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Effects of Sterilization Methods on the Physical Characteristics of Soil: Implications for Sorption Isotherm Analyses

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Laboratory studies of contaminant partitioning and transport behavior are dependent upon the exclusion of biological activity from the experimental system. Isotherm sorption methods usually sample the aqueous phase of an aqueous-soil mixture, and subsequently rely upon a closed system mass balance to determine the amount of material partitioning to the solid phase. Without the assurance of sterile conditions, losses due to biological activity can be misinterpreted as sorption to solid substrate, thus generating erroneous partitioning coefficients. Few studies to date indicate consideration of this effect. Of concern is the need to eliminate biological activity in a soil sample, without changing the intrinsic qualities of the soil which can affect the mechanisms of adsorption and desorption. Several techniques, including γ-irradiation, autoclaving, amendment with poisons (e.g. $HgCl_2$, NaN_3), and chemo-oxidants (ethylene oxide) have been employed to sterilize soil. While some methods of soil sterilization have been shown to change the nature of the soil (Walker and Thompson, 1949), it is clear that limited data are available which describe the effects of these processes on the soil structure, and the consequent effect on chemical partitioning.

Since many sterilization methods can disrupt the physical and chemical properties of the soil, it is therefore important to identify the least destructive and confounding methods in eliminating microbial activity in soil. In these experiments, the efficacy of autoclaving and γ-irradiation as sterilizing methods were compared, and the effect of these methods on the physical and chemical properties (surface area, cation exchange capacity, organic matter) of the treated soil were evaluated. In addition, the adsorption of 1,1,2-trichloroethylene (TCE) on untreated and autoclaved soil was examined to determine if autoclaving affected the chemical partitioning behavior of the soil. TCE was selected as the solute for investigation because (i) the mechanism of its partitioning has been well studied (Mokrauer and Kosson, 1989) and offers a frame of reference for experimental results, and (ii) no significant aerobic biodegradation for TCE has been described, making biological losses unlikely under these experimental conditions. Other solutes such as benzene, toluene, and the xylenes are potentially subject to significant aerobic biodegradation under the typical adsorption isotherm conditions (Cerniglia, 1981).

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MATERIALS AND METHODS

The soil studied was collected from the B/C horizon of an uncontaminated agricultural site in Quakertown, N.J. Prior to use, all soil was sieved (2 mm) and air dried. Autoclaving cycles consisted of 30 min at 121°C. Irradiated soils were exposed to 10 kGy of γ-irradiation in a commercial laboratory. In addition, several soil treatments (i.e. dehydrated, hydrated, hydrated and pre-incubated) were also examined for their effect on sterility. Hydrated soils were amended with 1 or 2 ml H_2O per 3 g soil.

Immediately following soil treatment, sterility was assessed by aseptically mixing 6.0 g of treated (autoclaved or γ-irradiated) soil with 7 mL of sterilized tryptic soy broth (30 g/L distilled H_2O) in 16 mL Pyrex culture tubes, which were capped and vortexed for 30-60 seconds. Following 3-5 days incubation at 28°C, the broth was assessed for turbidity and plated (0.25 mL) onto tryptic soy agar plates (20 g Bacto agar + 30 g tryptic soy broth in 1 L distilled H_2O) using the spread plate technique. Agar plates were observed daily for 5-10 days, and single colony isolates were counted on plates positive for growth. All cultures and plates were established in replicate (n=5). When the media blanks showed turbidity and growth, the experiment was repeated. No growth implies no growth in any replicate in either broth or on solid media. Positive growth implies growth in at least 1 of 5 replicates in either broth, solid media, or both. On all occasions, growth in broth produced growth on solid agar, and on no occasion was growth found on solid agar without turbidity in the broth. Positive controls (media plus unsterilized soil) and media blanks were incubated with all soil cultures.

Following sterilization, soils were forwarded to either the Rutgers University Soil Testing Laboratory for compositional analysis, or the Dept. of Ceramic Engineering, Rutgers University, for BET surface area analysis. Compositional analysis included mechanical sieve separation, organic matter content, cation exchange capacity, and pH determination.

