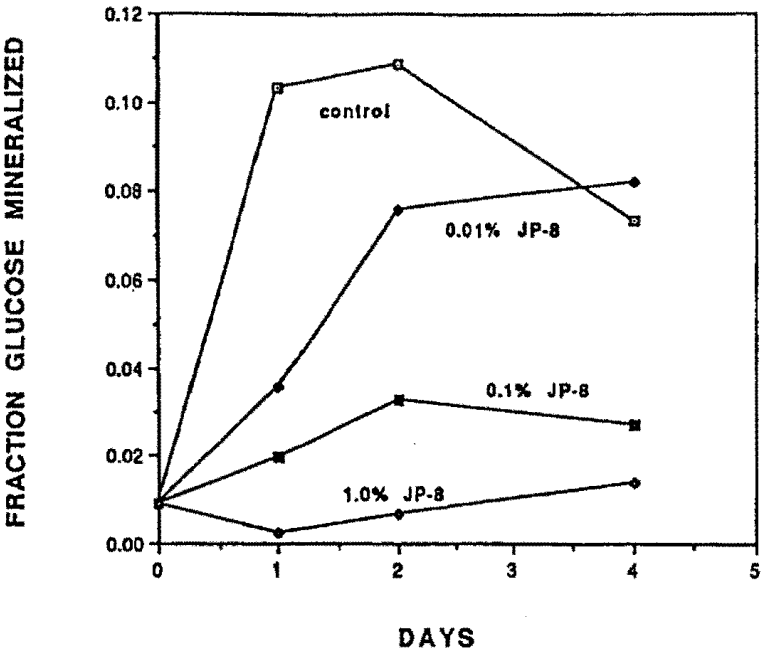

Figure 66. Effects of Varying Amounts of JP-8 on Microorganisms as Measured by Glucose Mineralization

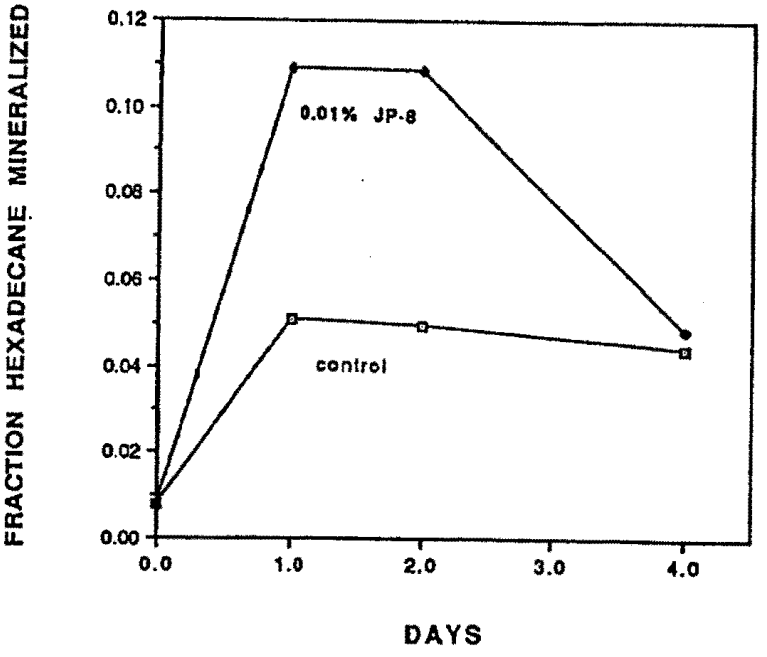

Figure 67. Effects of Varying Amounts of JP-8 on Microorganisms as Measured by Hexadecane Mineralization

