EFFECT OF ORGANIC MATTER AND CONTACT TIME ON THE SORPTION AND BIOAVAILABILITY OF CHLOROPHENOLS

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(ABSTRACT)

Chlorophenols represent a class of organic contaminants that are commonly used and widely distributed in the environment. Adsorption to soils may inhibit the transport of these chemicals, while slow desorption rates can create a potential source for long-term contamination of groundwater supplies. Microbial degradation of these compounds may also play an important role in their environmental fate. The purpose of this study was to investigate how the processes of sorption, desorption, and biodegradation interact to effect the distribution of pentachlorophenol (PCP) and 4-monochlorophenol (4-MCP) in two soils with different soil organic matter (SOM) content.

Batch soil microcosms were used to measure the sorption of both test compounds at three concentrations for each, exposed to two soils with different SOM levels. An ultrafiltration study was designed to quantify partitioning to dissolved organic matter (DOM), while batch desorption experiments were performed to measure chemical release from the soils. Finally, biodegradation experiments were carried out under aerobic conditions to evaluate microbial interactions with PCP and 4-MCP in both the liquid and solid phases.

Sorption of both compounds was rapid, with 60-80% occurring within one day, but the process appeared to continue at a slower rate over several months. Statistical analysis showed that there were no differences in sorption due to SOM content or chemical concentration, for the

two silty loam soils used in this study. Desorption followed a similar pattern of a fast and then a slow stage, and a significant difference was measured between the two soils. Higher levels of SOM resulted in slower desorption and a lower total release of the contaminants, but both soils retained a large percentage of unextractable compounds. Contact time was found to have the greatest effect on the amount of this nondesorbable fraction.

The ultrafiltration study suggested that DOM polymerized into larger molecules after sufficient mixing time (3 months) in solution, which also increased the amount of ^{14}C compounds that were bound to these humic substances. The biodegradation study suggested that natural soil microbes could utilize PCP and 4-MCP from both the liquid and solid phases, and that sorption to dissolved organics and colloidal matter could protect these chlorophenols from microbial degradation. Some data also indicated a possible correlation between desorption rates and bioavailability.

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Chapter 1

Introduction

Due to their widespread use, there is concern over environmental contamination caused by chlorinated phenols. Their presence is due to the application of pesticides and wood preservatives, contaminated industrial waste waters, and accidental spills, to name a few of the major sources. In the United States alone over 95% of the usable freshwater is stored below ground, and the harmful effects that may result from its continuing pollution can no longer be dismissed (Morris and Novak, 1989).

The World Health Organization (1987) reported that approximately 30,000 metric tons of pentachlorophenol (PCP) was produced annually in the late 1980's. Because of its low cost and efficiency as a pesticide, herbicide, bactericide, fungicide, etc., its usage worldwide has resulted in the pollution of many soils and water systems. Low-level human exposure can come from treated textiles, paper, or wood products, but high concentrations of PCP have been found at many spill or waste dump sites where drinking water sources are in danger of contamination. In addition to direct application as a pesticide or preservative, environmental exposure from chlorinated phenols may occur during the biodegradation of other herbicides and pesticides, such as 2,4,5-T, 2,4-D, lindane and silvex (Sittig, 1985).

PCP is a very effective biocide that is mainly used to preserve wood products (Crosby, 1981), and has been labelled as a priority pollutant by the U. S. Environmental Protection Agency (Sittig, 1985). There are many sites within the United States that are contaminated with wood-preserving chemicals, and over 500 of these contain PCP as the primary pollutant (Cirelli, 1978). This chemical was also found in the U. S. in 80% of over 100 tested sources of drinking water. PCP is a highly toxic compound, and can cause adverse health effects in humans even at low concentrations. However, its commercial form can contain even more toxic impurities such as polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (Crosby, 1981).

Due to its reported hazards and generally recalcitrant nature, PCP can remain concentrated in soils or be transported to surface or ground waters where it may enter the food chain and threaten wildlife and humans (Lee *et al.*, 1978). Since chlorinated phenols can persist in aqueous environments at levels hazardous to human health (Smith and Novak, 1987), it is important to understand the fate and transport processes occurring in the subsurface environment. Calculations by Hattemer-Frey and Travis (1989), based on reported partition coefficients, suggest that the majority of PCP (96.5%) adsorbs to soils, 2.5% remains in the liquid phase, and <1% partitions into the atmosphere. By inhibiting the transport of environmental pollutants, the sorption-desorption mechanism can effect other processes which may occur underground, such as hydrolysis, chemical oxidation-reduction, or biodegradation (Sabatini and Austin, 1990). The kinetics of a pollutants sorption and desorption may greatly influence its rate of removal from a contaminated site, and thereby control the economic feasibility of employing remediation technologies.

In order to identify the factors controlling these fate and transport mechanisms, many researchers perform lab experiments using a wide variety of techniques. Unfortunately, "real world" conditions are so varied and complex that they cannot all be duplicated in a laboratory. Also, experiments which incorporate too many variables would make it very difficult to isolate the causes of the results obtained. Therefore, the scope of this research was limited in order to

more fully and accurately understand the in situ processes controlling the environmental fate of chemical contaminants. Specifically, this purpose of this study was to characterize the relative effects of soil organic matter, pollutant concentration and contact time on the partitioning and bioavailability of two chlorinated phenols, pentachlorophenol (PCP) and 4-monochlorophenol (4-MCP).

Chapter 2

Literature Review

2.1 Chemical Properties

The primary test chemical, PCP, represents a widely distributed primary pollutant that can also be classified as a hydrophobic organic compound (HOC) due to its relatively low aqueous solubility. The secondary test chemical, 4-MCP, is used in the manufacture of various chlorophenol-based pesticides, and is also a biodegradation product of PCP. It represents a similar compound physically, but with some important chemical differences. Neutral chlorophenol species generally show increasing levels of hydrophobicity as chlorine atoms are substituted onto the phenol ring structure (Lee *et al.*, 1990). As a result, the 4-MCP was included in this study in order to compare the PCP results with another contaminant that has a higher aqueous solubility and is more readily biodegradable in the subsurface environment.

The chemical structures of the PCP and 4-MCP are shown in Figure 1. The radiolabelled forms of these compounds are both uniformly labelled, which means that each of the six carbon atoms in the ring is a carbon-14 isotope. This will allow each individual carbon atom from a PCP

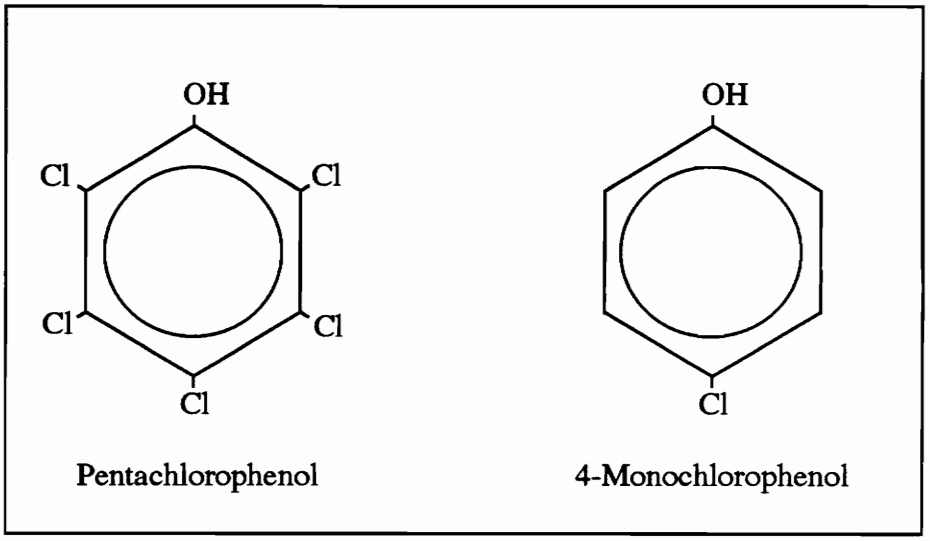


Figure 1. Chemical structures of the test compounds.

or 4-MCP molecule to be tracked, even if the ring has been broken by a degradation process. Experimentally determined properties of these compounds are shown in Table 1.

2.2 Sorption Processes

There are many theories and models that have been used to both describe or predict the fate of pollutants in the environment. Some researchers using hydrophobic organic compounds (HOCs) indicate a linear relationship between values of log K_{ow} (the octanol-water partition coefficient) and log K_{oc} (the partition coefficient normalized for soil organic carbon) (Karickhoff, 1981; Leo *et al.*, 1971). Correlation's have also been shown to exist between log K_{oc} and a compounds molecular connectivity (Sabljic, 1987) or its hydrophobic surface area (Rao *et al.*, 1985). The nature of these relationships is primarily determined by the physical and chemical characteristics of the sorbate and the fraction of organic carbon (f_{oc}) of the soil (Karickhoff *et al.*, 1979). Other soil properties such as particle size distribution, cation-exchange capacity (CEC), anion-exchange capacity (AEC), and ionic strength (μ) of the solution have little effect on the sorption processes (Karickhoff *et al.*, 1979; Lee *et al.*, 1990), while soil pH can play an important role when the p*K*a (acid dissociation constant) of the sorbate is also considered (Lee *et al.*, 1990). Probably the major property of a sorbate that determines its partitioning in a soil-liquid environment is its aqueous solubility, which is also related to its K_{ow}. A lower solubility generally indicates a higher K_{ow} value and increased sorption to the solid phase (Chiou *et al.*, 1983; Karickhoff, 1984).

Chlorophenols can also be classified as ionizable organic compounds (IOCs). Lee *et al.* (1990) defined the fraction of neutral compound as a function of the solution pH and the chemicals p*K*a, which can be used to determine the amount of neutral species present for a given set of conditions. Calculations based on these factors showed that PCP is >99% ionized at a pH of 6.8, while 4-MCP is >99% neutral at the same pH. PCP is a weak organic acid that is strongly

Table 1. Physical and chemical properties of PCP and 4-MCP.

Characteristic	Pentachlorophenol	4-Chlorophenol
Molecular Wt. (g/mole)	266.35[A]	128.56[A]
Boiling Point (°C)	310[D]	217[A]
Melting Point (°C)	191[D]	43[D]
Aqueous Solubility (mg/L)	14 (@ 20°C)[A,B]	27,100 (@ 20°C)[A]
Log K_{ow}	5.01[A]	2.39[A]
Vapor Pressure (mm Hg)	1.7×10^{-4} (@ 20°C)[B] 1.1×10^{-4} (@ 20°C)[A]	0.10 (@ 20°C)[A] 0.25 (@ 30°C)[A]
p*K*a	4.93 (@ 20°C)[C] 4.70 (@ 20°C)[B]	9.70 (@ 20°C)[C]
Vapor Density	9.20[A]	4.4[A]
Specific Gravity	1.978[A]	1.306[A]
Biodegradation * (days)	>72[D]	9[D]

References:

A Verschueren, 1983.
B Crosby, 1981.
C Li *et al.*, 1991.
D Woodcock, 1971.

* Indicates the time required for complete decomposition in a soil suspension study.

hydrophobic in its neutral state, but displays increased solubility in its ionized form. As for the characteristics of the sorbent, most soils have a net negative surface charge at pH values between 4 and 8 (Lee *et al.*, 1990), which would tend to create repulsive forces between the ionized PCP and the soil surfaces, leading to decreased sorption.

However, Bjerrum's theory of ionic association describes how the formation of ion pairs in the liquid phase can allow otherwise soluble compounds to transport to the solid phase as a neutral, metal-ion complex (Harned and Owen, 1958). Sorption of both the pentachlorophenolate anion (PCP^-) and the neutral metal-phenolate complex has been reported (Schellenberg *et al.*, 1984). These ion pairs can form at the solid-liquid interface, or completely in the liquid solution. As a result, the degree of dissociation can control the sorption process by changing the ratio of neutral to ionized species (Harned and Owen, 1958). Using many different forms of polychlorinated phenols, including PCP, Schellenberg *et al.* (1984) observed that as solution pH decreased, sorption of the test compounds increased in every case.

For both species, the sorption process is mainly governed by interactions between solvent and solute. Anion exchange is practically non-existent, while sorption of the neutral, hydrophobic chlorophenols and/or ion pairs is the predominant mechanism. The ionized species of a weak organic acid, such as PCP^-, is more soluble than its neutral form, and thus less likely to sorb to a solid surface (Lee *et al.*, 1990). As the various phenolates become the dominant species, they may play an important role in the overall sorption process, and their partitioning has also been found to be directly related to sorbent f_{oc} (Schellenberg *et al.*, 1984). Features of the minerals which may influence the sorption process include surface charge and mineral composition. Even with a positively charged clay surface, the PCP^- in solution does not display any electrostatic attraction for these solids, possibly due to the abundance of Cl^- in the test solution (Lee *et al.*, 1990).

Increasing levels of dissolved salts in solution generally increase the amount of sorption that is measured. Lee *et al.* (1990) found a 30% increase in PCP's sorption coefficient over the

range of μ tested. Increasing ionic strength can also effect the p*K*a of PCP, resulting in slightly lower levels of sorption than expected. However, the total effect of ionic strength on the fate of PCP appears to be minimal at typical environmental values near 10^{-3} M.

Banerji *et al.* (1986) also found that, in addition to SOM content, soil pH can play an important role in the degree and mechanisms of PCP sorption. As pH drops, PCP binding to soils can increase significantly. Whereas high SOM content soils generally have much larger sorption coefficients that low SOM soils, their K_{oc} values are often much smaller than those for the lower SOM soils (Hamaker and Thompson, 1972, as cited by Banerji *et al.*, 1986). This effect may be caused by the organic matter existing in multiple layers on the mineral surfaces, which should limit access to their sorption sites and thus reduce their overall sorptive capacities on an f_{oc} basis.

In laboratory experiments, the addition of NaOH to raise pH of soil solutions tends to lower the ionic strength of the liquid, and increase the amount of dissolved organic carbon (DOC). Koskinin and Cheng (1983) suggested that pesticide binding to DOC could reduce sorption to the solid phase. However, Clay *et al.* (1988) found no correlation between DOC concentration and sorption of two herbicides to various soils.

When hydrophobic compounds exist at equilibrium concentrations of <50% of their maximum aqueous solubility, sorption isotherms have been found to be linear (Karickhoff *et al.*, 1979; Means *et al.*, 1980; Rodgers *et al.*, 1980). This effect has also been witnessed when nonhydrophobic bonding mechanisms dominate the sorption process (Mingelgrin and Gerstl, 1983). Rao and Davidson (1979) showed that enhanced mobility of certain pesticides occurred at high loading rates, indicating that adsorption isotherms will not always remain linear over a wide range of chemical concentrations. From numerous studies using various pesticides and soils, many physical and chemical soil characteristics (pH, f_{oc}, CEC, particle size distribution, types of clay minerals present, etc.) are known to control the sorption process to a large degree. However, f_{oc} has been repeatedly identified as the one soil property that has the greatest effect on pesticide sorption (Harris and Sheets, 1965; Karickhoff and Brown, 1978; Mingelgrin and Gerstl, 1983;

Wahid and Sethunathan, 1978).

Research has shown that it may take weeks or months for some soil-pollutant systems to reach a true state of equilibrium, with one possible cause being limited access of the solute to the sorption sites (Karickhoff, 1984). This effect is believed to be due to soil-bound organic matter effectively blocking some of the binding sites on mineral surfaces, thus inhibiting the sorption process. The observed result is a "two-stage" binding process, with the "fast-stage" representing sorption to readily available solids and the "slow-stage" being caused by limited access to SOM and/or mineral sites in soil aggregates. Karickhoff (1980) found that the sorption process for several polyaromatic hydrocarbons (PAHs) could be divided into such fast and slow stages, with about 50% of the sorption occurring within minutes to a few hours. Uptake of the remaining chemicals appeared to need many days or even weeks to approach a true state of equilibrium.

Karickhoff (1984) found that in many situations, both the sorptive and desorptive processes can be greatly effected by the solids concentration that is used in the soil suspensions. He suggests that even at 1 g/L, aggregates can form which will delay both the uptake and release of the target compound. Other studies have shown that when very low concentrations of solids are used (<100 ppm) the results may show large increases in the measured K_{oc} values. These are possibly due to the minimization of soil aggregates, which increases the exposure of SOM and mineral surfaces to the liquid-phase sorbate (O'Conner and Connolly, 1980; Weber *et al.*, 1982). Hassett *et al.* (1980) showed that with PAHs, larger molecular sizes decreased the compounds availability to the sorbent.

The sorptive capacities of most minerals is largely controlled by the surface area they present to the sorbate. As a result, swelling clays such as montmorillonite have been found to dominate the mineral-phase sorption process in soils containing solids of various sizes and composition (Hassett *et al.*, 1980; Karickhoff and Brown, 1978). While f_{OM} typically controls the sorption process, there appears to be a point where the contributions by these swelling clays can dominate those of the organic matter. This level has been found to occur when the ratio of swelling clays to organic carbon exceeded 30:1 (Hassett *et al.*, 1980). When this situation occurs,

the removal of organic carbon can result in increased sorption for some polar organic compounds. This effect also should appear to support the theory of organic matter blockage of mineral surfaces. If it is assumed that the SOM exists in a single layer on the surface of the soil minerals, it has been estimated that a f_{oc} of 0.01 would decrease that surface area by 32 m^2/g (Walker and Crawford, 1968, as cited by Karickhoff, 1984). This would result in a 20-30% reduction in available surface area for most swelling clays. When aquifer materials were studied having an $f_{oc} \leq 0.001$, no correlation could be found between the measured sorption coefficients (K) and f_{oc} values. However, reasonable correlation's were found between K values and CEC, silt content, and 1:1 clay content (Stauffer *et al.*, 1989).

Theoretically, increasing system temperature should result in smaller partition coefficients due to increased solubilities for most organic chemicals. However, Weber *et al.* (1982) found that some HOCs showed significantly greater levels of sorption with higher temperatures. If the sorption process is limited by physical access to binding sites, the increase in molecular kinetic energy caused by raising the temperature may allow more rapid intraparticle transfer of these compounds.

While studying sorption of various HOCs to soil fractions separated by particle size, Karickhoff *et al.* (1979) found that when sorption to each size fraction was corrected for organic carbon content, the K_{oc} values were approximately equal between the silt and clay fractions. However, the sand fraction showed a 50-90% reduction in K_{oc} when compared to the silt and clay, suggesting physically and/or chemically assisted sorption for these fines, probably due to their increased surface areas. Karickhoff *et al.* (1979) found it possible to make a fairly accurate estimation of HOC sorption knowing only a soils particle size distribution, the f_{oc} associated with each size fraction, and the K_{ow} of the target pollutant.

Uniformity of the composition and reactivity of soil organic matter cannot be taken for granted, as it may display very different physical and chemical characteristics depending on the formation processes and the degree of weathering it has undergone (Grathwol, 1990). Other

researchers have found that some organic chemicals can sorb preferentially to fulvic acids, humic acids, humins or lipids (Garbarini and Lion, 1986; Stauffer *et al.*, 1988, as cited by Grathwol, 1990). Results such as these suggest that K_{oc} values found in the literature are dependent on the characteristics of the organic matter that was used in the respective experiments. Grathwol (1990) performed a vapor-phase sorption study using several volatile, chlorinated aliphatic hydrocarbons. The sorption process was found to be controlled to a large degree by the nature of the organic matter in the tested soils. These properties were determined by the level of weathering or coal forming processes to which the soil or sediment was exposed. Since the weathering process tends to increase the polarity, and thus decrease the hydrophobicity of SOM, it should also lower the sorptive capacities of these organic polymers for HOCs. This is exactly the effect that Grathwol (1990) observed, noting decreases in K^{G}_{oc} values (vapor-phase sorption coefficient normalized for f_{oc}) of 1-2 log units between unweathered and highly weathered shales and sandstones. These results seem to indicate that a direct relationship exists between the hydrogen to oxygen (H/O) atomic ratio of SOM and the K_{oc} that can be expected when using neutral HOCs as sorbates (Grathwol, 1990).

Boyd *et al.* (1988) studied the sorption of PCP onto clays that were supplemented with organic cations of varying hydrophobicities. He found that the greater the hydrophobic nature of these cations, the higher the level of sorption that was observed. Solution pH was found to have little effect on the sorptive capacities of these clay-organic complexes, as they sorbed the neutral and ionized PCP species equally. These facts seem to indicate that non-polar or hydrophobic mechanisms are dominating the uptake of PCP by treated clays. It has also been shown that increasing the level of chlorination of the basic phenol structure, and as a result its hydrophobicity, increased the amount of sorption to different organo-clay complexes (Mortland *et al.*, 1986).

In any soil system, sorption of various compounds can occur by the resident microbial biomass, even if sterilization procedures have been followed. Tsezos and Bell (1989) found that

for compounds which are easily degradable or hydrophobic in nature, the live biomass will uptake them to a greater extent than dead biomass. Using PCP as one of their test chemicals, they also found that this biosorption was not completely reversible in all cases. Other research has indicated that physical interaction comprise the major mechanisms of biosorption of PCP and several other pesticides, and K_{ow} is a fairly good predictor of the extent of this process (Bell and Tsezos, 1987).

The development of mathematical models to simulate the transport processes of sorption and desorption, has been undertaken by many researchers. The last several decades, especially, have seen great strides taken towards the understanding of these mechanisms. Recurring deviations from expected sorption equilibrium, were shown to be due to more than just chemical nonequilibrium effects (van Genuchten *et al.*, 1974). van Genuchten and Wierenga (1976) found that physical processes, such as mass transfer and diffusion, were better predictors of nonequilibrium. These findings enabled them to develop a two-phase model, which incorporated their hypothesis of relatively mobile and immobile stages during the solute transport process. More recently, a dual resistance model was proposed as a more accurate descriptor of physical nonequilibrium. In this model, a contaminant molecule was seen as diffusing from a free liquid phase through a hydrodynamic film layer, to the surface of the sorbent. From there, intraparticle diffusion was responsible for the transport of the contaminant to a sorption site within the solid phase (Miller, 1984, as cited by Sabatini 1990). This theory was supported by later research, when diffusion within an organic sorbent was found to be the cause of sorption nonequilibrium during column studies (Bouchard *et al.*, 1988).

2.3 Desorption Processes

HOCs can appear to sorb rapidly in soil suspensions, but often are very difficult to extract from the solid phase. A hexachlorobiphenyl compound was found to sorb within hours to various soil fractions, but approximately 50% of the bound chemical would not desorb from the sorbent

(Di Toro and Horzempa, 1982). Karickhoff (1980) found that the two-stage sorption theory would also apply to and have a significant impact on the desorption process. Compounds sorbed during the "fast" stage were easily removed by solvent extraction, but the slowly bound chemicals often required many days to desorb. He also observed that a relationship existed between contact time and the desorption process. When the compounds were mixed with the soils for less than five minutes, removal efficiencies of >90% were achieved. However, when this contact time increased to 4-5 days, only 20-40% of the sorbed PAHs could be extracted. Karickhoff (1984) suggests that the intraparticle movement of the chemicals within pore structures to internal binding sites, can account for the two stages observed for both the sorption and desorption processes. Other researchers have also found evidence of this "nonlabile" nature displayed during chemical release from the solid to the liquid phase (Di Toro and Horzempa, 1982).

Banerji et al. (1986) found that, although PCP sorption to soils is largely reversible, a fraction of the compound may be irreversibly sorbed, especially at lower concentrations. As a result, a contaminant plume consisting of PCP would be slowed and diluted by this sorption-desorption process, but would also continue a relatively long-term release of low-level concentrations of the pollutant. The desorption process was shown to occur at a slower rate than sorption for phenol, 2-MCP, and 2.4-dichlorophenol (Isaacson and Frink, 1984). They also found that a portion of these test compounds was irreversibly bound to the solid phase.

It has been observed by various researchers, when plotting sorption-desorption isotherms, that a degree of asymmetry is often found in the data. In these cases, the desorption data do not follow the sorption isotherms, but often remain above them. This effect, often called hysteresis, indicates that desorption can occur at a much slower rate than adsorption (Sabatini and Austin, 1990). Also, if these isotherms do not meet at the graph's point of origin (0,0), the data may suggest that some of the test compounds have been irreversibly bound to the sorbent.

Desorption rates in soil-water systems are highly dependent on the concentration gradient between the solid and liquid phases, and the average path length from the sorption sites to the bulk liquid (Rijnaarts *et al.*, 1990). Other factors which may control desorption include chemical

reaction with the sorbent (Lapidus and Amundson, 1952), diffusion out of the SOM fraction (Karickhoff and Morris, 1985), or transport rates through a stationary layer of liquid surrounding the solid surfaces (Miller and Weber, 1988). Since contaminants can bind to soils with mechanisms of varying strengths (e.g. hydrogen bonds, covalent bonds, ionic bonds, van der Waals forces, hydrophobic bonds, etc.) the extent and rate of their release can differ significantly (Dec and Bollag, 1988). The sorption of various pollutants to SOM is often reversible to some degree, and this suggests that the primary mechanisms of attachment are physical (physisorption) rather than chemical (chemisorption) in nature. The rationale for this theory comes from the lower bonding energies that are involved in the physisorption process (Sabatini and Austin, 1990).

Freeman and Cheung (1981) proposed that some of the "sorbed" chemicals may simply be physically trapped within the structure of the organic matter. For optimum extraction in this situation, the solvent used should closely approximate the solubility characteristics of the SOM's humin-kerogen polymer, enabling it to "swell" the organics and release these compounds more efficiently.

2.4 Soil-Bound Organic Matter

The primary sources of SOM include both terrestrial plants and microorganisms from the marine environment. Once they are deposited onto a soil surface or the bottom of a body of water, various natural processes eventually incorporate them into the subsurface layers. Grathwol (1990) briefly described some of the transformation processes in which the original biopolymers (proteins, carbohydrates, lignins, lipids, etc.) are degraded into geopolymers (fulvic and humic acids, humin, kerogen). These changes generally lower both the oxygen and hydrogen to carbon ratios (O/C and H/C), and raise the levels of organic carbon through polymerization effects. The reverse process, or weathering, causes oxidation of the functional groups contained on the organic polymers. This results in increased numbers of carboxyl and hydroxyl groups, and a decrease in

the hydrogen to oxygen (H/O) atomic ratio. The net effect of weathering is to increase the polarity of these large, organic molecules, which can decrease their affinity, and thus their sorptive capacities for neutral, hydrophobic compounds in solution (Grathwol, 1990).

Soil humates are often divided into fractions (humin, humic acid and fulvic acid) based on their solubilities in different acidic and basic solution. Soils are extracted with sodium hydroxide to remove the humic and fulvic acids, leaving the insoluble humins on the mineral surfaces. Then, by dropping the solution pH below 2, the humic acids precipitate out and the fulvics will stay in solution. Due to their widely varying characteristics, this operational definition is the only generally accepted method for fractionating humic materials (Wershaw, 1986). Humic acids isolated in this manner, can be further separated using gel chromatography on the basic of physical and chemical differences (Wershaw and Pinckney, 1973). The heterogeneous nature of humic acids has been researched, showing that some are aliphatic while others are mostly aromatic, and all may contain widely varying types and quantities of substituent functional groups (Wershaw, 1986). These macromolecules can separate during gel fractionation, evidence that their larger components are held together by relatively weak mechanisms, such as hydrogen or pi bonding (Wershaw, 1986). Wershaw and Pinckney (1973) found that humic acids were bound to clay minerals by the electrostatic charges of proteins, amino acids or metal ions.

Some researchers believe that humic acids are created from the polymerization or condensation of fulvic acids and even lower molecular weight (MW) compounds. Others have suggested that the larger, more complex molecules (humins and humic acids) are created initially from organic matter, and that their physical, chemical, and/or biological degradation results in the formation of fulvic substances (Hatcher, 1985; Schnitzer, 1978). Still other believe that humins are simply larger, stable polycondensates of humic acid molecules. Radiocarbon dating techniques, measuring ^{14}C activity, have been used to determine the ages of SOM fractions. Balesdent (1987) found that the newest organic fractions (<15 years) consisted mainly of plant debris. The oldest (280 years) consisted of the non-hydrolyzable humins that were bound to clay

minerals. From each soil sample, the humic and fulvic extracts were of approximately equal age. Therefore, these findings do not support the theoretical creation of humic acids through the polycondensation of fulvic acids.

More recently, Ramunni et al. (1987) found that microbial activity on an isolated sample of each class of humates (humin, humic acid, fulvic acid) could generate a small amount of the other two forms. This process occurred under both aerobic and anaerobic conditions, although the presence of oxygen enhanced the substrate degradation and the formation of byproducts. These results suggest that humic substances are not formed solely on the basis of molecular size, and that the processes of formation and degradation are reversible to some degree.

SOM was reported to have a high surface area (approximately 560-800 m^2/g) by Bower and Gschwend (1952), using a modified ethylene glycol-retention method. This fact was thought to account for much of the large sorptive capacities reported for this organic fraction. Throughout the 1980's, however, more researchers began to believe that partitioning rather than sorption was the mechanism of uptake of neutral organic compounds by SOM (Chiou *et al.*, 1983). Using the standard BET (Brunauer-Emmet-Teller) method of nitrogen adsorption, Chiou *et al.* (1990) measured the surface areas of two highly organic soils ($f_{OM} > 0.85$) and two humic acid extracts. Their findings showed surface areas of 0.61-0.73 m^2/g for these humic substances, except for one humic acid extract (18 m^2/g) which probably was artificially enhanced through a freeze-drying process. The results of Bower and Gschwend were discounted due to their analytical method, which measured the uptake of ethylene glycol by SOM. The polar nature of the ethylene glycol essentially makes it soluble within the organic macromolecular structures, resulting in false high levels of apparent surface area (Chiou *et al.*, 1990). This problem does not occur with nitrogen adsorption, and thus the BET surface area measurements of SOM are significantly lower and more reliable. Therefore, it should be concluded that significant surface adsorption of organic contaminants by the relatively low surface area of SOM is highly unlikely, and the partitioning mechanisms currently proposed have a much stronger scientific foundation (Chiou *et al.*, 1990).

Fendler (1982) suggested that the degradation of organic matter into lipids, carbohydrates, phenols and other components, also results in their aggregation into micelle-like structures with hydrophilic exteriors and hydrophobic interiors. In this respect, humic materials can display characteristics similar to biological membranes. In 1969, Wershaw *et al.* suggested that their test compound, DDT, was not adsorbed by humic compounds but rather was solubilized and partitioned into their interiors. Changes in pH can ionize acid groups in these structures, and thereby alter their solubility. Higher pH values will disrupt these humic micelles and release the humates into solution, while low pH will enhance their aggregation and precipitation (Wershaw, 1986). Depending upon the HOC in question and the environmental conditions, pollutant transport or remediation efforts can be either hindered or made easier by partitioning into SOM.

Catroux and Schnitzer (1987) used ^{13}C nuclear magnetic resonance (NMR) to analyze humic acids extracted from soil samples, and the residual humins left on the minerals. They found that the humic acids which came from the clay fraction, were significantly more aliphatic in nature than those from either the sand or silt fractions. This effect appears to be due to a high content of alkanoic or hydroxyalkanoic acids in the clay-associated humic acids. Conversely, the other extracted humic acids contained more aromatic structures and both phenolic and carboxylic acid groups. An analysis of the humins showed that they are even more aromatic in nature, larger, more homogeneous, have less carbohydrates and proteins, and contain higher concentrations of phenolic and carboxylic acid groups. These findings suggest that humin structures are more complex, and yet more stable than extractable humic acids.

NMR measurements of ^{13}C in liquid samples, using magic-angle spinning (MAS) and cross polarization (CP), has demonstrated the ability to provide additional insights into the structures of humic and fulvic materials (Hatcher *et al.*, 1980). This technique has made it possible to identify aromatic compounds and various functional groups present in the samples. Hatcher *et al.* (1981) found a much lower degree of aromaticity present in soil humic acids using CPMAS ^{13}C NMR, than was previously found using various oxidation techniques. These results suggest that

aliphatic structures may play a more important role in defining the chemical and physical nature of humic materials. His results also showed that the humic acid fractions contained a greater percentage of aromatic structures than the fulvic acid samples. However, in a more detailed study, Saiz-Jimenez *et al.* (1986) found the greatest aromaticities in the fulvic acids (39-44%), lower amounts in the humic acids (25-34%), and the least in the humins (20-31%) that were tested. Analysis of the humin samples showed them to be strikingly similar to humic acids in both the types and amounts of constituents present. This suggests that humins simply may be humic acids that have bonded to the fine soil fractions in such a way as to inhibit their removal using standard fractionation techniques.

While these studies appear to have conflicting results, Lobartini and Tan (1988) also used ^{13}C NMR, infrared spectroscopy and a scanning electron microscope to show that humic acids extracted from soils of various geographic and climatic origins, can exhibit significantly different characteristics of aromaticity or in the type and concentration of attached functional groups. It has also been shown that the isolation method used for fulvic acids can change the nature of the sample (Saiz-Jimenez *et al.*, 1979). Therefore, the use of different sorbents, such as activated carbon or Polyclor AT, may result in lower or higher aromaticities measured for this organic fraction (Saiz-Jimenez *et al.*, 1986).

Bollag and Liu (1985) suggested that a fraction of the strongly bound polychlorinated phenols studied, were not due to either adsorption or partitioning into the interiors of micelles or macromolecules. Instead, they proposed that the aromatic xenobiotics were incorporated into the humates during their formation by copolymerization reactions. They also believed these reactions to be controlled by microbial enzymes, such as the extracellular laccases that were tested. This process created covalent bonds between the chlorinated phenols and syringic acid, and no dehalogenation was observed. As a result, the contaminants were essentially detoxified and eliminated as a further hazard to water supplies, unless future reactions were to release these bound residues. Martin and Haider (1971) proposed that microbial activity can either synthesize

phenolic compounds, or produce them from the degradation of organic matter. These basic components are then oxidatively coupled from reactions with natural enzymes, producing large humic polymers that are relatively stable in the environment. In later research, Martin and Haider (1980) reported the use of oxidative enzymes to duplicate the creation of humic compounds, thus verifying the process of enzymatic polymerization. Stevenson (1982) reported that in addition to phenols, other compounds such as proteins and aromatic amines, could be incorporated into these macromolecules during their synthesis.

The analysis and description of soil-bound organics is a complex process that is continually being updated and refined as new technologies become available. There have been many theories proposed concerning the creation, structures, and physical and chemical properties of SOM, but definitive answers to these questions remain elusive. However, such research must continue because a thorough understanding of the nature of soil organics is essential to predicting its response to exposure to the numerous potential contaminants that are currently in use.

2.5 Dissolved Organic Matter

An operational definition of dissolved organic matter (DOM) that is widely accepted, is those soluble organics which pass through a 0.45 μm filter. Analysis of DOM from various ground water systems has shown that humics constitute a large fraction of the soluble organic matter. These materials appear to be structurally different from those found in surface waters or soils, having lower oxygen and higher carbon contents (Wassenaar *et al.*, 1990). A major source of DOM in shallow aquifers is from the transport of organics originating in surface soils, while deeper aquifers receive dissolved organic carbon (DOC) mainly from bacterial degradation of kerogen, or fossilized organic matter (Thurman, 1985). Another possible source is from buried organic sources, such as peat or kerogen, that is already present in the aquifer. This organic matter may be chemically or biologically degraded, and form larger humic molecules through

polymerization (Stevenson, 1985). Terrestrial plant and animal species (high oxygen, low carbon content) generally decompose into complex humate structures as an intermediate step in the process of forming sedimentary kerogen (low oxygen, high carbon content) (Wassenaar *et al.*, 1990). Biochemical processes play an important role in these changes by increasing the level of intermolecular binding and removing functional groups from the humic structures (Barnes *et al.*, 1984).

Radiocarbon dating methods have been shown to be effective in evaluating the age, origin and transport of DOM (Murphy *et al.*, 1989; O'Brien and Stout, 1978). The ^{14}C measured in the ground water samples taken, suggests that most of the DOC in shallow aquifers originated from surface soils, with rapid transport rates (Wassenaar *et al.*, 1990). However, not all organics travel quickly, even through shallow, sandy aquifers. In these cases, ^{14}C dating suggests typical ages for the soluble humates ranging from several hundred to several thousand years. Much deeper or clay aquifers showed the ages of the aquatic humics ranging from 10,000 to 30,000 years (Wassenaar *et al.*, 1990).

DOM consists of macromolecules with widely varying base structures and attached functional groups. Two organic acids commonly found on them are phenolic and carboxylic acids, with the latter being the much stronger acid (Clair *et al.*, 1989). I was also shown that microorganisms were able to increase the levels of pH and DOC in batch microcosms, due to their interaction with the humic compounds. The major structural change observed was the complete disappearance of the carboxyl groups over the two month study, and a significant increase in the amount of phenolic acids (Clair *et al.*, 1989). These results support other findings where microbial activity has been identified as having an important role in the modification of DOM.

Soluble humics can also mitigate, in some cases, the effect that organic pollutants will have on aquatic organisms. The accumulation of both benzo(a)pyrene and dehydroabietic acid (DHAA) into *Daphnia magna* was greatly reduced when they were first contacted with DOM

from a surface water (Kukkonen and Oikari, 1987). However, the humics did not reduce the bioaccumulation of PCP, probably due to the ionization of the PCP (>99%) at the test pH. They also found that the acute toxicity of 2,4,6-trichlorophenol was greatly reduced by exposure to DOM, but the toxicity of DHAA was increased. One other test compound, methylparathion, showed no change in toxicity between the humic and control water samples (Kukkonen and Oikari, 1987).

Using gel permeation chromatography to fractionate humic acid samples, and by making surface tension measurements on the various size separates, Yonebayashi and Hattori (1987) found that the largest macromolecules possessed the majority of the surface active properties found in the entire sample. As a result, the greater molecular size and surface activity of these humic acids allows them to form into micelle-like macrostructures. By comparing a structural analysis of the humic compounds with the physical and chemical properties they displayed, Yonebayashi and Hattori (1987) proposed that highly surface active humic acids contain a nucleus of loosely associated aromatic compounds, with long aliphatic side chains.

Chiou *et al.* (1986) showed that DOM can enhance the apparent water solubility (S_w) of certain organic compounds. Linear partitioning relationships were shown to exist between S_w and DOM concentration for test chemicals with very low solubility (<30 µg/L). This effect increased with a decrease in solute S_w, or with higher K_{ow} values. However, other chemicals (S_w >8 mg/L) showed no solubility enhancement with increasing levels of dissolved organics. The partitioning coefficients into DOM showed that the humic acid fractions were up to four times as effective as the fulvic acids in the uptake of these HOCs. The polarity and molecular size of the humates in solution, appear to be the major factors controlling their enhancement of aqueous solubilities. Investigations of humic and fulvic acids using scanning electron microscopy and X-ray analysis, have shown their structures to be relatively flexible and porous, containing many voids that could serve to hold compatible compounds (Schnitzer, 1978).

Stirred-cell ultrafiltration units are often used to separate organic compounds in solution, on the basis of molecular size. Amy *et al.* (1987) reported that many factors will control the

effectiveness of the ultrafiltration process, including temperature, ionic strength, the size, shape, and concentration of the solute molecules, and their affinity for the membranes themselves. Logan and Jiang (1990) found that very large errors in a size distribution analysis could be caused by membrane rejection of the organic molecules, and developed a model to correct for this problem. Amy *et al.* (1992) tested 12 ground water sources and found an average DOC concentration of about 4 mg/L, with humic substances making up 55-94% of the total DOC.

Chin *et al.* (1990) found that humic polymers in solution have little effect on the sorption characteristics of low MW, relatively soluble organic chemicals (log K_{ow} <5), but very hydrophobic compounds can be highly sensitive to even low concentrations of these humates. As a result, the solids effect often reported in batch sorption experiments may be partially due to the release of DOM, depending on the nature and amounts of both the soils and chemicals that are tested. Therefore, experimentally determined partition coefficients for organic contaminants like PCBs, various PAHs, and some pesticides, may be highly dependent on experimental solid:liquid ratios (Chin *et al.*, 1990). Also, such results may not accurately reflect the fate and transport mechanisms in a specific environment due to characteristic differences. Voice and Weber (1985) clearly demonstrated the effect of solids concentration on contaminant partitioning, and suggested that the many factors which control the nature and amount of DOM in a natural system may also effect a pollutants transport. They further hypothesize that complexation with DOM may determine a compounds susceptibility to photolysis, volatilization, chemical reactions, biodegradation and bioaccumulation.

Research has shown that nonpolar organic contaminants (NPOC) can be very difficult to remove from soils or aquifer materials (Mackay *et al.*, 1983). Abdul *et al.* (1990) found that the use of dilute humic acid solutions (29 mg/L) provided enhanced removal of several test compounds from low-carbon aquifer material, as compared to water washing only. The more hydrophobic NPOC showed greater improvements in removal efficiencies than did the more soluble compounds. Partitioning into the hydrophobic interiors of humic aggregates or micelles

in solution is believed to be the mechanism which allowed increased transport of the HOCs from the solid to the liquid phase. These results are consistent with other research on the interaction of HOCs with aqueous organic macromolecules, which also predicted enhanced transport in the presence of DOM (Enfield and Bengtsson, 1988).

2.6 Biodegradation Processes

Various studies have identified aerobic bacteria capable of degrading chlorophenols, some of which are fairly effective at decontaminating soils. A strain of *Flavobacterium* worked well on a soil contaminated with 300 mg/kg PCP (Crawford and Mohn, 1985), and Edgehill and Finn (1983) isolated an *Arthrobacter* strain that provided comparable results. Unfortunately, these experiments generally resulted in residual chlorophenols left in the soils in a range of 10-30 ppm. Mikesell and Boyd (1985) showed that anaerobic microbes from municipal sewage sludge were able to reductively dechlorinate PCP. In 1988, Mikesell and Boyd demonstrated that anaerobic biodegradation was able to reduce soil concentrations of 10 and 30 ppm PCP to less that 0.5 ppm in approximately one month. However, 60-80% of the original PCP was found remaining in the systems as lesser chlorophenols. Their proposed degradation pathway was as follows:

PCP —> 2,3,4,5-tetrachlorophenol —> 3,4,5-trichlorophenol —>

3,5-dichlorophenol & 3,4-dichlorophenol —> 3-chlorophenol

This process of reductive dechlorination is environmentally important, since each successive compound generally has a lower toxicity, a lower bioaccumulation factor, and is more likely to be mineralized through other aerobic and/or anaerobic processes (Mikesell and Boyd, 1988).

Studying PCP biodegradation in field soils that had been previously exposed to this pesticide, Kuwatsuka and Igarishi (1975) found that PCP degradation rates correlated highly with SOM content and, to a lesser extent, with CEC. They found no correlation between biodegradation rates and soil pH or clay content, but soil microbial activity and prior exposure to

PCP both appeared to be important factors in the mineralization process. They were also able to identify PCP degradation products, which consisted mainly of various isomers of tetra- and trichlorophenols. Lin *et al.* (1990) found that both intra- and extracellular enzymes were utilized during PCP mineralization by *P. chrysosporium*.