Adsorption of chlorinated hydrocarbons under water saturated conditions were measured via a solid-liquid equilibrium batch method. The serum bottle method for isotherm analysis is presented in detail elsewhere (Kong et al., 1986; Mokrauer and Kosson, 1989), with modifications as described by Lam (1994). In brief, 50 g of soil were placed in 125 mL serum bottles, autoclaved once, and cooled to room temperature. Each bottle was then filled with a $CaCl_2$ solution to minimize headspace volume, and sealed with a teflon butyl rubber septum and aluminum crimp.

After the bottles were sealed and weighed, various volumes of trichloroethylene/methanol solution were delivered to the bottles via gas-tight syringes. It has been shown that methanol concentrations up to 0.1% by volume in soil/water mixtures do not affect the partitioning effects of organic solutes (Kong et al., 1986). Initial solute concentrations of 15-1000 mg/L (0.03-8 mM) in serum bottles were established. All bottles were shaken and allowed to equilibrate for 48-55 hours (Lam, 1994). After equilibration, the aqueous phase was sampled

via a gas-tight syringe and analyzed by gas chromatography (GC). Blank controls containing aqueous solution and no soil were established in parallel to account for any solute vapor loss during the equilibration phase.

TCE concentrations were determined using gas chromatography (Hewlett-Packard 5890 or 5880 gas chromatograph). Operational parameters have been described in detail elsewhere (Lam, 1994).

RESULTS AND DISCUSSION

Several methods for eliminating biological activity in soil have been investigated. Wang et al. (1991) used streptomycin sulfate and chlorotetracycline to inhibit microbial activity in sediments where they were studying the transport of 2,4,6-trichlorophenol. Lion et al. (1990) used 0.02% NaN_3 to inhibit biological degradation in column studies examining the sorption of polynuclear aromatic hydrocarbons on to low carbon aquifer materials. Gore et al. (1984) used two drops of metallic mercury in column studies examining the differential transport of chlorinated solvents through compacted clays. These authors found that columns run for only two days without the addition of mercury exhibited significant biological activity, and that even in short term experiments, inhibition of biological activity was essential. Potential disadvantages of these approaches include: competitive sorption of biological inhibitor and contaminant; formation of toxic methyl mercury; sorptive removal of antibiotics, thus mitigating their biological effectiveness; and incomplete inhibition.

In studies on the effect of sewage sludge on the adsorption of polychlorinated biphenyls, Fairbanks and O'Connor (1984) used 10 kGy of γ-irradiation to successfully reduce biological activity.

Table 1 presents the efficacies of the sterilization methods. These data show that at least three separate autoclaving cycles were required to achieve sterility regardless of hydration. Preincubation for 7 days prior to autoclaving increased the sensitivity of the soil to sterilization. While it is generally recognized that dry cells are more heat resistant than moist cells, hydration did not appear to have any effect on the efficacy of sterilization. The decreased number of cycles necessary to achieve sterility in the pre-incubated samples suggests that pre-incubation of hydrated samples may have facilitated germination of spores, rendering the soil sample more heat sensitive from the beginning. Preincubation of samples for less than 7 days did not achieve sterilization even when autoclaved multiple times. γ-Irradiation was more effective than autoclaving in producing sterilization, as only a single treatment with approximately 10 kGy was sufficient. Hydration and pre-incubation had no effect on the efficacy of this treatment. While multiple autoclavings were necessary to achieve complete sterility, single treatments successfully increased the incubation time required to produce growth (data not shown). In general, 1-2 autoclavings increased the time required to produce growth by 2-4 days. These data suggest that while complete sterility may not have been achieved, the ability to reduce biological activity for the duration of the equilibration phase was achieved.

