

**Use of Soil Column Test Apparatus
for Evaluation of In Situ Bioremediation Potential**

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An apparatus has been constructed to test for biodegradation potential in field samples of subsurface soil directly as taken from a contaminated site. Field cores are taken using a split-spoon hollow-core auger with a removable and sterilizable core barrel insert. The insert containing the soil sample is transported to the laboratory and placed directly into the pre-sterilized apparatus. Disturbance of the soil sample is thus limited to the compaction incurred during procurement of the sample. The apparatus provides axial flow along the core barrel under a constant head, samples are removable from influent and effluent, flow rate may be continuously monitored during incubation, and nutrient, electron acceptor, and contaminant addition are accomplished through mixing in the head tank. The overall recirculation pattern minimizes effluent generated to roughly 1/100 of the total volume of water passed through the column. Refinement of proposed *in situ* bioremediation schemes may also be carried out using microcosm studies in conjunction with soil column testing. Applicability of the procedure for determining potential for *in situ* bioremediation is expected to be high, and the cost associated with use of such an apparatus is low enough that numerous samples may be tested simultaneously. Primary expense of operation is in the cost of sample analysis.

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INTRODUCTION

Historically, evaluation of bioremediation strategies at hazardous waste sites has been limited to either batch microcosm studies^{4,9} or pilot scale tests using estimates of nutrient requirements with little or no thought to kinetic and other limitations present in the soil environment¹. Soil column studies have been widely applied in areas such as transport of pesticides in surface soils and in ion transport in saturated flow^{2,5-8}, but have not been used widely in studying conditions for optimal bioremediation of hazardous waste³. Reasons for this limitation may be: 1) the increased volume of hazardous material that must be handled with a soil column study, 2) lack of confidence in relationship of soil column data to actual field conditions, 3) difficulty in maintaining field core samples intact in a testing apparatus. The methods proposed in this paper may alleviate some of these concerns by offering alternative modes of operation.

The traditional mode of operation of a soil column is characterized by a once-through apparatus. Influent is provided to the head end of the column via a pump, and effluent is collected from the bottom. Sampling ports may be provided along the length of the column. Typically, the unit is operated by filling the column with soil of interest (a "disturbed" column) or by procuring a field sample or subcoring (an "undisturbed" core) and operating under constant flowrate conditions until some desired endpoint is reached.

The use of a disturbed column must be regarded with some caution; the relationships between mass transfer, hydraulic conductivity, biotransformation rate, and soil heterogeneity are strongly influenced by characteristics such as packing density and soil handling. The use of "undisturbed" columns is an improvement, but longer core samples may be compacted significantly, resulting in significantly different hydraulic characteristics. In monitoring the dynamics of the flow of through such a system, operation under constant flowrate conditions may also not be representative. If bacterial growth in the column is significant, the actual and average pore velocities will change due to decreased pore volume. Operation under constant hydraulic gradient (as proposed here) will result in less variation in pore velocity (although pore velocity will NOT be constant), and changes in the hydraulic conductivity can be determined by monitoring the changes in flowrate.

In the proposed work, direct field core samples will be used to monitor transport/leaching of free pentachlorophenol (PCP) and PCP associated with adsorbed non-aqueous phase liquid (NAPL) under constant hydraulic gradient. Small or short cores will be used to control conditions within the core, and studies will be carried out to qualitatively describe the effects of soil compaction, heterogeneity, and nutrient enrichment schemes on leaching and biotransformation of the NAPL and PCP.

MICROCOSM STUDIES

To develop baseline kinetic data and to determine nutrient requirements for organisms in the site of interest, the microcosm study provides a convenient method for screening a number of treatment methodologies in a laboratory environment. A site in Butte, MT was chosen for study which historically received a significant spill of pentachlorophenol in #6 fuel oil. The resulting plume floated on the groundwater and flowed about 700 m until discharging to surface water. The resulting contaminated areas vary in condition from continuous contamination from ground surface to water table near the original point of the spill to a thin layer on the groundwater surface alone. Microcosm studies have been carried out on soils from several such areas, using a dilution of 5 g of soil (wet weight) in 100 ml of solution. Solutions tested included distilled water and groundwater as a base, with varying amounts of nitrate, ammonia, phosphate, and mineral salts as additives, all supplemented with 25 mg/L of pentachlorophenol.