Travnik and Höfle (1987) showed that bacterial cultures from humic lake waters were able to degrade natural phenols, such as those found in humic acids. By using these same enzyme systems, it has been demonstrated that they also can cleave the phenolic rings of some chlorinated, aromatic hydrocarbons (Larsson *et al.*, 1988). In studies with both clear and humic lake waters, di- and trichlorophenols (DCP and TCP) were degraded to a greater extent in the humic waters, while PCP was degraded slightly more in the clear lake waters. However, when sediments were added to these same systems, PCP mineralization increased significantly for both water types while DCP and TCP mineralization dropped (Larsson *et al.*, 1988; Larsson and Lemkemeier, 1989). Research conducted by Pignatello *et al.* (1983) showed that microbes attached to solid surfaces were much more effective at degrading PCP. This finding may explain why sediment-associated bacteria were capable of higher PCP degradation rates in the previously mentioned studies (Larsson and Lemkemeier, 1989).

Murthy *et al.* (1979) conducted batch biodegradation studies using PCP amended moist soils (10 ppm) under both aerobic and anaerobic conditions. They detected no $^{14}CO_2$ evolution from the anaerobic systems, while approximately 40% of the radiolabelled PCP disappeared from the aerobic systems, presumably as $^{14}CO_2$. They also found that about 45% of the original PCP addition remained sorbed to the soil despite multiple solvent extractions. Most of this residual was bound to the fulvic and humic fractions of the SOM, while a smaller amount was found to be associated with the humin fraction.

Smith and Novak (1987) studied the biodegradation of phenol and some chlorinated phenols in various soil-water systems, and found that phenol and 2-MCP were degraded most quickly. Lower degradation rates were measured for 2,4,6-TCP, PCP, and 2,4-DCP, in that order.

All rates were directly proportional to the equilibrium concentration of each chemical tested, and they appeared to follow first-order kinetics. The findings of Smith and Novak (1987) indicated that phenols with increasing numbers of chlorine atoms substituted on the aromatic ring are not necessarily more resistant to biodegradation. Supporting research was conducted by Tabak *et al.* (1964), which measured higher biodegradation rates in the order of phenol, 2,4,6-TCP, then 2,4-DCP. Banerjee *et al.* (1984) also reported this same trend, and showed that PCP was biodegraded faster than 2,4,5-TCP, which had a higher degradation rate than 2,4-DCP. Smith and Novak (1987) also found higher degradation rates for chlorophenols in soils with larger populations of soil microbes.

Ruckdeschel *et al.* (1987) tested PCP and over 30 metabolites as a bactericide against 30 different bacterial species. They found that PCP was generally the most lethal agent tested, with the exception of two of the tetrachlorophenol (TeCP) isomers. They also noticed a common trend, suggesting that antibacterial effectiveness tends to increase from the "ortho-", to the "meta-", to the "para-" isomers of all the chlorophenol species.

Various species of bacteria have been shown to be capable of degrading PCP in contaminated soils. There are many factors which can effect the metabolism of PCP by soil microorganisms, and one important factor may be a source of readily metabolizable carbon (Topp *et al.*, 1988). It has been shown that the biodegradation of a test compound may be increased or reduced by the presence of other compounds (Harder and Dijkhuizen, 1982, as cited by Topp *et al.*, 1988). Topp *et al.* (1988) used a *Flavobacterium* sp. to show that additional carbon sources often improved, but was not required for, the metabolism of PCP. He was also able to identify instances where the addition of multiple carbon sources resulted in significantly lower PCP biodegradation rates. The application of concentrated PCP caused considerable damage to the microbial populations, but they were generally able to acclimate to and then utilize the PCP for cellular repairs and growth. As a result of their research, Topp *et al.* (1988) suggest that the use of a secondary source of metabolizable carbon may improve the bioremediation of PCP

contaminated soils.

Klecka and Maier (1988) found that PCP biodegradation decreased when unusable carbon compounds were added to the test microcosms. The addition of readily degradable compounds, such as phenol, also decreased the PCP degradation rate initially, but increased the overall rate of PCP removal due to a larger microbial population. They also found that higher PCP concentrations did not always improve microbial growth rates, and observed a threshold level which inhibited biodegradation. This effect most likely was due to the biocidal nature of PCP.

Some pollutants become highly toxic to microorganisms at concentrated levels, and thus can inhibit biodegradation processes (Jones *et al.*, 1973; Tyler and Finn, 1974). Brown *et al.* (1990) found no inhibition of microbial growth at 20 mg/L of 4-MCP, however it was detected at a concentration of 100 mg/L. Their results also suggest that 4-MCP is the most easily biodegradable of the three monochlorophenol compounds. Topp and Hanson (1990) showed that PCP exposure could cause a significant drop in microbial populations, but that recovery to initial levels generally occurred within 2-3 days. The addition of glucose usually enhanced PCP biodegradation in these tests, and the limitation of various nutrients (sulfate, nitrogen, phosphate, and ammonium) was found to be able to enhance or inhibit PCP biodegradation rates. The biodegradation of various hydrocarbons, including phenolic compounds, has been shown to be improved by the addition of nitrate or molybdate, depending on the types of soil and the microbial population (Morris and Novak, 1989). Research has even shown that the bioremediation of polluted soils can lower the toxicity of some contaminants (Dasappa and Loehr, 1991; Wang *et al.*, 1990). Also, the initial concentration of the contaminant can effect the rates at which both biodegradation and toxicity reduction will occur (Dasappa and Loehr, 1991).

Middledorp *et al.* (1990) showed that PCP could be degraded by adding an appropriate biomass to natural soils. However, they also found that by adding nutrients or an oxygen source to stimulate the natural soil microbes, or by the addition of an uncontrolled mixture of microorganisms, the contamination could be worsened through the production of more toxic and resistant compounds such as polychlorinated dibenzo-p-dioxins (PCDDs). Therefore, a complete

understanding of all the processes and important variables controlling microbial degradation of various pollutants, can be critical to the safe and effective bioremediation of contaminated soil systems.

2.7 Bioavailability of Sorbed Substrates

Klecka *et al.* (1990) conducted on-site sampling and laboratory studies to characterize the fate and transport of various organic pollutants (mainly phenol, methyl-substituted phenols, and napthalene) at a Superfund Site. Using adsorption and biodegradation studies and computer modeling, they found that the high solubility compounds (phenols) were not retarded by adsorption onto the shallow aquifer material, but were significantly removed through aerobic biological mineralization. Conversely, the PAHs, with much lower aqueous solubilities, were largely attenuated by adsorption processes. This effect helped to compensate for the lower biodegradation rates of these compounds, also enabling their removal prior to significant spreading of the plume of contaminants. Their results show that even strongly adsorbed hydrocarbons will still be accessible to natural microorganisms.

The commercial usage of pesticides usually has little or no effect on soil microbes (Smith, 1988). However, where pesticides are concentrated in soils, such as at spill or waste disposal sites, they can either stimulate or inhibit the biological processes of one or many species of microorganisms (Johnston and Camper, 1991). Adaptation may allow soil microbes to use such organic compounds as carbon sources, and research has shown that their populations can actually increase at hazardous waste sites (Dean-Ross, 1989). Johnston and Camper (1991) conducted research with soils that had been exposed to pesticides over a number of years, and that still retained an active population of microbes. They found that one test compound, propanil, was partially degraded by these soil bacteria, whereas 4-chlorophenol was almost completely mineralized. However, two other pesticides, trifluralin and diuron, were not significantly degraded over a five month test period. These results highlight the problems which may arise

when trying to make generalizations concerning the effects of pesticides on soil microorganisms.

Many studies have documented the biodegradation of organic compounds, but they are often unable to identify whether the chemicals were metabolized in the free or sorbed states (Smith and Novak, 1987). Chakravarty *et al.* (1972) suggested that only free aromatic hydrocarbons in the liquid phase were available for biodegradation by bacteria. Other researchers have found that sorption to solids (Ogram *et al.*, 1985) and humic compounds (Martin *et al.*, 1978) can decrease bioavailability for certain compounds. However, the results of Remberger *et al.* (1986) suggest that even strongly sorbed organic compounds can still be altered or degraded to some degree. Ogram *et al.* (1985) conducted research which suggested that the pesticide 2,4-D was not bioavailable while in the sorbed state, however no studies have proven that chlorophenols are not biodegradable while bound to solids. Robinson *et al.* (1986) observed a rapid initial sorption phase of toluene onto soils, followed by a lower sorption rate over an extended period of time. Desorption occurred in a similar two-stage process, with >90% of the bound toluene being rapidly removed. However the remaining fraction of the sorbed compound was highly resistant to extraction. Interestingly, they found that the mineralization of the toluene paralleled the desorption rate, suggesting that biodegradation occurred in the aqueous phase and that sorbed compounds were not bioavailable to the microbial population.

If a compound is highly toxic to microorganisms, Subba-Rao and Alexander (1982) found that sorption to solids could enhance biodegradation by decreasing the chemical's aqueous concentration. However, they also observed that the more common effect was to inhibit the mineralization of sorbed compounds by limiting their bioavailability to microbes. Ogram *et al.* (1985) found that microorganisms primarily degrade pesticides in their dissolved state in solution. Other researchers have suggested the the sorption-desorption processes can limit substrate bioavailability, and thus inhibit biodegradation by: (1) lowering the initial aqueous concentration of the substrate (Alexander, 1985), or (2) decreasing the rate of substrate desorption (Bouwer and McCarty, 1982). Microbes that are located on or near the sorption surfaces, and are able to

metabolize the substrate, can increase the gradient between the solid- and liquid-phase concentrations and thus increase the desorption rate (van Loosdrecht *et al.*, 1990). Rijnaarts *et al.* (1990) found that the biodegradation of α-hexachlorocyclohexane in their soil suspensions was controlled mainly by intraaggregate mass transfer processes. When the mixing system was changed to reduce the size of aggregates in their microcosms, higher desorption and biodegradation rates were measured. This effect was attributed to the reduction in average path length from the solid to the liquid phase.

Sorption of soil contaminants to SOM may effectively prevent their further transport in the environment, but it may also hinder efforts to bioremediate a given site (Dec and Bollag, 1988). It has also been shown that certain soil fungi can release bound xenobiotic compounds during the metabolization of humic substances, extending their hazard potential for many years (Mathur and Paul, 1967). Dec and Bollag (1988) researched the biodegradation of chlorophenols bound to synthetic humic acids with a peroxidase catalyst, and found decreasing mineralization with increasing chlorination of the phenol ring structure. Their results also suggest that only a small fraction of each compound was bioavailable, while the bulk of the chlorophenol was not accessible to the microbes. As a result, they found very limited release of 4-MCP and PCP by the added microbes, suggesting that enzymatic coupling to humates can greatly reduce the potential hazard of these compounds.

Berry and Boyd (1984) found that the type and position of substituent groups on anilines and phenolic compounds, would significantly effect the extent to which they could be polymerized. Using a peroxidase as the oxidative enzyme, they showed that chlorine groups on the aromatic rings would slightly inhibit the oxidative coupling process. These results suggest that a polychlorinated phenol, such as PCP, would be incorporated into humic polymers to a lesser degree than MCP through microbial activity. Bollag (1983) suggested that the process of oxidative coupling may result in the formation of covalent bonds between some contaminants and SOM. Such pollutants are not easily separated from the humic polymers, and as a result they become relatively immobile, less toxic, and are generally not bioavailable to the microbial

population (Freitag *et al.*, 1984). Berry and Boyd (1985a) researched the binding of dichlorobenzidine (DCB) to humic materials, and found that additions of ferulic acid and H_2O_2 could significantly lower the levels of extractable DCB. Their results indicate that it may be economically feasible to artificially enhance the process of enzymatic cross-coupling to decontaminate soils.

Chapter 3

Methods and Materials

3.1 Introduction

The purpose of this study was to determine the impact of contact time on the adsorption, desorption, and biodegradation of two chlorinated phenols (PCP and 4-MCP). Other contributing factors that were examined included soil organic matter content and the aqueous concentration of the test compounds. In order to evaluate these effects, two silty loam soils with different SOM contents were exposed to dilute aqueous solutions of each chemical. Three concentrations and four contact times were used in the sorption tests, following standard guidelines for batch, shake-flask experiments. The desorption study was carried out by performing sequential "washings" of the previously exposed soils, to monitor the various patterns of chemical release. Ultrafiltration techniques were also used to identify whether the compounds remaining in solution existed in a free or bound state. Finally, a biodegradation study was set up using cultured microbes and CO_2 trapping to identify what effects the tested variables would have on substrate bioavailability under aerobic conditions. Controls were used during all stages of this research to verify the accuracy of the experimental methods.

3.2 Chemical Compounds and Stock Solutions

Radiolabelled pentachlorophenol, $^{14}C_6Cl_5OH$, was obtained from SIGMA Chemical Co. (Lot No. 031H9208) and was uniformly ring-labelled (specific activity = 11.9 mCi/mmol). It was received as 50 μCi shipped in 0.05 ml of toluene in a glass ampule. This volume was transferred to a 10 ml volumetric flask using distilled-deionized (d-d) water that was adjusted to pH 10 with 1 N NaOH, to ensure the PCP remained in the aqueous phase. This solution was warmed on a hot plate and exposed to a steady stream of air for approximately 24 hours in a ventilated hood. The purpose of this process was to remove as much of the toluene as possible, using the greater volatility of the toluene (BP of 110°C) to evaporate it from the solution. The volumetric flask was then sealed with Teflon tape and stored at 5°C until needed. The uniformly ring-labelled 4-monochlorophenol was also obtained from SIGMA (Lot No. 128F9211; specific activity = 7.4 mCi/mmol), and the 100 μCi of this chemical was prepared and stored similarly to the ^{14}C-PCP.

In order to economize usage of the radiolabelled compounds, they were mixed with unlabelled compounds prior to use in these experiments. The stock solution of unlabelled pentachlorophenol was prepared using crystalline PCP manufactured by SIGMA (Lot No. 127F3439). A mass of 4.5 mg of the crystals was weighed out on a Mettler H10 analytical balance, and added to 500 ml of d-d water in a Pyrex reagent bottle. This solution was then mixed using a magnetic stir plate until all of the crystals were dissolved.

An unlabelled stock solution of 4-monochlorophenol was prepared from crystals also purchased from SIGMA (Lot No. 119F3531). A Mettler AC 100 balance was used to weigh out 50.0 mg of 4-MCP. These crystals were placed in a Pyrex beaker, and 0.5 ml of 1 N NaOH was added to make a paste. Next, 200 ml of d-d water was added and the mixture was stirred until the 4-MCP was completely dissolved. During this mixing, approximately 0.35 ml of 1 N H_2SO_4 was added to lower the solution pH from 9.5 to 7. This solution of 200 mg/L of 4-MCP was transferred to an acid cleaned Pyrex reagent bottle for storage. Both of the unlabelled stock

solutions were kept under refrigeration at 5°C between uses.

In order to saturate the test soils and simulate the ionic strength of a typical ground water system, a solution of 0.01 M $CaCl_2$ was prepared. Use of a dilute calcium chloride solution is common in soil system sorption-desorption experiments (Brusseau *et al.*, 1990; Lee *et al.*, 1990). A 1 M phosphate buffer solution, made from technical grade monobasic potassium phosphate, was used to maintain the pH at a constant level (6.83 ± 0.03) in the test flasks.

3.3 Soil Samples

Another requirement of this experiment was to attempt to isolate SOM as the only variable between the soils tested. One method often used to compare the relative effects of SOM is to remove part of the organic matter with hydrogen peroxide. The result is two soil samples that have virtually identical mineral compositions, with the only apparent difference being SOM content. However, such fractionation techniques can alter other physical and chemical properties in the soil matrix, possibly affecting the sorption mechanisms (Karickhoff, 1984). Therefore, the method of soil collection chosen was to select a site where a wooded area has existed next to an open field, and both have remained relatively undisturbed for a number of years. Ideally, the mineral composition at both sites would be very similar, but the soil bound organic matter should be greater on the wooded soils due to their long-term exposure to decaying vegetation.

3.3.1 Collection and Preparation

Soil samples were collected on the Virginia Tech campus in an undeveloped area, were a field had been cleared and the adjacent woods were undisturbed. Soil samples were removed from a 0.5 m^2 area at a depth of 15-25 cm from both the wooded area and the open field. These sites were approximately 15 m apart. All soils were collected in plastic sample bags, and taken immediately to the lab for further processing. There they were passed through a No. 10 Standard

Testing Sieve (2.00 mm) at least five times to remove most of the visible organic matter. Each soil type was allowed to air dry for approximately 8 hours, mixed thoroughly and stored in clean mason jars with Teflon lids at 10°C. The sample collected from the open field is hereafter referred to as soil S1, and the sample taken from the wooded area is designated soil S2.

3.3.2 Measurement of Soil Organic Matter

There are several methods that are often used to analyze the organic matter content of soils. One relatively quick and simple procedure is the thermal, loss-on-ignition technique. This procedure measures organic matter by burning off the volatile solids, and has been found to provide accurate and reliable results (Davies, 1974). It differs from traditional wet oxidation methods in that a slightly greater level of organic matter (1-2%) is typically reported, depending on the type of soil. Following the general procedure outlined by Davies (1974), four random samples from each soil type, of approximately 10 grams each, were placed in a drying oven at 110°C for 8 hours. Eight aluminum pans were preheated in a muffle furnace at 430°C for two hours. The dry soils were placed in the prepared pans, weighed on an analytical balance, then put into the muffle furnace at 430°C for 12 hours. At this temperature, all of the organic matter should have been oxidized, while leaving unchanged the carbonates and clay minerals containing structural water. The weight differences before and after this process were used to calculate the f_{OM}, with the following results: SOIL S1 f_{OM} = 3.04%, SOIL S2 f_{OM} = 4.94%

A second method of testing SOM content was performed by the Soil Testing and Plant Analysis Laboratory at Virginia Tech. The procedure used was a modified Walkley-Black method, which oxidized the organics through the addition of sodium dichromate and sulfuric acid solutions. A colorimeter was used to analyze the resulting liquid sample, and comparison to a previously prepared standard curve was used to determine the percent organic matter of the soil (Donohue, 1988). The findings of the Soil Testing Lab were slightly lower than the previously reported values, and are as follows: SOIL S1 f_{OM} = 2.7%, SOIL S2 f_{OM} = 4.4%

3.3.3 Particle Size Analysis

Another important area of soil testing involves separating the samples into standard size fractions. This procedure was performed at Virginia Tech's Soil Characterization Lab. Table 2 shows four classification systems used to identify soil fractions, and the actual particle size ranges used during the analysis. The results are shown in Table 3. This table shows that the percentages of sand, silt and clay for each soil type are very similar.

3.3.4 Soil Mineralogy

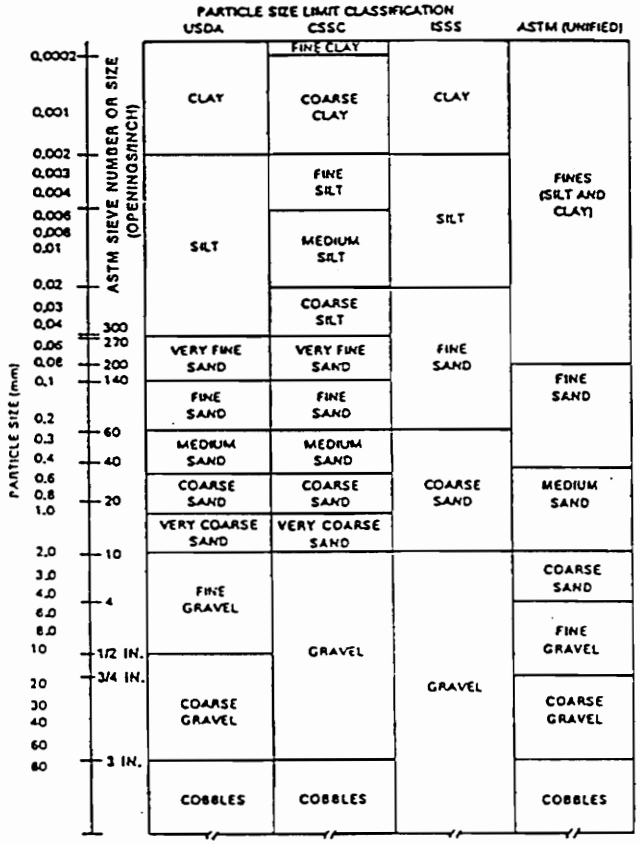
One other soil test was conducted at Virginia Tech's Soil Testing Lab. The purpose was to analyze natural soil pH and concentrations of some of the major minerals of each soil sample. The results of this examination are shown in Table 4, and the methods used were outlined by Donohue (1988). Soil S1 had a higher pH, which is generally expected with higher f_{OM}, due to the acidic nature of humic and fulvic molecules. This soil also had higher concentrations of most of the minerals tested. However, the experimental results should not be effected, since adsorption to mineral surfaces is much lower than to soil-bound organics when the f_{OM} is at the relatively high level found in these soils (Lion *et al.*, 1990; Murphy *et al.*, 1990).

3.4 Microcosms

Batch or shake-flask tests offer some definite advantages over other commonly used methods such as column studies. Due to the requirement to test two chemicals, two soils and three aqueous concentrations over four different exposure times, all at the same pH, it would have been nearly impossible to perform column tests with the space and equipment limitations imposed. Newer techniques like gas-purge or miscible-displacement would have created similar complications. Therefore, using the batch method by making individual microcosms of soils,

Table 2. Soil classification systems.

PARTICLE SIZE RANGES	SIEVE NUMBER
VCS - 1.000mm - 2.000mm	18
CS - 0.500mm - 1.000mm	35
MS - 0.250mm - 0.500mm	60
FS - 0.100mm - 0.250mm	140
VFS - 0.050mm - 0.100mm	325
CSi - 0.020mm - 0.050mm	
MSi - 0.005mm - 0.020mm	
FSi - 0.002mm - 0.005mm	
C - <0.002mm	



USDA—U.S. DEPARTMENT OF AGRICULTURE, (SOIL SURVEY STAFF, 1975)
CSSC—CANADA SOIL SURVEY COMMITTEE, (McKEAGUE, 1978)
ISSS—INTERNATIONAL SOIL SCI. SOC. (YONG AND WARKENTIN, 1966)
ASTM (UNIFIED)—AMERICAN SOCIETY FOR TESTING & MATERIALS (ASTM, D-2487, 1985a)

Table 3. Particle size analysis.

SOIL FRACTION	SOIL S1	SOIL S2
% Very Coarse Sand	1.2	0.9
% Coarse Sand	2.3	2.4
% Medium Sand	2.9	3.4
% Fine Sand	8.0	8.4
% Very Fine Sand	11.6	10.0
TOTAL % SAND	**26.0**	**25.0**
% Coarse Silt	24.1	25.6
% Medium Silt	26.6	27.7
% Fine Silt	9.5	9.3
TOTAL % SILT	**60.2**	**62.6**
TOTAL % CLAY	**13.8**	**12.4**
TEXTURAL CLASS	**Silty Loam**	**Silty Loam**

Table 4. Soil mineralogy.

SOIL PARAMETER	SOIL S1	SOIL S2
Soil pH	6.9	4.5
Calcium (ppm)	1116	240
Magnesium (ppm)	120	55
Zinc (ppm)	1.6	2.4
Manganese (ppm)	14.8	16.1
Potassium (ppm)	157	34
Phosphorous (ppm)	35	2

liquid solutions and test chemicals, provided the most effective process for analyzing the variables of this study.

3.4.1 Preparation of Microcosms

All microcosms were mixed within Pyrex or Kimax 250 ml screw-top Erlenmeyer flasks. Twenty grams of soil was weighed in an aluminum pan and added to each flask, and then 50 ml of the previously prepared 0.01 M $CaCl_2$ solution was combined with the soils. In order to equalize the pH of both soil types, 1.0 N NaOH was added to each flask. Based on prior tests, 0.1 ml of the sodium hydroxide was added to the S1 flasks and 0.9 ml was added to the S2 flasks to provide a pH of 6.8 to 7.0. This pH was selected to minimize the change from the natural soil pH, while providing an optimum environment for the addition of cultured microorganisms during later experiments. Each microcosm was then labelled and weighed on a balance to ±0.01 g.

After mixing the contents of each flask thoroughly by hand, their tops were covered tightly with aluminum foil and they were autoclaved at 122°C and 16 psi for 20 minutes. The flasks were allowed to cool overnight and then autoclaved once per day on the two following days. After the third autoclaving, the pH of each soil slurry was checked with Baxter Scientific Products S/P pH Indicator Strips with a range of 5.1 to 7.2. Use of electrical pH probes was not desirable due to the high soil:liquid ratio and the possibility of greater loss of solids. Based on these results, 0.05 ml of 1 N NaOH was added to each S1 flask, and 0.25 ml was added to each S2 mixture to raise the pH close to 7. All microcosms were then weighed, and d-d water was added to bring them back to their original weights. This step was necessary due to slight losses from evaporation during the sterilization process.

The flasks were autoclaved a fourth and fifth time on the next two consecutive days, and after cooling the solution pH and the weight of each microcosm was checked as before. Distilled-deionized water was added to bring them back to their original weights, then 0.05 ml and 0.20 ml of 1 N NaOH was added to the S1 and S2 flasks respectively, raising pH values to 6.8-7.0.

Finally, 2.00 ml of the phosphate buffer solution was added to each flask to stabilize their pH levels for the duration of the experiments.

The purpose of slowly mixing in small amounts of sodium hydroxide, was to eliminate the natural buffering capability of each soil so that the pH of the soil slurry could be controlled and equalized for both soil types. This method also prevented the solution pH from rising high enough to promote excessive detachment of soil-bound organics into solution. At the same time, autoclaving the microcosms five times over five days was designed to eliminate as much of the microbial activity as possible in the flasks. Excessive interference by soil microbes could interfere with the sorption-desorption experiments if biodegradation of the substrates was allowed to take place.

Other possible sources of substrate losses include volatilization, adsorption and photodecomposition of the PCP and 4-MCP. Since the soil slurry is mostly opaque, there is probably very little penetration of light into the bulk of its mass. Also, the lab where radioactive compounds are used has restricted access and therefore the lab is darkened for most of the day. When they were not being used, all flasks were kept covered to minimize exposure to light sources. Due to these efforts, losses from photodecomposition were probably extremely small and inconsequential to these experiments.

Losses due to volatilization and adsorption to the container surface were measured by preparing and monitoring control flasks. 50 ml of 0.01 M $CaCl_2$ and 2 ml of phosphate buffer were placed in each of two clean flasks. To one flask, 5.8 ml of 200 mg/L 4-MCP stock solution and 50 µl of ^{14}C-MCP were added to make an overall concentration of 20 mg/L 4-MCP. To the other flask, 6.5 ml of 9 mg/L PCP stock solution and 130 µl of ^{14}C-PCP were added for a 1.0 mg/L PCP concentration. These represent the highest concentrations used of each of the chemical compounds. The control flasks were sampled at the same time as those microcosms used in the sorption study (1, 3, 21 and 91 days) to check for losses of the radiolabelled compounds from solution.

3.4.2 Use of Radiolabelled Compounds

Once the microcosms had been prepared by sterilization and pH modification, the chemical substrates had to be added in a manner such that the final concentration was at the required level, and the initial amount of radiolabelled compound was known. The ^{14}C stock solutions were checked by diluting 100 µl of each with 900 µl of d-d water, and taking three 250 µl samples. These were counted on a Beckman LS-230 Liquid Scintillation Beta Spectrometer, and the three samples were averaged for each chemical to obtain the initial level of radioactive compound in each ^{14}C stock solution.

Knowing this amount, it was possible to calculate the volume of radiolabelled PCP and 4-MCP that needed to be added to the unlabelled stock solutions (9 mg/L PCP, and 200 mg/L MCP) to obtain a starting DPM that could still be measured effectively after sorption of the substrate had occurred. At a level of 500 DPM, the possible range of error from the scintillation counter is 3%, which was the lowest desired level of accuracy for the sorption study. Since it was determined from pilot studies that the maximum amount of sorption that could be expected was approximately 90%, the goal for mixing these chemicals was to achieve a concentration of around 5000 DPM per 250 µl in each microcosm. This method realized an acceptable compromise between experimental accuracy, and an efficient rationing of the labelled compounds.

3.4.3 Addition of Substrates

Since the S1 and S2 soils needed to be treated with different amounts of sodium hydroxide, the final volumes of liquid in their respective microcosms were also different. For the S1 flasks this came to 52.1 ml, and it was 53.1 ml for the S2 flasks. However, this difference was less than 2% of the total volume, and it was accounted for when the final calculations and data analysis were performed. Because three different concentrations of each chemical were created in each set of flasks, different volumes of the labelled and unlabelled chemical mixtures had to be added to

every microcosm. Table 5 shows the resulting mixture of solutions that was needed to create the desired final aqueous concentrations in each flask.

One small drawback to this method was that, although the final concentrations were correct, every microcosm had a slightly different ultimate volume of liquid. The only problem this created was that each flask had a slightly different solids concentration which, due to the large amount of soils used, was insignificant to the final results. The only way to get around this requirement would have been to make many dilutions of the PCP and 4-MCP stock solutions, and mix in the ^{14}C compounds for each flask individually. Such a procedure would have been dramatically more difficult to control with an adequate level of precision.

3.5 Sorption Study

In order to test the effects of soil organic matter and aqueous concentration on the environmental fate of PCP and 4-MCP, each soil-chemical combination was prepared at three concentrations. This test protocol, as defined in Table 5, showed how these twelve microcosms were created. For the sorption-desorption experiments, four complete and identical sets of these microcosms were prepared. They were exposed to PCP or 4-MCP for 1, 3, 21 and 91 days to test for the effect of exposure time on these processes. Since this test design required the preparation of 48 microcosms, they were not duplicated.

After the flasks were prepared, they were placed on a Fisher Model 129 shaker table set at 90 cycles per minute. The microcosms were shaken by hand periodically to break up any soil aggregates and to try to expose them evenly to the aqueous phase. However, some solids still "stuck" to the bottom of the flasks, which could limit solute access to the soils, showing up as kinetic inhibition of the sorption process (Karickhoff, 1984). After the required contact time, most of the contents of each flask was poured into a 50 ml polycarbonate tube. These tubes were placed eight at a time into a International Equipment Co. Model CS centrifuge, and spun for 40 minutes at 2000 rpm (1000xg). The supernatant was then poured off into a different, labelled

Table 5. Preparation of microcosms for sorption study.

CHEMICAL	SOIL TYPE	INITIAL VOL (ml)	[^{12}C-] VOL (ml)	[^{14}C-] VOL (µl)	FINAL CONC (ppm)
PCP	S2	53.1	0.60	100	0.10
"	"	"	1.84	"	0.30
"	"	"	6.66	"	1.00
PCP	S1	52.1	0.59	100	0.10
"	"	"	1.80	"	0.30
"	"	"	6.52	"	1.00
MCP	S2	53.1	0.27	50	1.0
"	"	"	1.09	"	4.0
"	"	"	5.92	"	20.0
MCP	S1	52.1	0.26	50	1.0
"	"	"	1.06	"	4.0
"	"	"	5.80	"	20.0

polycarbonate tube for use in later tests. The soil residue in each original flask was rinsed out with a small amount of 0.01 M $CaCl_2$ solution, and this mixture was then transferred to the appropriate tube to be centrifuged again. The purpose of this step was to ensure that all of the solids were transferred from the flasks to the polycarbonate tubes. The diluted supernatant from this final spin was discarded to waste and the soil "pellet" was saved for the desorption experiments. All tests were carried out at approximately 25°C.

The large, International Co. centrifuge was not able to remove all of the visible colloids from the supernatant, so it needed to be processed further prior to scintillation counting to prevent interference from these solids. Three 0.5 ml samples from each saved supernatant were placed into 0.6 ml polypropylene microcentrifuge tubes (Fisher Scientific). They were spun in a Fisher Scientific Model 235C microcentrifuge with a 45° rotor, at approximately 13,000 rpm (14,000xg) for five minutes each. This procedure removed the remaining visible solids from each sample. Previous research showed that the proper use of a centrifuge gave comparable results to filtering methods while studying the adsorption of various organic compounds (Karickhoff *et al.*, 1979). Finally, using a 250 µl Eppendorff fixed-volume pipet, one sample was taken from each centrifuge tube and put into a liquid scintillation vial for counting. The results from these three samples were averaged determine the amount of radiolabelled compound remaining in solution in each microcosm after the specified contact time.

3.6 Ultrafiltration

During the sorption study, samples were taken from the centrifuged liquid to determine the amount of PCP and 4-MCP that was left in the liquid phase. From this value, and knowing the volume of liquid in each flask, it was possible to determine the quantity of chemical that had sorbed to the solid phase. However, this method was not able to discriminate between ^{14}C compounds that were free in solution or bound to dissolved organic matter (DOM) or colloidal matter. Since the fate and transport of pollutants can be largely dependent on their tendency to

bind to macromolecules in solution, a short study was devised to try to separate free and bound test compounds in solution.

3.6.1 Ultrafiltration Units

Stirred-cell ultrafiltration systems have been used in various studies for the separation of DOM (Amy *et al.*, 1992; Logan and Jiang, 1990). The ultrafiltration unit chosen was the Amicon MPS-1 micropartition system, due primarily to the small sample volumes. Two sizes of Diaflo ultrafilters, 500 MW and 10,000 MW, were selected as the best sizes available to perform the desired tests. The goal was to separate the samples into three size fractions, with the free PCP and 4-MCP being measured from the filtrate using the 500 MW filter. The size fraction between 500 - 10,000 MW represented chemicals bound to smaller colloids and DOM, and the >10,000 MW filtrate could indicate chemicals bound to larger substances in solution. The distinction between fulvic and humic acids is not a precise one. An operational definition of the humic acid fraction consists of DOM that is insoluble at pH=2, while the fulvic acid fraction remains soluble (Stevenson, 1982; Bourbonniere, 1989). The larger membrane was chosen as close as possible to this "cut-off" between humics and fulvics to try and distinguish between sorption to these two components of DOM.

3.6.2 Filter Preparation

The YM10 (10,000 MW) and YC05 (500 MW) membranes have cutoff ratings based on molecular weight, but there are several other factors that can greatly affect filter performance. The foremost of these is molecular size, which determines the compounds ability to fit through the pore openings. Because of the manufacturing process, these filters are created with little variance in the pore sizes, resulting in a high level of permeate selectivity. However, adsorption to the membrane can occur depending on solution temperature, pH and the chemical properties of the solutes (Amicon MPS-1 Technical Manual, 1990). Therefore, it was necessary to conduct a

pilot study to check for adsorption of PCP or 4-MCP to either membrane.

Dilute solutions of both chemicals were centrifuged through each size ultrafilter three times, with 250 µl samples being taken from the filtrate each time. An increasing amount of radiolabelled compounds was measured after every trial, with 65-85% of the initial concentration passing through the membrane from the third spin. In order to boost this percentage, the ultrafilters were pre saturated by passing two, 1 ml aliquots of each unlabelled stock solution (9 ppm PCP, 200 ppm MCP) through one pair of both size membranes. After this treatment, 85-98% of the compounds were able to pass through the filters. As a result, all filters used in subsequent test were prepared in this manner.

3.6.3 Separation of Dissolved Organic Matter

Ultrafiltration tests were performed on microcosms made in an identical manner to those used for the sorption-desorption study. Each soil type was combined with each test chemical at the highest concentration (1 mg/L PCP, 20 mg/L MCP) to make four different flasks. Four sets of these flasks were duplicated, and they were exposed for 1, 3, 21 and 91 days.

After the required contact time, the soil slurry from each flask was transferred to a 50 ml polycarbonate tube and centrifuged at 1000xg for 10 minutes. From this supernatant, seven 1 ml samples were spun an a microcentrifuge (14,000xg) for another 10 minutes to remove all settleable solids. Three 250 µl samples were taken from one microcentrifuge tube to quantify the total initial radioactivity, and the other six were processed through the ultrafiltration units (three for each size membrane). These results were used to determine the amount of PCP or 4-MCP that remained in each size fraction. For purpose of analysis, it was assumed that any test chemicals that passed through 500 MW filters were in an unbound state in solution.

3.6.4 Total Organic Carbon Analysis

In order to determine if the distribution of PCP and 4-MCP was related to the amount of

DOM in each size fraction, it was necessary to quantify the DOM content. The method used was to ultrafilter the soil solutions as before, and measure the organic matter as total organic carbon (TOC) on a Dohrmann DC-80 Carbon Analyzer. Since radiolabelled compounds would contaminate that equipment, eight new flasks were created exactly as the others (four of each soil type) except that no PCP or 4-MCP compounds were added. Each pair of flasks were placed on the shaker table for 1, 3, 21 or 91 days, after which the liquid phase was separated by microcentrifuging the soil slurry.

The ultrafilters were prepared by soaking and rinsing them to remove their glycerin coating, as described in their manual. Three 1 ml samples were run through each size membrane, and then combined for the TOC analysis. Another volume of the unfiltered liquid phase was taken from each flask to determine the initial levels of DOM. All of the samples were acidified to ≤ pH 2 with one drop of 85% phosphoric acid, and then purged of all inorganic carbon using 99.8% pure oxygen for 5 minutes. All samples were tested twice, and the results were averaged together. The resulting TOC within each size fraction was calculated by subtraction of the smaller size fraction(s). These findings were compared to those from the ultrafiltration of the radiolabelled compounds to characterize the distribution of PCP and 4-MCP in the liquid phase.

3.7 Desorption

The desorption process involves the release of the test chemicals from the sorbed or solid phase, back into the liquid phase. As with the sorption process, a state of equilibrium between these two phases is approached that depends upon the physical and chemical properties of the sorbent, sorbate, and the solvent. In order to allow some type of comparisons to be made between the sorption and desorption rates, a batch process using similar conditions of temperature, pH and ionic strength was used to perform the desorption study.

After the soil slurry was transferred from each flask into a polycarbonate tube, centrifuged, and the supernatant was removed, the soil samples were ready to be processed. The first step involved the addition of 25 ml of 0.01 M $CaCl_2$ to each tube. Each soil "pellet" was mixed gently

with a clean stainless steel spatula in order to suspend the soil, and allow it to mix freely with the uncontaminated solution. Two ml of the phosphate buffer was added to maintain the pH as before, and the tubes were placed horizontally on the shaker table, set at 90 cycles/min.

After a period of time, generally 1 or 2 days, all tubes were taken off the shaker table and centrifuged at 1000xg for 20 minutes. A 0.5 ml sample was taken from the supernatant of each tube, using an Eppendorff pipet. The remaining liquid was disposed of as waste, and the 0.5 ml samples were centrifuged at 14,000xg to remove any suspended particles. Then, 250 µl was transferred from each microcentrifuge tube into a liquid scintillation vial to analyze their levels of radioactivity. This process was repeated until little or no more desorption could be detected.

3.8 Biodegradation

The final study of this experiment was designed to measure the biodegradation of the PCP and 4-MCP by cultured microorganisms. An attempt was then made to correlate that bioavailability with the variables of f_{OM} and exposure time. Since it is believed that these are the major environmental factors controlling the desorption process in this study, favorable results would show how critical this process is to the successful promotion of biodegradation.

3.8.1 Preparation of Microbes

Two sets of microorganisms had to be cultured for this study, one using PCP and one using 4-MCP. A pair of 750 ml flasks were filled with 300 ml of d-d water, 5 g of each soil type and 200 ml of a mineral salts solution. The contents of this solution are shown in Table 6.

An aeration system was set up using compressed air passed through an activated carbon filter, and going to each culture flask in parallel. A post-filtration system consisting of a KOH solution and another carbon filter was used to trap any volatilized compounds before discharge to the atmosphere. Crystalline PCP was added to one flask to a concentration of 5 mg/L, and

Table 6. Preparation of the mineral salts medium.

COMPOUND	AMOUNT (g)
KH_2PO_4	2.00
K_2HPO_4	6.00
$(NH_4)_2HPO_4$	0.50
$MgSO_4$	2.00
H_3BO_3	0.02
$Na_2HPO_4 \cdot 7H_2O$	0.20
$FeSO_4 \cdot 7H_2O$	0.10
$MnSO_4 \cdot H_2O$	0.10
$CaCl_2$	0.10
$ZnSO_4 \cdot 7H_2O$	0.02
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.02

* The above compounds were dissolved in 2 L of distilled-deionized water.

4-MCP was added to a 30 mg/L concentration. This design is shown in Figure 2. The growth of the microbes was monitored by observing liquid samples through a 100X microscope. The substrate compounds had to be supplemented periodically due to losses from biodegradation and volatilization. Finally, solution pH was maintained at approximately 6.8 by adding small quantities of phosphate buffer as needed.

After a period of 5-6 weeks, these cultures were enriched to separate the microbes from the soils in the initial flasks. A duplicate aeration system was set up identical to the first, and 200 ml each of distilled water and mineral salts solution was added to these new culture flasks. Crystalline PCP and 4-MCP was also added as before. Then, after allowing the soil particles to settle for about 2 hours in the original flasks, the top 200 ml was removed and transferred to the new cultures. Growth was monitored until a sufficient population of microbes had developed, and was ready for use in the biodegradation experiment.

3.8.2 Preparation of Microcosms

Due to the complexity of this experiment, only twelve flasks were created, consisting of three sets of each soil-chemical combination. They were prepared exactly as the others, ensuring pH was stabilized at 6.8. Although the autoclaving process was probably not as efficient as using a dilute sodium azide solution, proven effective by Kale and Raghu (1982), the addition of such a biocide would have also inhibited those microbes added later in this experiment. The labelled and unlabelled compounds were combined prior to addition to these flasks, making final concentrations of 1 mg/L PCP and 20 mg/L 4-MCP. All of the flasks were placed on the shaker table at 90 cycles per minute, which appeared sufficient to maintain aerobic conditions within the soil slurry. Each set of four flasks was left undisturbed for either 1, 3 or 21 days.

