Treating Soil Solution Samplers To Prevent Microbial Removal of Analytes

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Soil microorganisms colonizing soil water sampling devices (lysimeters) reduced concentrations of biodegradable organic chemicals, including 2,4-dichlorophenoxyacetic acid methyl ester, alachlor, methyl m-chlorobenzoate, and metolachlor as water entered through porous ceramic cups. In some cases, losses exceeded 99%. Additions of either a biocide (sodium hypochlorite) or a bacteriostat (copper salt) prevented microbial activity so that concentrations of test chemicals inside lysimeters equaled those outside. Field studies further indicated that treating lysimeters with a copper salt effectively prevented microbial activity. Thus, chemically treating soil water samplers could improve the accuracy of soil water data for a wide variety of analytes, including environmentally important organics, such as pesticides and industrial wastes, and inorganics, such as ammonia and nitrate.

Soil solution samplers (tension and suction lysimeters) have been used to test for a variety of dissolved chemicals, including nutrients, pesticides, and other pollutants, in the vadose (unsaturated) zone. These devices, which consist of a tube fitted with a porous cup at the end, are inserted in soil at various depths to monitor chemical movement through the soil column. Samplers vary in size and construction materials (4, 7, 9). Cups, which are generally made of ceramics, Teflon, or stainless steel, may either be in direct contact with soil or enclosed with silica flour. Silica provides a filtration medium between the soil and the cup. When the sampler is placed in the field, soil water enters the porous cup by gravitational flow or by suction and is removed via a suction tube inserted in the lysimeter during sampling.

When comparing bromide concentrations in the soil column with those from lysimeter samples, Smith et al. (8) reported concentrations three to five times lower in water collected in lysimeters. The authors suggested that silica flour may have impeded ion transport, that higher moisture content in the flour may have diluted the bromide, or that the ceramic cup may have adsorbed it. Similar discrepancies have been noted by others (1).

Another explanation, which is the object of the present studies, is that analytes may be removed by sorption, transformation, or other processes mediated by microorganisms colonizing porous cups. Even in cases in which biotransformation may be negligible for soil water containing free-living microbes, highly concentrated microbiota associated with biofilms formed on surfaces can rapidly reduce the concentrations of chemicals contacting them. In studies with methyl m-chlorobenzoate, for example, no microbiologically mediated loss of the chemical could be detected over several days in the water phase, but the half-life of the chemical in contact with biofilm was less than 10 min (3). Biofilms associated with the exterior surfaces of lysimeter cups may not provide much more biomass than microbes attached to soil particles. Thus, one might not expect microbially mediated analyte losses from cup surfaces to exceed losses attributable to the surrounding soil particulate microbes. However, colonization in and around pores of the ceramic walls through which water is channeled may provide increased contact surface area between microbes and dissolved analytes.

Perhaps a more important consideration is that biotransformation rates of dissolved chemicals moving slowly through soils are likely to be mass-transport limited. Under these conditions, transformation rates are accelerated by increased water flow rates (2). Thus, water moving across and through biofilms under suction used to withdraw soil water would further enhance biotransformation rates associated with lysimeter cups. Suction-enhanced biotransformation rates might result not only from microbes colonizing cups but also from those associated with silica flour or soil in the immediate vicinity of cups.

Many of the analytes monitored with soil water samplers are adsorbed or transformed by microorganisms living in soil environments. Therefore, we conducted laboratory studies to determine whether microbes colonizing lysimeter cups could cause measurable decreases in the concentrations of various chemicals passing through the porous ceramic walls. Test chemicals included 2,4-dichlorophenoxyacetic acid methyl ester (2,4-DME), 2-chloro-2',6'-diethyl-N-(methoxyethyl)acetanilide (alachlor), methyl m-chlorobenzoate, and 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide (metolachlor). These chemicals were selected so that a suite of compounds exhibiting a wide range of biodegradation rates would be investigated. We also carried out laboratory and field tests using biocidal concentrations of sodium hypochlorite and bacteriostatic concentrations of two copper salts to prevent microbial activities in and around soil water samplers.
MATERIALS AND METHODS