Growth curves for organisms in one such study are shown in Figure 2. The corresponding growth rates vary from 0.13/hr for sample 1 to 0.30/hr for sample 4. Pentachlorophenol analyses were done on several samples from the beginning and end of the study, and disappearance of PCP was not detected; final samples had concentrations which were not significantly different from the initial samples. In these same samples, however, a significant disappearance of non-aqueous phase liquid (NAPL) was noted, as determined by the appearance of the samples. Studies are now underway (a) to isolate PCP degrading organisms from the site, for the purpose of determining whether PCP degradation is even theoretically possible at the site, and (b) to determine the rate of NAPL degradation, if any, so that rates of release of PCP from disappearance of the NAPL can be estimated.

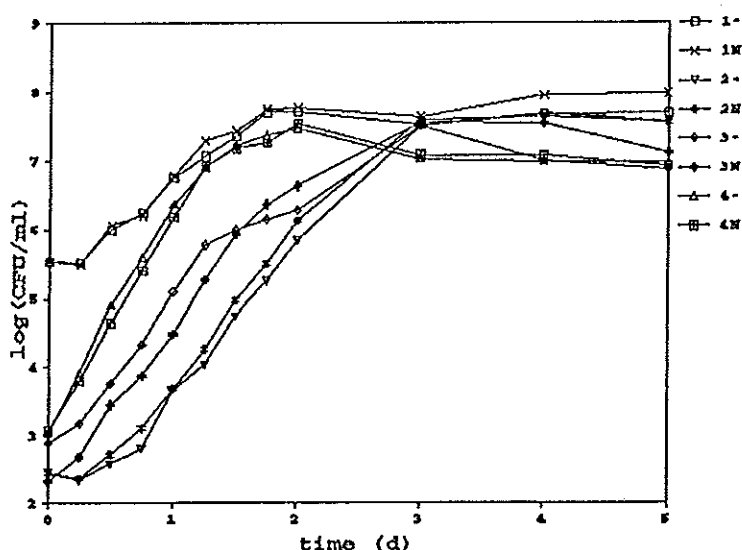


Figure 1. Results of plate counts on soil from contaminated source zone. (-): no enhancement, (N): amended with 25 ppm $\text{NH}_3\text{-N}$. Sample 1: surface; Sample 2: 1 m deep; Sample 3: 4 m deep (above GW table); Sample 4: 5 m deep (below GW table).

METHODS AND MATERIALS

A soil column apparatus has been constructed to contain a liner from a split spoon sampler so that field samples can be tested directly. A cap system was designed to fit snugly over each end of the liner barrel, with parallel plates on either end secured with all-thread passed through holes on the outer end of the retaining plates. This system is shown in Figure 2. A sealing system consisting of a series of two O-rings within the cap provides significant protection against leakage due to pressure increases within the column and against rough handling.