Table 1. Effectiveness of autoclaving and γ-irradiation on the sterilization of soil

Treatment [a]	Bacterial Growth Observed[b]
I. AUTOCLAVING	
A. Dry soil	
Autoclaved 1 time	+ (5/5)
Autoclaved 2 times	+ (4/5)
Autoclaved 3 times	-
Autoclaved 4 times	-
B. Hydrated soil [c]	
Autoclaved 1 time	+ (5/5)
Autoclaved 2 times	+ (4/5)
Autoclaved 3 times	-
Autoclaved 4 times	-
C. Hydrated soil with pre-incubation [d]	
Pre-incubated 1 day; autoclaved 1 time	+ (5/5)
Pre-incubated 2 days; autoclaved 1 time	+ (5/5)
Pre-incubated 5 days; autoclaved 1 time	+ (5/5)
Pre-incubated 7 days; autoclaved 1 time	+ (5/5)
Pre-incubated 7 days; autoclaved 2 times	-
II. γ-IRRADIATION	
Dry soil	-
Hydrated soil - 3 g soil:1 mL H_2O	+ (1/5)
Hydrated soil - 3 g soil:2 mL H_2O	-
Hydrated soil with 7 day Pre-Incubation [c,d]	-

[a] See text for methods. Each sample was run in replicate (n= 5). In all cases, sterile controls produced no growth on solid media. Each autoclave cycle was 30 minutes.

[b] (-) Bacterial growth implies no growth in either broth or solid media or in any replicate, while (+) implies growth in either media or in both, in at least 1 of the 5 replicates. See text for detail.

[c] 3 g soil to 1 mL H_2O.

[d] Tubes were hydrated and pre-incubated at 30°C for the number of days indicated.

Salonius et al. (1967) demonstrated that irradiation and autoclaving of moistened soils increased the soluble fraction of NH_4-N, carbohydrate and organic matter compared to treated dry samples. In addition, they found that solubilized carbohydrate and organic matter were increased more in the autoclaved samples. Other studies support the observation that irradiation alters the soil to a lesser extent when compared with

autoclaving and other forms of heat treatment (Stotzky and Mortensen, 1959).

Tables 2 and 3 present the effects of sterilization on several properties of the soil. These data reveal that γ-irradiation had minimal effect on the size distribution of the soil particles, while autoclaving appeared to increase the percent sand and decrease the percent clay fractions, probably by causing increased aggregation of soil particulates. Consistent with this observation, Table 2 also shows that autoclaving decreased the BET surface area of the soil sample by 55%, while γ-irradiation decreased it by only 16%. This change in surface area, which may be secondary to changes in the ionic composition of the media (increase in dissolved electrolytes), has been postulated to have a significant affect on the adsorption of organic compounds. Table 3 shows that both sterilization methods had minimal effect on the chemical properties of the soil. In general, however, both methods slightly increased the pH and cation exchange capacity of the soil, but had little to no effect on the organic matter content. Salonius et al. (1967) demonstrated that γ-irradiation and autoclaving increased the conductance of the samples by increasing the concentration of dissolved electrolytes, especially Ca^{+2} and Mg^{+2}, while Na^{+} changed only in autoclaved samples. While these changes alone are not likely to have a significant affect on the adsorptive characteristics of the soil, they may have a significant impact on the ability of microorganisms to grow and compete in the soil matrix treated (Salonius et al., 1967; Salonius et al., 1970).

Table 2. Effect of sterilization method on selected physical properties of soil

Soil Treatment	sand [%]	silt [%]	clay [%]	Surface Area	
				$[m^2/g]$	Change [%]
CONTROL	25	50	25	14.3	0
AUTOCLAVED					
1 time, Dry	39	46	15	6.4	55
3 times, Dry	33	50	17		
1 time, Hydrated [a]	33	50	17		
γ-IRRADIATED					
Dry	25	44	31	12.0	16
Hydrated [a]	21	50	29		
Hydrated [b]	23	50	27		

[a] 3 g soil to 1 mL H_2O.

[b] 3 g soil to 2 mL H_2O.

Table 3. Effect of sterilization method on selected chemical properties of soil

Soil Treatment	Organic Matter [%]	Cation Exchange Capacity [meq/g]	pH
CONTROL	3.5	12.7	6.6
AUTOCLAVED			
1 time, Dry	3.4	12.7	6.3
3 times, Dry	3.7	15.1	6.8
1 time, Hydrated [a]	3.5	13.1	7.0
γ-IRRADIATED			
Dry	3.3	15.1	6.7
Hydrated [a]	3.3	12.7	6.9
Hydrated [b]	3.4	12.6	7.4

[a] 3 g soil to 1 mL H_2O.
[b] 3 g soil to 2 mL H_2O.