Field conditions. Field experiments were carried out at North American Farms in Jackson County, Fla., in an uncropped area adjacent to cultivated peanuts. Soil at the test site was identified as Lakeland sand, a type of quarto-psamment, occurring to a minimum depth of 2 m. Soil water samplers were 1-m polyvinyl chloride suction lysimeters with ceramic cups having a pore size of 2.5 μm (Soil Moisture Equipment Co., Santa Barbara, Calif.). Duplicate lysimeters were incubated in the field and removed for laboratory studies of biofilm activity. Field incubations lasted for various lengths of time between July 1990 and January 1991. A source of soil water was provided in the field by a center-pivot irrigation system used on the adjacent peanut crop. Triplicate treated and untreated lysimeters used in studies with copper salts were incubated for 42 days, from 30 April to 11 June 1991. During the latter part of the period, rainfall in the area was excessive (almost daily, ranging from approximately 0.5 to 6 in. [ca. 1 to 15 cm]/day).

Lysimeters were buried 1 m deep in augured holes, and cups were enveloped with approximately 100 g of silica flour (Timco Manufacturing, Inc., Prairie du Sac, Wis.) as a slurry. The remaining space around polyvinyl chloride tubes was backfilled with soil removed during auguring. For sampling soil water, 5 × 10⁶ Pa (ch-centibars) vacuum for 24 h was used to withdraw water from surrounding soil. For determining biofilm activities associated with lysimeter cups and for water sample analyses, untreated lysimeters and water samples (approximately 5 to 20 ml) were shipped in cooled, insulated containers by overnight mail to the U.S. Environmental Protection Agency (EPA) laboratory in Athens, Ga.

Laboratory experiments. Upon arrival of the soil water samplers, excess attached soil was scraped away. Then the outside of each lysimeter was rinsed with sterile Payne-Feisal basal salts solution (5). Duplicate rinsed lysimeters were placed in 2-liter graduated cylinders containing 1.5 liters of sterile basal salts solution (pH 6.8) with dissolved organic test chemical. A Beckman Zeromatic SS-3 pH meter was used for pH determinations. Lysimeters were held in place with ringstands such that the cups were slightly above the bottoms of the graduated cylinders. Sampling was accomplished by inserting a Teflon tube near the outside of the lysimeter cup and emptying the lysimeters at each sampling time. For controls, duplicate lysimeters that had not been incubated in soil were used.

Alachlor and metolachlor were obtained as analytical standards from the U.S. EPA (Research Triangle Park, N.C.); methyl m-chlorobenzoate was from Chem Service (West Chester, Penn.). 2,4-DME was prepared by methyllating 2,4-dichlorophenoxyacetic acid with acidic methanol, extracting the ester with methane chloride, and evaporating the solvent. Gas chromatographic analysis indicated no detectable impurities in the 2,4-DME. Sodium hypochlorite solution (5.25%) was obtained from The Clorox Company, Oakland, Calif. 2,2,4-Trimethyl pentane (isooctane), methanol, and methane chloride were high-pressure liquid chromatography grade (Burdick & Jackson, Muskegon, Mich.).

Treated silica flour was prepared by dissolving 20 g of CuCl₂ or CuSO₄ (Fisher Scientific Co., Fair Lawn, N.J.) in 300 ml of distilled water, adding 980 g of silica flour, and oven drying with frequent mixing for several days at approximately 60°C. In the field, treated silica was rehydrated to the consistency of a thick paste before the lysimeters were coated.