3.8.3 Addition of Microbes

Once a sufficient population of PCP and 4-MCP degraders had developed, they needed to

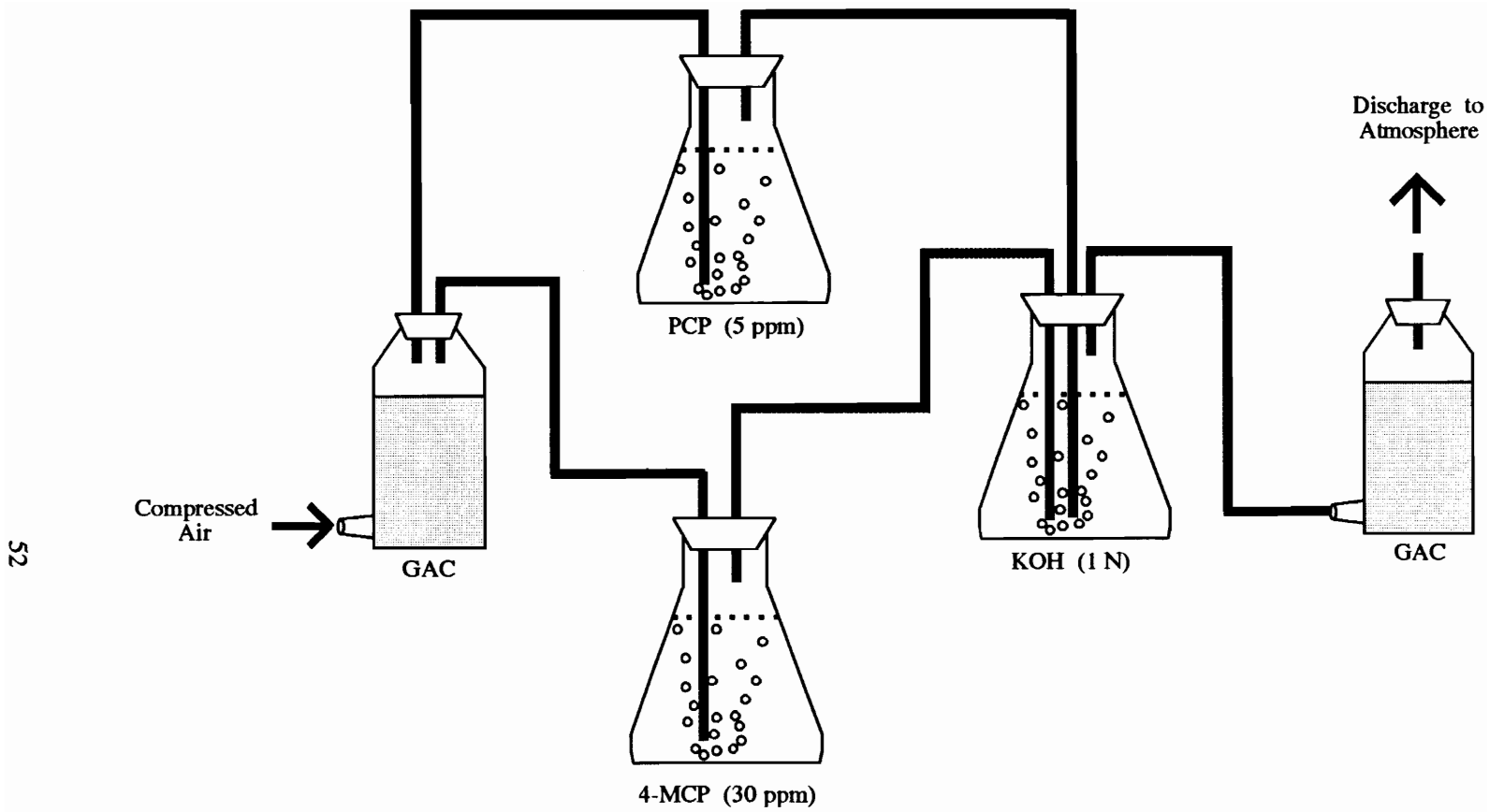


Figure 2. System design for culturing microorganisms.

be transferred to the microcosms. This had to be done in such a manner as to not dilute the solutions excessively, and not add PCP or 4-MCP from the culture solutions to the flasks. Therefore, when the exposure time was reached for each set of four flasks, two 50 ml volumes were taken from each culture and placed into clean centrifuge tubes. They were concentrated on a Beckman J-21C Centrifuge at 15,000 RPM (10,000xg) for 45 minutes. The supernatant, approximately 49 ml, was removed with a pipet and checked under a 100X microscope. There were very few microbes visible in this liquid, however a check of the solid deposits in the remaining 1 ml showed considerable microbial activity. Then, 20 ml of a freshly prepared mineral salts dilution was added to each tube. The solid deposits were resuspended by vortex mixing and the tubes were centrifuged again as before. The top 20 ml was removed with a pipet, and the remaining 1 ml was added to the appropriate flask for the biodegradation study. This last step ensured removal of most of the PCP or 4-MCP before adding the microbes to the microcosms.

3.8.4 Sampling of Microcosms

In order to keep a close track of the distribution of the test chemicals within the flasks, samples were generally taken every day in the beginning, tapering off to every 3-4 days at the end of the experiments. Samples were taken to measure the level of radioactivity associated with the solid, liquid and gaseous phases.

When the microbes were added to each flask, a polypropylene center well (Kontes) was mounted in each cap. 100 µl of a 1 N KOH solution was pipetted into these center wells, to trap carbon dioxide evolved from the biodegradation process. The use of radiolabelled compounds and the trapping of $^{14}CO_2$ as a measure of biodegradation is a widely accepted procedure (Dec and Bollag, 1988; Larsson and Lemkemeier, 1989; Murthy *et al.*, 1979). A diagram of this setup is shown in Figure 3. Although normal atmospheric carbon dioxide was also trapped, only the radiolabelled molecules from the test chemicals would be measured on the scintillation counter.

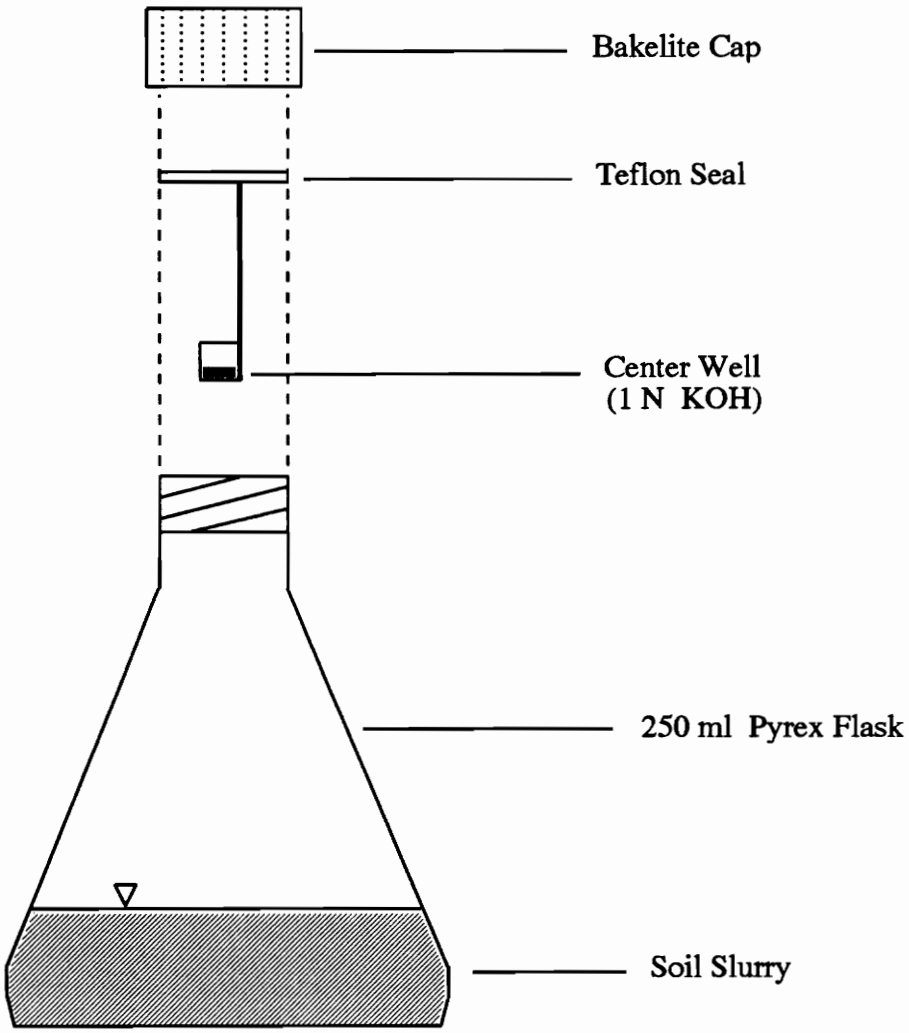


Figure 3. Microcosm set up for the biodegradation study.

When each sample was taken, the bottom portion of the center wells were cut off and placed into individual scintillation vials. These were shaken to ensure complete mixing of the KOH with the scintillation fluid. Afterwards, a new center well with KOH was placed into each flask for the next sampling. Some interference could be caused by volatilized, non-degraded compounds, but this process would be monitored and accounted for by separate control flasks.

The soil and liquid samples were taken using a 250 µl Eppendorff pipet, and pipet tips that had approximately 5 mm cut off their ends to enlarge the openings. This procedure allowed the soil slurry to be taken evenly into the pipet tip, without excluding any of the solids. The flasks were shaken by hand to create a uniform solution, and the pipet tip was placed several millimeters above the bottom of each flask to ensure that a representative sample was taken. However, some fluctuations in the results from the soil samples would be expected due to variations in the sand, silt and clay fractions collected.

The next step was to transfer the 250 µl of soil slurry into a 0.65 ml microcentrifuge tube. These tubes were pre weighed to ± 0.1 mg on a Mettler H34 analytical balance. After all samples were taken, the tubes were placed in the Fisher Scientific microcentrifuge and spun at 14,000xg for 5 minutes, to separate the solid and liquid fractions. A fixed-volume Eppendorff pipet was used to take a 100 µl sample from the supernatant, which was placed into a scintillation vial for counting. These results were used to calculate the aqueous concentration of test chemical in each flask. There were probably many different degradation products coexisting in the microcosms, such as various tri-, di-, monochloropenols, and others. However, since only the radioactivity of each sample is measured and not its true chemical identity, all ^{14}C in the liquid and solid phases is attributed to undegraded PCP.

Next, the remaining free liquid in each microcentrifuge tube was removed by pipet, and disposed of as waste. These tubes were then reweighed to allow calculation of the mass of saturated soil in each, and then the samples were transferred to scintillation vials. This was done by first mixing the compacted soil with a clean, stainless steel needle, and then adding d-d water with a pipet to suspend the soil particles. These solutions could then be poured into scintillation

vials, and a final rinse was performed so that no visible soil remained within the tubes. Approximately 0.5 ml of d-d water was needed to complete the transfer of each soil sample.

3.8.5 Controls

In order to measure the effectiveness of both the sterilization process and the addition of microbes to these microcosms, a series of control flasks was created using the same procedures as before. PCP or 4-MCP was added to two flasks each, one with S1 soil and one with S2 soil. The evolution of radiolabelled CO_2 was monitored using center wells with KOH, and any resulting biodegradation would be due solely to the surviving natural soil microbes.

Another pair of microcosms was created using the pH stabilization technique, but without autoclaving. 4-MCP was added to these flasks, and the biodegradation measured was due to the original population of soil microorganisms. Comparing these results to those from the other biodegradation studies should indicate how well the cultured microbes worked, and how effectively the autoclave was able to sterilize the soils.

Finally, two flasks were made using 50 ml of 0.01 M $CaCl_2$ and 2 ml of phosphate buffer, but with no soils added. PCP was added to one flask to create a 1 ppm solution, and 4-MCP was mixed into the other to make a 20 ppm solution. Center wells with KOH were mounted in both caps, and were sampled at the same time as the biodegradation microcosms. The purpose of these flasks was to measure the maximum expected volatilization of the test chemicals. However, this amount would be less in the actual microcosms, due to sorption to soil particles and DOM in solution.

3.9 Liquid Scintillation Counting

All of the radioactive samples taken during this experiment were placed into 15 ml of Scintiverse BD scintillation fluid (Fisher Scientific) that was measured out into 20 ml borosilicate

liquid scintillation vials. These samples were tested on a Beckman LS-230 Beta Spectrometer, using a 10 minute counting time per vial. A kinetic study of some of these samples showed possible quenching with KOH samples (Figure 4) and evidence of chemiluminesence from soil solution samples (Figure 5), which can introduce large errors into the data analysis from false high or low measurements of the sample radioactivity. Since these effects diminished over time, all experimental samples were stored in the dark and tested 24-48 hours after they were taken.

3.9.1 Soil Samples

The biodegradation study required the taking of soil samples as described earlier. However, these also included pore water within the soil matrix, which had to be accounted for when calculating the soil-bound fractions of PCP and 4-MCP. By weighing many microcentrifuged soil "pellets" before and after drying at 110°C, the percent pore water was computed to be 39% for soil S1 and 42% for the S2 soil. These results were used to subtract the liquid-phase contribution to the total sample DPM.

Unfortunately, these samples could not be read accurately by the liquid scintillation counter until the soils were allowed to settle completely to the bottom of the vials. This requirement only served to increase the shielding of the beta particles, resulting in lower radioactivity measurements than were actually present on the soils. No compensation was made to these results as only the relative values are critical to this analysis, but this effect showed up as a low mass balance for the biodegradation studies.

3.9.1 CO_2 Trapping Efficiency Test

In order to determine the effectiveness of this CO_2 trapping method, two flasks were set up with 50 ml of d-d water, 1 ml of 1 N NaOH and different initial concentrations of radiolabelled sodium bicarbonate ($Na_2H^{14}CO_3$). Duplicate samples were taken to calculate the total amount of

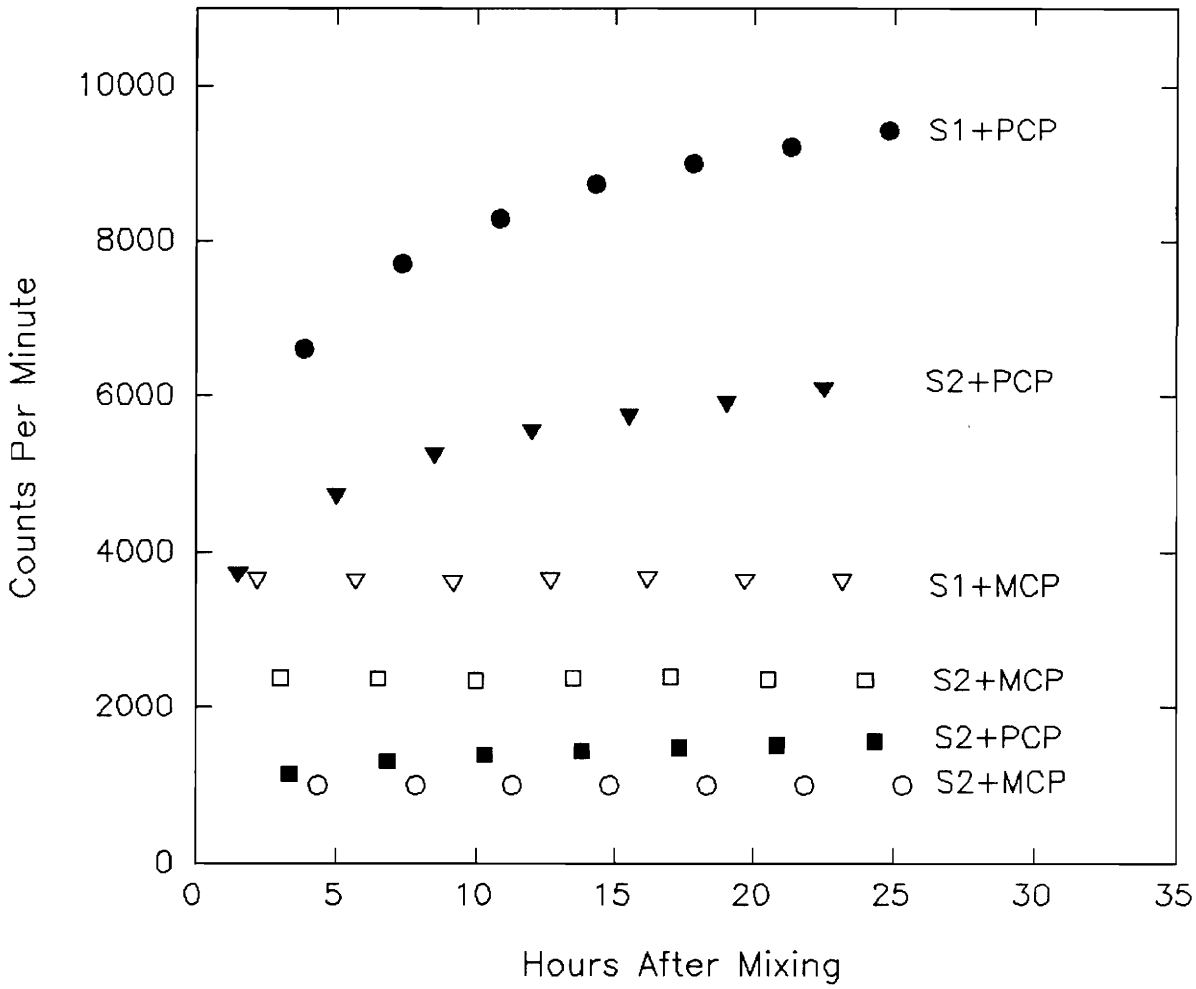


Figure 4. Kinetic test of random, 100 µl KOH samples taken from various soil-chemical combinations to monitor quenching and/or chemiluminesence effects.

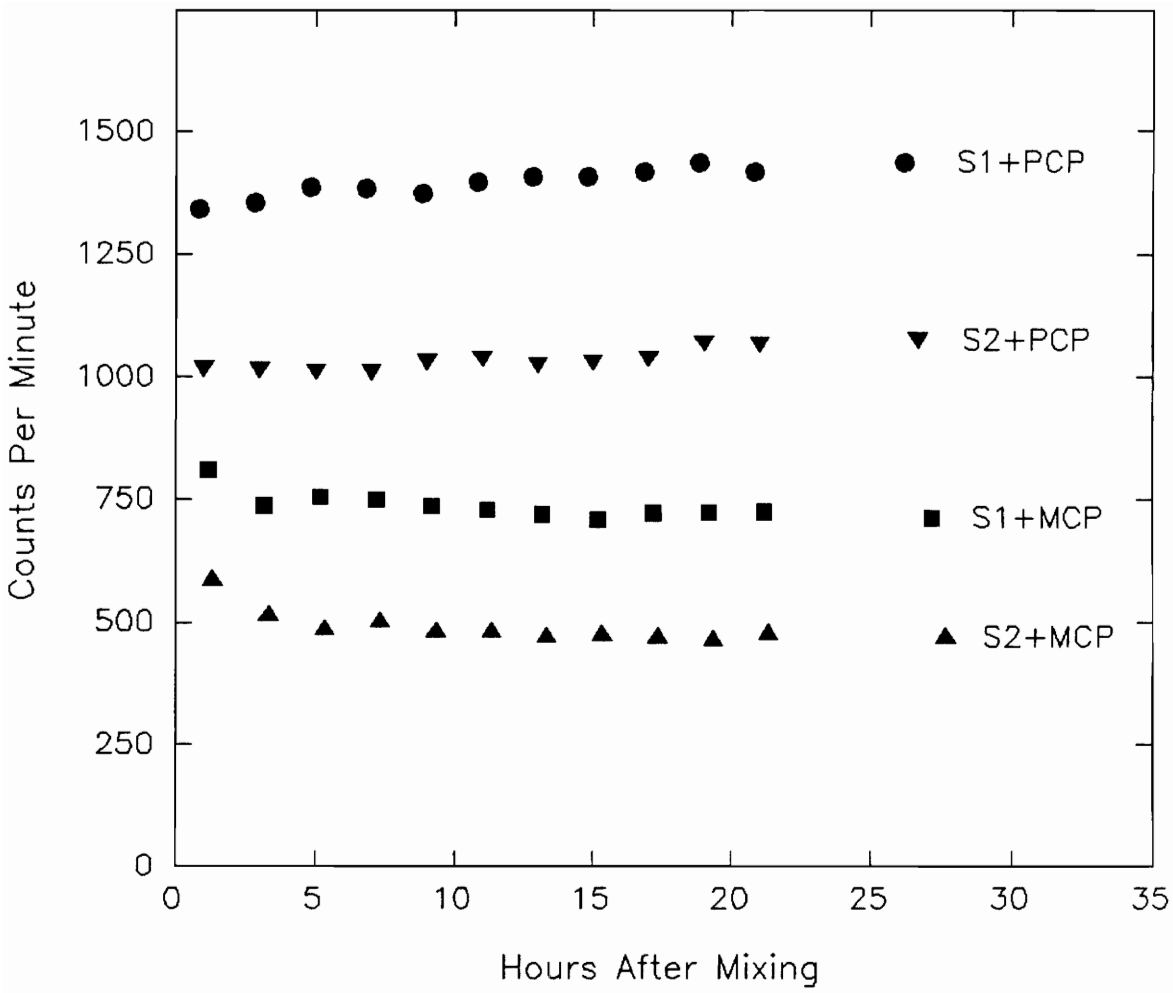


Figure 5. Kinetic test of random soil solution samples taken from each soil-chemical combination to monitor quenching and/or chemiluminesence effects.

radioactivity in solution, and then each flask was acidified to pH < 2 with 1 ml of concentrated (37%) HCl. Both caps were immediately screwed on, with a center well containing 100 µl of 1 N KOH mounted in each one. These flasks were placed on the shaker table for 24 hours, and then liquid samples were taken along with the KOH samples. These results were used to calculate the amounts of $^{14}CO_2$ that were trapped by this system, which were 99.3% and 101.1%. This test proved the ability of this method to trap CO_2 efficiently during the biodegradation experiments.

3.9 Statistical Analysis of the Data

In order to accurately and quickly analyze the large amount of sorption and desorption data, a three-parameter nonlinear regression model was used to fit these curves for a computer-aided statistical analysis. A SAS(R) program was written to calculate these three parameters for each data set, and then the PROC NLIN procedure in SAS(R) was used to perform pairwise comparisons of various curves. This method allowed statistical verification of whether or not any actual differences in the sorption-desorption responses were due to SOM content, contact time, or the initial aqueous concentration of the test compounds, assuming all other factors were the same. These analyses were calculated at a confidence level of $\alpha = 0.05$.

The computer codes and a more detailed explanation of this technique was written by Chism *et al.* (1991). While more traditional analytical techniques involve two-stage or diffusion models, the basis for this nonlinear regression model was the Miterlich equation, which is a three-parameter exponential function. The variables were modified slightly for use in this study, and the equation was used in the following form:

$$C_{aq} = B_0 + (B_1)e^{(-B_2 \cdot T)} \qquad \text{(Equation 1)}$$

where C_{aq} represents the aqueous concentration of the test compounds in µg/L, and T is the time

in days at which either the sorption or desorption data was taken. The three parameters used to analyze and compare data sets (B_0, B_1 and B_2), are defined in Figure 6. The variables used for the sorption curves are shown in Diagram 6(A), while the desorption variables are shown in 6(B).

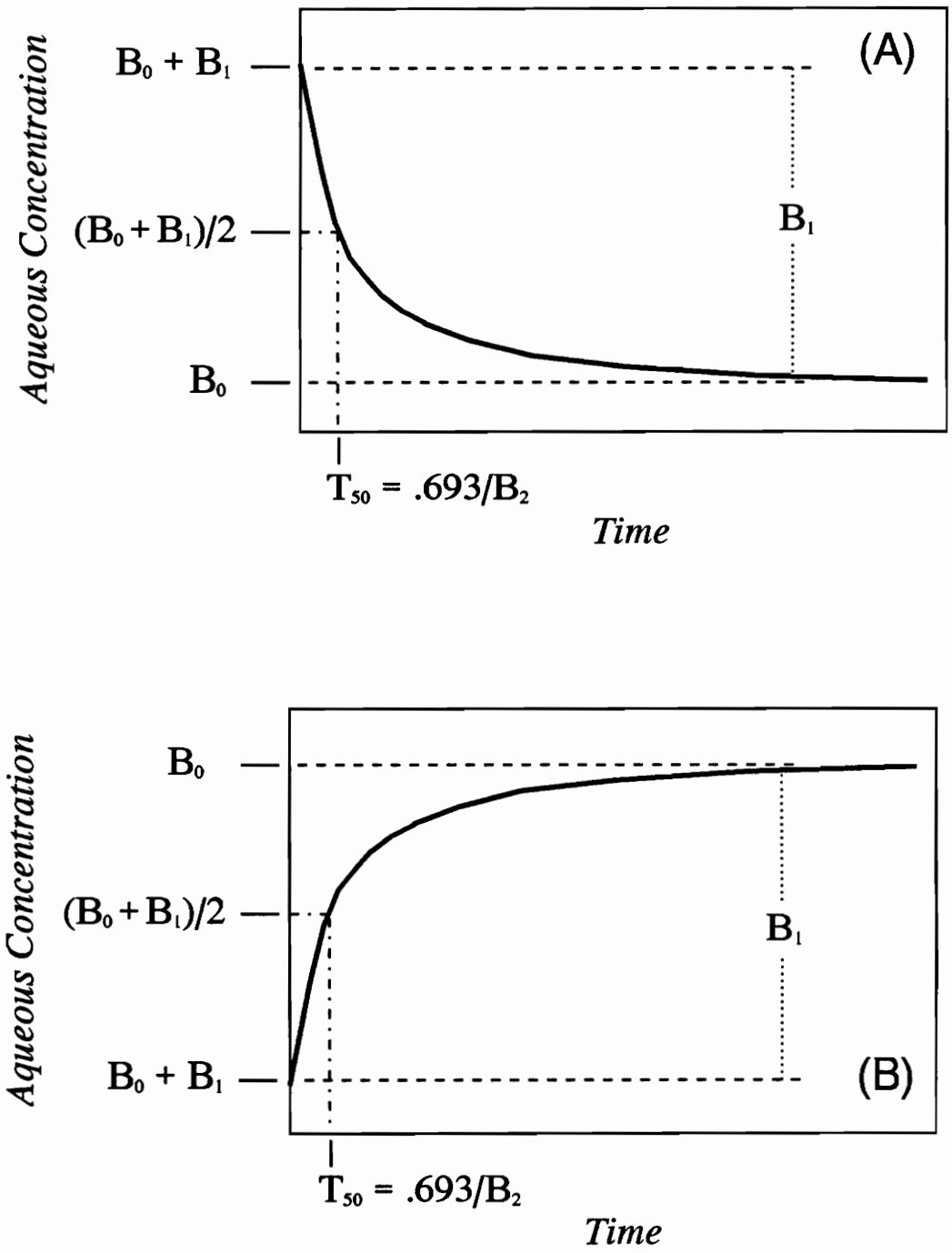


Figure 6. Variables used for the nonlinear regression analysis of the sorption-desorption data.

Chapter 4

Results and Discussion

4.1 Sorption

The sorption experiments were designed to characterize the adsorption of PCP and 4-MCP that could be attributed to SOM content or initial aqueous chemical concentration. They were carried out as described in Methods and Materials, and the data are presented in Figures 7 and 8. Although there are small deviations in the "curves" obtained from this study, this is to be expected from the use of separate soil-water systems for each data point on the graphs. However, the results generally follow the expected pattern of a high initial sorption rate followed by a much slower binding rate of the test compounds to the solids over the remainder of the experiment. Only the lowest concentration of 4-MCP appeared to reach true equilibrium prior to the 91 day samples.

The control flasks, which contained only a $CaCl_2$ solution and phosphate buffer, were intended to quantify any losses of the test compounds due to volatilization, photodecomposition, or sorption to the flask surfaces. The results show that a small fraction of the 4-MCP disappeared

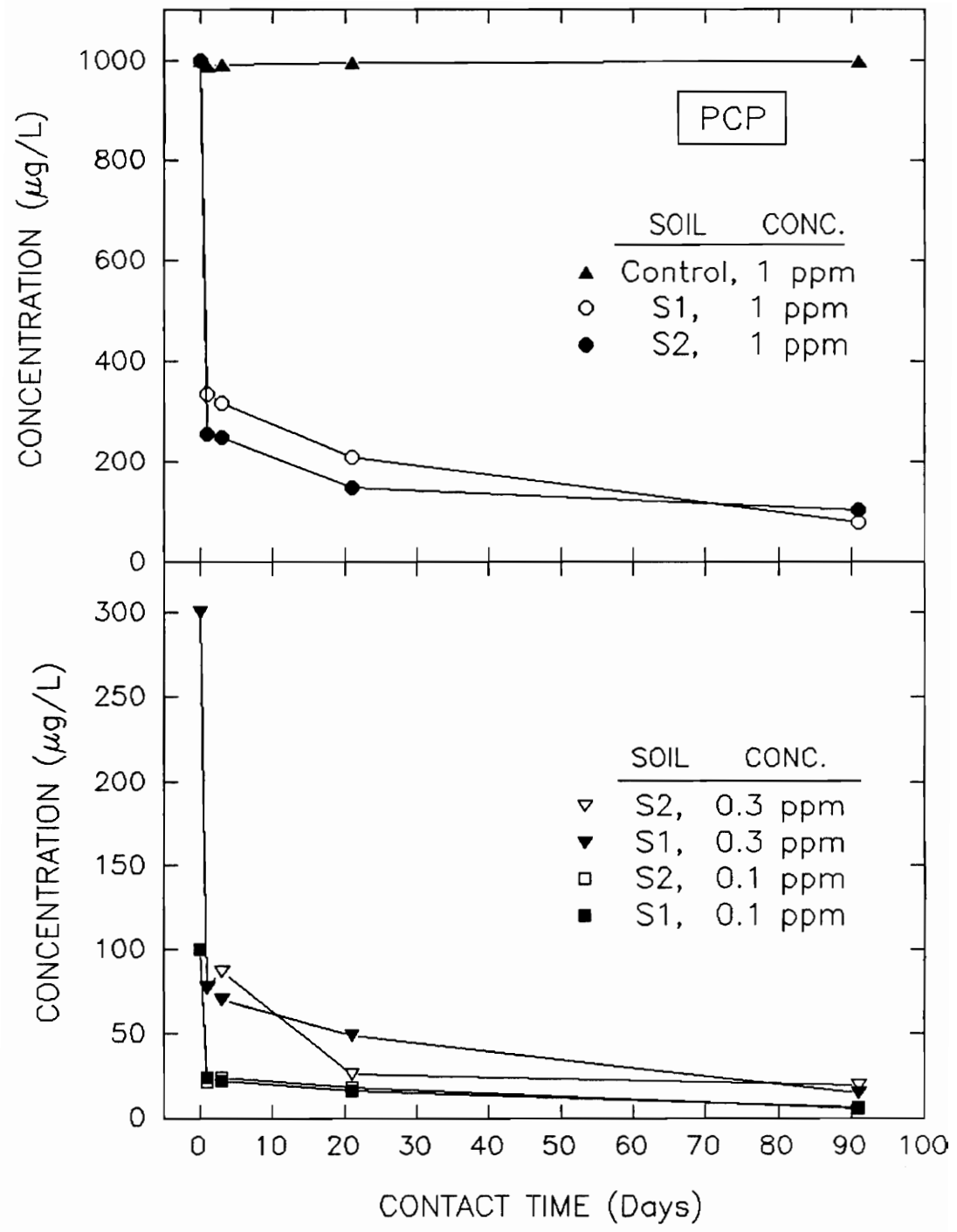


Figure 7. Sorption of PCP using various soils, concentrations, and contact times.

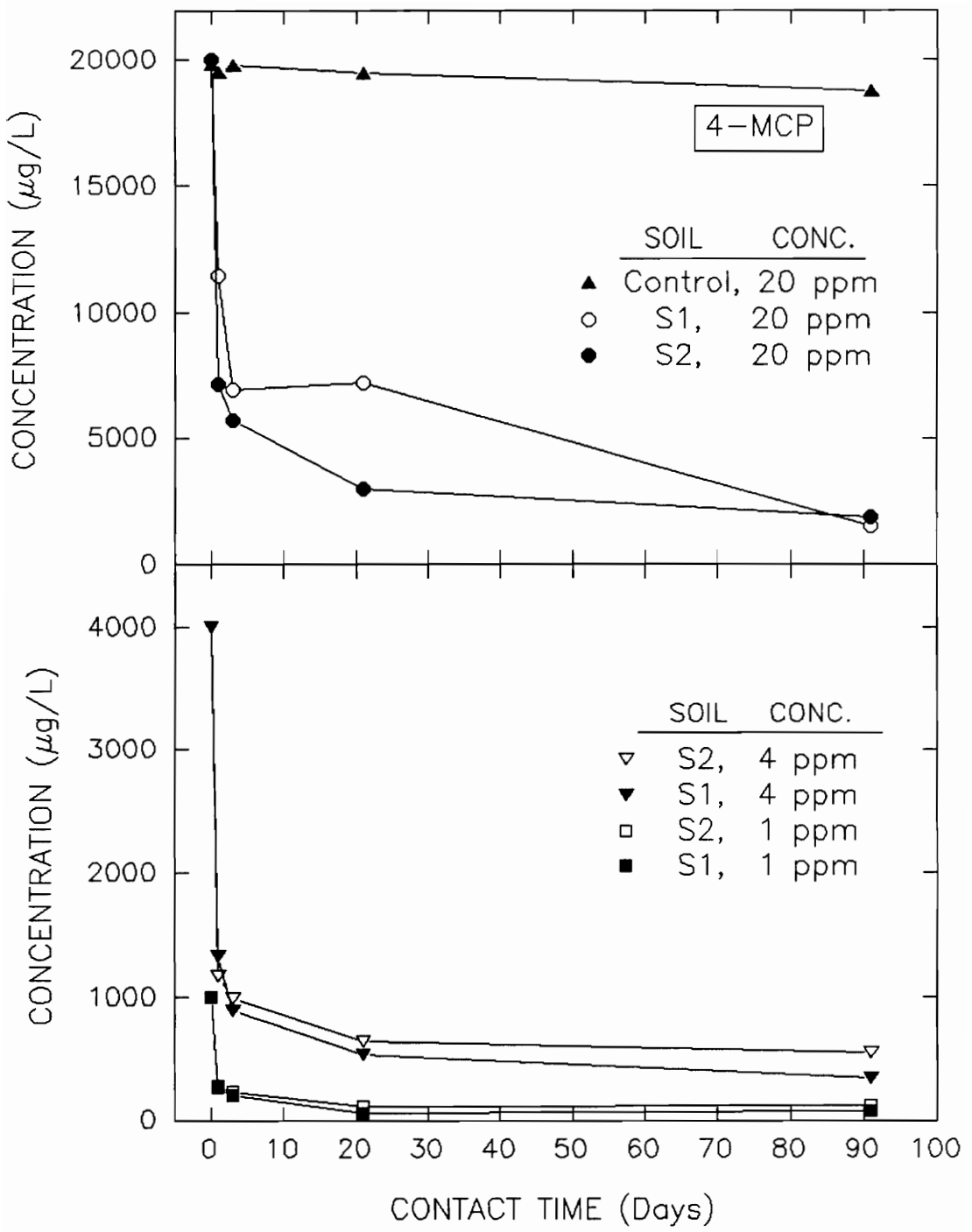


Figure 8. Sorption of 4-MCP using various soils, concentrations, and contact times.

from the liquid phase, probably due to its relatively high volatility, but essentially none of the PCP was lost from solution. While it has been demonstrated that hydrocarbons can undergo many physical, chemical and biological changes in soil systems, all of the ^{14}C in the liquid and solid phases is attributed to the original, unaltered compounds.

A fairly large mass of soil (20 g) was used in these experiments, so that there would be sufficient solids available for the desorption studies. Since the amount of liquid used had to be minimized due to the small size of the flasks, the soil:liquid ratio was rather high. Since solids concentrations of even 1 g/L have been shown to delay sorption due to the "solids effect" of soil aggregates (Karickhoff, 1984), it was anticipated that a long time period would be required to approach equilibrium. The results showed a very high initial rate of adsorption, with approximately 60-80% of the sorption measured over the 91 day test occurring within the first day. These data conform to previous studies, and appear to follow a "two-stage" sorption process as described by Karickhoff (1980).

As mentioned earlier, many studies have indicated that the fraction of organic matter (f_{OM}) is a primary factor in determining the sorption characteristics of a given soil (Karickhoff and Brown, 1978; Mingelgrin and Gerstl, 1983). While it appears that the higher SOM content soil (S2) sorbed more of the chemicals in these tests, a statistical analysis of the data indicated that there was no significant difference between each paired set of data for both PCP and 4-MCP, at an $\alpha = 0.05$ level of significance. In other words, the f_{OM} of these soils did not significantly influence the kinetics of the sorption processes which occurred in the flasks.

While this result seems to be contrary to published reports, there are many other factors which research has shown to effect contaminant adsorption to soils. Grathwol (1990) showed that SOM can display very different physical and chemical characteristics, depending on the source of origin and the weathering processes involved. While the soil-bound organics on both of these surface soils were probably of a relatively recent origin, the sources of organic matter in a wooded area and a grassy field would have different characteristics. His studies with other chlorinated hydrocarbons indicated that it was the nature of the organic matter which largely

controlled the sorption processes, rather than the overall amounts.

Other research has verified the heterogeneous nature of humic acids, suggesting that some are aliphatic while others are mostly aromatic, and all may contain widely varying types and quantities of substituent functional groups (Wershaw, 1986). Each fraction of SOM (humins, humic acids, fulvic acids) can also have differing percentages of aromatic compounds (Saiz-Jimenez *et al.*, 1986). Since Chiou *et al.* (1990) showed that significant surface adsorption of organic contaminants by SOM is highly unlikely, and that the partitioning mechanisms currently proposed have a much stronger scientific foundation, this may indicate that the nature of humic compounds play an even more important role in the sorption processes than previously believed. As a result, the characteristics of the SOM from soil S1 may have allowed equivalent amounts of sorption despite the lower total content (2.7% vs. 4.4%).

Another possible cause of these results could be the effect of DOM released into solution by the processes of pH adjustment, autoclaving, and shaker-table mixing. Visual observations indicated that autoclaving was the major factor contributing to this release. Koskinin and Cheng (1983) suggested that pesticide binding to dissolved organics could reduce sorption to the solid phase, and Chin *et al.* (1990) found that very hydrophobic compounds could be highly sensitive to even low concentrations of humic polymers in solution. When total organic carbon (TOC) in solution was measured as a function of soil type and contact time, the results shown in Figure 9 indicate that soil S2 released over twice as much DOM as did soil S1. Additionally, these levels of TOC are 25-70 times greater than the 4 mg/L average found by Amy *et al.* (1992) in various groundwater sources. The net effect could be a greater level of binding of the test compounds to these dissolved humic compounds, where they would be measured as aqueous-phase contaminants. This process could reduce sorption to the solid phase in the S2 flasks and create the appearance that f_{OM} was not significant to the overall sorption process.

Any of these effects, combined with the small sample size, might have resulted in more sorption to soil S1 than could be explained by SOM content alone. As a result, there was not a sufficient level of statistical resolution available to differentiate between the sorption patterns of

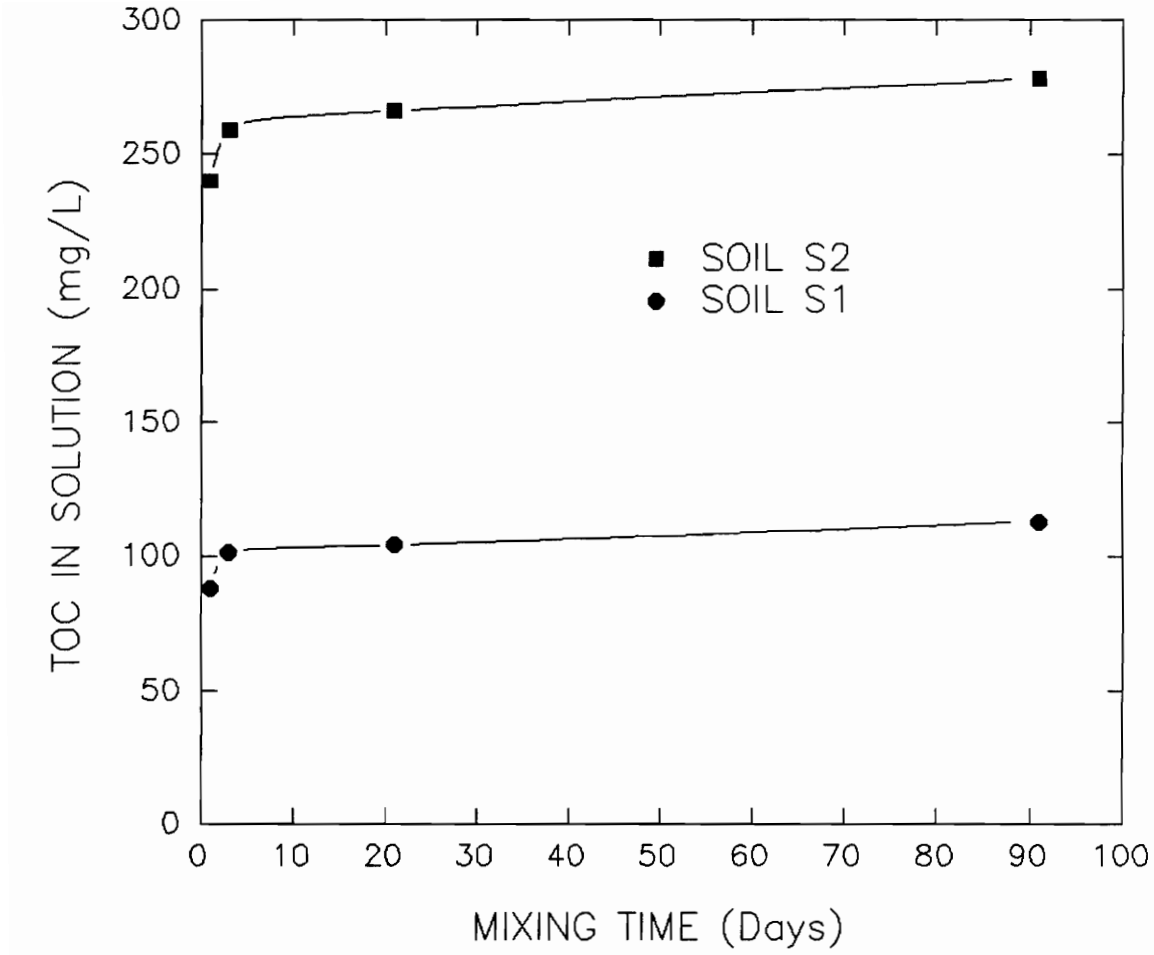


Figure 9. DOM measurements for both soil types.

soils S1 and S2.

Using the three-variable regression model described in Methods and Materials, it also was possible to analyze whether the initial chemical concentration influenced sorption behavior. A comparison of the highest and lowest concentrations of each test compound and soil type also showed no statistical difference between the measured data sets. This means that the initial concentration had no effect on the relative amounts of sorption that were measured. However, linear isotherms have often been reported at concentrations <50% of a compounds maximum solubility (Karickhoff *et al.*, 1979; Means *et al.*, 1980) which indicates that these results are in agreement with previous research.

Finally, an attempt was made to compare the sorption of PCP to 4-MCP, since they were both tested at a concentration of 1 ppm. These data were combined and are presented in Figure 10. Since 4-MCP has a much higher aqueous solubility and a lower K_{ow} value, it would be expected to partition into the solid phase to a lesser degree than PCP (Chiou *et al.*, 1983; Karickhoff, 1984). However, these results suggest that 4-MCP sorbed to the soils to at least an equal, if not a greater degree than did PCP. As a result the statistical analysis again found no significant difference between these curves, indicating that sorption was occurring at approximately equal rates for both chemicals.