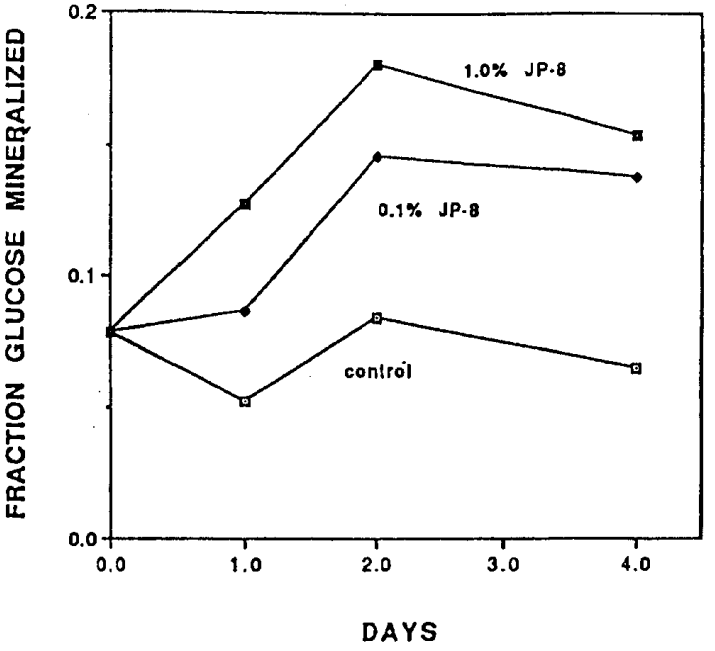

Figure 68. Toxicity of Varying Amounts of JP-8 to Microorganisms in Water/sediment Slurries as Measured by Glucose Mineralization

SECTION IV
CONCLUSIONS

The major removal process of JP-8 in the aquatic environment is evaporation. The more volatile components of the fuel evaporated within the initial 5 days of the experiment; significant removal of all components occurred by the end of the experiment. Some components were still present in significant amounts at the end of the experimental period, particularly n-alkanes such as tetradecane, pentadecane and hexadecane. Addition of sediments to water inhibited the evaporative removal of JP-8, apparently by adsorbing the components of JP-8 thus rendering them unavailable for evaporation.

One possible explanation for the lack of biodegradation of JP-8 in water samples is the toxicity the fuel exerts towards microorganisms. The concentration of fuel used in the quiescent bottle test (1 percent) was inhibitory to microbial heterotrophic activity and hydrocarbon-degrading activity. Thus the persistence of some components of JP-8 until the end of the experimental period may be due to severe inhibition of microbial activity within the test bottles.

As measured by glucose mineralization, JP-8 was not toxic to sediment microorganisms. Nonetheless, hydrocarbon-degrading capabilities of the population were below the detection limit even in the control flasks. One possible explanation may be that sediment-hydrocarbon binding may sequester the hydrocarbon, making it less available for microbial metabolism.

Biodegradation contributed to the removal of JP-8 from the terrestrial environment. Eight of the components disappeared faster in the active treatments than in the sterile treatments. For other components, there was a reduction of the slope in active treatments, but not to a statistically significant extent within the experimental design. This suggests that manipulation of conditions to enhance biodegradation may increase the rate of removal of JP-8 from the terrestrial environment. This agrees well with literature reports on land farming of waste hydrocarbons (Bartha and Bossert, 1984) and the results of a recent study on the biodegradation of JP-4 in a contaminated aquifer (Aelion and Bradley, 1991). These authors confirmed our findings that biodegradation was compound-specific and was limited by availability of nitrogen.

SECTION V
RECOMMENDATIONS

Because sorption on sediments reduced evaporation, remedial actions which increase the contact between fuel and sediment should be investigated further.

Biodegradation can contribute to the removal of some components of JP-8 in the soil. Strategies to enhance biodegradation in this environment, such as fertilization and aeration, may be useful in achieving maximum rates of removal of JP-8. Further investigation of enhanced biodegradation seems warranted.

JP-8 showed less of a potential for biodegradation than JP-4. This may be due to increased toxicity of the fuel to microorganisms. Further investigation to determine the toxic components of JP-8 is recommended.

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