RESULTS

When soil water samplers were untreated and placed in basal salts solution containing 2,4-DME, substantial differences between concentrations inside lysimeters in samples taken at 4.5 h and outside concentrations were observed even after 1 week of field incubation (Fig. 1). After 2 months, little to none of the chemical passed through the lysimeter cups. Alachlor, methyl m-chlorobenzoate, and metolachlor concentrations were also consistently lower in samples taken at 4.5 h from inside the lysimeters (Fig. 2). Concentrations remained lower inside the lysimeters when all of the water was emptied from lysimeters at 4.5 h and concentrations were determined for water entering from 4.5 to 8.5 h. For all of the data, percentages of inside versus outside concentrations ranged from 0.0 to 56 for 2,4-DME (21 ± 4.3; n, 20), 61 to 91 for alachlor (79 ± 5.5; n, 4), 47 to 85 for methyl m-chlorobenzoate (63 ± 8.5; n, 4), and 22 to 100 for metolachlor (77 ± 16; n, 4). Percentages above are means ± standard errors of the means; n was the number of lysimeters tested. Lysimeters that were not incubated in soil did not exhibit differences between inside and outside chemical concentrations.

When sodium hypochlorite was added as a biocide, concentrations of two of the test chemicals in soil-incubated
lysimeters equalized with outside concentrations as the lysimeters were periodically emptied (Fig. 3). In other trials (data not shown), 2,4-DME concentrations also equalized. Thus, the disparities between inside and outside concentrations for these chemicals could be completely accounted for by microbial transformation rather than adsorption to the polyvinyl chloride lysimeter pipes or ceramic cups. Metolachlor reacted chemically with hypochlorite; therefore, data for which the biocide was used were not obtained for it.

In laboratory tests, microbial colonization of porous lysimeter cups buried in soil transformed the organic chemicals, and the problem appeared to be remedied by treatment with a biocide. However, for field application, we needed a chemical agent that was less reactive than hypochlorites, which would be quickly quenched by soil organic matter or may chemically transform any of the analytes of interest. On the basis of previous work in which very low concentrations of metals inhibited biodegradation activity in environmental samples (6), we decided to test the effectiveness of a metal salt.

Of the toxic metals tested in the previous studies, copper had certain practical advantages. It is inexpensive, relatively nontoxic to animals compared with some of the other metals tested, and is approved by the EPA in various formulations for agricultural and environmental applications. Moreover, previous studies (6) showed that concentrations of the metal as low as 0.1 mM (0.017 g of CuCl₂ liter⁻¹) could completely inhibit biodegradation activities in sediments. For treating lysimeters, we decided to envelope the cups with approximately 100 g of silica flour containing 2% (wt/wt) cupric chloride as a slurry. This high concentration (exceeding the minimum concentration required to fully inhibit biodegradation by more than 100-fold) was used to allow for losses from leaching after rainfall and from suction sampling. Because chlorides are sometimes monitored as tracers in environmental studies, cupric chloride could interfere with chemical analyses, so we also tested copper sulfate. The inhibitory activity of treated silica was demonstrated by placing small amounts of soil and treated silica on nutrient agar plates near soil samples (Fig. 4).

As an indicator of progressive microbial colonization of treated and untreated soil water samplers in the field, we determined CFU in water samples taken over time. Copper-treated silica envelopes reduced microbial activity associated with the lysimeters over the 42-day field trial (Fig. 5). The addition of 100 ml of 2% copper sulfate (12.5 mmol/liter) to the treated lysimeters on day 30 further reduced CFU. Thus, copper salt treatments could be administered either by using treated silica when lysimeters were installed or afterwards by adding the salt solution to the lysimeter.

Colonies formed by plating water taken from copper sulfate-treated lysimeters were very small and difficult to count. Moreover, the numbers of CFU in serial dilutions of copper-containing samples were higher at some higher dilutions. From these results, it appeared that copper was acting as a bacteriostat, preventing metabolic activity in the presence of the metal but not killing the microorganisms so that they could not grow when the metal was diluted. Because colonies observed in copper-containing samples were very
tiny, and considering that copper concentrations originally present in the lysimeters were much higher than that in the nutrient agar, we concluded that microbes in treated lysimeters were inactive. Therefore, CFU for treated lysimeters did not indicate that active microbial colonization had occurred. Instead, these microbes were carried in from surrounding soil water and inactivated by the copper treatment.