While the pH in solutions was maintained at a constant level to eliminate this variable, the chemical differences between the test compounds were also enhanced. At the test pH of 6.8, PCP was >99% ionized while the 4-MCP was >99% neutral in form. Lee *et al.* (1990) showed that sorption of the neutral, hydrophobic chlorophenols and/or ion pairs was the predominant mechanism of binding. The ionized species of a weak organic acid, such as PCP^-, was found to be more soluble than its neutral form, and thus less likely to sorb to solids. Additional research verified that for many polychlorinated phenols, including PCP, sorption of the test compounds increased as the solution pH decreased (Schellenberg *et al.*, 1984). Therefore, while it was anticipated that PCP would sorb to SOM to a greater degree than 4-MCP, the pH level in the

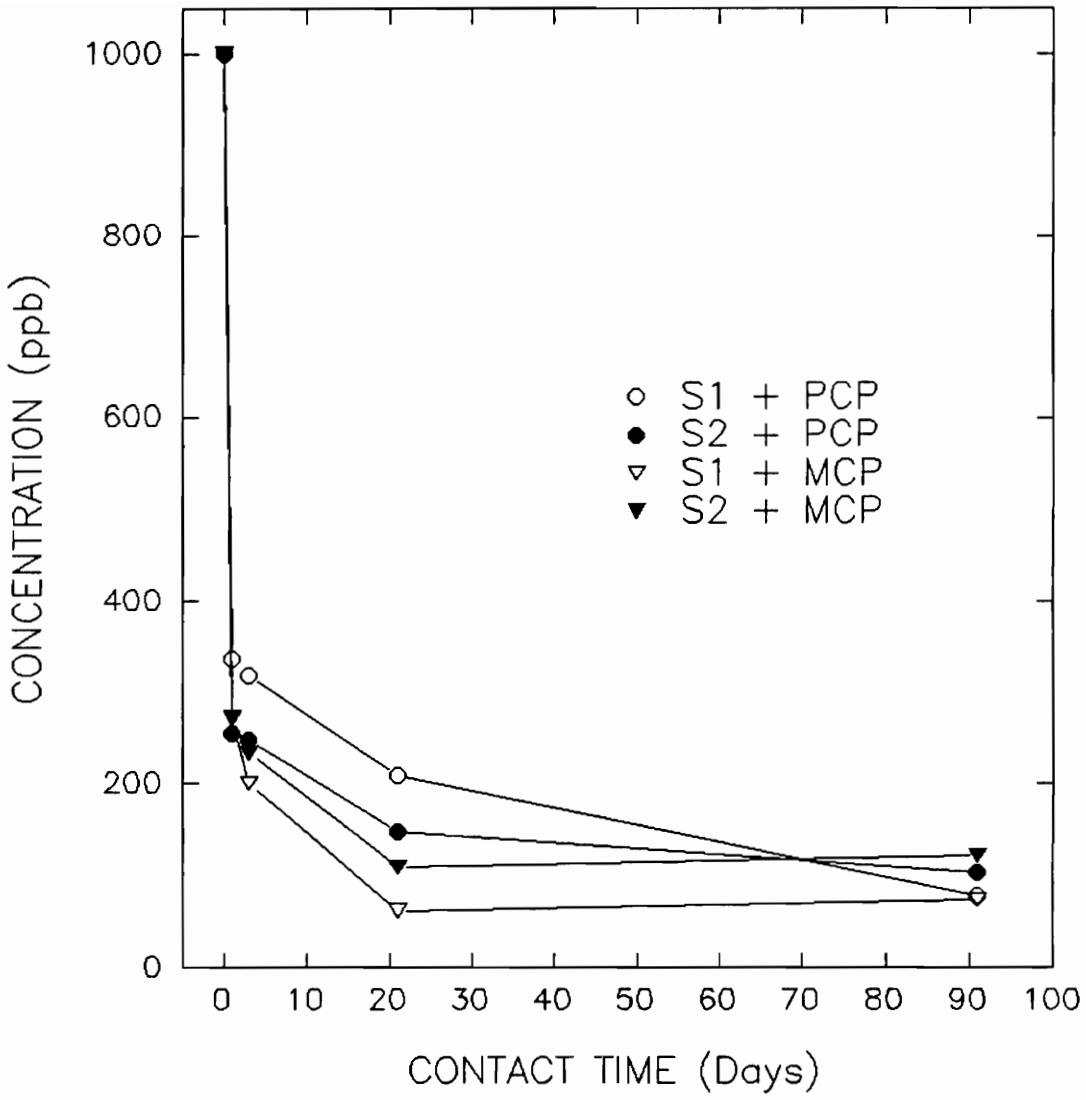


Figure 10. A comparison of PCP and 4-MCP sorption at equal concentrations.

flasks explains much of the relative decrease in PCP's affinity for the organic, solid phase.

Another explanation for the similarity in sorption characteristics might be due to microbial activity. Berry and Boyd (1985b) found that enzymatic cross-coupling could bind various contaminants to soil organic matter. Also, they found that a polychlorinated phenol, such as PCP, would be incorporated into humic polymers to a lesser degree than MCP by these microorganisms. If significant microbial utilization of 4-MCP was occurring in these flasks, that may help to explain the high level of 4-MCP sorption relative to PCP.

4.2 Desorption

After exposing the soils to the test compounds for the predetermined contact times, the liquid portion was removed and the soils were transferred to polycarbonate tubes for the desorption experiments. The soil washings were generally performed every 1-3 days, and the results for all 48 flasks are shown in Appendix A in Figures A1-A8. From these graphs it appears that the desorption process occurs at a much slower "rate" than adsorption. While most of the sorption occurred within a few days, desorption often required several weeks to complete. Karickhoff (1980) found that desorption often followed a "two-stage" pattern of fast and then slow rates, while Isaacson and Frink (1984) also showed that the desorption process occurred at a slower rate than sorption for various chlorophenols.

However, the use of batch desorption experiments creates a problem in that the soil washings can be performed at various time intervals, but the time required for these systems to reach chemical equilibrium may be entirely different from those intervals. Unless additional studies are conducted, this time to equilibrium may remain unknown, and calculations of desorption rates based on such data may be misleading. Unpublished research conducted at Virginia Tech using similar techniques has shown that desorption equilibrium using soil systems can be achieved in less than one minute in some cases. In this case, the presention of these results relative to the time intervals between washings would be inappropriate. Therefore, figures which

show desorption data are based on the number of soil washings that were performed, and not the time intervals between those washings. Since each set of samples was taken at the same time, it was possible to analyze and compare these data relative to each other, although absolute desorption rate information could not be determined.

In almost every case, soil S2 appeared to desorb its contaminant at a slower rate and to a lesser total extent than soil S1. This observation was verified by statistical analysis for all PCP concentrations for the 1, 3, and 21 day contact times. For the soils exposed to 4-MCP, most of the desorption patterns for S1 and S2 were found to be significantly different. However, several of these comparisons were not found to be different, but only at the lower chemical concentrations. These results suggest that for 4-MCP, there exists a relatively low aqueous concentration at which the SOM content will no longer influence the desorption kinetics. All of these results are summarized in Table 7. In each case, a significant difference indicates that soil S2 either desorbed a lesser total amount of contaminant (D_0), or took a longer period of time to release 50% of that total amount (D_2).

While statistical analysis provided evidence of the effect of SOM on the desorption process, graphical presentations gave further insight into the nature of this process. A comparison of the soil concentrations following each washing of the two soils, paired with the cumulative desorption curves, makes the differences due to SOM even more apparent. Figures A9-A13 in Appendix A show that soil S1 typically released more of the test chemicals during the first several days, but in each case the individual desorption samples from soil S2 eventually contained higher levels of PCP or 4-MCP than those from S1. Since both soils sorbed similar quantities of the contaminants, these results suggest that higher SOM content soils may release a lesser total amount of the bound chemicals over a longer period of time, than will lower SOM content soils.

It is interesting to note that this "cross-over" in desorption rates from the two soils appears to occur much sooner when 4-MCP is used than with PCP. This effect may indicate that the hydrophobic partitioning mechanisms associated with PCP are stronger than the bonds formed between soil-bound organics and a more soluble compound, such as 4-MCP, as was previously

Table 7. Statistical analysis of soil S1 and soil S2 desorption data for the given conditions.

Effect of SOM Content on Desorption Kinetics

Test Compound	Contact Time (Days)	Aqueous Conc. (ppm)	D_0	D_2
PCP	1	0.1	SIG	SIG
		0.3	SIG	SIG
		1.0	SIG	SIG
	3	0.1	SIG	SIG
		0.3	SIG	SIG
		1.0	SIG	SIG
	21	0.1	SIG	SIG
		0.3	SIG	SIG
		1.0	SIG	SIG
4-MCP	1	1	SIG	n.s.
		4	n.s.	n.s.
		20	SIG	SIG
	3	1	SIG	SIG
		4	SIG	SIG
		20	SIG	SIG
	21	1	n.s.	n.s.
		4	n.s.	SIG
		20	SIG	SIG

D_0 : represents the total amount of test compound that was desorbed
SIG : indicates that soil S2 released a lesser total amount of test compound
n.s. : indicates that no significant difference was found for this variable

D_2 : represents the time at which 50% of the total amount of test compound was desorbed
SIG : indicates that soil S2 took a longer time to release 50% of the test compound
n.s. : indicates that no significant difference was found for this variable

proposed by Mortland *et al.* (1986). Further evidence supporting this suggestion can be found in Figures 11 and 12, which show that in every case, soil S2 released a lower percentage of the initially bound contaminant than did soil S1.

These graphs also show how contact time can inhibit the ultimate release of soil-bound compounds. As the contact time increased from several days to several weeks, there was approximately a 50% decrease in the amount of sorbed PCP that was released by the water-washing technique. Karickhoff (1980) previously observed that a relationship existed between contact time and the extent of desorption. When he mixed PAHs with soils for less than five minutes removal efficiencies of >90% were achieved, but when this contact time increased to 4-5 days only 20-40% of the sorbed compounds could be extracted. Again, statistical analysis confirmed the differences in desorption kinetics caused by contact time, and these results are given in Table 8.

Additionally, Figures 11 and 12 show that little or none of the sorbed ^{14}C was released back into solution after the 91 day contact time. Murthy *et al.* (1979) found that about 45% of PCP added to soil remained sorbed to the solids despite multiple solvent extractions, and Banerji *et al.* (1986) showed that a fraction of PCP may be irreversibly sorbed, especially at lower concentrations. The results from the 91 day contact flasks are more extreme than those studies, and may be due to differences in amounts and characteristics of the soils, the soil-bound humics, and possible interaction with the microbial population. Bollag and Liu (1985) suggested that a fraction of the strongly bound polychlorinated phenols studied was not due to either adsorption or partitioning into the interiors of micelles or macromolecules. Instead, they proposed that the compounds were incorporated into the humates during their formation by copolymerization reactions. This process was controlled by microbial enzymes, and resulted in the formation of covalent bonds between the chlorophenols and the other organic molecules.

The effect of initial chemical concentration on the desorption process was also investigated but no significant differences could be attributed to the concentrations within the range tested.

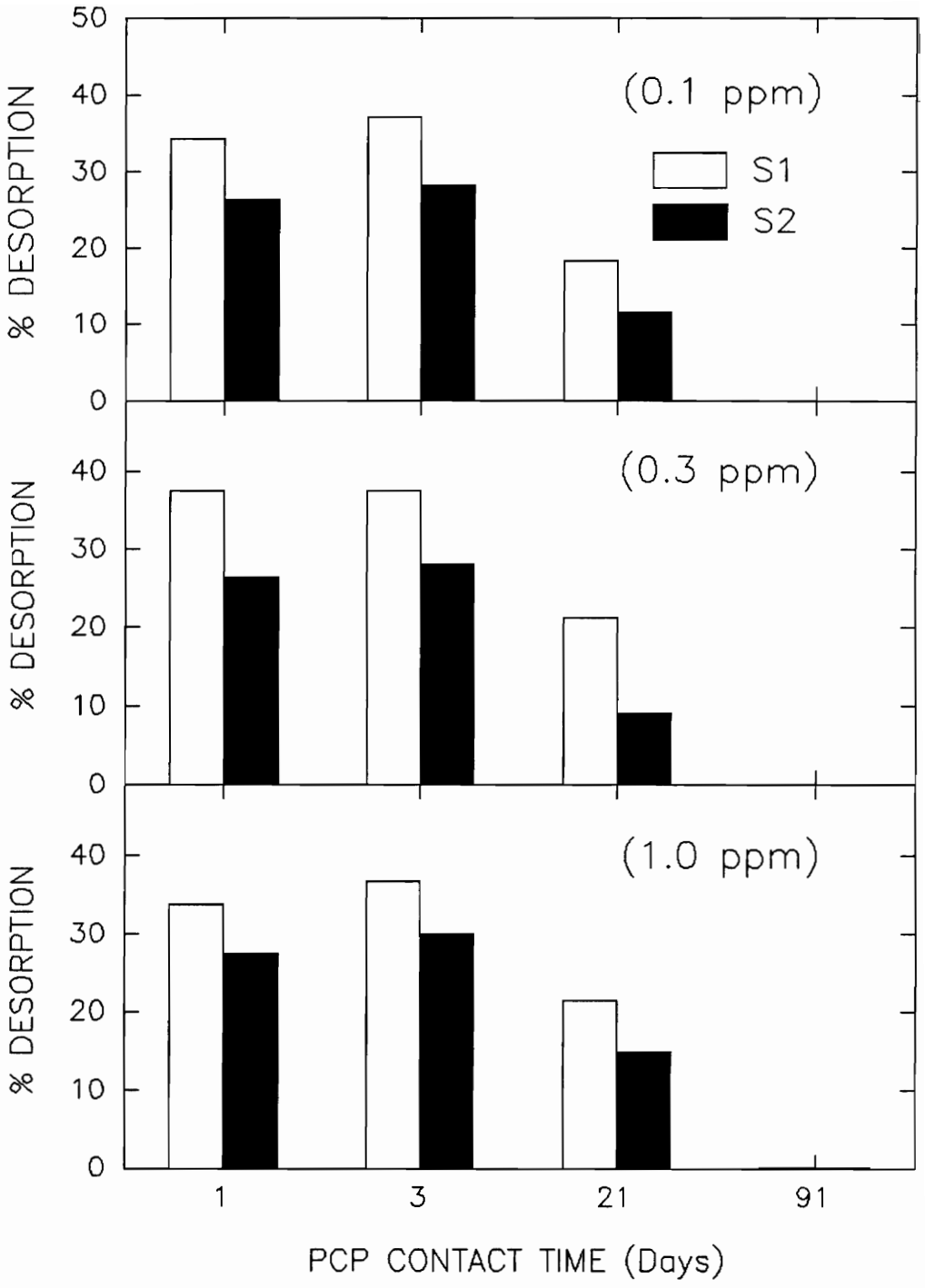


Figure 11. Percentages of the sorbed PCP that were released during desorption.

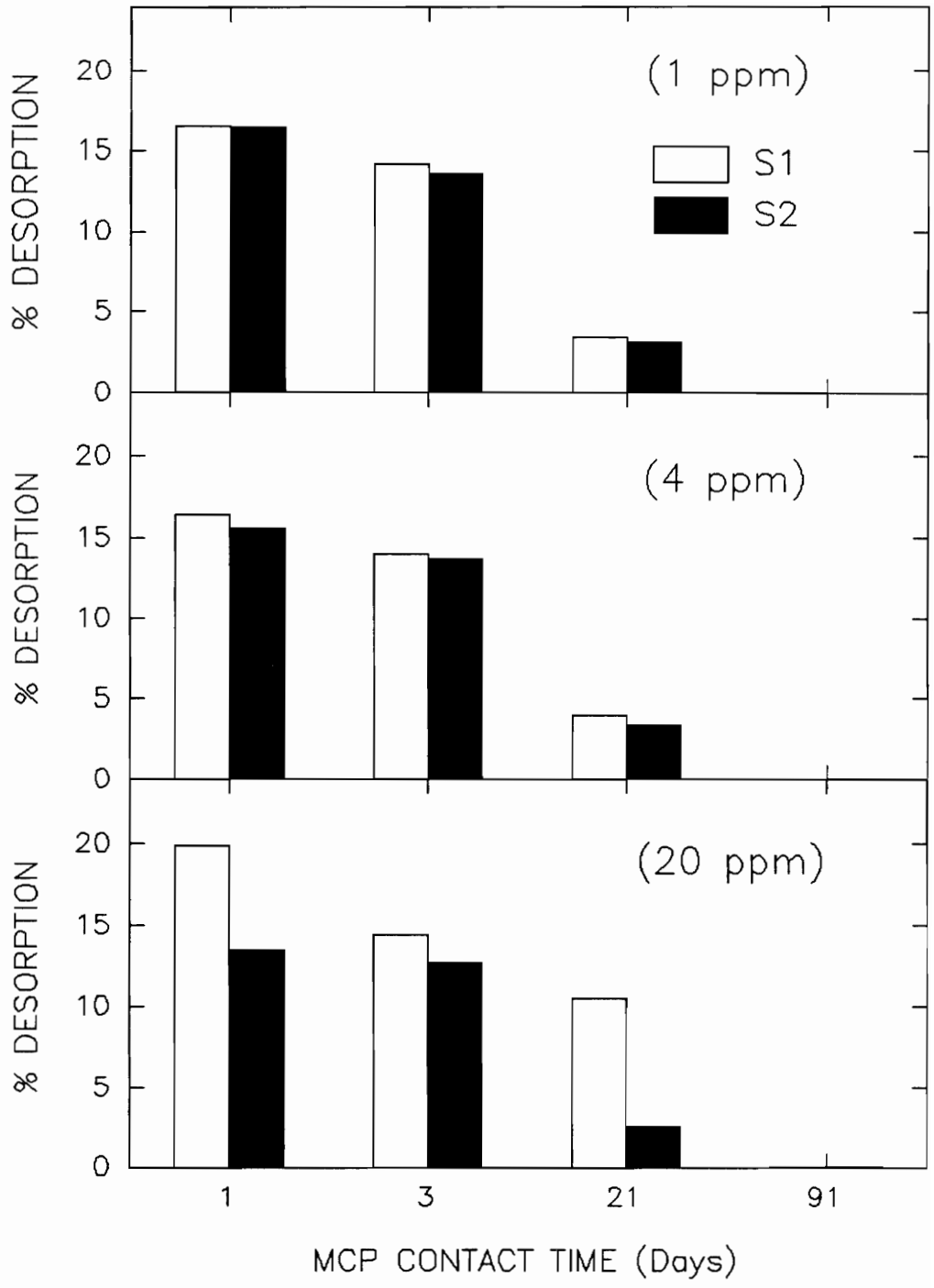


Figure 12. Percentages of the sorbed 4-MCP that were released during desorption.

Table 8. Statistical analysis of soils contacted for 1 and 21 days for the given conditions.

Effect of Contact Time on Desorption Kinetics

Test Compound	Aqueous Conc. (ppm)	Soil Type	D_0	D_2
PCP	0.1	S1	SIG	SIG(+)
		S2	SIG	SIG(+)
	0.3	S1	SIG	SIG(+)
		S2	SIG	n.s.
	1.0	S1	SIG	SIG(+)
		S2	SIG	SIG(+)
4-MCP	1	S1	SIG	SIG(-)
		S2	SIG	SIG(-)
	4	S1	SIG	SIG(-)
		S2	SIG	SIG(-)
	20	S1	SIG	SIG(+)
		S2	SIG	SIG(-)

D_0: represents the total amount of test compound that was desorbed
SIG: indicates that the soil contacted for 21 days released a lower amount of test compound
n.s. : indicates that no significant difference was found for this variable

D_2: represents the time at which 50% of the total amount of test compound was desorbed
SIG(-): indicates that the soil contacted for 21 days took a longer time to release 50% of the test compound
SIG(+): indicates that the soil contacted for 21 days took a shorter time to release 50% of the test compound
n.s. : indicates that no significant difference was found for this variable

However, the scope of this analysis was rather limited in that the only common variable which could be compared for the higher and lower concentrations was the B_2 term, which represents the time at which 50% of the total level of desorption was achieved. In other words, the statistical procedure used was to compare the B_2 values for the highest and lowest concentration of both compounds and soil types, for the 1, 3, and 21 day contact times. Of the twelve possible combinations, no significant differences were found between the any of the pairs of B_2 terms. Therefore, it was concluded that the initial aqueous concentrations of these contaminants did not influence desorption rates, within the ranges tested (0.1-1 mg/L PCP, and 1-20 mg/L 4-MCP).

Finally, a comparison of the 1 mg/L PCP and 4-MCP desorption curves, resulted in some interesting findings. The data for all four contact times are shown in Figures 13 and 14, and they clearly show that much less 4-MCP desorbed from both soil types after 1, 3, and 21 day exposures. The statistical analysis also confirmed that the curves were significantly different. While some research suggests that a hydrophilic compound like 4-MCP should bind less strongly to, and thus desorb to a greater extent from SOM, there are other variables which could account for these results. Since it is possible that differences in the characteristics of the organic matter influenced the sorption of the contaminants, it is very likely that these differences would also effect the desorption process. Any property of the humic substances which would increase their affinity for 4-MCP, such as aromatic or aliphatic structures or specific functional groups, would also inhibit desorption to a similar extent. Since the sorption studied showed that 4-MCP partitioned into the solid phase equally to PCP, the mechanism which controlled this relatively high uptake probably also prevented a large amount of release of 4-MCP during the desorption experiments.

Voice and Weber (1985) clearly demonstrated the effect of solids concentration on contaminant partitioning, and suggested that the many factors which control the nature and amount of DOM in a natural system may also effect a pollutants transport. Later research found that the solids effect often reported in batch sorption experiments may be partially due to the

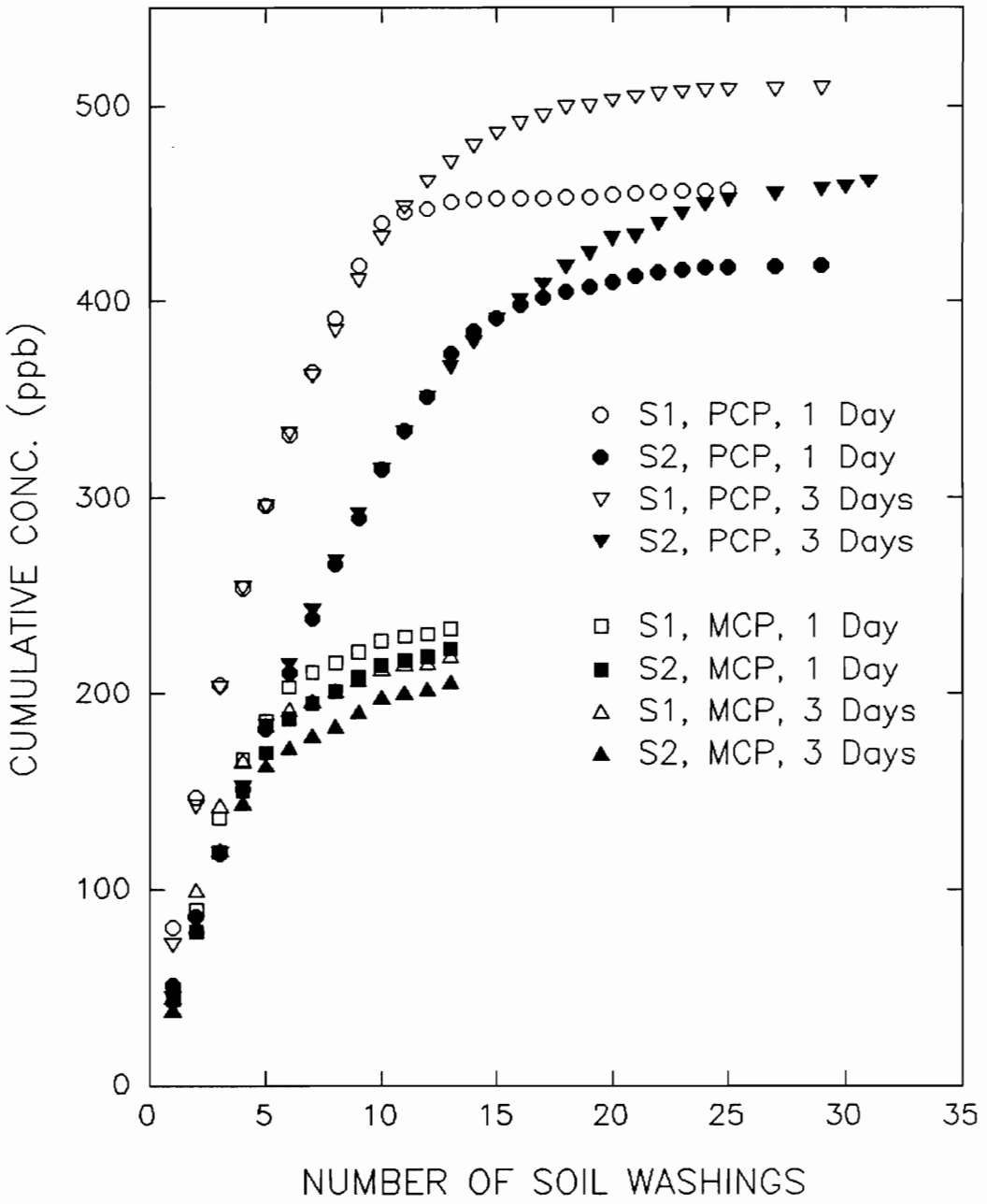


Figure 13. Comparison of 1 mg/L PCP and 4-MCP desorption curves for 1 and 3 day contact times.

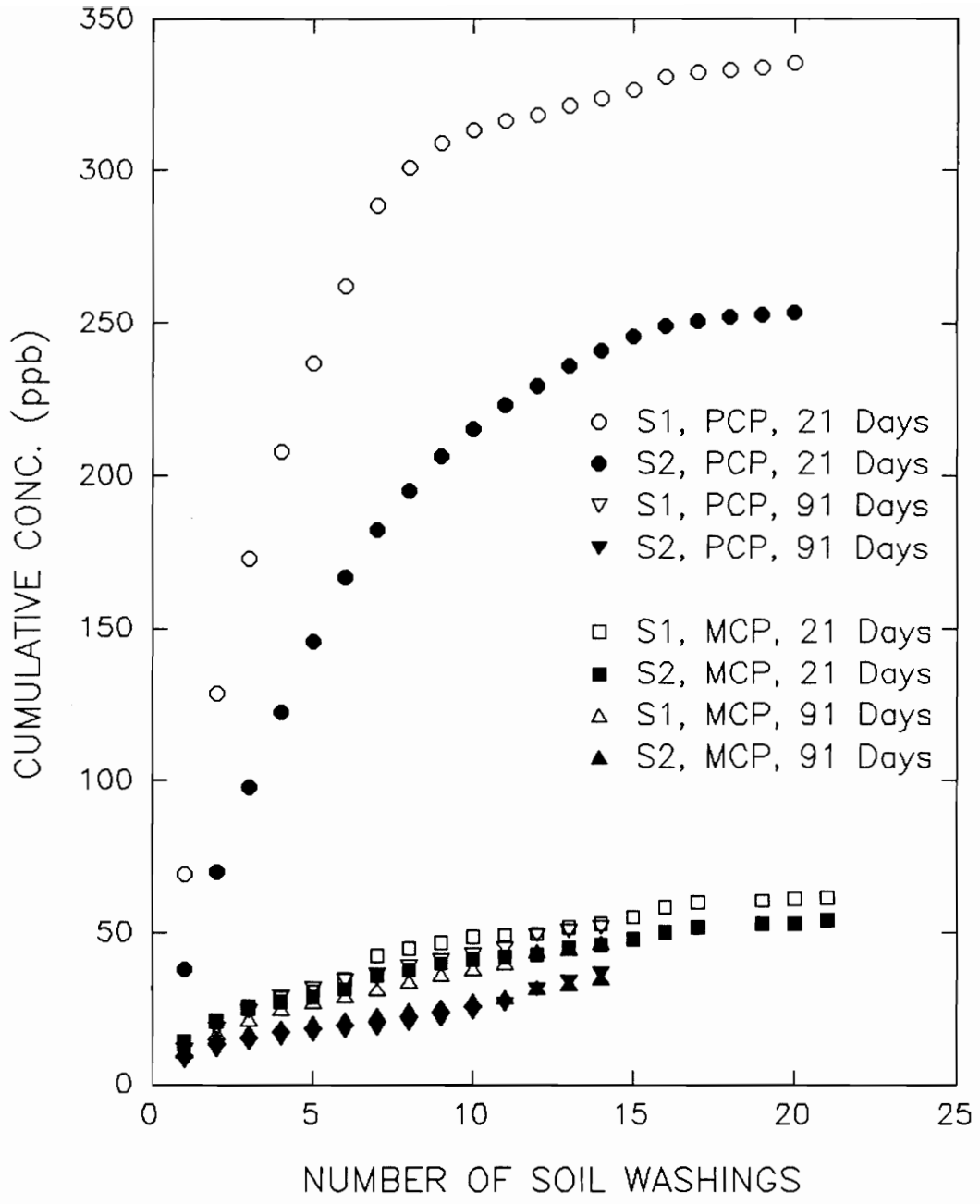


Figure 14. Comparison of 1 mg/L PCP and 4-MCP desorption curves for 21 and 91 day contact times.

release of DOM (Chin *et al.*, 1990). Enfield and Bengtsson (1988) predicted enhanced transport of HOCs in the presence of dissolved organics, and Abdul *et al.* (1990) found that dilute humic acid solutions could enhance the removal efficiencies of hydrophobic contaminants compared to more soluble compounds. Figure 15 shows that the water-washing techniques used during the desorption experiments were able to remove some of the organic matter, especially from soil S2. When these results are related to the previous research cited above, they suggest that hydrophobic partitioning of PCP into DOM may be enhancing its desorption from the solids into the liquid phase. This process of transferring some of the soil-bound organics into solution may also be responsible for a portion of the higher "slow-phase" desorption rate that was displayed by soil S2.

A number of researchers have shown that microbial activity is capable of coupling chlorophenols to humic compounds in a relatively irreversible process (Bollag and Liu, 1985; Dec and Bollag, 1988). Additionally, Berry and Boyd (1984) found that chlorine substituent groups were slightly inhibitive to the oxidative coupling process, suggesting that PCP would be incorporated into humic polymers to a lesser degree than 4-MCP. Therefore, through these enzymatically controlled coupling processes, soil microorganisms could also be partially responsible for the significantly lower desorption of 4-MCP relative to PCP.

4.3 Sorption-Desorption Isotherms

In order to evaluate the overall processes of sorption and desorption, the data are often presented as isotherms, where the mass of sorbate relative to the mass of sorbent is plotted against the aqueous concentration of the test compound. Figure 16 shows an example of a linear adsorption isotherm, followed by a nonlinear desorption isotherm. In the case of a "perfect" sorption-desorption process, the desorption data would follow the same path as the sorption data, indicating that the test compound was desorbed at the same rate that it had sorbed to the solids. Also, by returning to the graph's origin during this ideal process, the data would indicate that

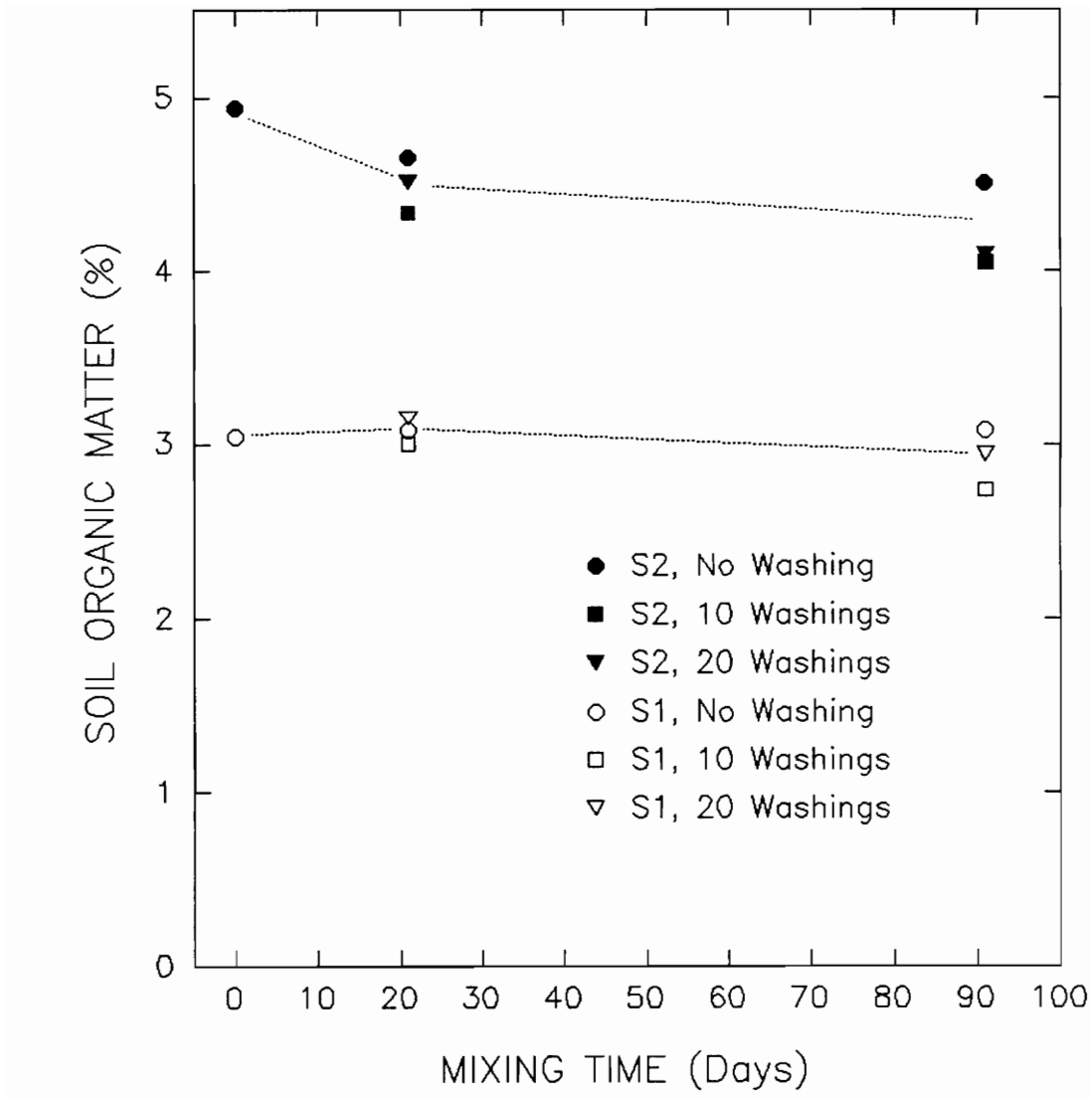


Figure 15. The effect of soil mixing and washing on SOM content.

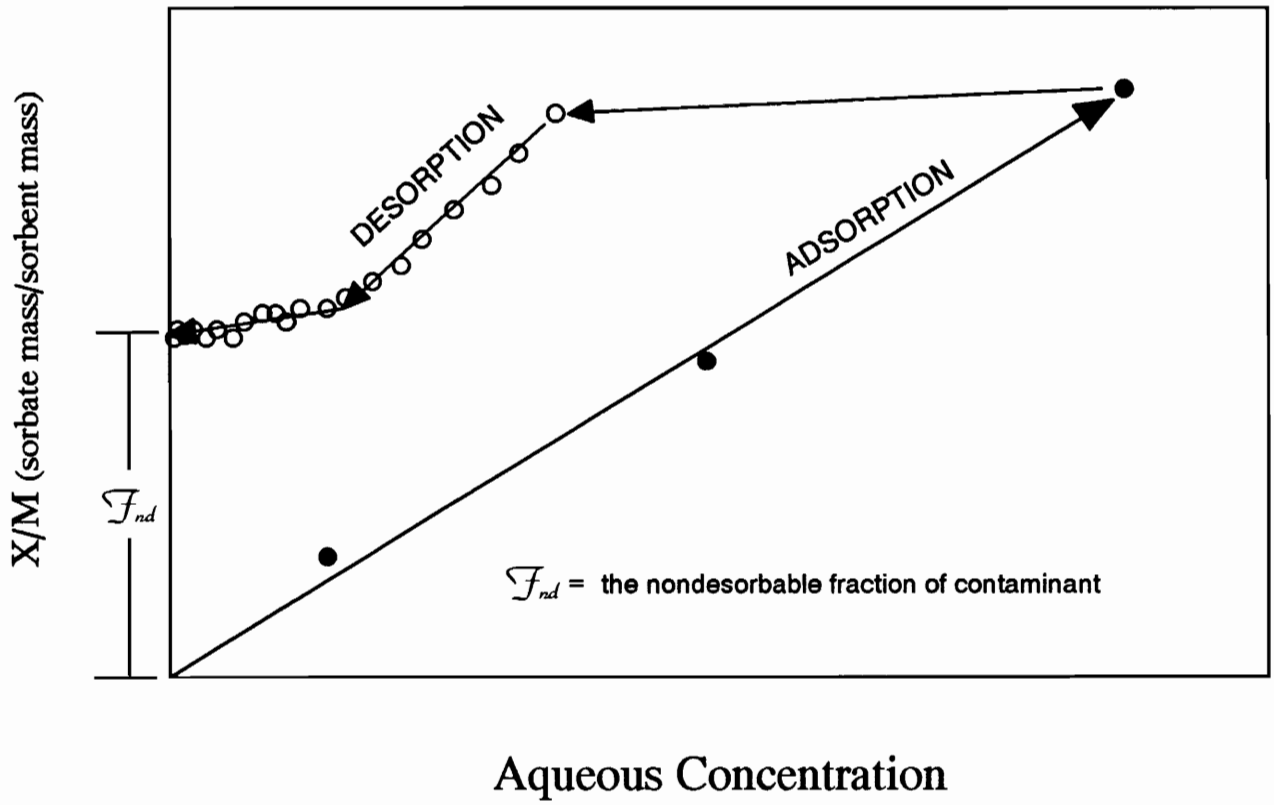


Figure 16. Example of a typical adsorption-desorption isotherm

100% of the bound compound was released from the sorbent.

However, contaminants rarely exhibit such behavior in the environment, and they usually display asymmetrical desorption patterns as shown in Figure 16. This effect, often called hysteresis, indicates that desorption can occur at a much slower rate than adsorption (Sabatini and Austin, 1990). Also, if these isotherms do not meet at the graph's point of origin, the data may suggest that some of the test compounds have been irreversibly bound to the sorbent.

The results from all 48 microcosms are shown for each soil-chemical combination in Appendix A in Figures A14-A21. From these data it appears that desorption occured at a considerably slower rate than adsorption, and that a nondesorbable fraction may have remained bound to the solid phase in spite of continuing the water-washing process. Previous research also showed that PCP and MCP could bind irreversibly to soils, especially at lower concentrations (Banerji *et al.*, 1986; Isaacson and Frink, 1984). Therefore, the sorption mechanisms which were binding these contaminants were apparently strong enough to overcome the concentration gradient established during the desorption experiments.

It is also possible to use these isotherms to compare the relative effects of contact time on the desorption process. By plotting the desorption data for one chemical concentration over all four contact times, the changes in each desorption pattern became readily apparent. Typical graphs of these data are shown in Figures 17 and 18, and a comparison of their relative slopes and y-intercept values indicate that increased contact times will decrease the desorption rates and result in a larger fraction of nondesorbable contaminant. As a result, these data suggest that longer exposure times for these environmental contaminants may make their removal from soils a more difficult process to accomplish.

4.4 Biodegradation

The biodegradation study was conducted using each chemical at one concentration (20 mg/L 4-MCP and 1 mg/L PCP), combined with each soil type, and contacted for 1, 3 and 21 days.

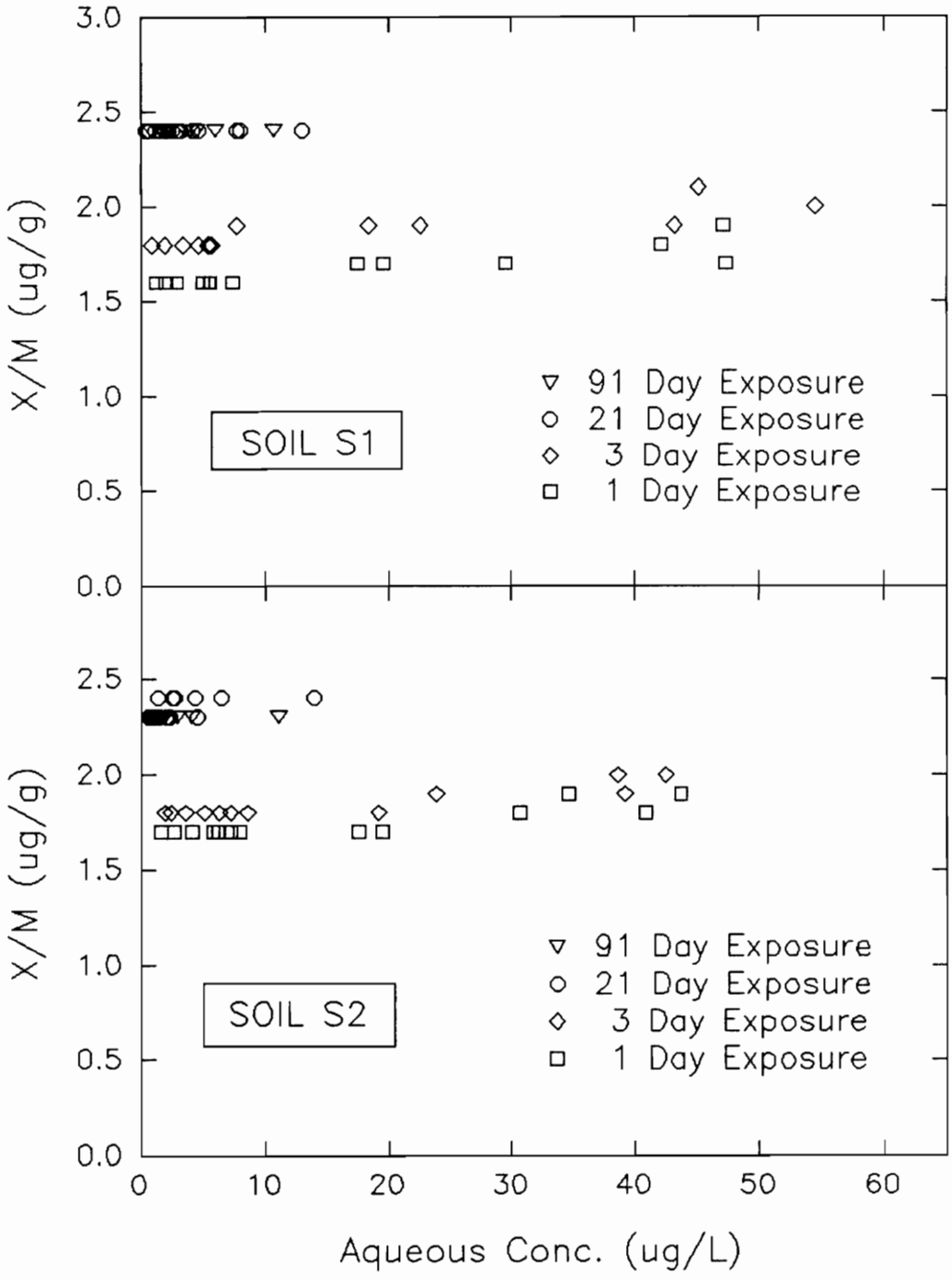


Figure 17. Effect of contact time on the desorption of 1 mg/L 4-MCP.