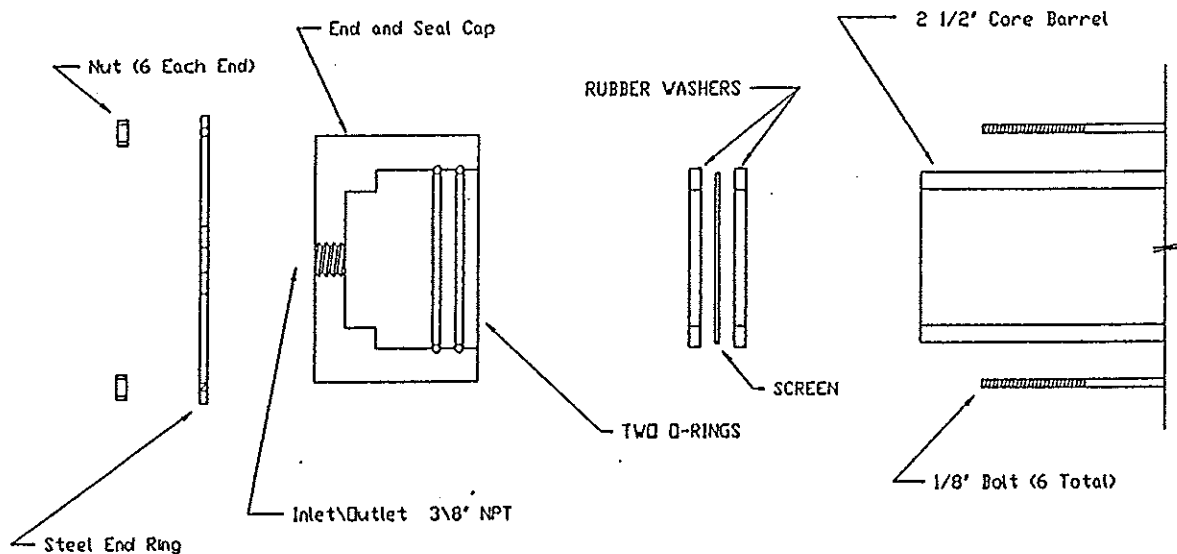


Figure 2 End cap system to contain a liner barrel from a split spoon sampler.

Soil is retained within the liner by insertion of a fine screen into the base of the liner cap, between the end of the liner and the inside end of the cap. This system can be refined somewhat if necessary by the addition of 1 to 2 cm of sand. End caps and fittings are constructed of polycarbonate and stainless steel to minimize the effects of adsorption to surfaces, and allow for autoclaving.

An overall flow diagram of the system is shown in Figure 3. Materials of construction include glass (constant head reservoirs and glass tubing in each), teflon (valves and T-connectors), and Neoprene rubber (flexible tubing). Flow tests on the apparatus while filled with disturbed uncontaminated soil indicated that it was operable under a variety of conditions. In particular, 1) the recycle rate had little to no effect on the column flow rate, 2) the stainless steel screen alone retained the bulk soil but 3) the fines within the soil column were poorly retained. In hydraulic tests of the apparatus, fluid frictional resistances through the various appurtenances were found to have no significant effect on the head loss through the column in comparison to the resistance of the soil material itself.

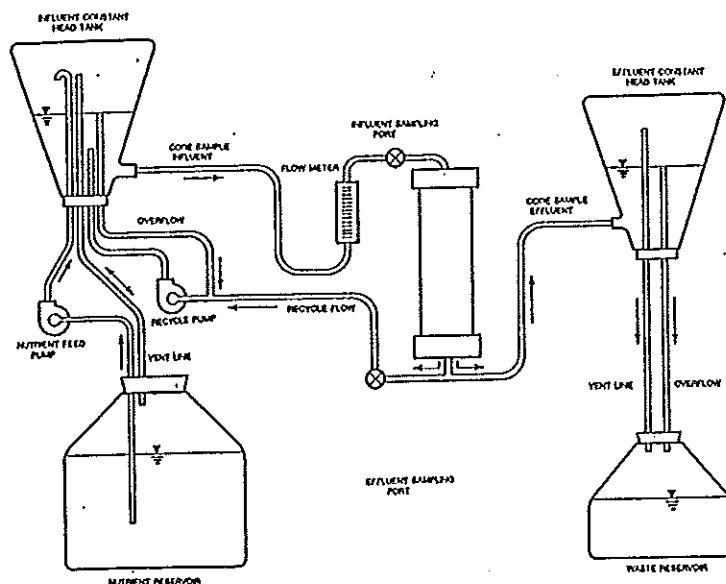


Figure 3 Flow diagram of the soil column apparatus utilizing recycle.

Dissolved oxygen in a test column filled with disturbed, uncontaminated soil run without oxygen enhancement is shown in Figure 4. These results indicate that mildly aerobic conditions can be maintained over a period of 1-2 weeks without O_2 addition other than that diffusing from the headspace in the constant head reservoirs. Where more aerobic conditions are desired throughout the system, pure O_2 will be bubbled into the upstream constant head reservoir. These high concentrations should inhibit biological activity in the reservoir while maximizing the potential for aerobic growth throughout the soil column. Where anaerobic conditions are desired, N_2 will be used to saturate the upstream constant head reservoir, purging the system of oxygen.

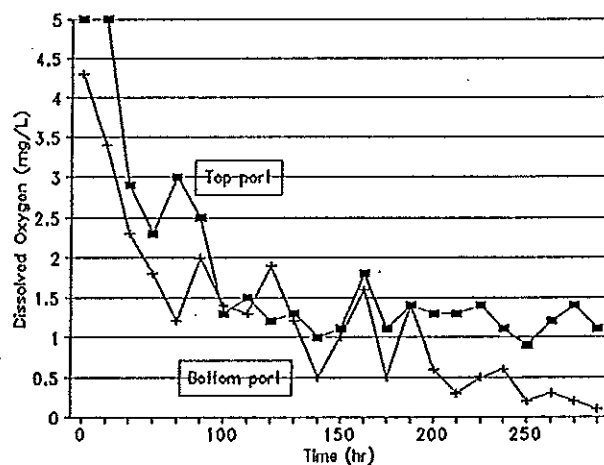


Figure 4 Dissolved oxygen at the top port (entrance) and bottom port (exit) of a 0.5 m column operated at 0.1 m head, containing disturbed garden soil and sand (50:50).