At 2 weeks, copper-treated lysimeters (Fig. 5) yielded more soil water than untreated lysimeters (463 ± 54 and 205 ± 68 ml, respectively [mean ± standard error of the mean; n, 3]). Similarly, at 30 days, treated and untreated lysimeters yielded 63 ± 13 and 32 ± 9.2 ml, respectively (mean ± standard error of the mean; n, 3). These observations indicated that microbial growth on cups was lessened, thus allowing soil water to move more freely through uncolonized ceramic walls.

**DISCUSSION**

The advantages of treating soil water samplers with either biocidal or bacteriostatic chemicals are twofold. First, chemical treatment prevents microbial colonization of lysimeter cups. Microbes colonizing the cups clog pores with biofilms that can sorb both biotransformable and nonbiotransformable chemicals. Some sorption, however, would occur even with inactivated bacteria suspended in soil water in and around cups. Nevertheless, inactivation prevents these microbes from growing in the lysimeters and exceeding the concentrations of microbiota in the surrounding soil water. Thus, sorption on inactivated microbes should be comparable to that which occurs with the normal concentrations of microorganisms in soil water nearby.

Second, because of mass-transport limitation of microbial activity in the soil water environment, very rapid biotransformation can occur when water is suctioned into the cup and through surrounding silica flour and soil. Inactivation of microbes in and around the cup should prevent this also. The degree to which microbes associated with lysimeters may affect chemical concentrations depends on several factors. Sorption generally increases with increasing analyte hydrophobicity. Various amounts of some analytes may also undergo ion exchange via ionic bonding with lysimeter-associated microbes. The extent to which this occurs depends upon chemical characteristics of analytes and upon the compositions of the cell surfaces of the particular soil microbes colonizing the lysimeters. Yet another process, biotransformation, will be increasingly important with higher specific transformation rates per cell and as the total numbers of microorganisms degrading the analyte increase. 2,4-DME, for example, which is degraded by almost all types of microorganisms (bacteria, fungi, and algae), was rapidly degraded by microbial growth in lysimeters (Fig. 2 and 3). The other chemicals tested may have been transformed by fewer microorganisms or at lower rates per cell, thus exhibiting lower biotransformation rates in our experiments.

Field tests were conducted under record-setting amounts of rainfall. In spite of the considerable leaching of the copper sulfate that occurred during the first 10 days (Fig. 5), the amount of copper sulfate remaining with the lysimeters was sufficient to inhibit microbial growth throughout the course of a 1-month trial. Future experience with chemically treated lysimeters may show that much lower concentrations of biocides or bacteriostats can give satisfactory results under less rainfall. To assess the effectiveness of treatments over time, water samples taken from lysimeters can be monitored, as was done in these studies, either for microbial activity or for concentrations of the biocide or bacteriostat. When used with copper, for example, lysimeters should be amended with additional copper salt before concentrations fall below 0.1 mM, the minimum concentration needed to fully inhibit biodegradation (6).

Overall, the results obtained in various experiments suggest that soil water samplers should be chemically treated to preclude microbial colonization of lysimeter cups and to prevent enhanced microbial activity during suctioning. Unless soil water samplers are treated, concentrations of analytes that are either microbially sorbed or transformed may be much lower in the lysimeters than in the surrounding soil water. Chemicals potentially affected by microorganisms include organics, such as pesticides and industrial wastes, and inorganic chemicals, such as ammonia and nitrate. Therefore, treating lysimeters is important for studies with these devices to ascertain nutrient concentrations in soil water. Also, errors in determining concentrations of pesticides and other environmental pollutants in soil water can lead to inaccurate assessments of environmental and human health hazards posed by environmental pollution.

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