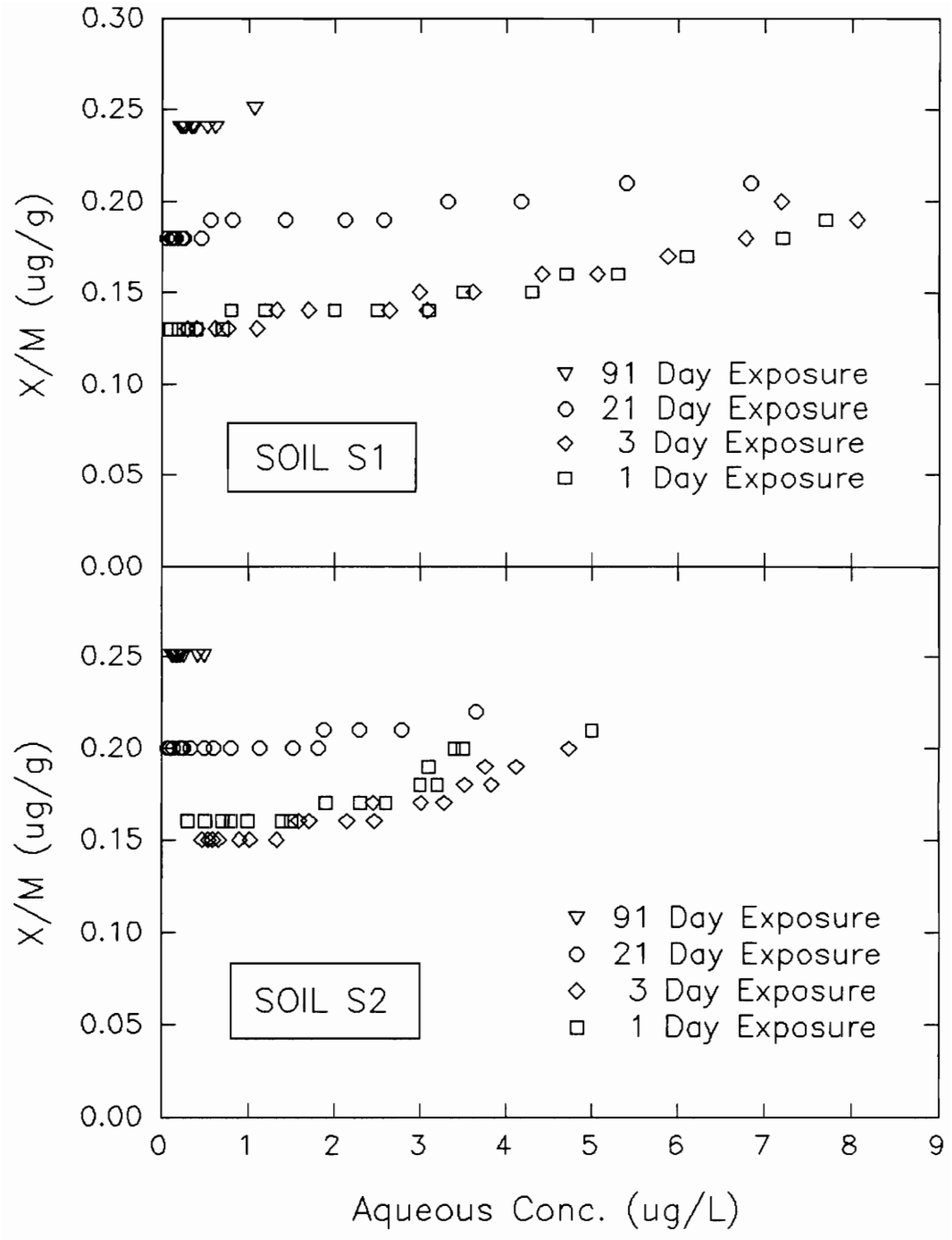


Figure 18. Effect of contact time on the desorption of 0.1 mg/L PCP.

This experimental matrix resulted in a total of 12 different microcosms that were to be sterilized, exposed to the appropriate contaminant, and then inoculated with cultured microbes. These tests were designed to determine whether differences in desorption patterns due to SOM content or contact time would also effect the bioavailability of these compounds.

To help in interpreting the results, a series of control flasks were created following the same autoclaving procedures. Then, without adding the cultured microorganisms, PCP or 4-MCP was added to several of these flasks and CO_2 traps were mounted inside them. The results were measured over the next 10-15 weeks and are shown in Figures 19 and 20, and they suggest that a significant microbial population was able to survive the autoclaving process and then metabolize both of the test compounds. In the four microcosms that were treated with 4-MCP, about 30% of the added radiolabelled compound was mineralized within 1-5 weeks. After a period of nine weeks, the maximum amount of observed mineralization of the 4-MCP was 57% for a flask with soil S1. Additionally, the microbes in soil S1 were able to degrade this chemical at a much faster rate and to a greater extent than the microorganisms in soil S2. Whether this result was due to differences in SOM content, the characteristics of the organic matter, initial pH levels or differences in the natural microbial populations, is not completely clear. Variations in the type and amount of soil-bound organics that could influence the sorption-desorption process, as discussed previously in this chapter, might also effect the bioavailability of these compounds.

Two additional flasks were prepared exactly as all of the others, except that they were not put through the autoclaving procedure. Next, they were dosed with both labelled and unlabelled 4-MCP and the resulting pattern of mineralization is also shown in Figure 19. These microcosms showed much lower levels of biodegradation than the previous four flasks that had been autoclaved. These results would suggest that a large percentage of the original microbial population was able to survive the autoclaving process, possibly due to the large soil mass within these flasks. The release of organic matter and nutrients into solution may have established conditions suitable for cometabolism of the test chemicals. It is also possible that the elimination of competing organisms or changes to the nature of the organic matter allowed higher levels of

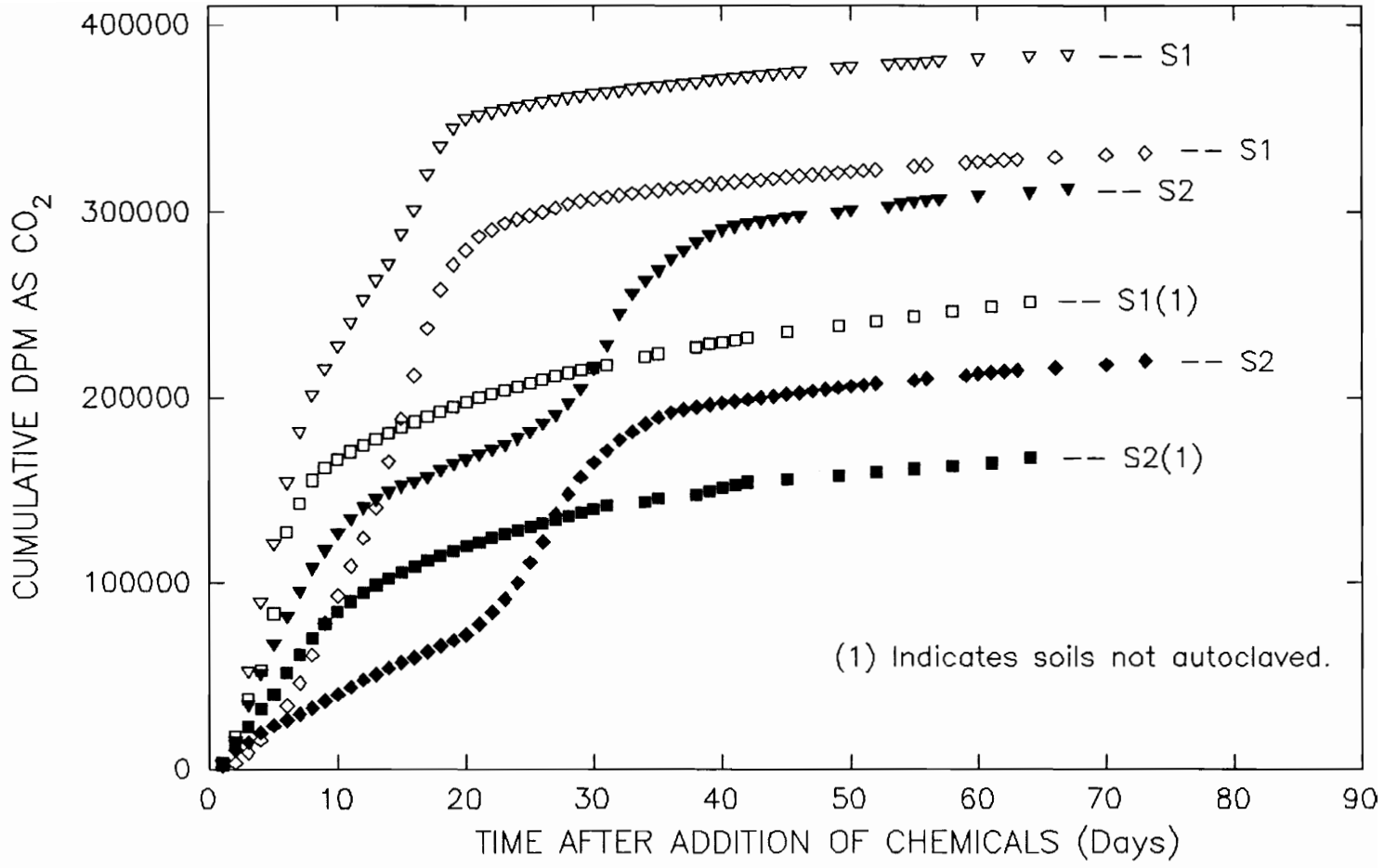


Figure 19. Biodegradation of 4-MCP measured in control flasks.

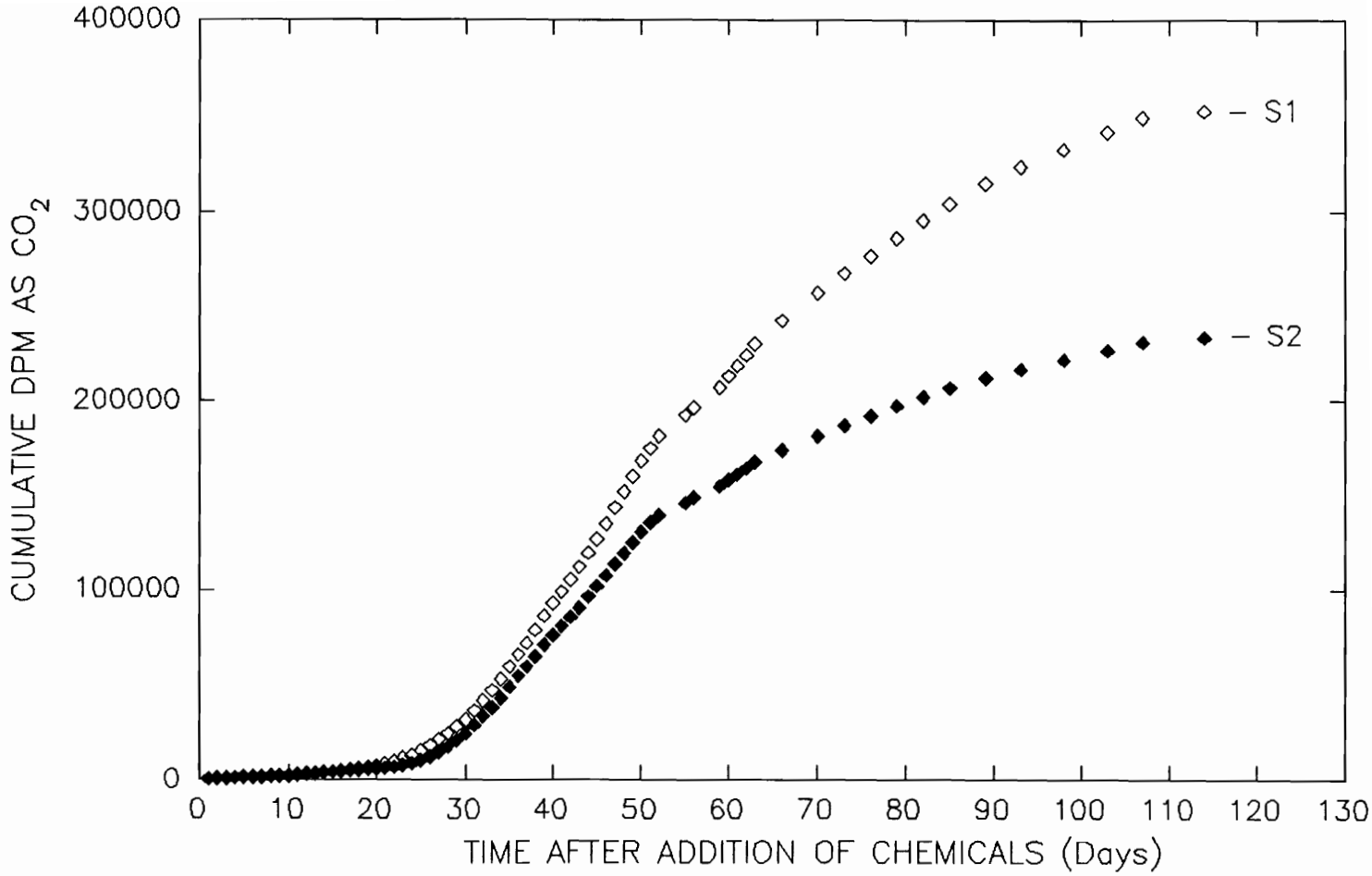


Figure 20. Biodegradation of PCP measured in control flasks.

biodegradation, but the lack of sufficient data on the environmental conditions within the flasks makes it difficult to draw specific conclusions about these systems.

Finally, the two "sterilized" flasks that were tested with PCP showed mineralization occurring from 3-15 weeks after chemical addition, and this pattern of biodegradation is shown in Figure 20. These results indicated that 30-45% of the initial PCP was metabolized into $^{14}CO_2$ during the sampling period. As evidenced with the 4-MCP control flasks, mineralization of PCP in soil S2 occurred at a slower rate than in soil S1. Again, while it is possible that the properties of the organics bound to these two soils influenced these biodegradation patterns, differences in the original microbial populations may make it difficult to isolate the effects of SOM content on the bioavailability of these compounds.

The first sets of biodegradation data are shown in Figures 21 and 22, indicating the amount of trapped $^{14}CO_2$ and the liquid- and solid-phase 4-MCP that was measured. Both of these microcosms were mixed for 21 days prior to beginning sampling, and both soil types were used. The final data points on these and all subsequent biodegradation graphs were taken after the microcosms were sacrificed by adjusting the pH to less than 2. While most of the degradation probably occurred prior to the start of sampling, 4-MCP utilization was continuing at a relatively slow rate. These data also provided the best evidence that the bioavailability of contaminants in soils may be related to the desorption process.

The rates of 4-MCP removal from the solid phase, the mineralization rate, and relative desorption rates during the final stage of each process were calculated from the appropriate data sets and are presented in Table 9. The trend shown by these data is that soil S1 releases 4-MCP at a slower rate than soil S2 during the latter stages of the desorption process, and that this compound is also biodegraded at a correspondingly lower rate. While previous research has also suggested that the microbial degradation of soil contaminants was related to the rate of desorption (Robinson *et al.*, 1986), these were the only two microcosms which clearly displayed this behavior, while the other microcosms provided data which was more difficult to interpret.

Many studies have documented the biodegradation of organic compounds in soil systems,

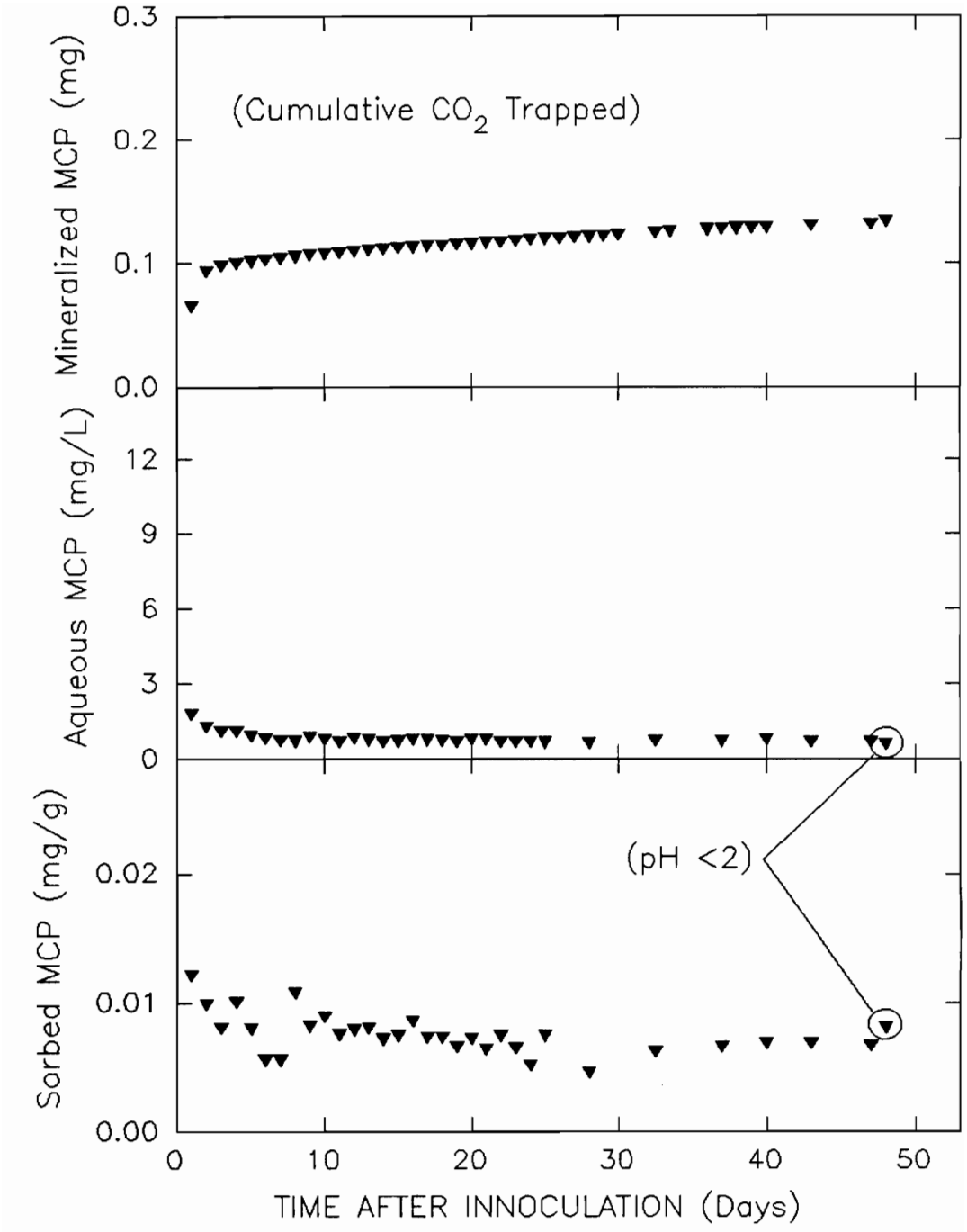


Figure 21. Biodegradation pattern for 4-MCP contacted with soil S1 for 21 days.

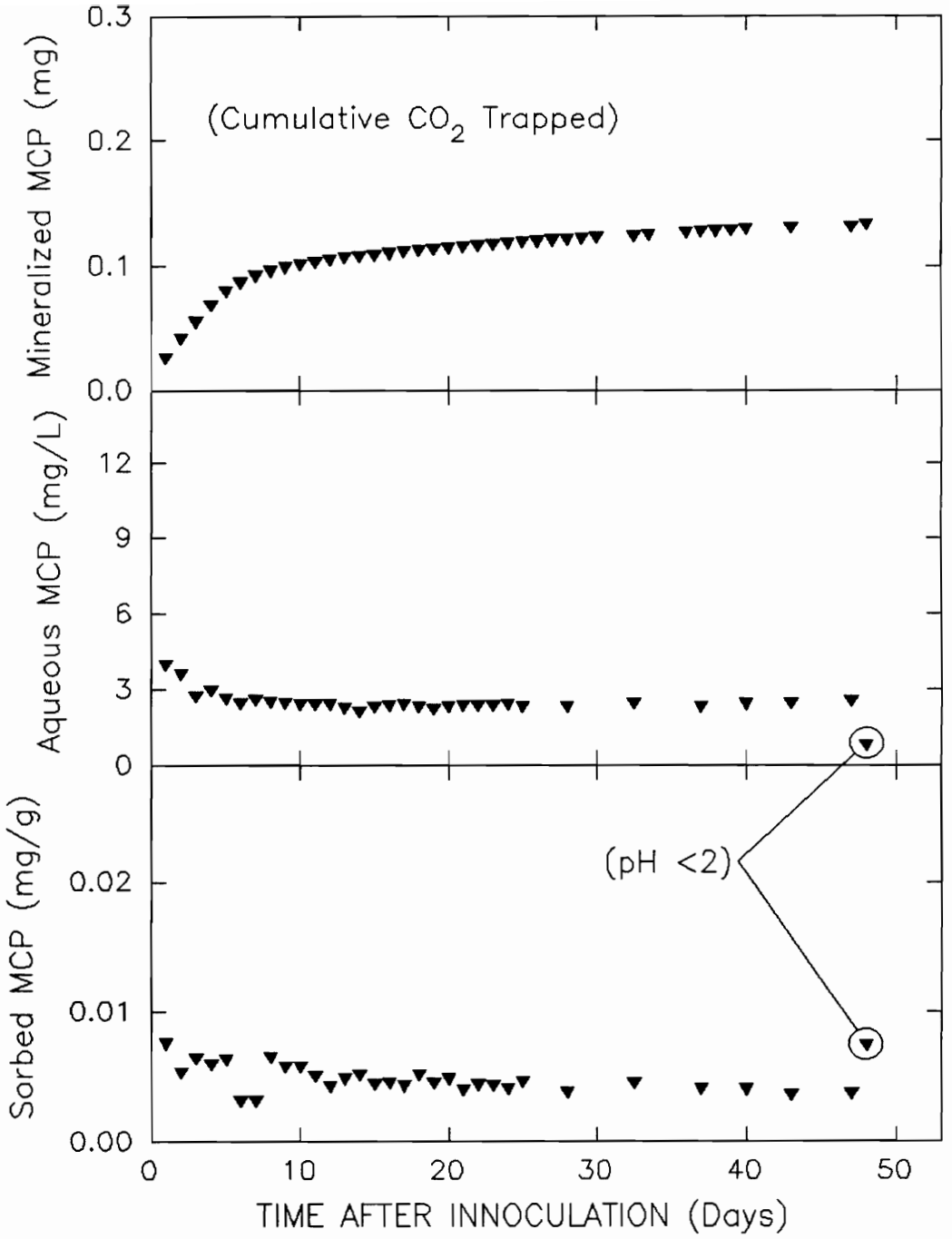


Figure 22. Biodegradation pattern for 4-MCP contacted with soil S2 for 21 days.

Table 9. Relationship of desorption to the bioavailability of 4-MCP in 21 day microcosms.

PROCESS	UNITS	SOIL S1	SOIL S2
Desorption Rate	µg/L/wash	19.8	26.1
Mineralization Rate	µg/day	0.604	0.808
Degradation of Sorbed Compound	µg/g/day	0.034	0.057

but they are often unable to identify whether the chemicals were metabolized in the free or sorbed states (Smith and Novak, 1986). Ogram et al. (1985) found that microorganisms primarily degrade pesticides in their dissolved state in solution, while the results of Remberger et al. (1986) suggest that even strongly sorbed compounds could still be biologically altered or degraded to some degree. Figure 23 shows a high correlation between the loss of aqueous 4-MCP and the collection of $^{14}CO_2$. Since the level of sorbed compound changed very little, this data set indicates that biodegradation was primarily occurring in the liquid phase.

The data presented in Figure 24 seems to support the above observation over the first three weeks of sampling, but the sorbed concentration of PCP eventually began to decrease at a rate which paralleled the loss of liquid-phase PCP. It is possible that biodegradation reduced the aqueous concentration to a low enough level to promote desorption from the solid phase, but it is also possible that the microorganisms began to metabolize sorbed PCP when the aqueous supply of this compound became too low for efficient utilization. Microbes located on or near the sorption surfaces, which can metabolize the substrate, can also increase the gradient between the solid- and liquid-phase concentrations and thus increase the desorption rate (van Loosedrecht *et al.*, 1990). However, the fact that the sorbed concentration was reduced by over 50% during this test could be interpreted as microbial activity releasing and then degrading PCP from the solid-phase, since the desorption experiments could remove only about 35% of the bound PCP. Figures 25 and 26 show this effect more clearly, with rapid losses of approximately 80% of both the sorbed 4-MCP and PCP.

Clair *et al.* (1989) identified microbial activity as having an important role in the modification of DOM. They showed that microorganisms were able to increase the levels of pH and dissolved organic carbon in batch microcosms due to their interaction with the humic compounds. Martin *et al.* (1978) found that sorption to humic substances could decrease the bioavailability of certain compounds, and Larsson *et al.* (1988) showed that PCP was degraded to a greater extent in clear lake waters than in humic waters. These studies indicate that DOM can

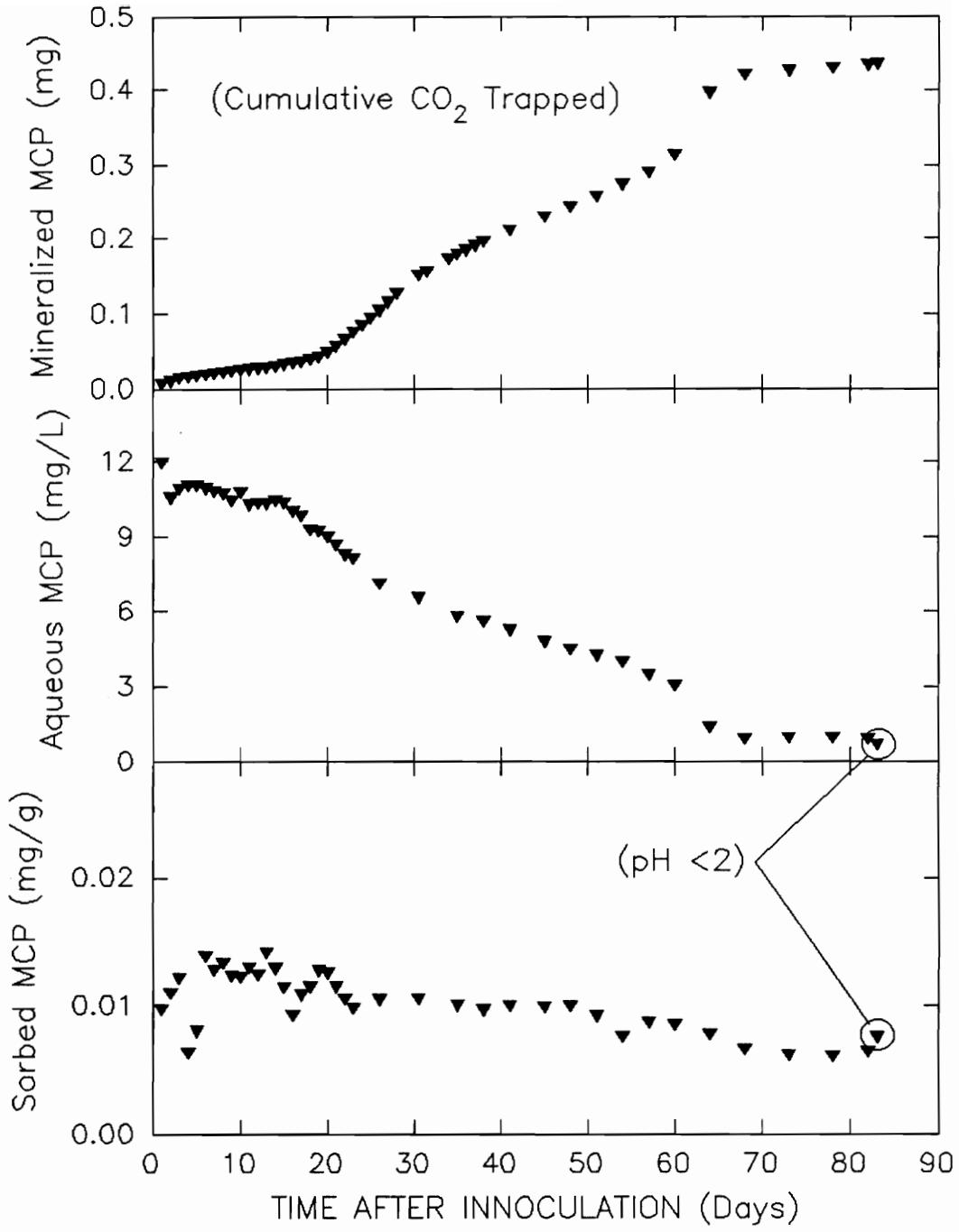


Figure 23. Biodegradation pattern for 4-MCP contacted with soil S1 for 3 days.

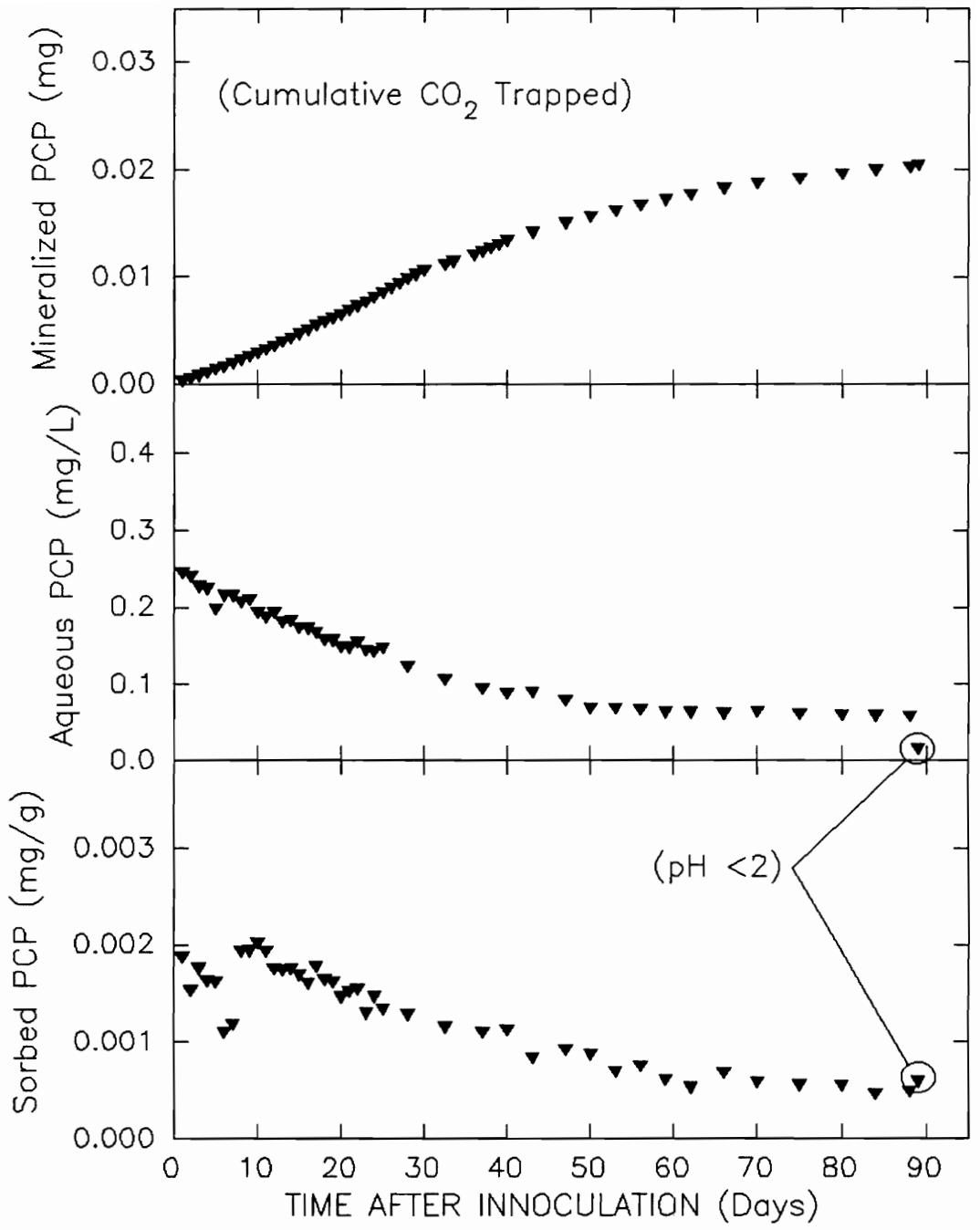


Figure 24. Biodegradation pattern for PCP contacted with soil S1 for 21 days.

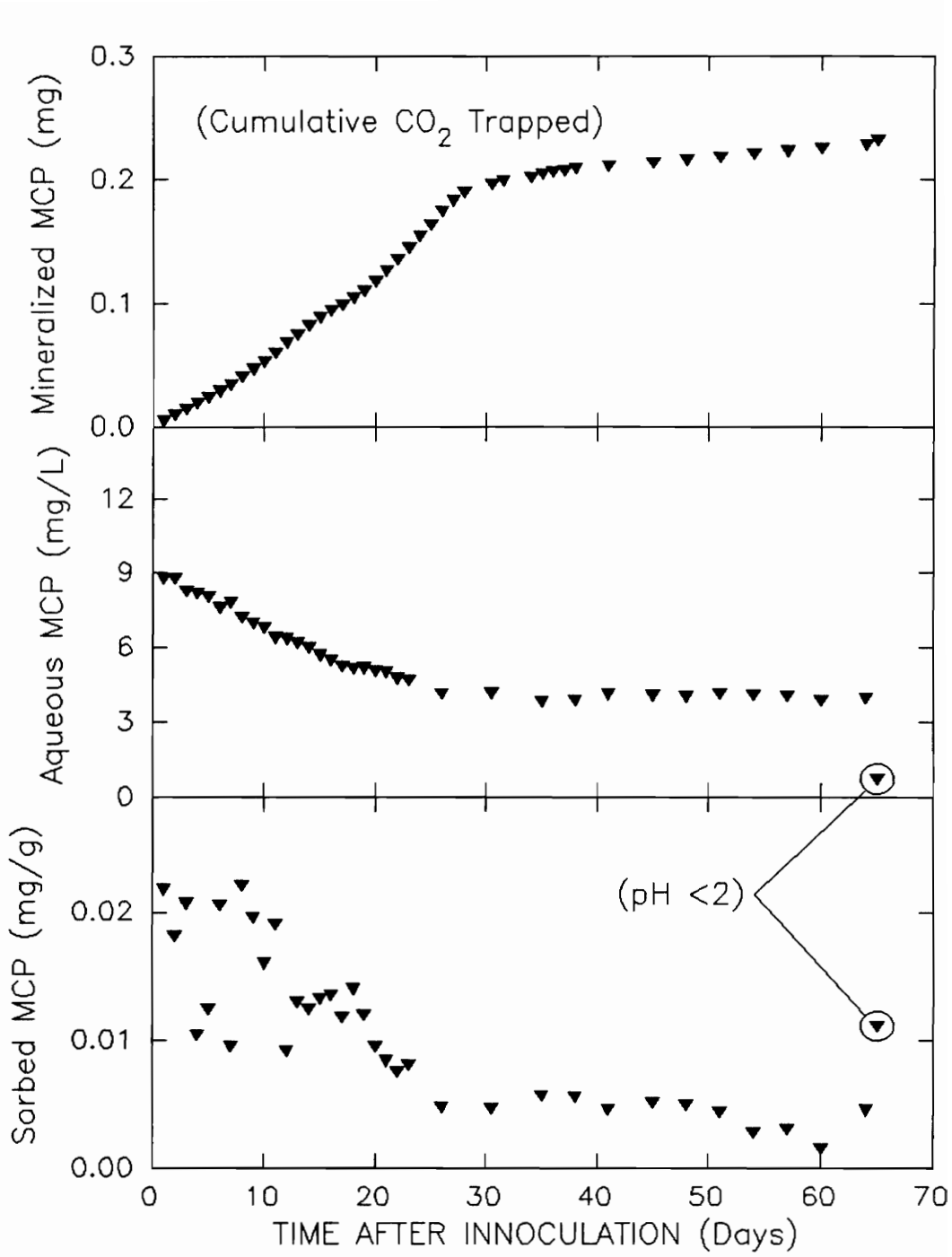


Figure 25. Biodegradation pattern for 4-MCP contacted with soil S2 for 3 days.

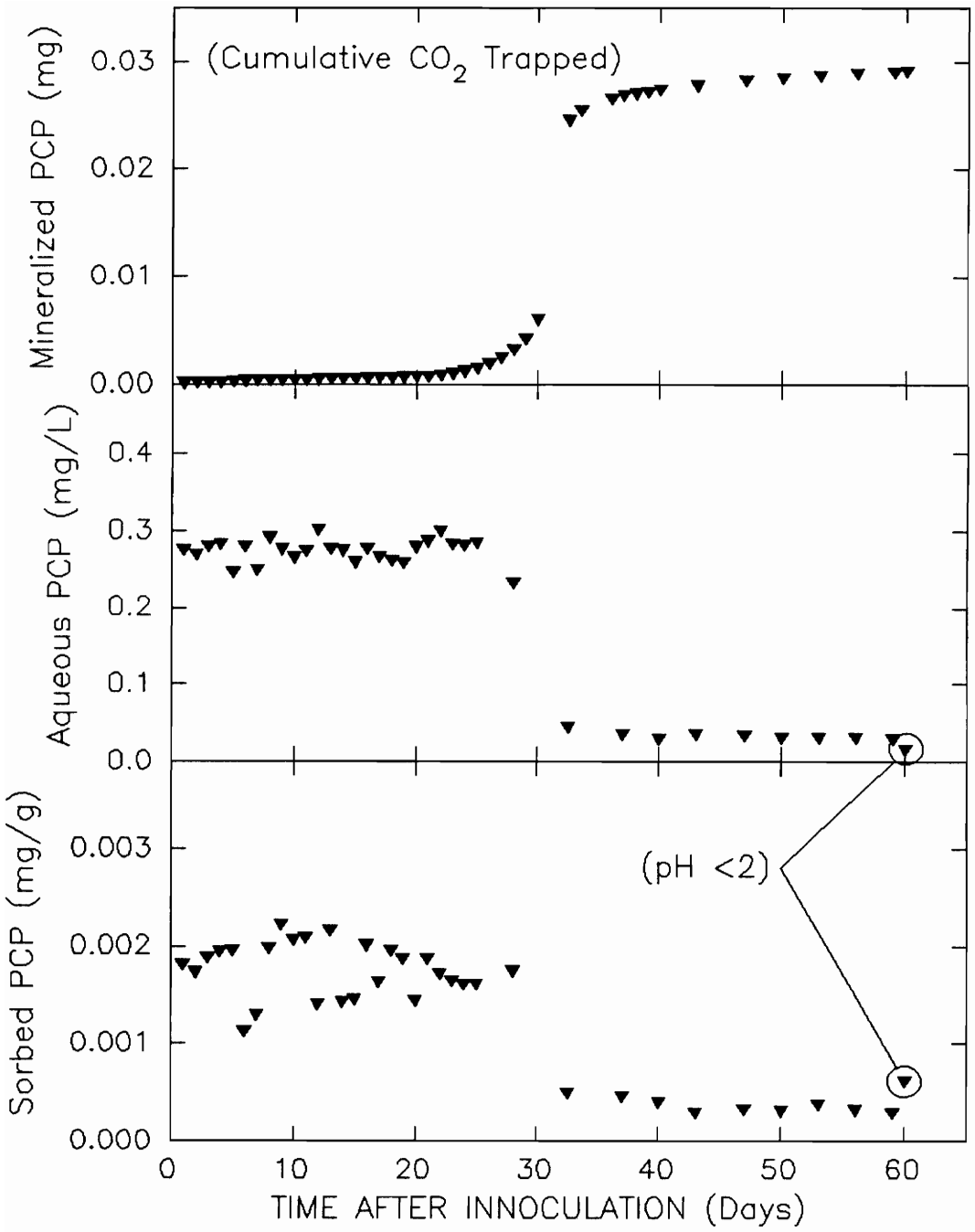


Figure 26. Biodegradation pattern for PCP contacted with soil S1 for 1 day.

protect hydophobic compounds in solution from biodegradation when they partition into this phase. Wershaw (1986) found that higher pH values would disrupt humic "micelles" and release the humates into solution, while a low pH would enhance their aggregation and precipitation. As a result of sacrificing the microcosms by acidification, some higher MW humic compounds appeared to precipitate while other lower MW humates were probably left in solution. In addition to releasing any aqueous $^{14}CO_2$, it was possible to observe the amount of binding which had occurred to dissolved organics which precipitated as a result of acidification.

While Figures 27-30 show relatively low levels of biodegradation in these microcosms, the data taken after they were sacrificed indicates that a large percentage of the aqueous PCP was bound to the humic acids in both soils S1 and S2. Conversely, Figures 31 and 32 suggest that 4-MCP did not partition as strongly into the dissolved humics, which was expected since this compound is much more soluble and has a lower K_{OW} than PCP. In addition, Dec and Bollag (1988) researched the biodegradation of chlorophenols that were enzymatically coupled to synthetic humic acids and found decreasing mineralization with increasing chlorination of the phenols. Whether this significant level of PCP binding to humic acids is due to hydrophobic partitioning mechanisms or oxidative coupling, the results suggest that the biodegradation of PCP can be inhibited in the presence of DOM. While this effect may reduce its toxicity to soil microbes, binding to DOM may also increase the effective solubility of PCP and enhance its transport within groundwater systems.