The soil column system contains approximately 3 m of neoprene tubing. Preventing biofilm growth on the interior surface of the tubing would require biocide addition or frequent physical removal; both of these are unacceptable alternatives. The contribution of any biofilm of contaminant removal is probably minimal considering the limited surface area in the tubing compared to the soil surface in the reactor. However, tubing samples will be taken following each experiment to determine biofilm and NAPL accumulation.

PERFORMANCE OF THE SYSTEM

Modelling of the Soil Column Reactor

It is difficult to quantify variations in contaminant concentration with depth within an undisturbed column without installing sampling ports. It is nevertheless possible to surmise a great deal from the overall column performance during operation. In particular, the material balances on these systems can be useful in describing the time required for achievement of steady-state, approximating the overall transformation rate within the system, and for predicting the overall quantity of readily removable material within the soil sample.

The material balances on a soil column system without any recycle can be written as follows:

$$\frac{dm}{dt} = F_c \cdot (c_0 - c_e) - \int_{V_c} r \, dV \quad (1)$$

where

V_c	=	total volume of soil core (L^3)
c_0	=	concentration of contaminant/substrate/biomass in the influent (ML^{-3})
c_e	=	concentration of contaminant/substrate/biomass in the effluent (ML^{-3})
F_c	=	flowrate of water through the column (L^3t^{-1})
r	=	rate of consumption within the soil core ($ML^{-3}t^{-1}$)
m	=	mass of contaminant/substrate/biomass within the column (M).

If the mass of contaminant within the core is treated as the product of an average concentration and the soil core volume, the rate of consumption within the core is similarly averaged, and the core volume is divided out of the equation, the following is obtained:

$$R \frac{d\hat{c}}{dt} = \frac{M_0}{V_c} - \frac{C_e}{\theta} - \bar{r} \quad (2)$$

where

M_0	=	mass loading of component to the system (Mt^{-1})
R	=	retardation factor (-), accounting for adsorption to soil and organics
θ	=	overall residence time within the system (t) = V_c/F_c
$\hat{c}$	=	indicator of volume average value

A similar approach to a mass balance around a soil column reactor system operated with recycle to the influent end constant head tank yields the following equation:

where

$$R \frac{d\hat{c}}{dt} = \frac{M_0}{V_c} - \frac{C_0}{\theta} - \frac{V_1}{V_c} \cdot \frac{dC_1}{dt} - f \quad (3)$$

V_1	=	influent constant head tank volume (L^3)
C_1	=	concentration of component in the influent reservoir (ML^{-3})
θ	=	system residence time (t) = V_1/F_0
F_0	=	overall system feed rate (L^3t^{-1})

The reaction rate term in the equations above pertains only to the actual degradation of a component within the system; mass transfer effects, etc. are included only insofar as they directly affect the reaction rate. As such, then, there is a ceiling on the reaction rate, as determined by the availability of substrate, electron acceptor, nutrients, and biomass within the system (e.g., the dual-substrate Monod model). The overall reaction rate will be maximized in a system such as a microcosm study where biomass is allowed to grow relatively unhindered by surrounding pore space, and optimal nutrients, substrate, and oxygen can be supplied. The actual reaction rate in a soil column will be significantly limited by mass transfer to a small fraction of that found in a microcosm study.

While equations (2) and (3) are quite similar, the residence times (θ) have different meanings. In the recycle system, the flowrate (F_0) is the flow applied to the entire system, which can be a very small fraction of the column flow (F_c). Thus, the time constant associated with bulk transport is much longer for the recycle system (equation 3) than for the once-through system (equation 2), even though the flow through the column (F_c) is the same in either case.