In the batch adsorption studies, the amount of solute adsorbed onto soil is calculated via mass balance and the concentration of solute in the fluid phase at equilibrium. Metabolism of dissolved adsorbates would result in overestimation of adsorption.

From the previous tables, it is clear that autoclaved dry soil had a smaller surface area than untreated soil, suggesting that autoclaving might have collapsed a portion of small pores in soil due to pressurization and depressurization cycling of the autoclaving process. In addition, autoclaving might also enhance aggregation, resulting in a smaller clay fraction and greater apparent sand fractions. However, it is important to note that the organic matter and cation exchange capacity of the autoclaved soil remained essentially unchanged. Figure 1 shows the adsorption isotherm obtained for TCE in both autoclaved and untreated soil. Since TCE is recalcitrant under the isotherm conditions employed, no loss of TCE under the unsterilized isotherm conditions would be anticipated. Hence, any difference in TCE adsorption between the two batch studies conducted should be entirely attributed to the effects of autoclaving. These data show that the TCE partition isotherms were comparable, suggesting that autoclaving had minimal effect on the behavior of the contaminant in these batch studies. These data support the conclusion that the availability of potential binding sites and not necessarily surface area, are important in defining the adsorptive capacity of a given soil. Since there was essentially minimal to no change in the characteristics of γ-irradiated or multiply autoclaved soil, batch isotherms were determined only for autoclaved samples. While γ-irradiation may disrupt the soil less, it may be considered less advantageous because of its higher cost and decreased accessibility

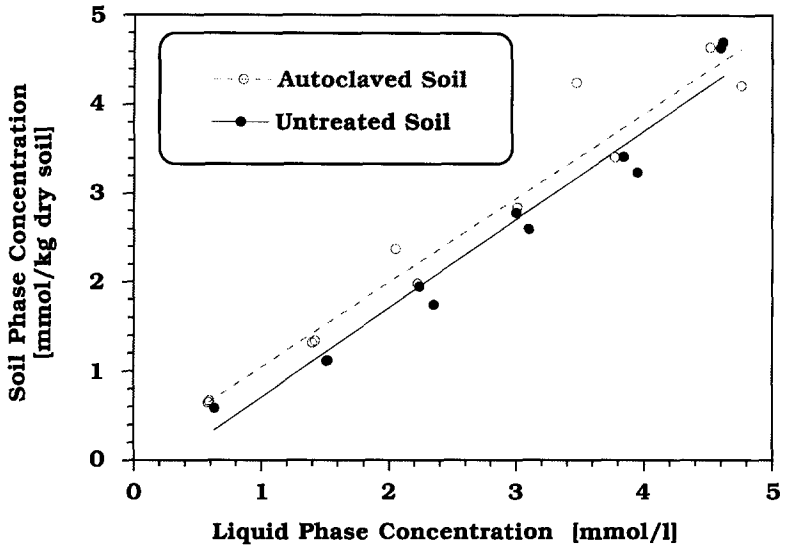

Figure 1. Adsorption isotherms for TCE on autoclaved and untreated soil

when compared to autoclaving.

Recent isotherm data collected in our lab suggests that the ionic strength of the solution, which might change following the addition of various poisons, can also affect sorptive capacity of the soil or the thermodynamic activity in solution of the organic solute. It is generally well accepted that Ca^{+2} and Mg^{+2} can increase the flocculation and aggregation of soil particulate, while Na^{+} may facilitate disaggregation. The addition of poisons, whether they be organic (antibiotics) or inorganic (NaN_3, $HgCl_2$), can affect the ionic strength of the medium, and may also bind non-specifically to sites on the particulates leading to altered substrate binding of other dissolved solutes, as has been demonstrated with aniline for example (Byrne, 1991).

Clearly, these studies are limited to the analysis of a single soil sample and single contaminant. While these data are suggestive of the fact that autoclaving may not have a large impact on TCE behavior in vitro, it cannot be extrapolated to all contaminants in all soils. Clearly, it would be difficult to perform such studies with easily metabolized substrates. These data do suggest that biodegradation should be considered in batch adsorption isotherm studies. It is recommended that procedures for the assessment of microbial activity be conducted in parallel with partitioning studies in order to assure that biological activity has not interfered with the partitioning results.

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