Finally, mass balances were calculated for each set of samples taken during the biodegradation study. The results for flasks that were contacted for 1 and 3 days are shown in Figures 33 and 34, respectively. While they appear to be fairly low and erratic, these findings are actually reasonable considering the small sample sizes and the potential shielding effects from the soil samples. However, Figure 35 shows a problem with the 21 day samples, in that the 4-MCP flasks experienced large losses of labelled compounds. The biodegradation data from these flasks indicated that approximately 40-50% of the initial 4-MCP was mineralized prior to beginning the sampling period, and the radiolabelled chemicals were probably lost as carbon dioxide when the

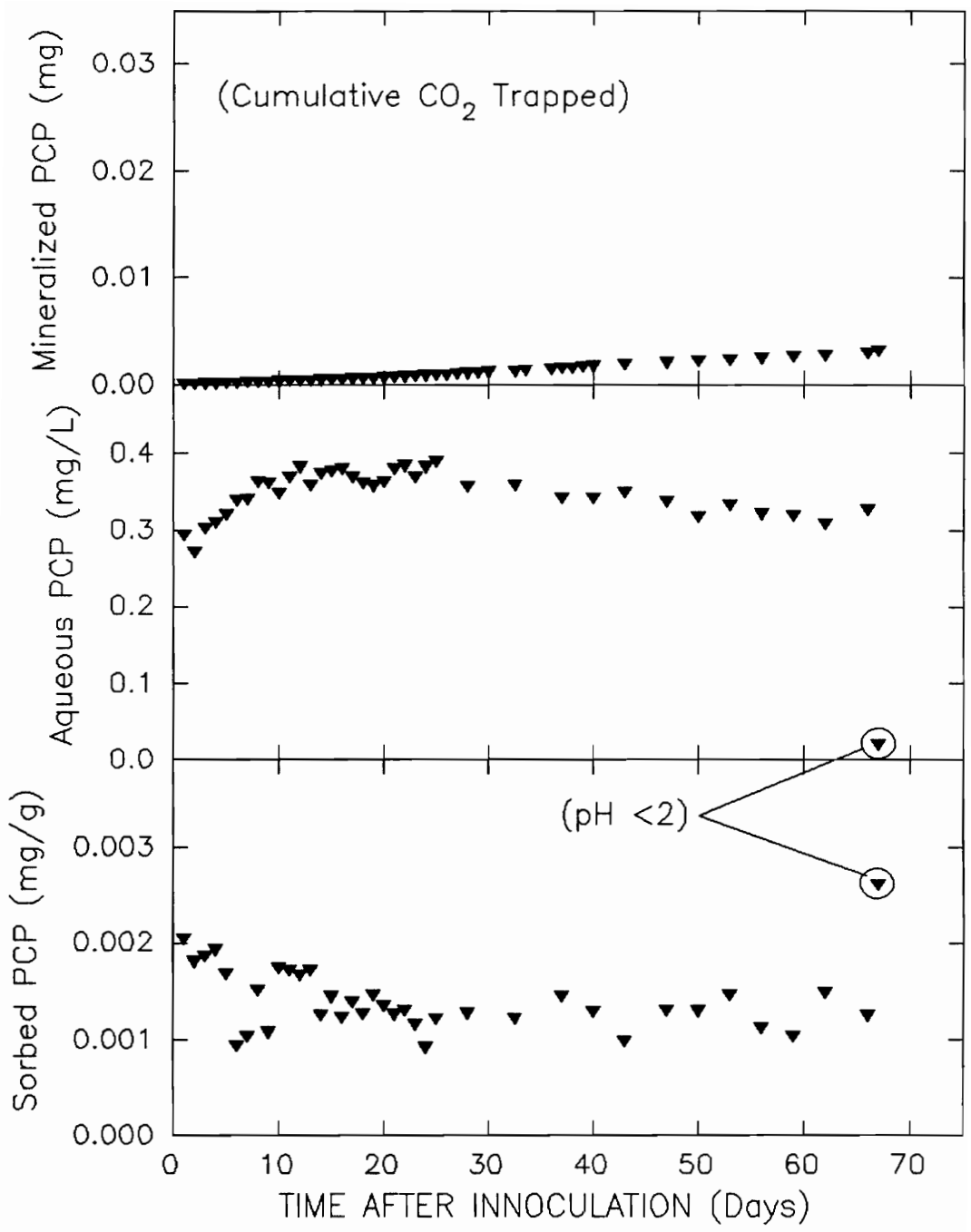


Figure 27. Biodegradation pattern for PCP contacted with soil S2 for 1 day.

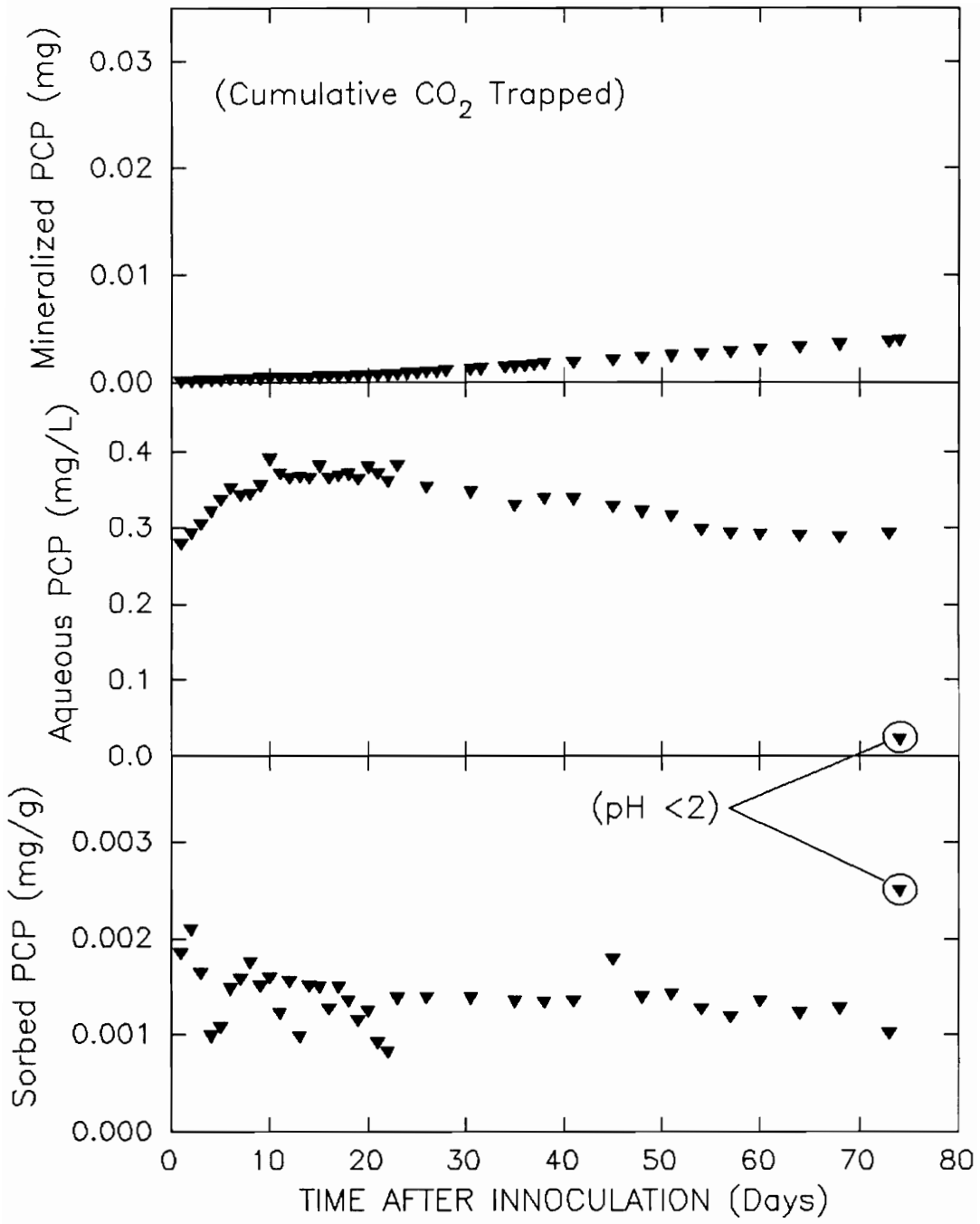


Figure 28. Biodegradation pattern for PCP contacted with soil S2 for 3 days.

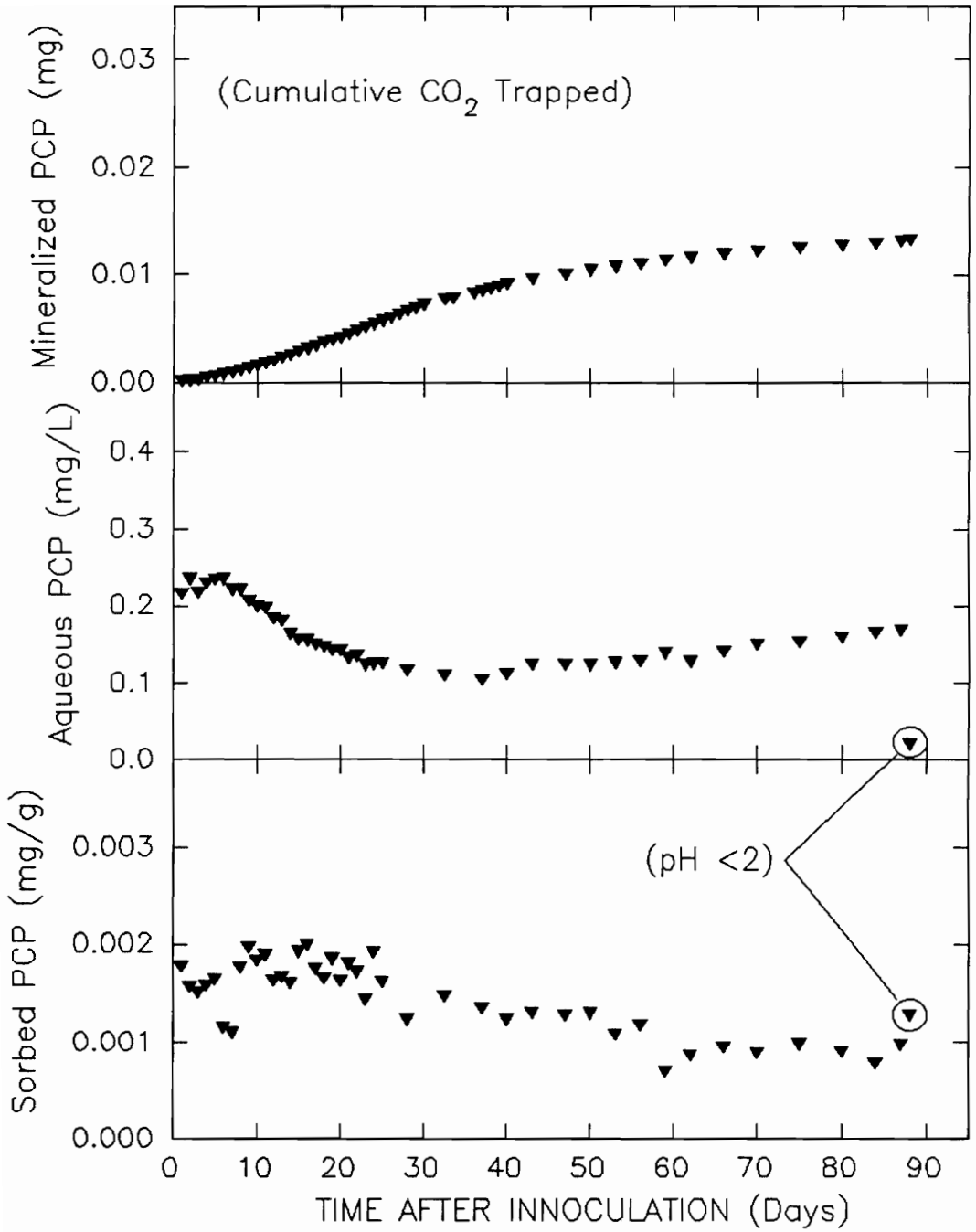


Figure 29. Biodegradation pattern for PCP contacted with soil S2 for 21 days.

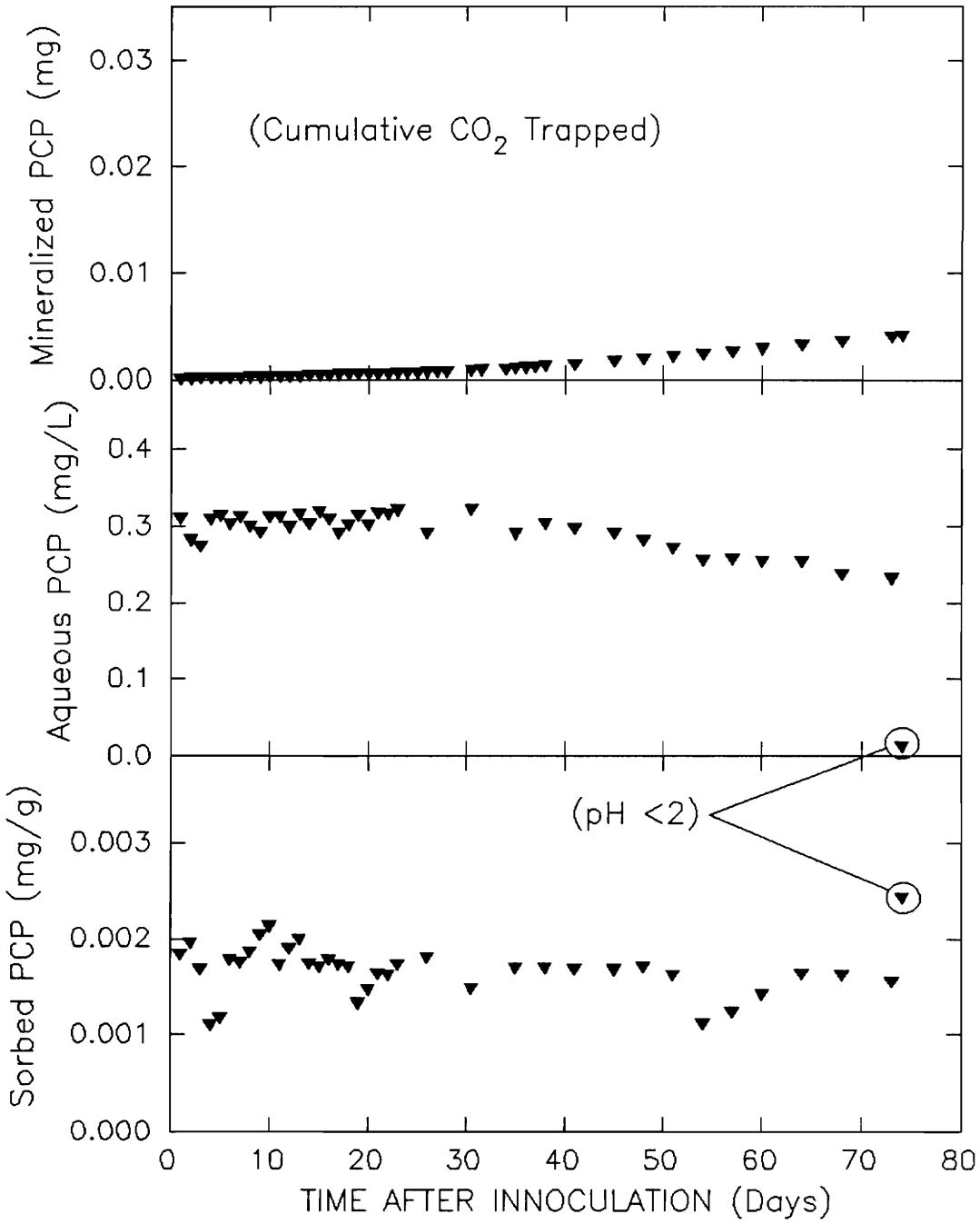


Figure 30. Biodegradation pattern for PCP contacted with soil S1 for 3 days.

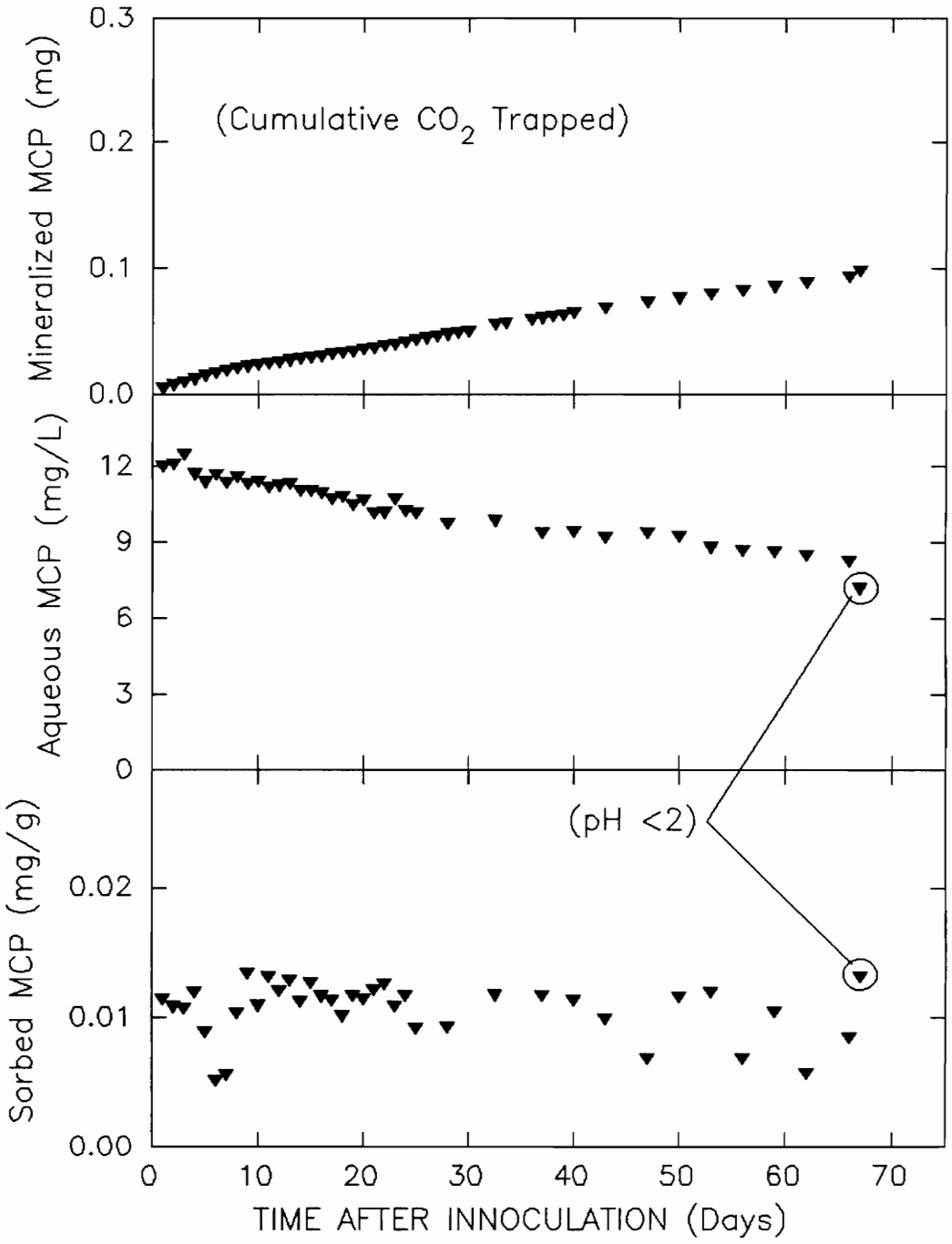


Figure 31. Biodegradation pattern for 4-MCP contacted with soil S1 for 1 day.

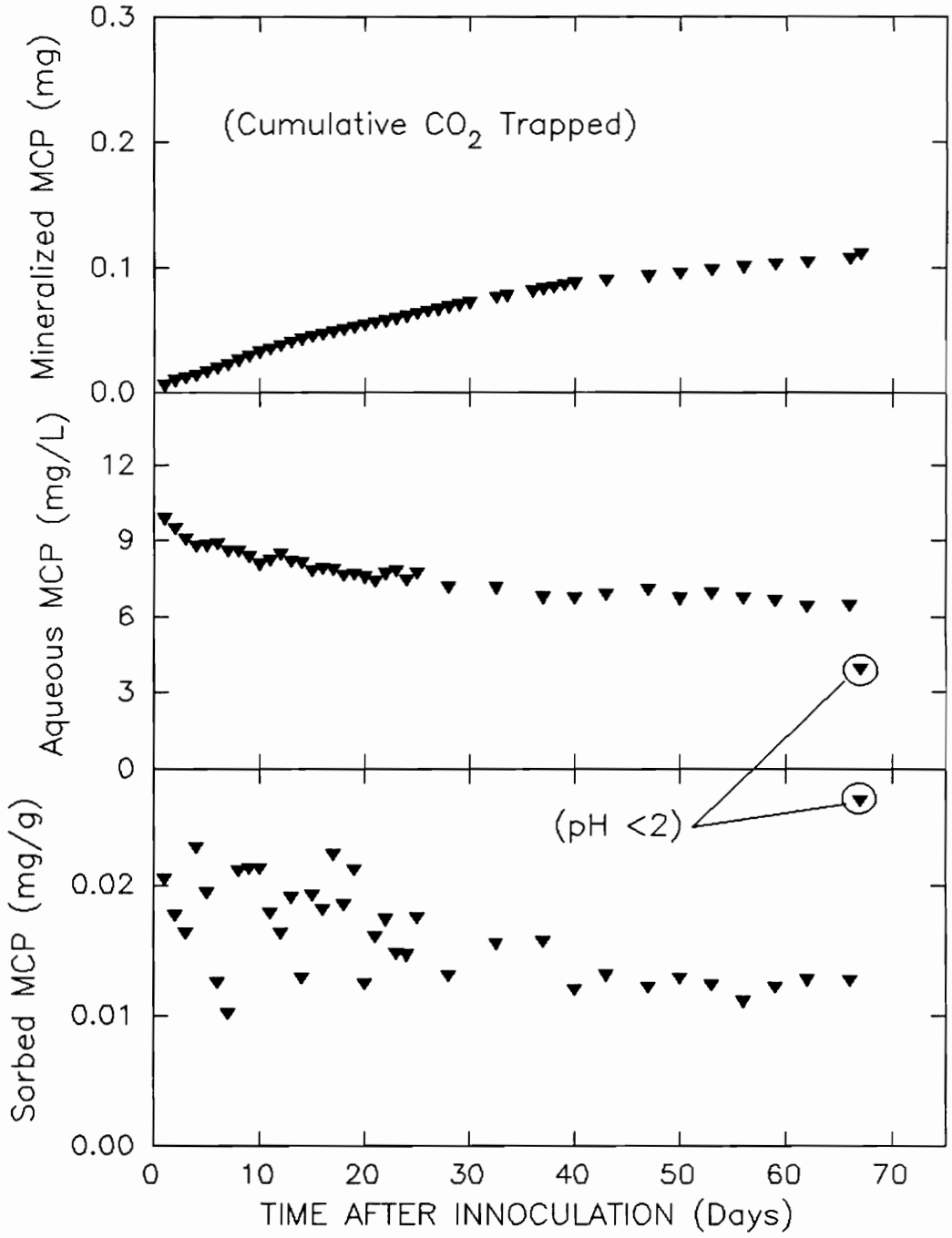


Figure 32. Biodegradation pattern for 4-MCP contacted with soil S2 for 1 day.

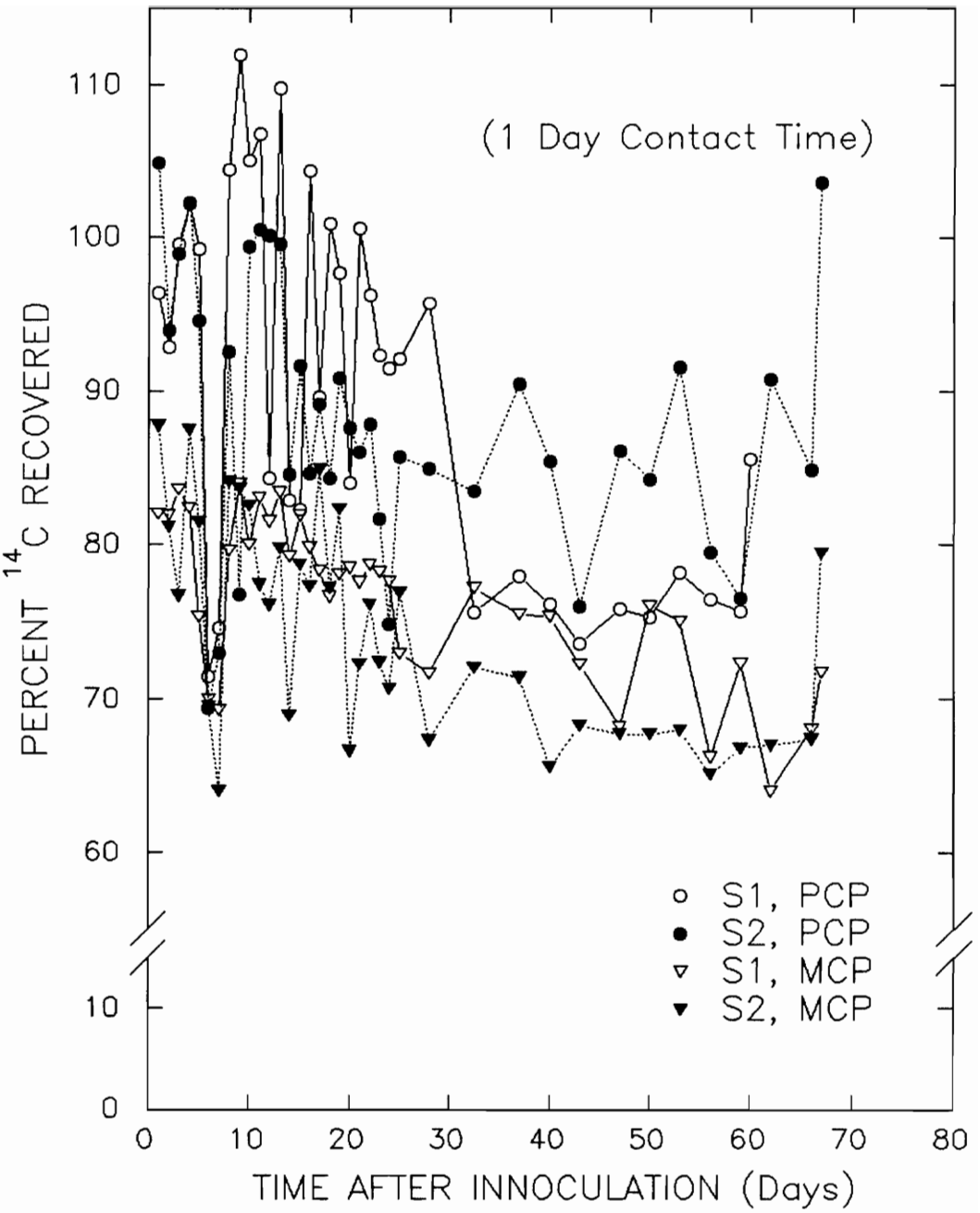


Figure 33. Mass balances for the 1 day biodegradation flasks.

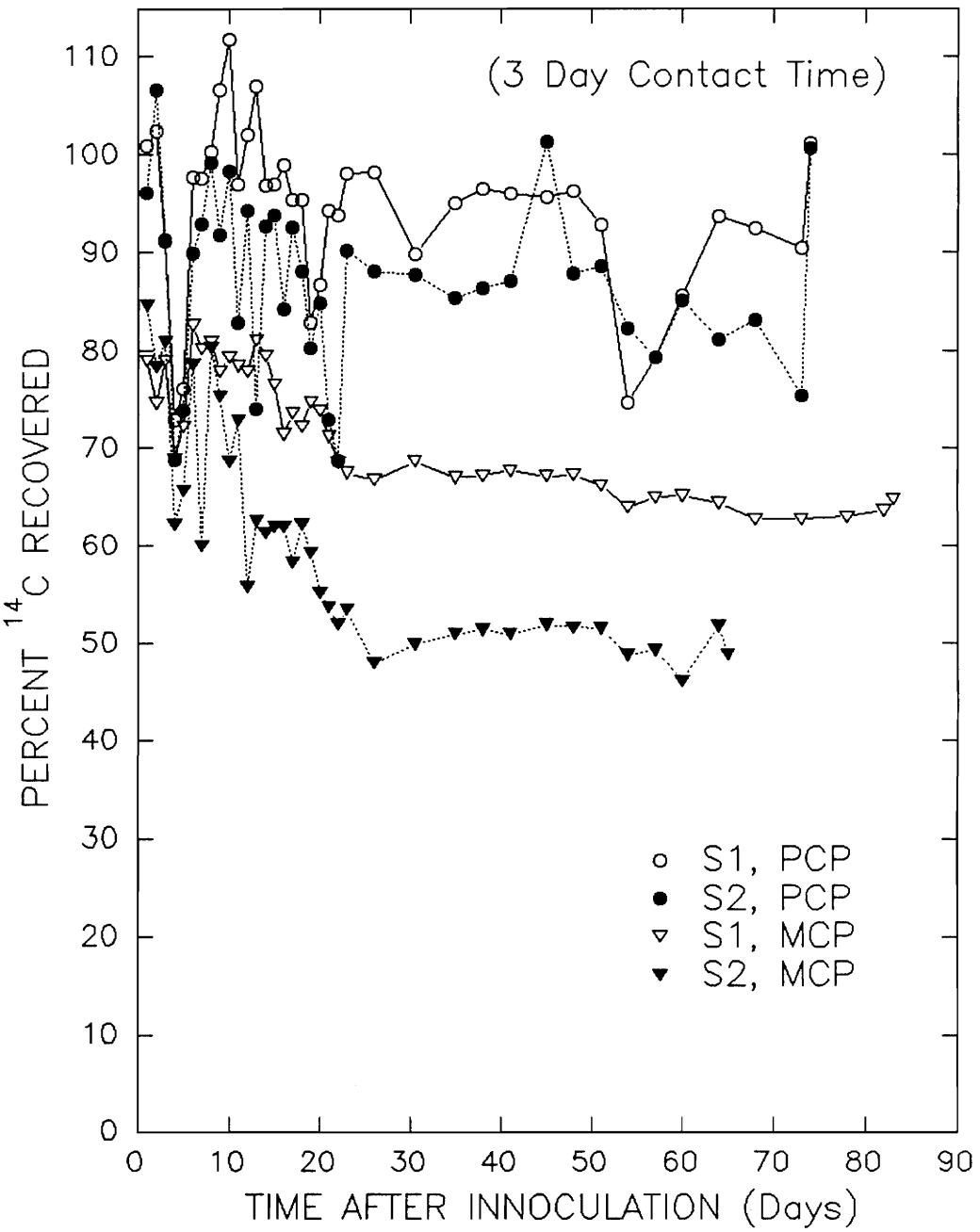


Figure 34. Mass balances for the 3 day biodegradation flasks.

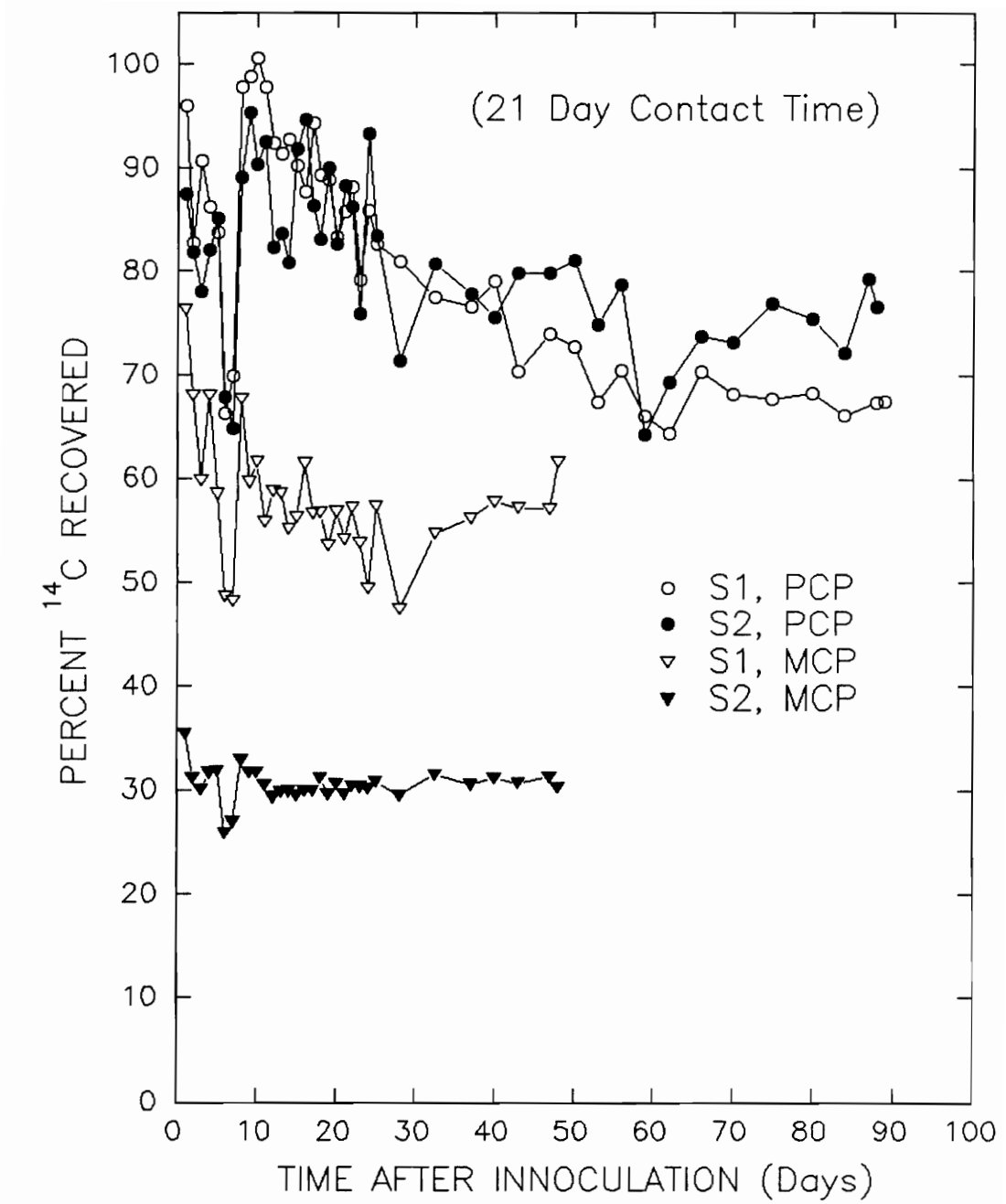


Figure 35. Mass balances for the 21 day biodegradation flasks.

flasks were opened to add the KOH traps. Since biodegradation was observed in the "sterilized" control flasks, some of the binding which appeared to be irreversible within the 91 day sorption flasks may have been due to mineralization of 30-60% of both test compounds, and covalent coupling of the remaining chemicals to soil-bound and dissolved organics in those microcosms.

4.5 Ultrafiltration

The liquid-phase measurements of the chlorophenols taken during the sorption study quantified the total amount of ^{14}C remaining in solution, but could not differentiate between compounds that were actually free in the liquid or bound to DOM. The ultrafiltration experiment was designed to separate dissolved chlorophenols from those associated with or bound to DOM. Also, this fractionation analysis could provide information about the molecular weights of the dissolved organics that were binding the test chemicals. The sampling method consisted of measuring both the ^{14}C and the TOC levels in each size fraction for all four contact times. The results are shown in Figures 36 and 37, for PCP and 4-MCP exposed to each soil types.

Both Figures 36 and 37 indicate that >50% of the chemicals were free in solution over the first 21 days of contact. However, there appeared to be a significant level of additional binding occurring to the 500-10,000 MW size fraction during the period from 21 to 91 days. It is also apparent that the majority of the TOC was in this range, too. This binding was probably caused by a combination of physical partitioning of the compounds into humic macrocolecules and microbial activity causing oxidative coupling of the phenolic compounds to the DOM, as discussed previously.

Martin and Haider (1971) proposed that microbial activity could either synthesize phenolic compounds or produce them from the degradation of organic matter. These basic components would then be oxidatively coupled from reactions with natural enzymes, producing large humic polymers that were relatively stable in the environment. In later research, Martin and Haider

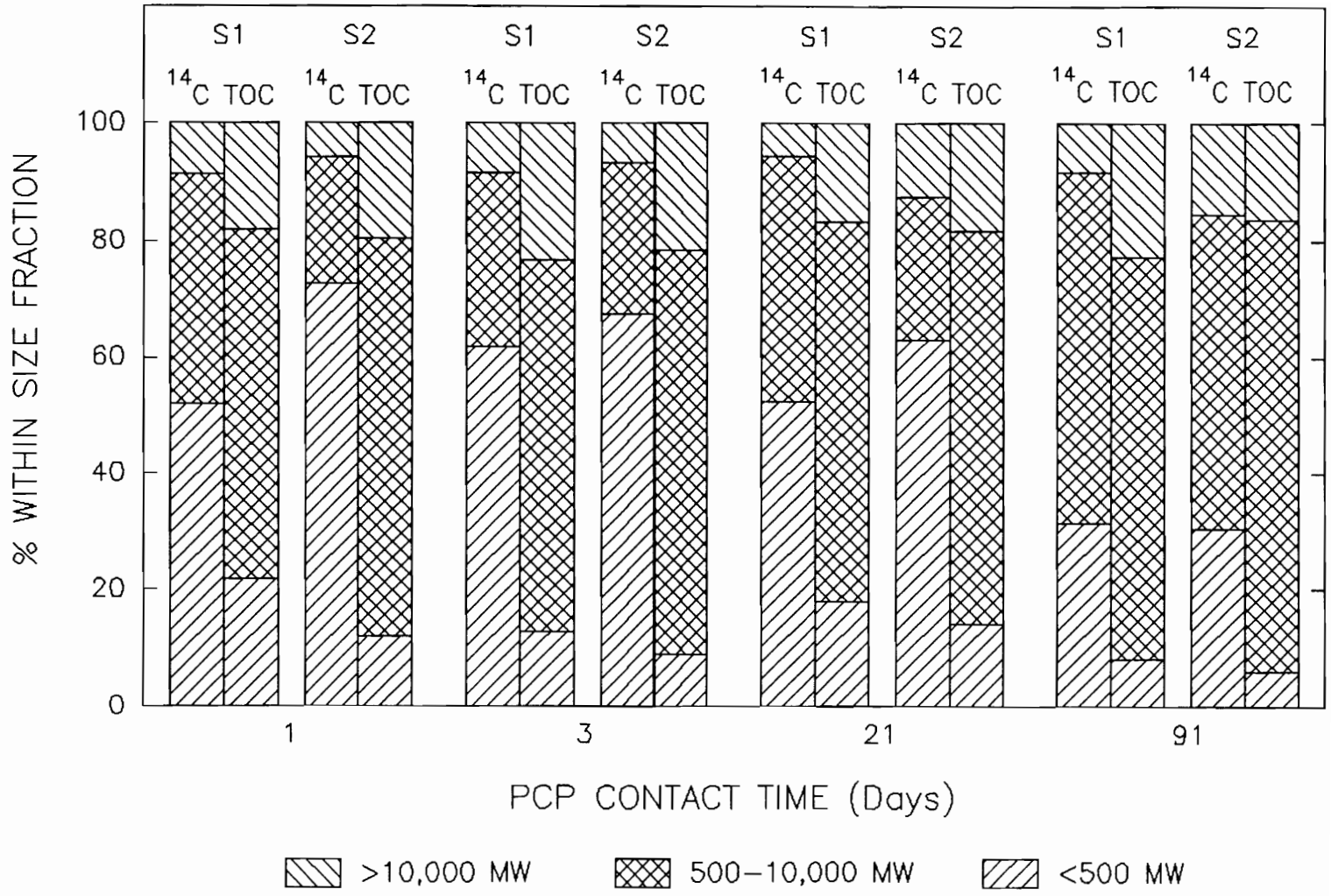


Figure 36. Ultrafiltration of the liquid phase in microcosms exposed to PCP.

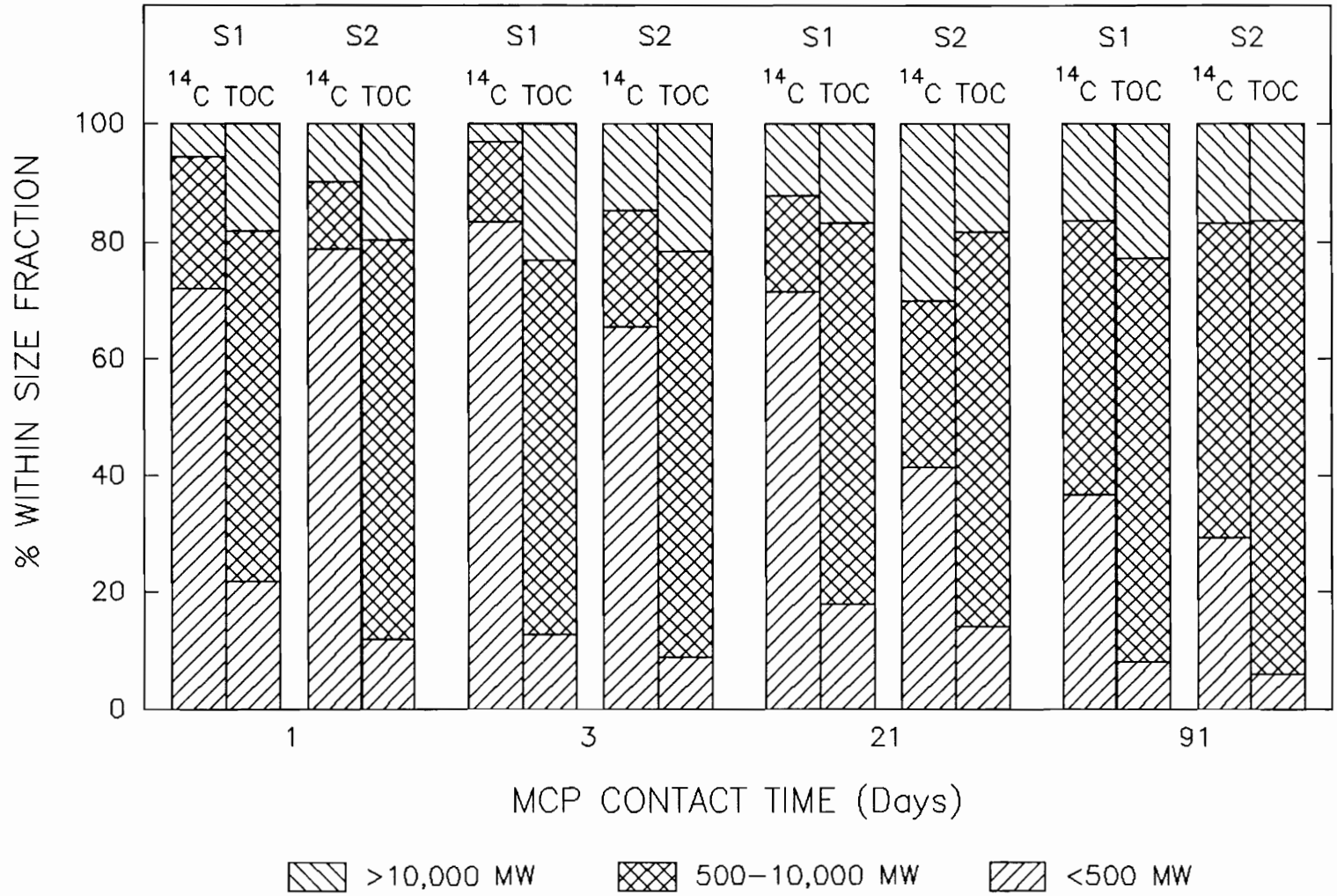


Figure 37. Ultrafiltration of the liquid phase in microcosms exposed to 4-MCP.