The primary disadvantage of the recycle system is that the use of a long overall residue time requires much more time to reach steady-state, and, longer experimental run times. The advantage of a longer residence time, however, is that the system remains relatively stable during acclimation of biomass within the system. Further, a longer time constant for the system is a more appropriate physical model of actual conditions. Conditions *in situ* are unlikely to undergo rapid change; except at the edges of the contaminant plume or the leading edge of a nutrient injection plume, a volume of soil is unlikely to see an abrupt change in conditions. A plug of soil water leaving a given soil volume is likely to continue to be replaced with very similar plugs for a significant time period.

An additional advantage of the recycle system is that critical components such as suspended biomass, inhibitive compounds, and other dissolved components will not be washed out of the system with the first displaced soil volume. Contaminants which originate in the soil sample will tend at least to remain in the system, so that the response of the system reflects the conditions likely to be encountered in the field.

Use with NAPLs

The material balances expressed in equations 1-3 become significantly more complex when applied to a soil core which comes into the laboratory contaminated with a non-aqueous phase liquid (NAPL). The NAPL will undergo transport via a variety of heterogeneous mechanisms, varying from bulk transport of macrodroplets to actual flow along the surface of the soil particles. With a heterogeneous soil, it becomes nearly impossible to predict the transport of the NAPL within and out of the column. The problem is complicated further by the fact that the tubing, glassware and other appurtenances within the system will tend to act as adsorbing surfaces for the NAPL, even if the most inert materials (glass, teflon) are used. The surface tension of the NAPL will be sufficient to provide the driving force for redeposition of material pulled from the column.

If the column is operated in once-through mode, these materials are lost, and there is a continuous but unpredictable disappearance of NAPL from the system. Operation through 3 to 4 residence times should be sufficient to remove material that is easily removed, and any material leaving the system after that time is likely to be removed gradually by forces that would normally act on a natural soil system. The result, however, is a significant "clean-up" of the test column before bacterial acclimation has had an opportunity to take place. The primary utility of the once-through column, then, is to determine the response of the system to the initial flow set up through the column, not for long-term studies.

In the recycle system, significant transport of NAPL droplets throughout the system can result in a fairly rapid loss of "modellable" conditions. NAPL will be found coating a number of surfaces within the system, including portions of the soil which were not previously coated. The usefulness of this type of operation, then, is limited to determining qualitative behavior of the system. A long lag time for growth of active organisms can be tolerated without significant loss of contaminant or biomass from the system. Biofilm growth and oxygen consumption can be allowed to reach a pseudo-steady state within the system. Degradation of the NAPL itself can be monitored via TPH "fingerprints" of material collected in the effluent and in the head tank. A critical question that can be answered qualitatively is that of the fate of toxic compounds dissolved in the NAPL: Does degradation of the NAPL precede degradation of other more toxic compounds, resulting in their release to the environment?

EXPERIMENTAL DESIGN

Sample wells drilled in July 1990 reveal substantial contamination still present in the form of a non-aqueous phase liquid (NAPL). Experimental cores were taken from (a) an area significantly removed from the source, but containing substantial subsurface contamination, and (b) near the predominant area of surface contamination. A high degree of variation in soil appearance and extent of contamination appeared over short distances. The following experiments attempt to determine the suitability of native

microbial populations for *in situ* bioremediation of the NAPL and PCP, and ascertain the effects of degradation of the NAPL on aqueous phase PCP concentration. Four sets of experiments will be conducted:

1. Leaching: The soil column will be run in once-through mode under anaerobic conditions. Site-collected, uncontaminated groundwater will be filter sterilized ($0.22\ \mu\text{m}$ filter) and used as a flow medium. Column effluent will be fraction collected and analyzed for PCP, TOC and total petroleum hydrocarbon (TPH). The use of anaerobic conditions will prevent aerobic activity, so that any effects of microbial activity will be a minimum.

2. Aerobic Degradation: Flow conditions will promote aerobic microbial activity. Replicates feed media will include:

- a. O_2 saturated, filter-sterilized groundwater.
- b. O_2 saturated, filter-sterilized groundwater + mineral salts + NH_3 .
- c. O_2 saturated, filter-sterilized groundwater + mineral salts + NH_3 + yeast extract.

Operation of the column apparatus will be in recycle mode. Sampling will consist of upstream and downstream samples analyzed for PCP, TOC, TPH, NH_3 , and NO_x .