(1980) reported the use of oxidative enzymes to duplicate the creation of humic compounds, thus verifying the process of enzymatic polymerization. Stevenson (1982) reported that in addition to phenols, other compounds such as proteins and aromatic amines could be incorporated into these macromolecules during their synthesis. The large increase in DOM molecular weight from the 21 to the 91 day sample could also be indicative of polymerization of the humic compounds, and the increase in the amount of ^{14}C bound to this size fraction would suggest coincidental coupling of the chlorophenols to the humates during this process.

While Figures 36 and 37 show the relative amounts of DOM within each microcosm, it is also important to know the actual levels. DOM, measured as mg/L of TOC in solution, was tested for each soil type at all four contact times. The results from these samples were shown previously in Figure 9, and indicated that soil S2 released over twice the amount of DOM into the flasks than soil S1. There was also a small rise in these levels over time, but it appears that the levels of dissolved organics was similar in all the flasks used for a specific soil type.

Chapter 5

Conclusions

The purpose of this study was to investigate the effects of SOM content, aqueous concentration and contact time on the sorption, desorption and bioavailability of PCP and 4-MCP under aerobic conditions. While it is highly likely that microbial activity caused some interference during these tests, it also made them more realistic and representative of what actually would occur in the subsurface environment. As a result, it was possible to observe how microbial activity could influence the patterns of sorption and desorption. The following conclusions were suggested by the results of this study:

1. In comparing the two silty loam soils used in this study, one from a field (2.7% SOM) and one from a wooded area (4.4% SOM), SOM content did not affect the overall levels of PCP and 4-MCP sorption at the chemical concentrations tested. These results were probably due to differences in the characteristics and properties of the soil-bound organics, PCP ionization, microbial metabolism and covalent coupling of the test compounds, and partitioning of the chemicals into dissolved organics and colloidal matter.

2. In contrast to the adsorption study, SOM content played a significant role in the desorption process with the higher f_{OM} soil releasing significantly lower amounts of contaminants, but over a longer time period.

3. For PCP, the desorption rate from soil S1 was approximately 50-70% higher than from soil S2 during the first five washings, but averaged 23% lower during the last five washings. At the highest concentration of 4-MCP, the desorption rate from soil S1 was about 18% greater than from S2 during the first five washings, and averaged 24% lower during the last five washings.

4. For the given contact time, the effects of initial aqueous concentration were proportionally similar to the patterns of both sorption and desorption for PCP and 4-MCP within the range of concentrations tested.

5. Increased contact times had a significant effect on the desorption kinetics, generally resulting in 40-50% lower levels of total contaminant release between the 3 and 21 day contact times.

6. A large fraction of these chlorophenols were bound very strongly to both the soil-bound and dissolved humic compounds, such that simple water-washing methods were not able to extract them.

7. Some of the data suggested that the bioavailabilty of 4-MCP was related to the desorption process for both soil types.

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Appendix A

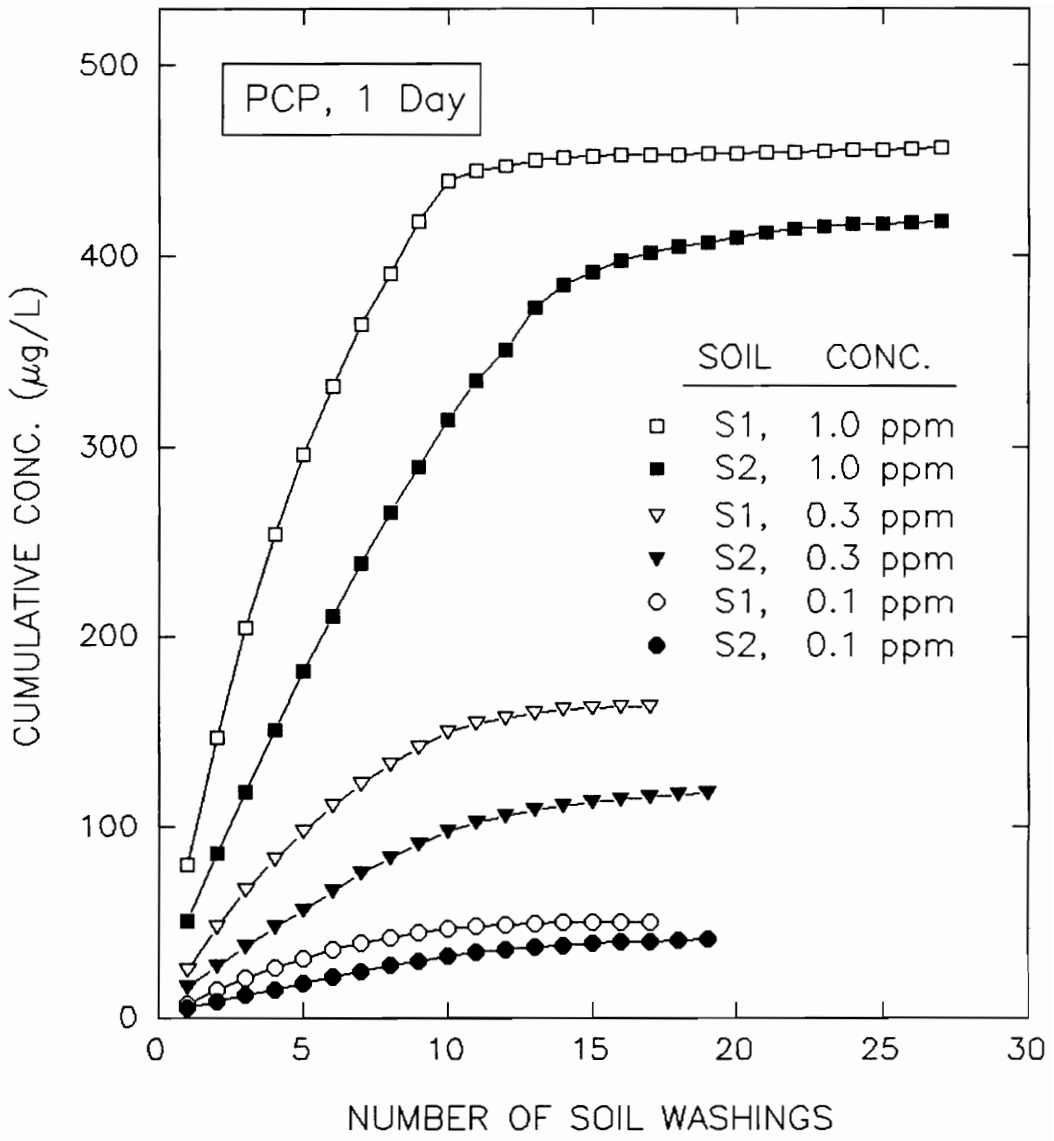


Figure A1. Desorption profiles for two soils contacted with PCP for 1 day.

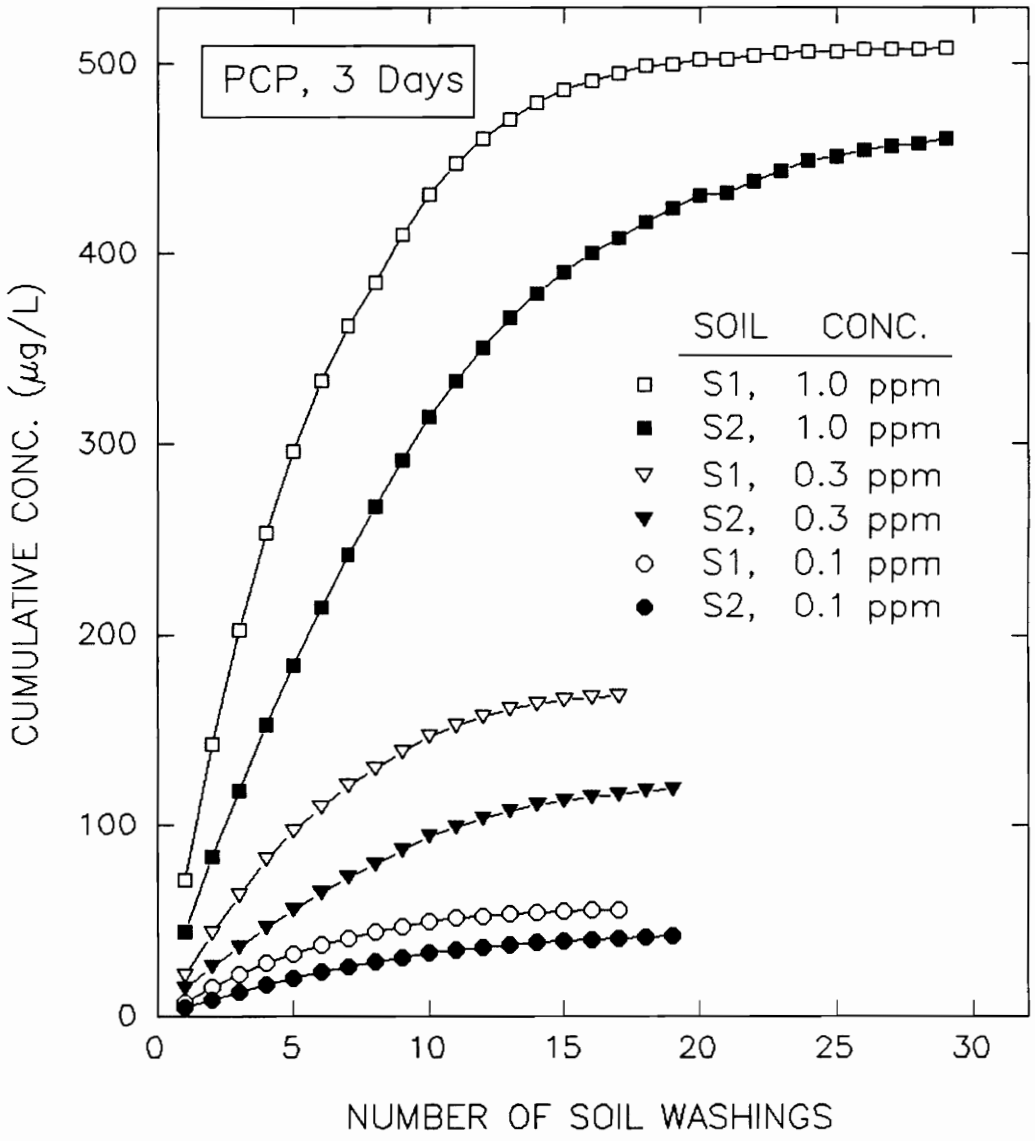


Figure A2. Desorption profiles for two soils contacted with PCP for 3 days.

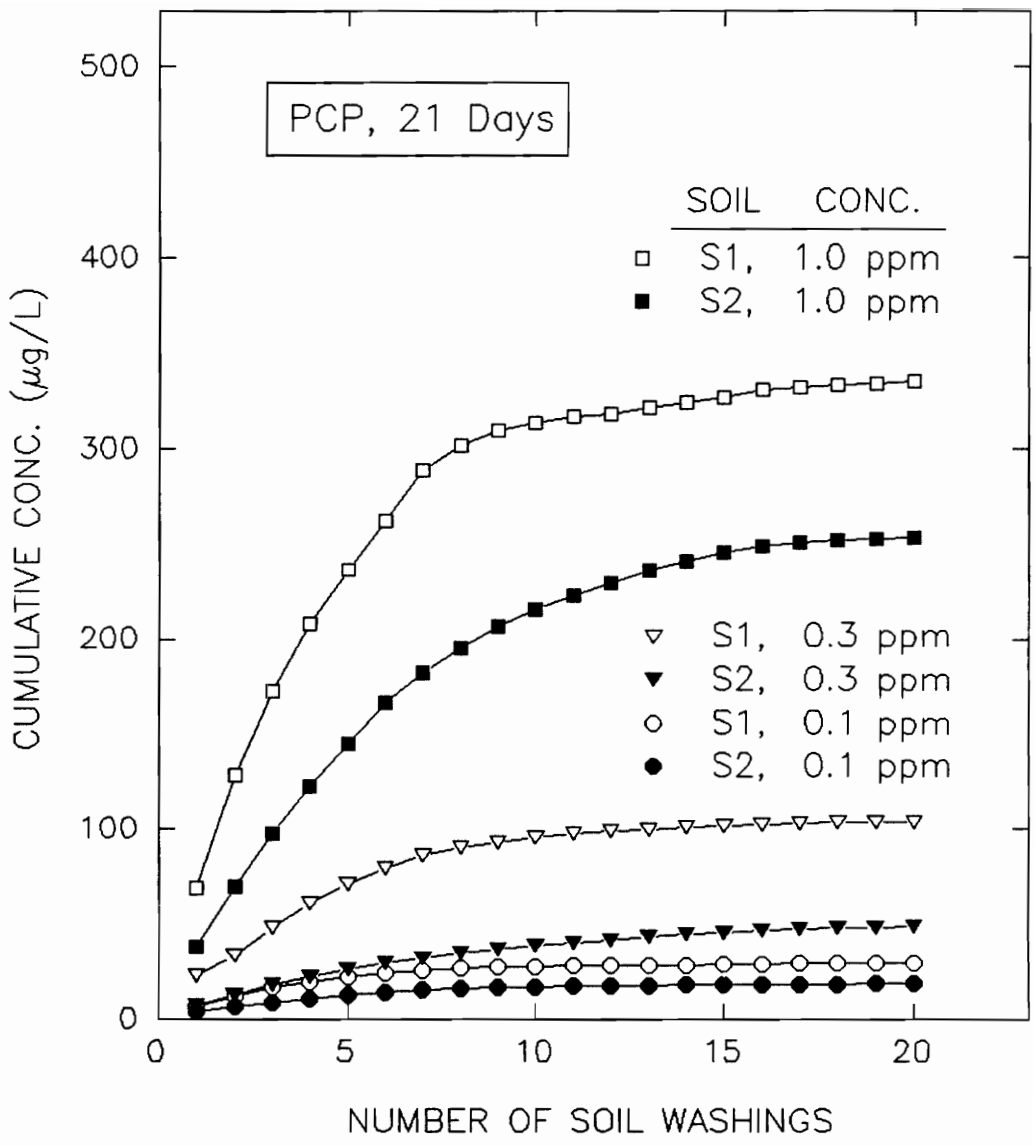


Figure A3. Desorption profiles for two soils contacted with PCP for 21 days.

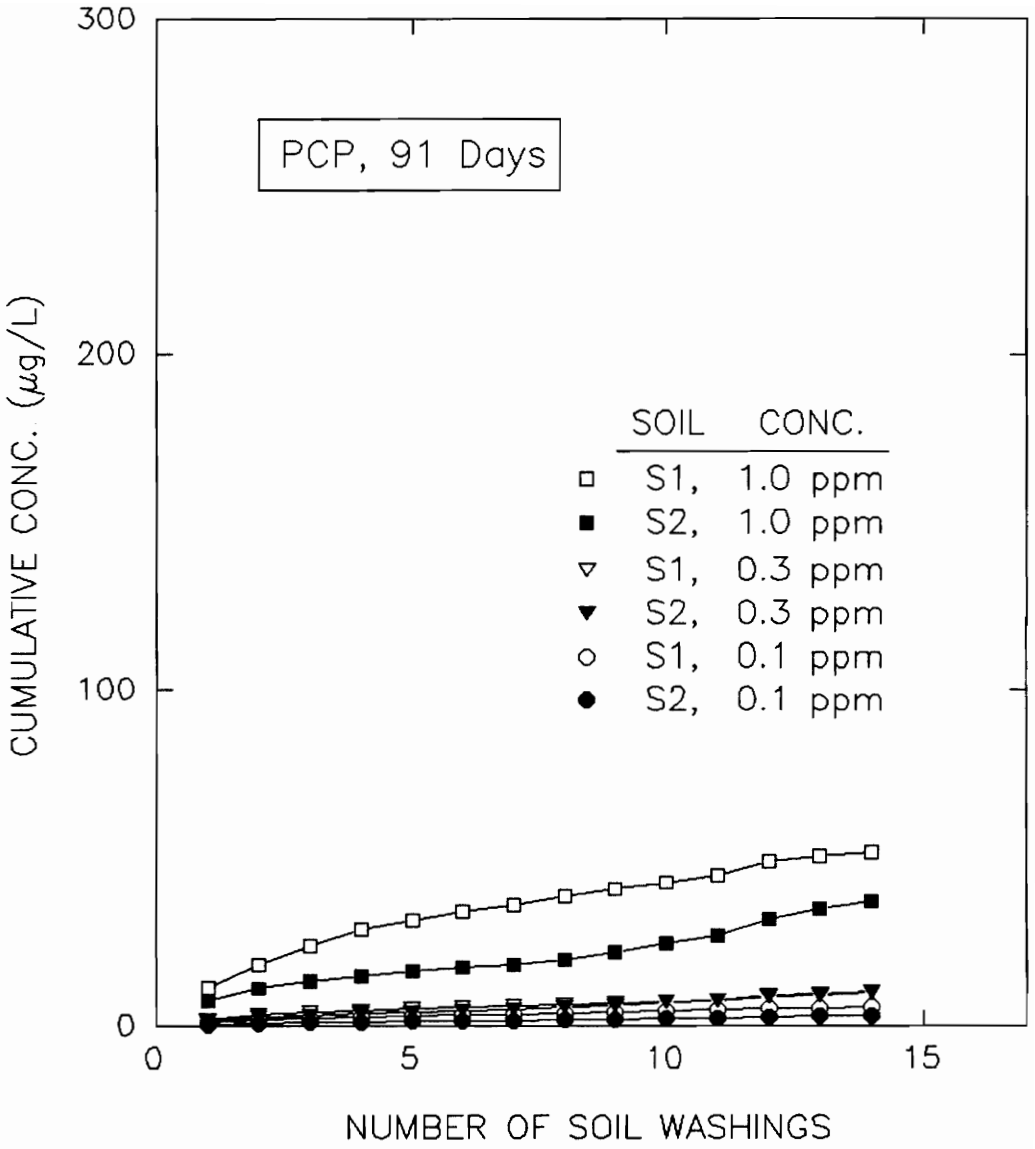


Figure A4. Desorption profiles for two soils contacted with PCP for 91 days.

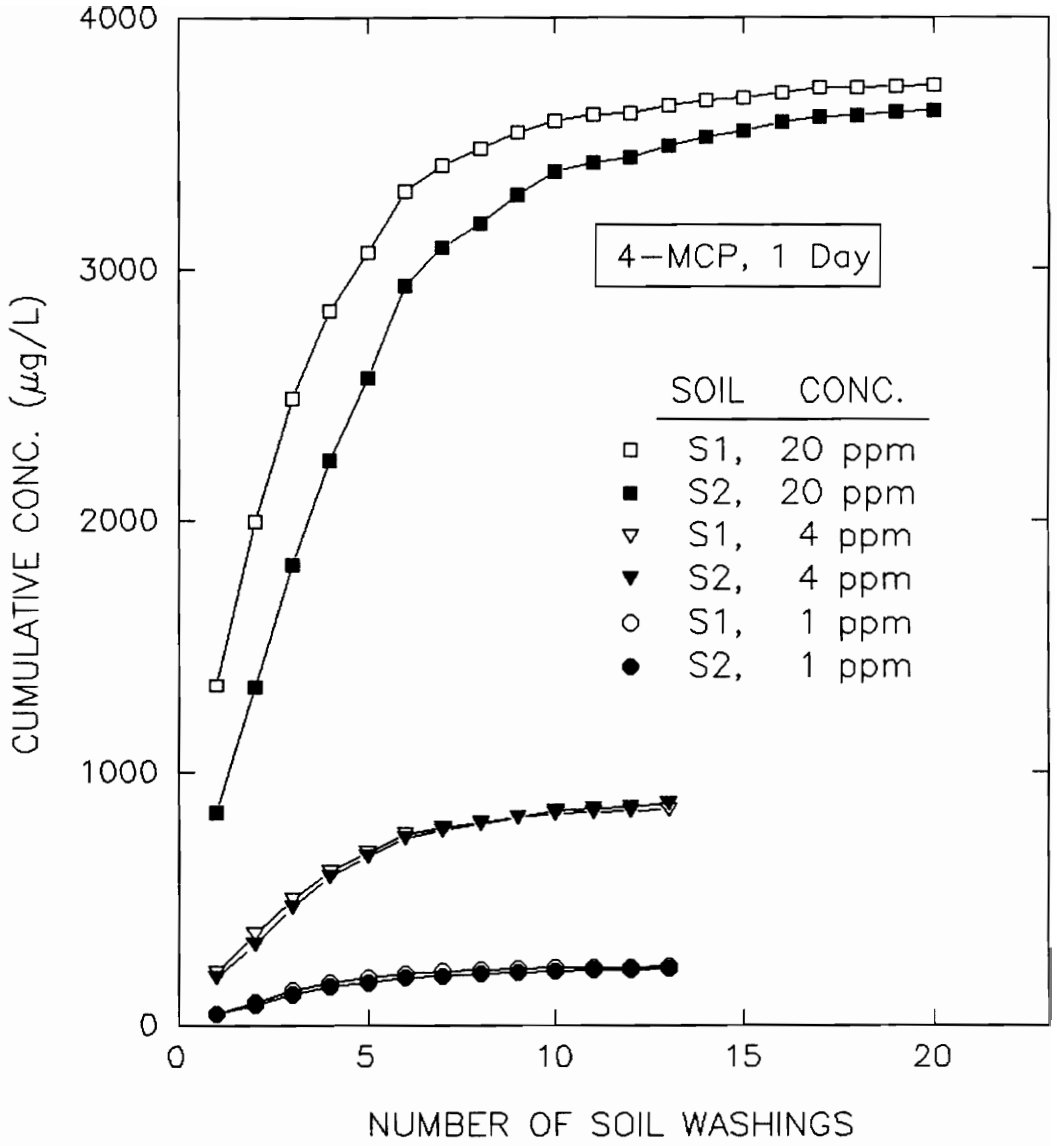


Figure A5. Desorption profiles for two soils contacted with 4-MCP for1 day.

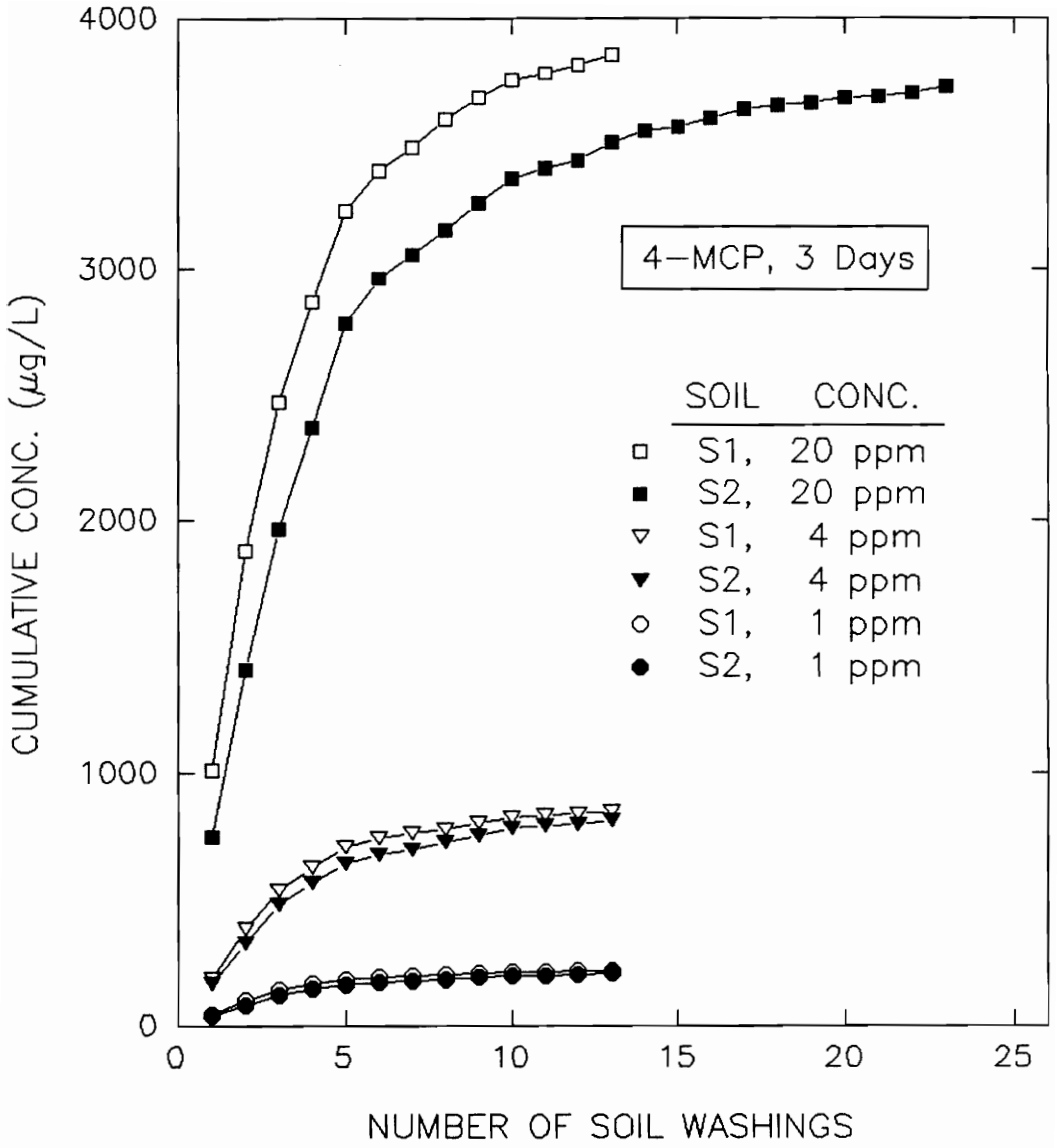


Figure A6. Desorption profiles for two soils contacted with 4-MCP for 3 days.

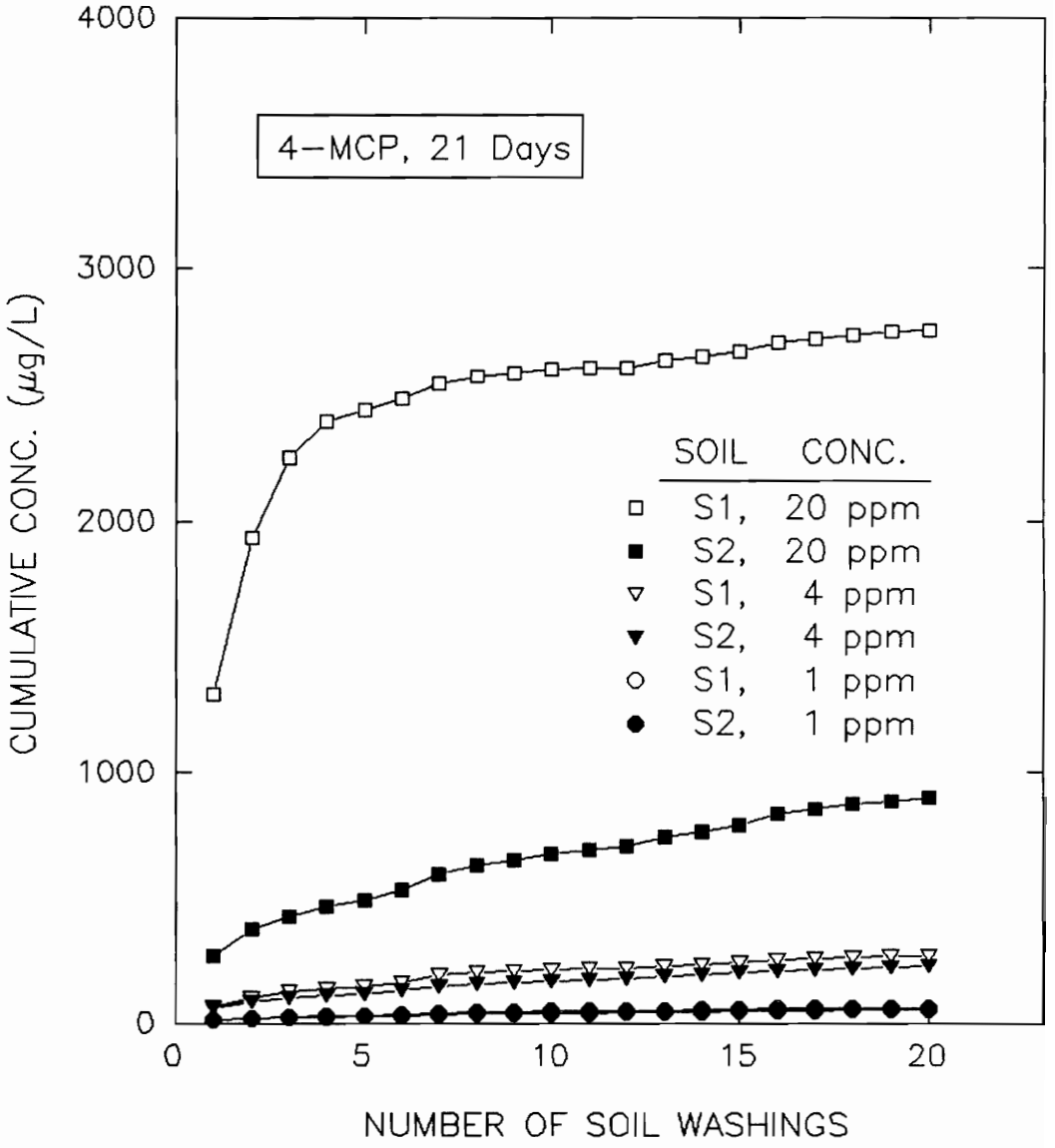


Figure A7. Desorption profiles for two soils contacted with 4-MCP for 21 days.

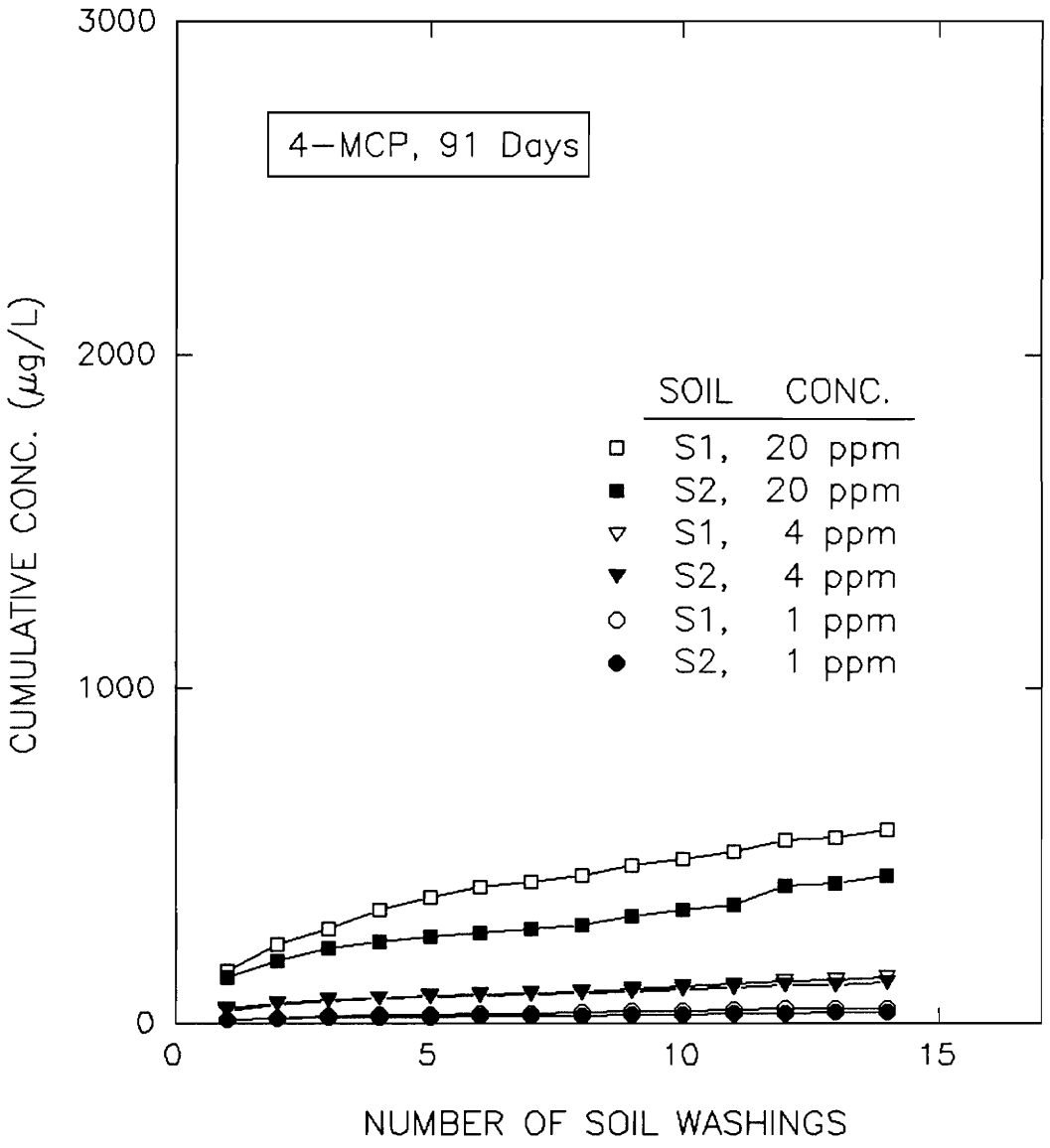


Figure A8. Desorption profiles for two soils contacted with 4-MCP for 91 days.

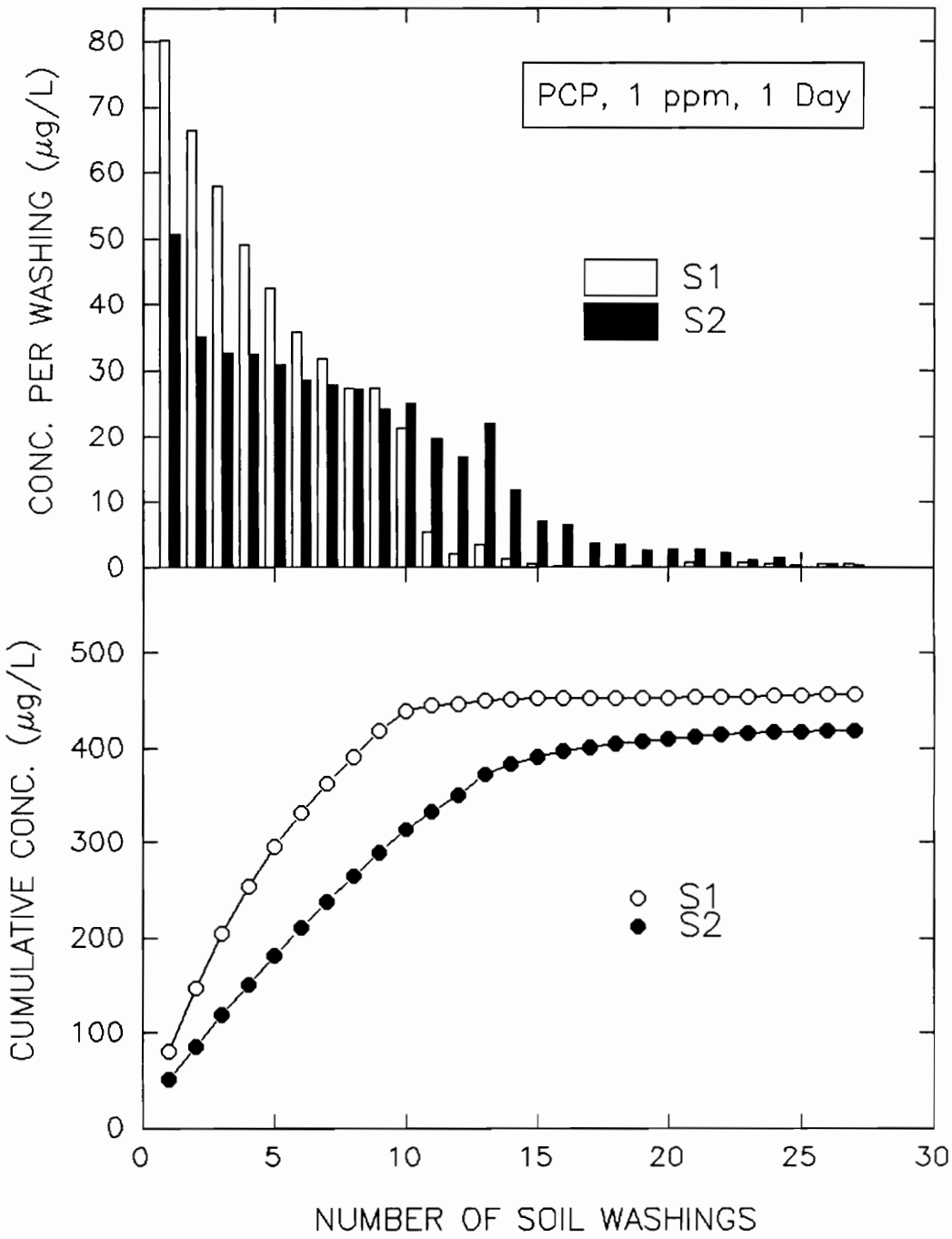


Figure A9. A comparison of the individual samples and cumulative levels of desorption for 1 mg/L PCP and a 1 day contact time.

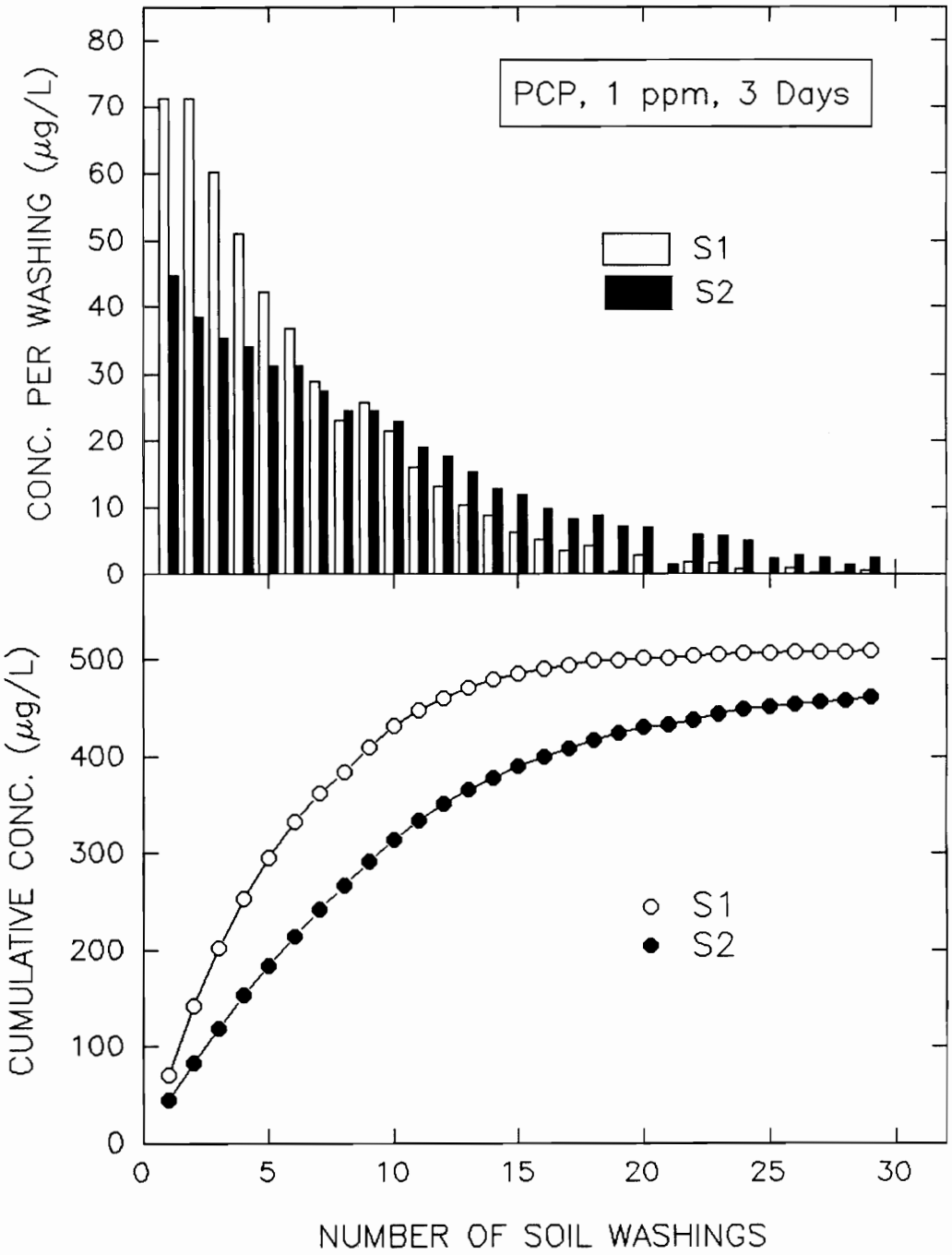


Figure A10. A comparison of the individual samples and cumulative levels of desorption for 1 mg/L PCP and a 3 day contact time.