3. Anaerobic Degradation: N_2 saturation of upstream and downstream constant head reservoirs will induce anaerobic conditions, which will be amended as in the following replicates:

- a. Filter sterilized groundwater (duplicates conditions in #1).
- b. Filter sterilized groundwater + mineral salts + NO_3 .
- c. Filter sterilized groundwater + mineral salts + NO_3 + yeast extract.

Sampling will take place as in #2 above.

4. Microbial Enhancement: Indigenous bacterial populations will be grown, isolated, and selected for their PCP degrading capability. Soil columns will be inoculated with these populations and tested under aerobic conditions as outlined in #2 above. Sampling will also follow as above.

EXPECTED RESULTS

To date, microcosm studies have revealed a microbial preference for the diesel fuel NAPL to the total exclusion of PCP. Both aerobic and anaerobic batch experiments have shown virtually no PCP degradation over an 8 week period. These results make it unlikely that the soil column experiments will produce measurable PCP degradation. However, even in the absence of biodegradation of PCP using unaltered native bacterial populations, these experiments should yield useful results.

Leaching experiments will provide a baseline from which NAPL and PCP biodegradation can be measured. In the once through mode, if PCP is added into the feed solution, effluent PCP concentration should behave as shown in Fig. 5. In recycle mode, with no PCP added in the influent, the effluent concentration should appear as in Figure 6. The shape and size of the response in Figure 5 can be used to predict portions of the response of the system when operated in recycle.

In experiments 2 and 3, several outcomes are possible. If no PCP biodegradation occurs, but NAPL biodegradation is significant, we may see an increase in PCP released to aqueous solution due to NAPL disappearance. If such an increase is not observed, yet NAPL degradation has occurred, PCP must be further concentrating in the remaining NAPL, or on soil adsorptive sites. Experiment 4 will demonstrate whether addition of microorganisms offers significant improvement of PCP biodegradation relative to *in situ* communities.

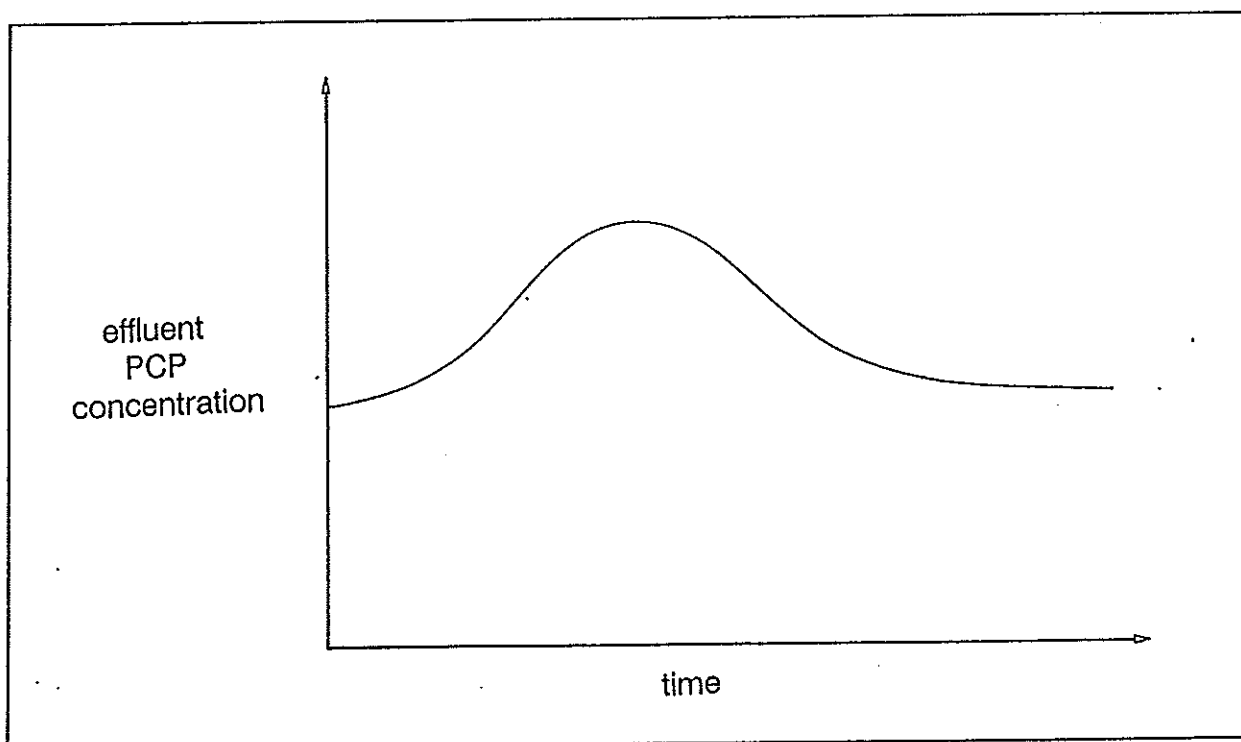


Figure 5 Anticipated effluent PCP concentration in once-through mode with influent PCP added.