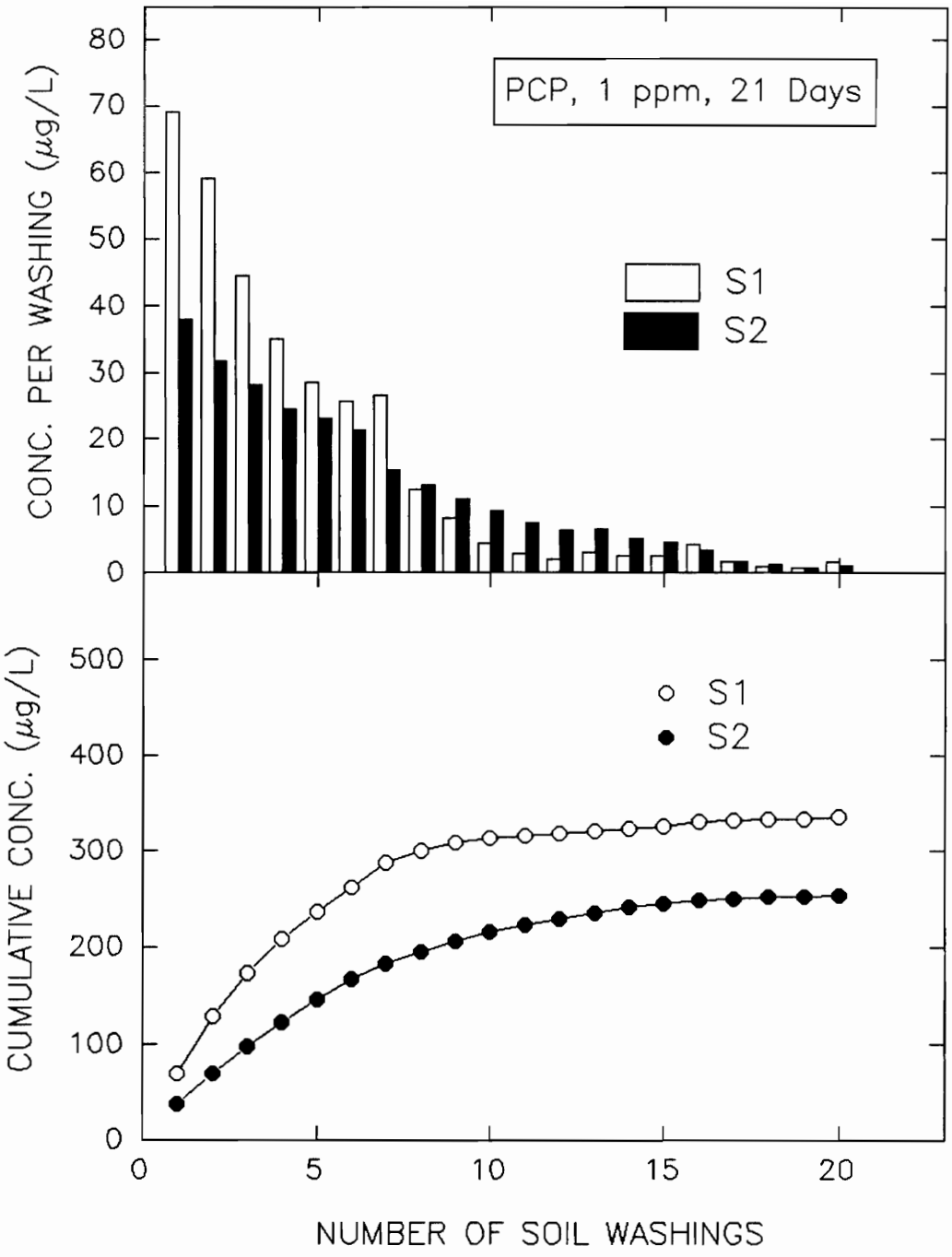


Figure A11. A comparison of the individual samples and cumulative levels of desorption for 1 mg/L PCP and a 21 day contact time.

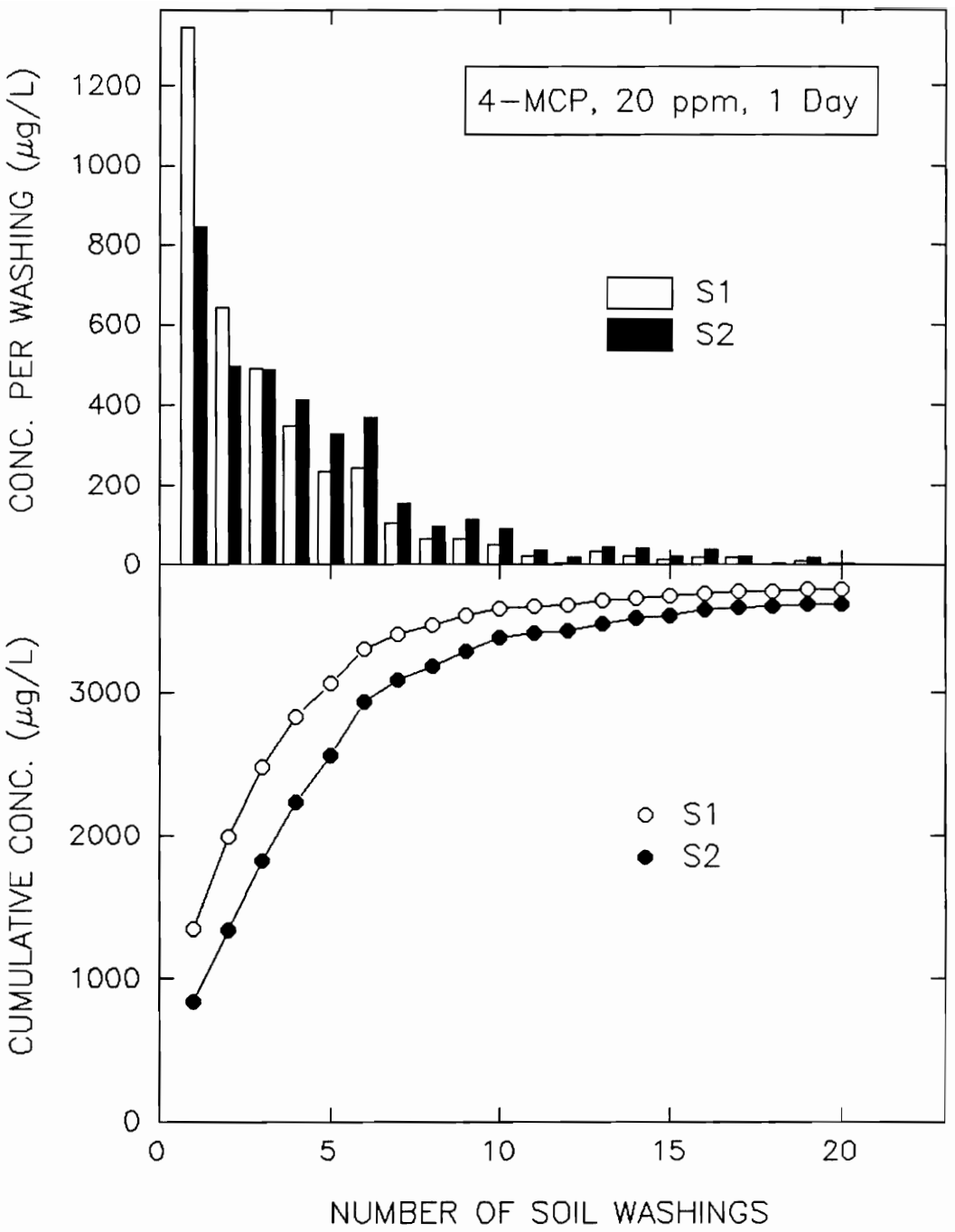


Figure A12. A comparison of the individual samples and cumulative levels of desorption for 20 mg/L 4-MCP and a 1 day contact time.

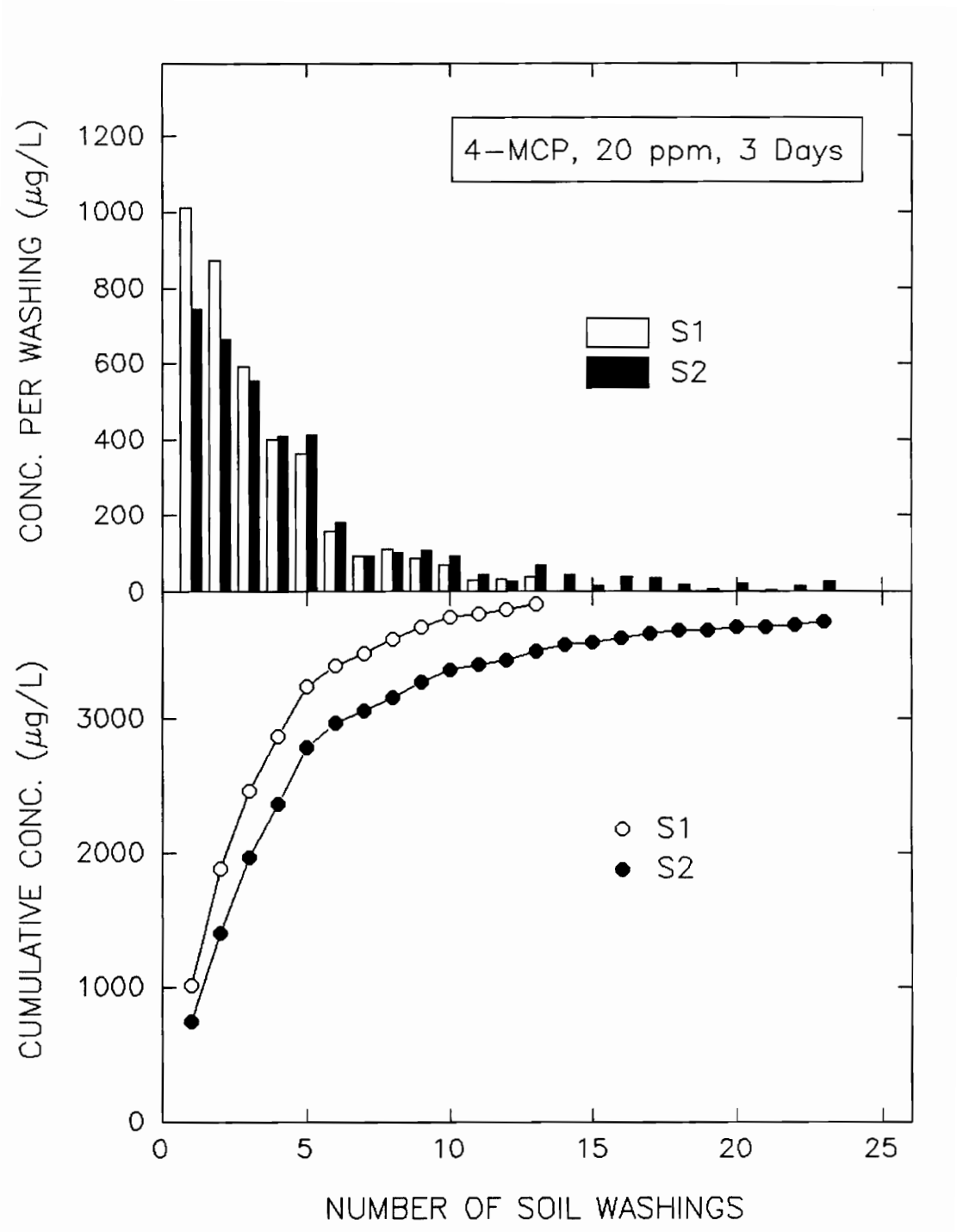


Figure A13. A comparison of the individual samples and cumulative levels of desorption for 20 mg/L 4-MCP and a 3 day contact time.

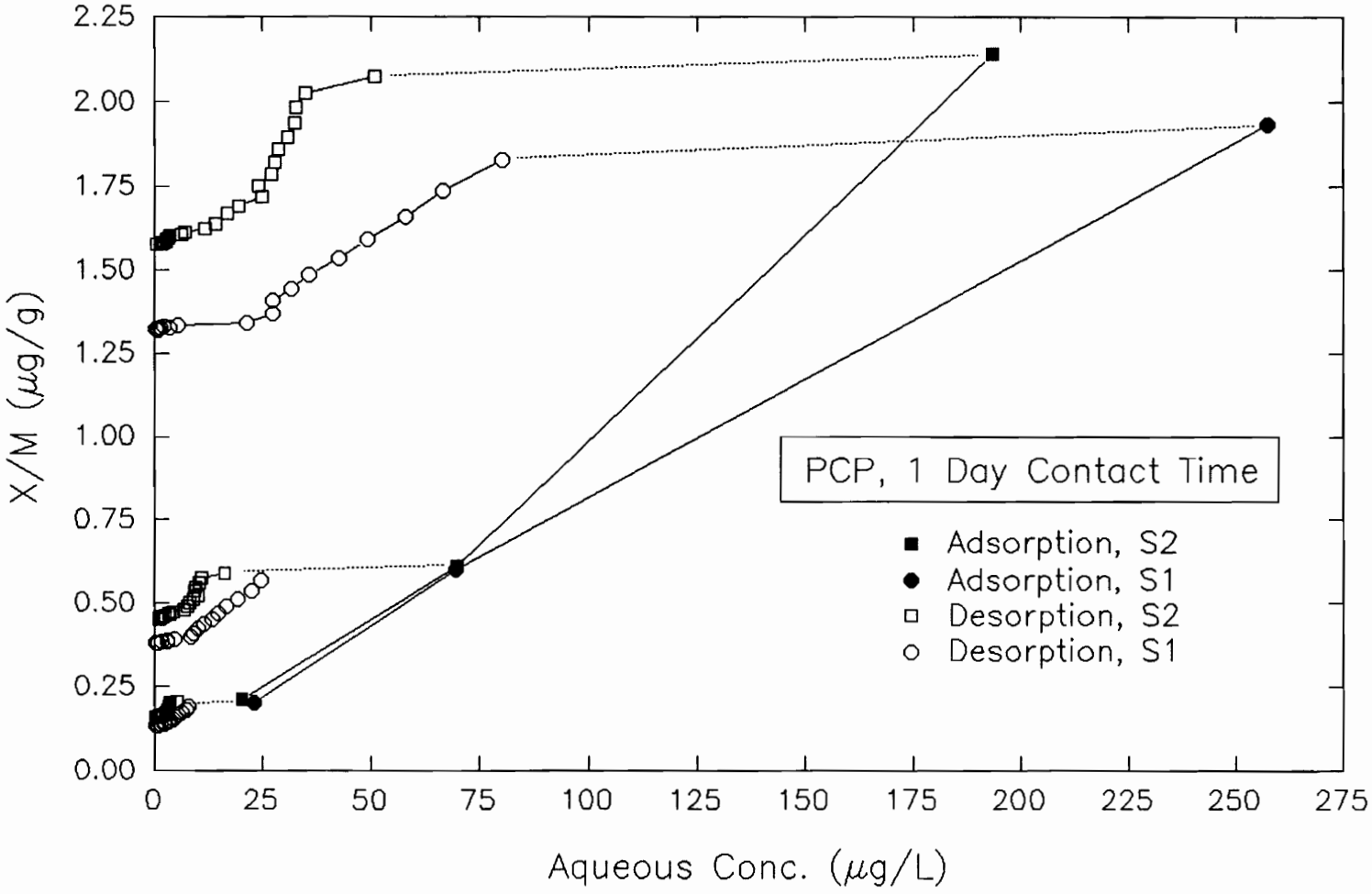


Figure A14. Sorption-desorption isotherms for both soils contacted with PCP for 1 day.

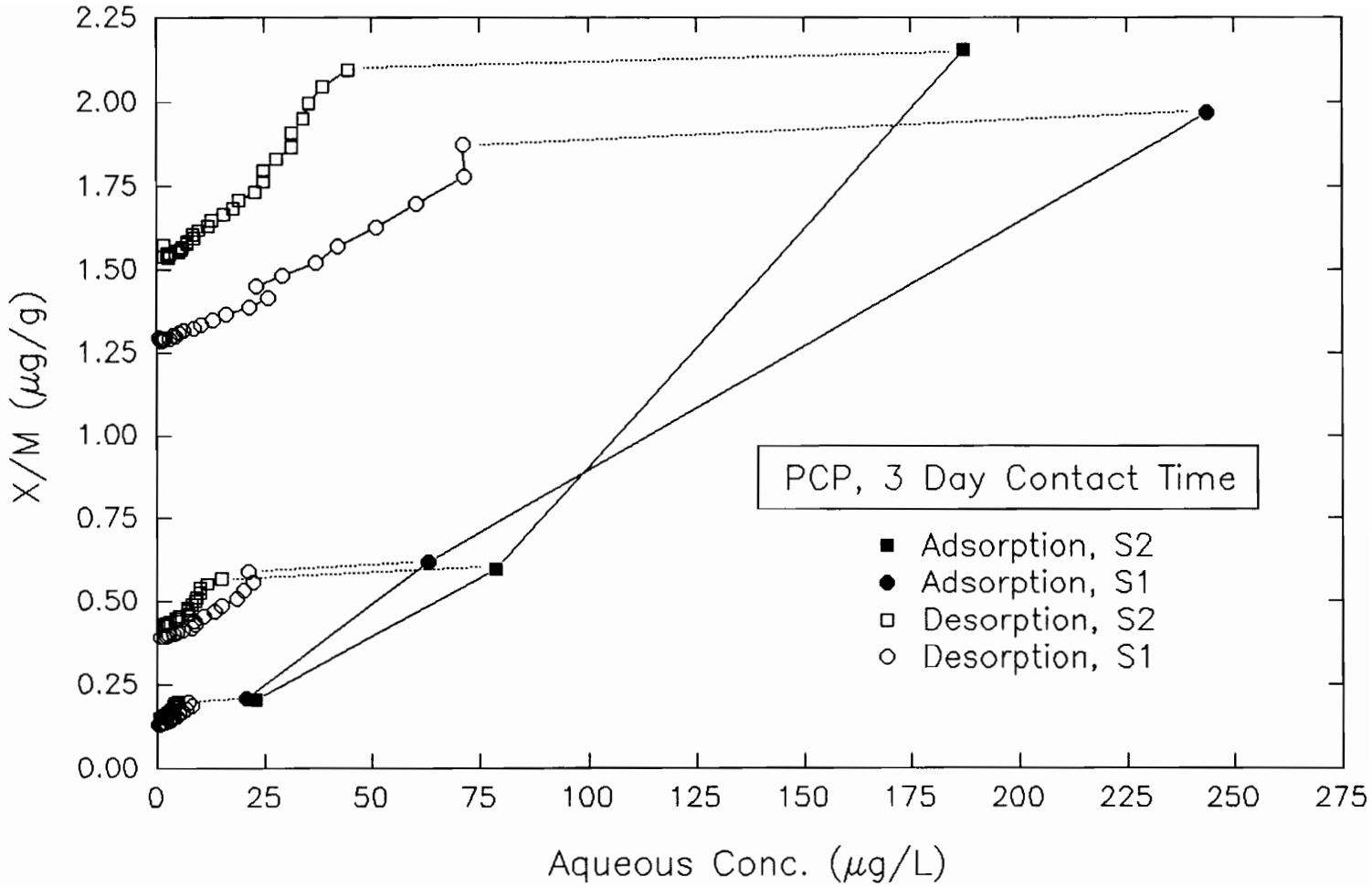


Figure A15. Sorption-desorption isotherms for both soils contacted with PCP for 3 days.

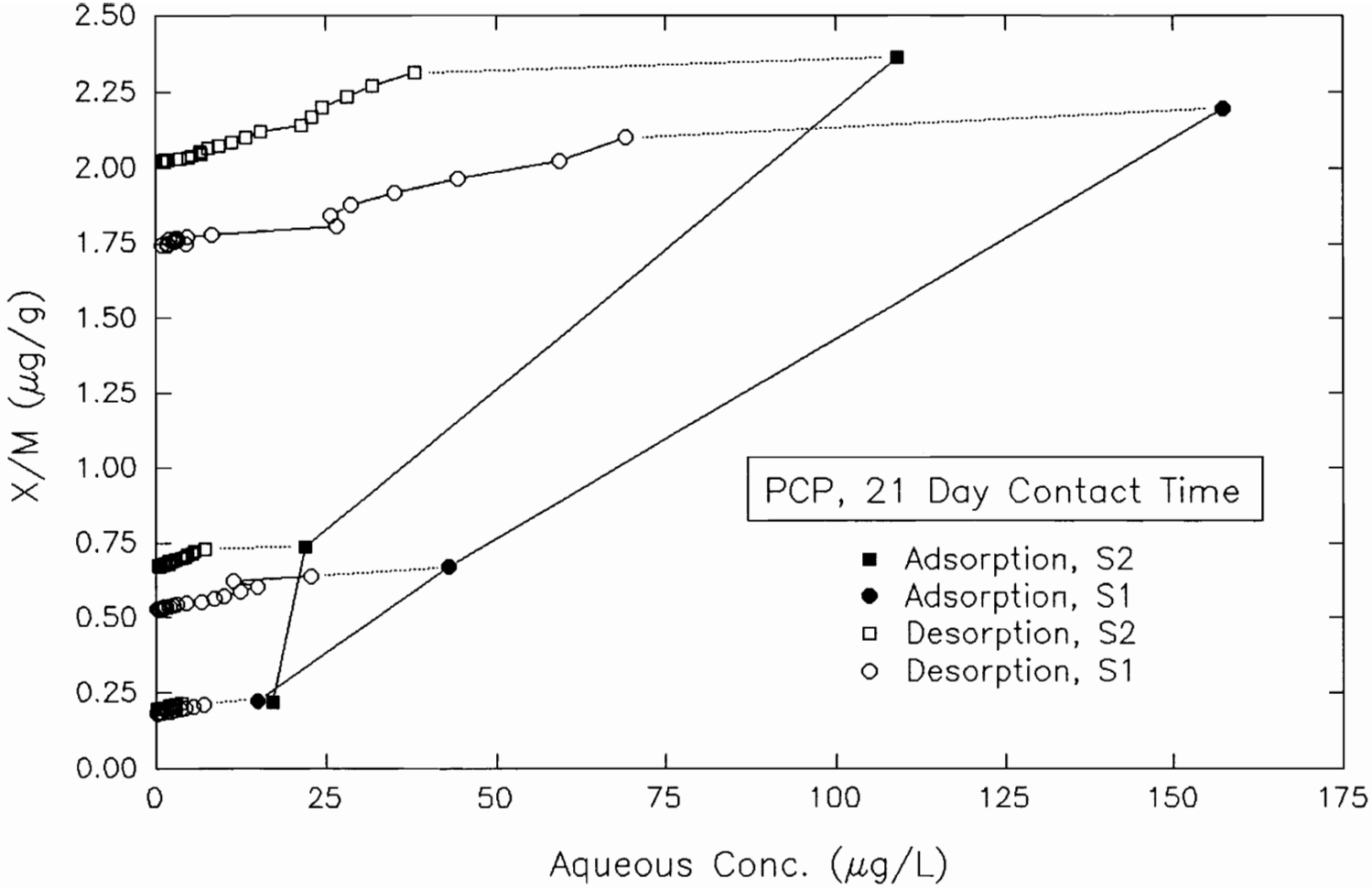


Figure A16. Sorption-desorption isotherms for both soils contacted with PCP for 21 days.

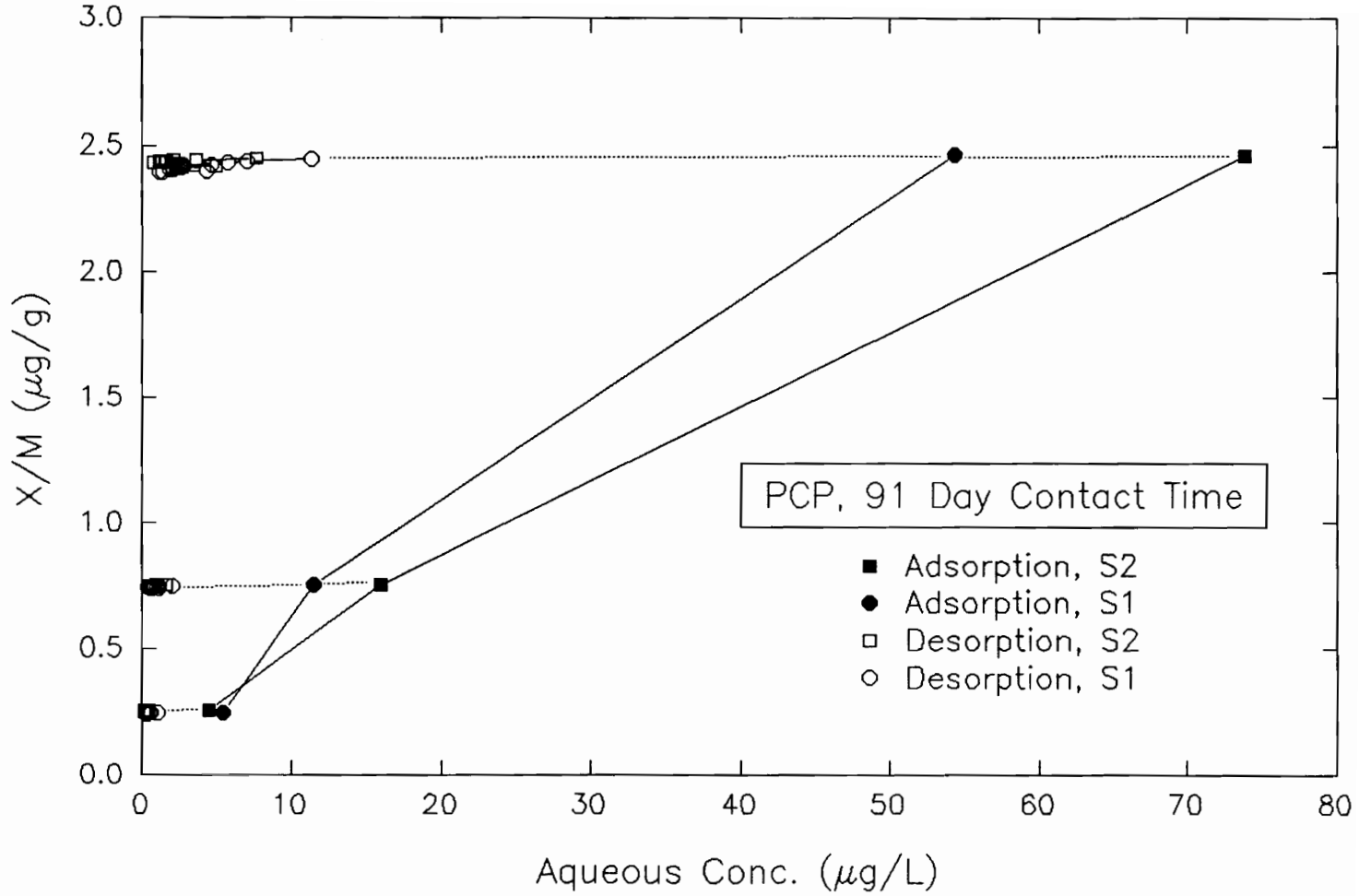


Figure A17. Sorption-desorption isotherms for both soils contacted with PCP for 91 days.

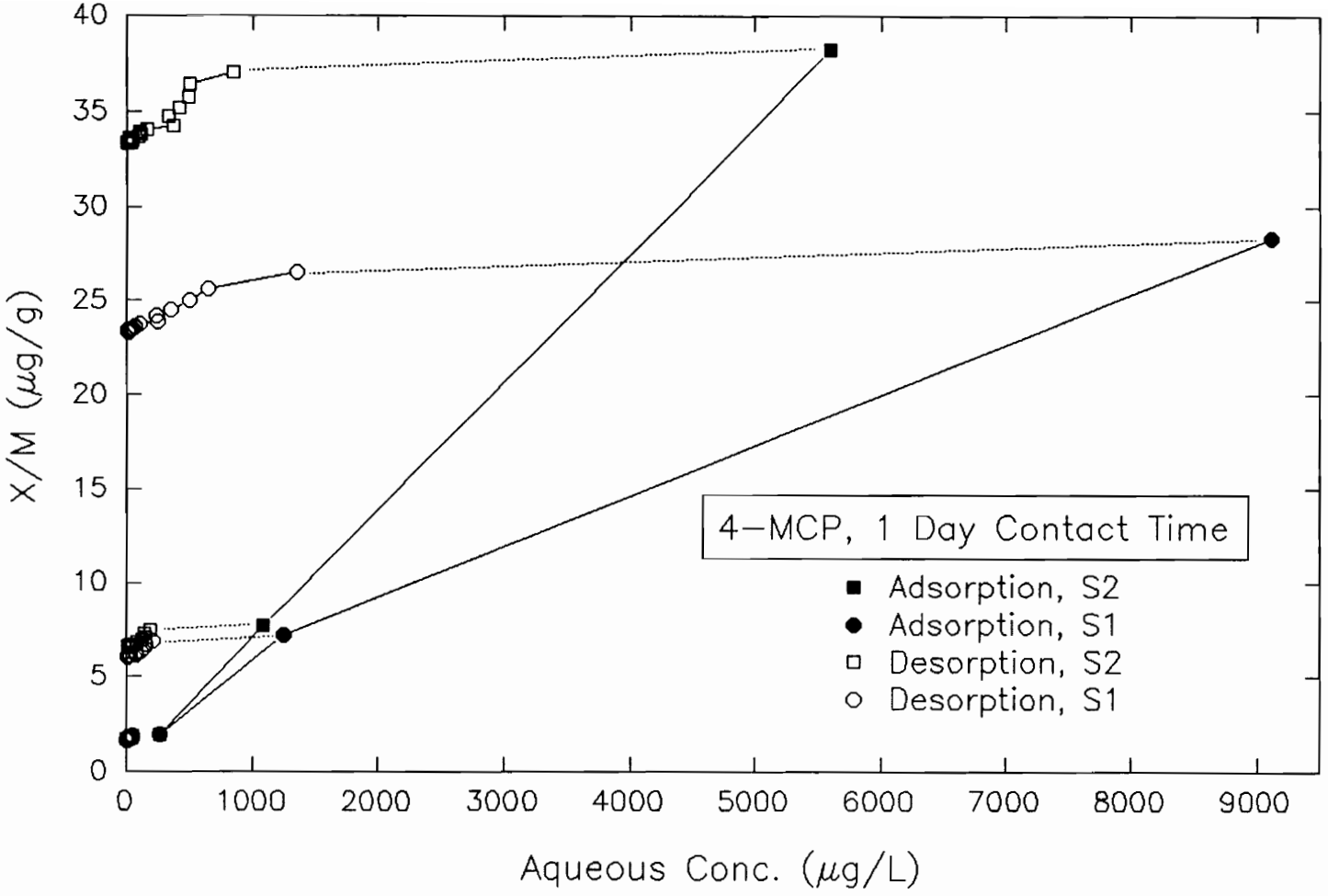


Figure A18. Sorption-desorption isotherms for both soils contacted with 4-MCP for 1 day.

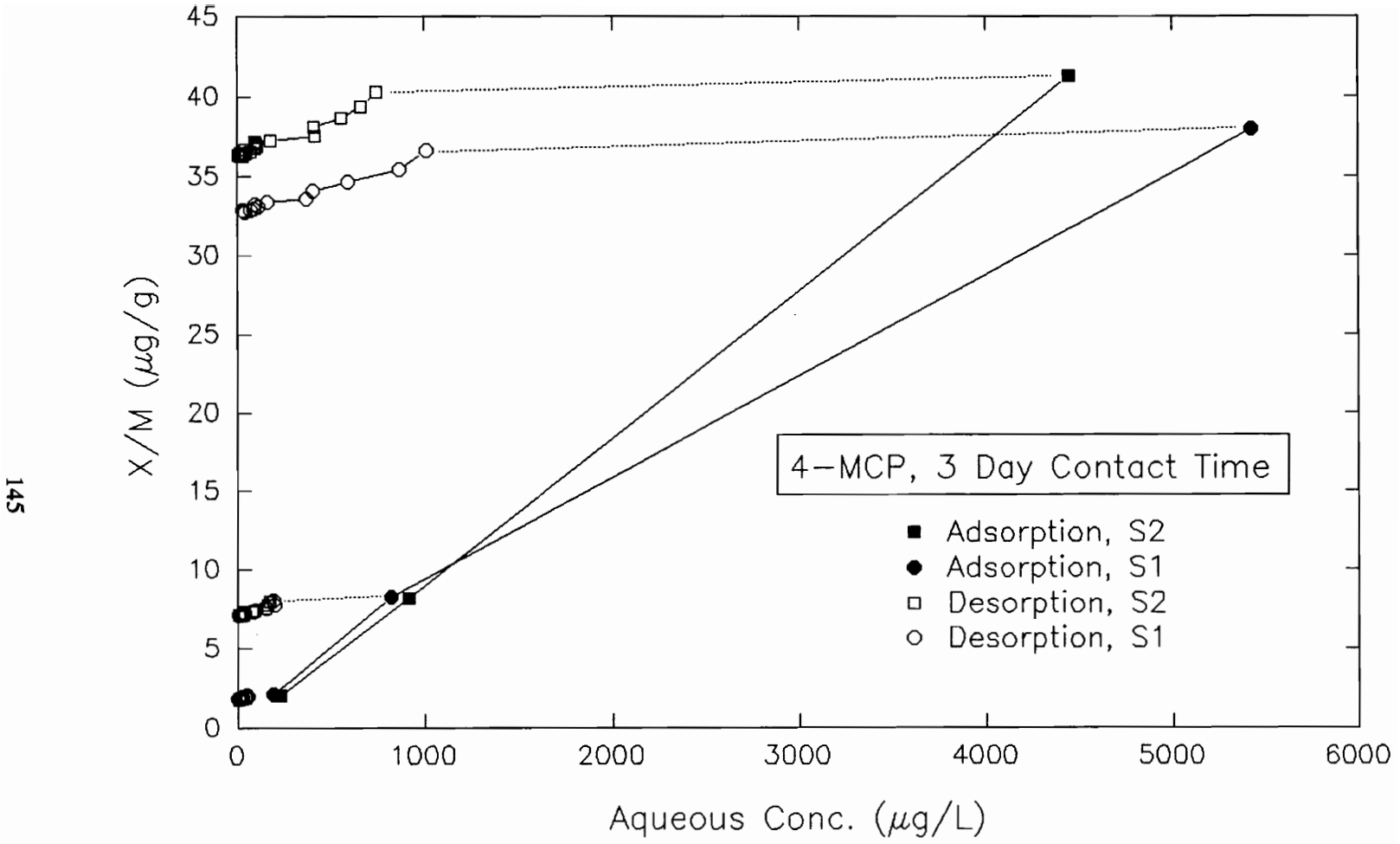


Figure A19. Sorption-desorption isotherms for both soils contacted with 4-MCP for 3 days.

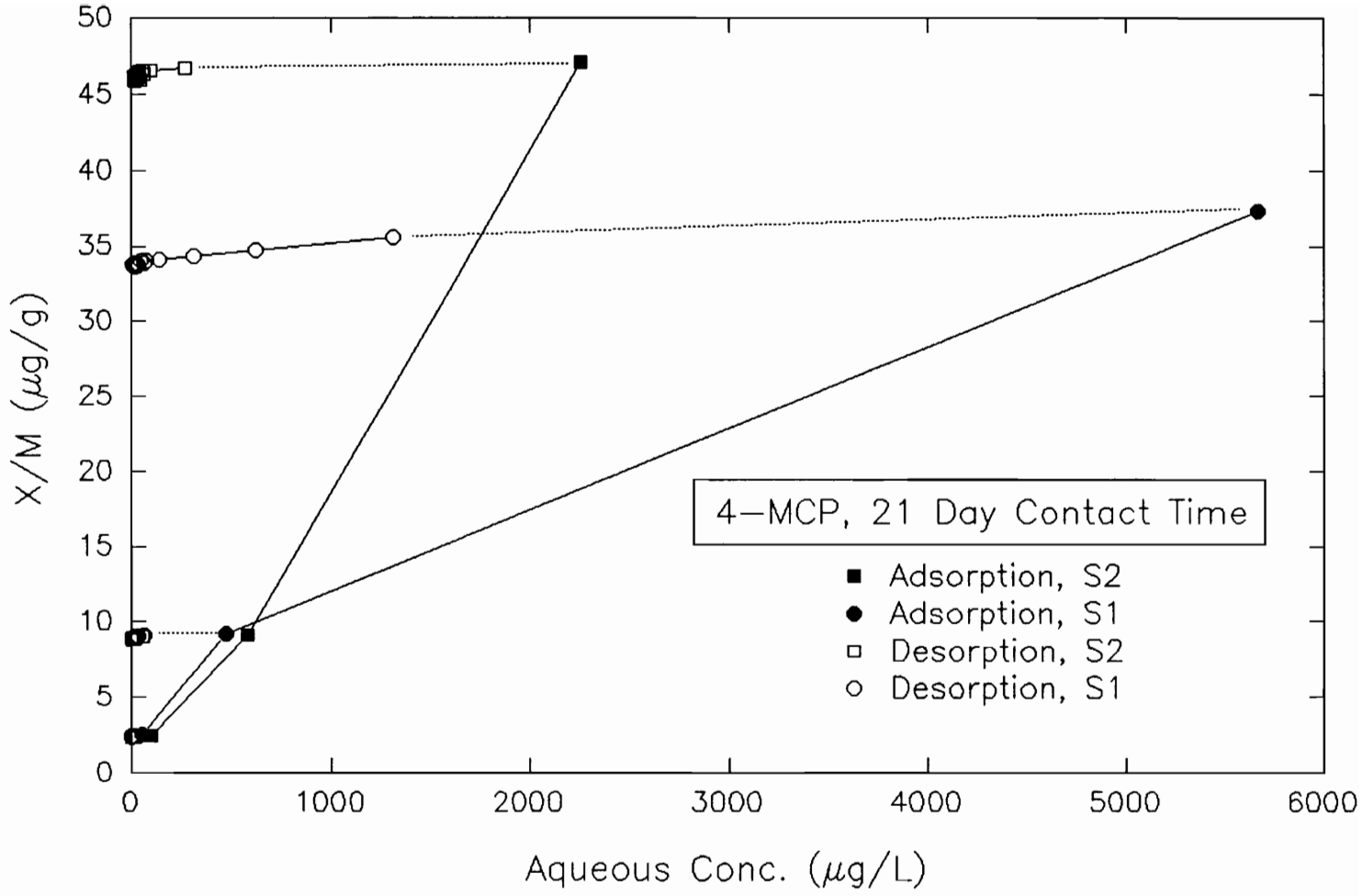


Figure A20. Sorption-desorption isotherms for both soils contacted with 4-MCP for 21 days.

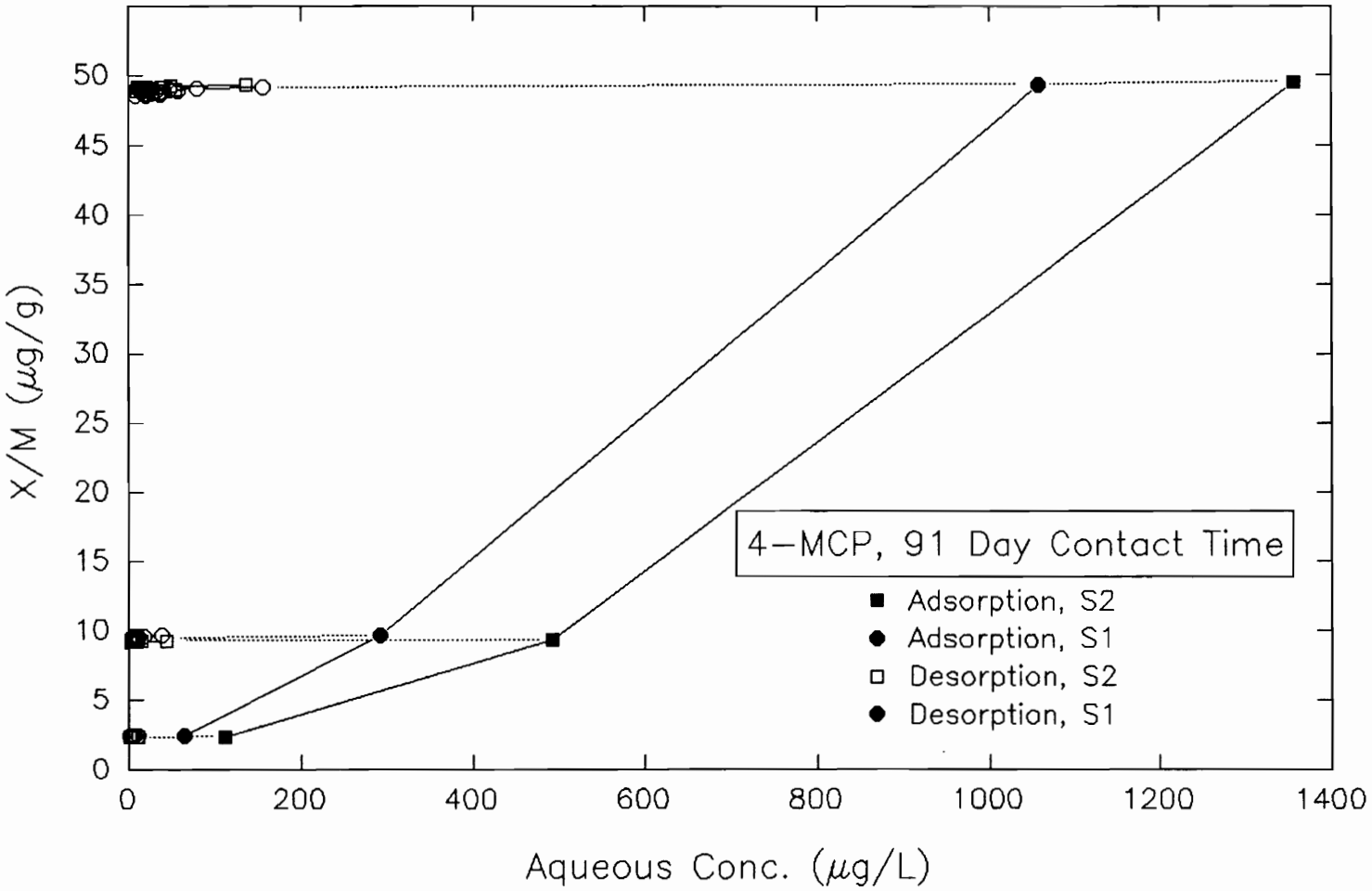
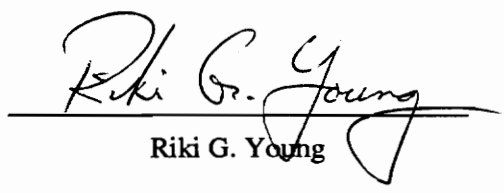


Figure A21. Sorption-desorption isotherms for both soils contacted with 4-MCP for 91 days.

Vita

Riki G. Young was born in Honolulu, Hawaii, on April 8, 1963, to Randall W. and Shizuko Young. He also resided in California and Virginia during his father's naval career, and finally settled in Williamsburg, Virginia in 1976. He graduated from Bruton High School in Williamsburg in 1981, and went on to earn a Bachelor of Science degree in Operations Analysis at the United States Naval Academy in Annapolis, Maryland. After graduating with distinction from USNA in 1985, he was received a commission in the U. S. Navy. On May 25, 1985, he married the former Dianne M. Phillips of Clarks Summit, Pennsylvania. His final three years were spent onboard the USS Paul (FF-1080) stationed in Mayport, Florida, where he served in various billets including Main Propulsion Assistant and Combat Information Center Officer. After separating from the service in 1990, he was accepted into the graduate school at Virginia Polytechnic Institute and State University, where he earned a Master of Science degree in Environmental Engineering in 1992. While attending VPI & SU, he was accepted into the Department of the Air Force's Palace Knight Program and he is currently employed at the Civil Engineering Support Activity at Tyndall Air Force Base, Florida as an Environmental Engineer.



Riki G. Young