ACKNOWLEDGEMENTS

Although the research described in this article has been funded in part by the U.S. Environmental Protection Agency under assistance agreement R-815709 to Montana State University through the Hazardous Substance Research Center for U.S. EPA Regions 7 and 8 headquartered at Kansas State University, and under assistance agreement R-815249-01-0 to MSU, it has not been subjected to the Agency's peer and administrative review and, therefore, may not necessarily reflect the views of the Agency, and no official endorsement should be inferred. This research was also partially supported by the U.S. Geological Survey (Project 14-08-0001-G1284), and the National Science Foundation (Grant ECE-8451060).

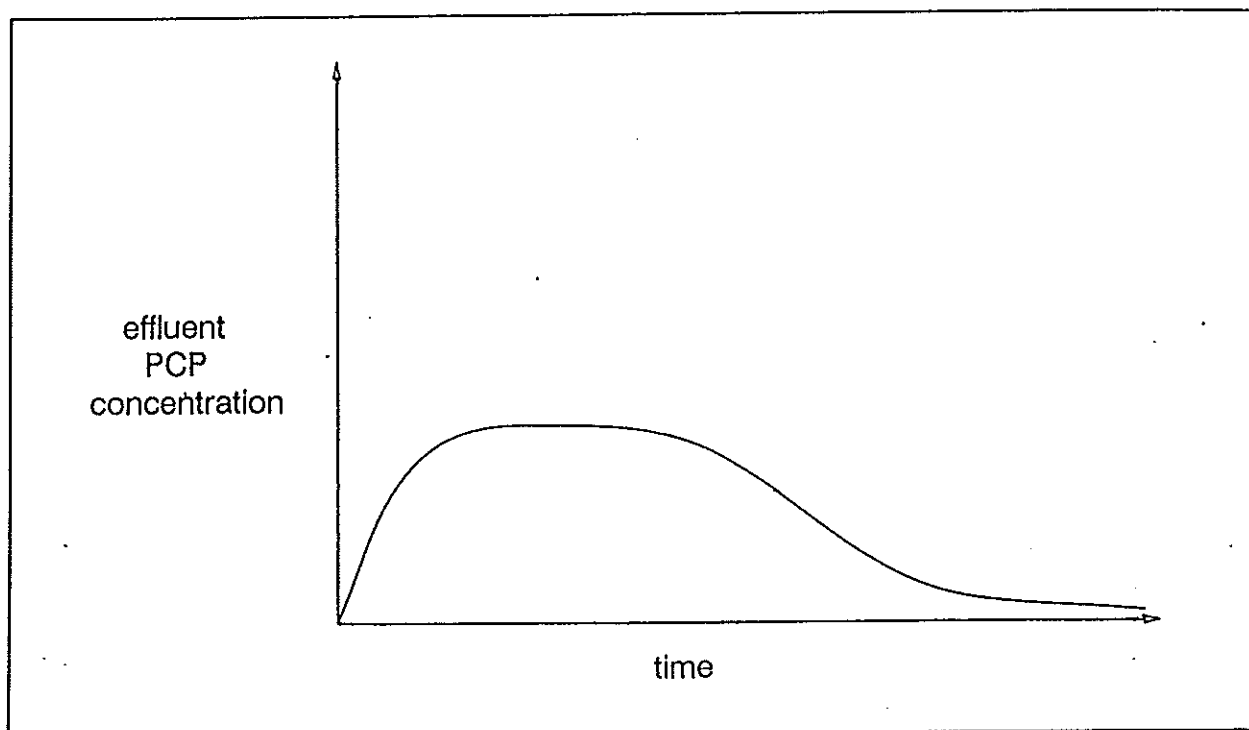


Figure 6 Anticipated effluent PCP concentration in recycle mode without PCP added.

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