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HIGHWAY RESEARCH PROGRAM REPORT

**305**

**ENVIRONMENTAL MONITORING AND  
EVALUATION OF  
CALCIUM MAGNESIUM ACETATE  
(CMA)**

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NATIONAL COOPERATIVE HIGHWAY RESEARCH PROGRAM  
REPORT

305

# ENVIRONMENTAL MONITORING AND EVALUATION OF CALCIUM MAGNESIUM ACETATE (CMA)

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RESEARCH SPONSORED BY THE AMERICAN  
ASSOCIATION OF STATE HIGHWAY AND  
TRANSPORTATION OFFICIALS IN COOPERATION  
WITH THE FEDERAL HIGHWAY ADMINISTRATION

## AREAS OF INTEREST:

Environmental Design  
General Materials  
Maintenance  
(Highway Transportation, Air Transportation)

TRANSPORTATION RESEARCH BOARD  
NATIONAL RESEARCH COUNCIL  
WASHINGTON, D.C.

APRIL 1988

## **NATIONAL COOPERATIVE HIGHWAY RESEARCH PROGRAM**

Systematic, well-designed research provides the most effective approach to the solution of many problems facing highway administrators and engineers. Often, highway problems are of local interest and can best be studied by highway departments individually or in cooperation with their state universities and others. However, the accelerating growth of highway transportation develops increasingly complex problems of wide interest to highway authorities. These problems are best studied through a coordinated program of cooperative research.

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The Transportation Research Board of the National Research Council was requested by the Association to administer the research program because of the Board's recognized objectivity and understanding of modern research practices. The Board is uniquely suited for this purpose as: it maintains an extensive committee structure from which authorities on any highway transportation subject may be drawn; it possesses avenues of communications and cooperation with federal, state, and local governmental agencies, universities, and industry; its relationship to the National Research Council is an insurance of objectivity; it maintains a full-time research correlation staff of specialists in highway transportation matters to bring the findings of research directly to those who are in a position to use them.

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The needs for highway research are many, and the National Cooperative Highway Research Program can make significant contributions to the solution of highway transportation problems of mutual concern to many responsible groups. The program, however, is intended to complement rather than to substitute for or duplicate other highway research programs.

## **NCHRP REPORT 305**

Project 4-17 FY '85

ISSN 0077-5614

ISBN 0-309-04602-5

L. C. Catalog Card No. 88-50329

**Price \$13.60**

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The members of the technical committee selected to monitor this project and to review this report were chosen for recognized scholarly competence and with due consideration for the balance of disciplines appropriate to the project. The opinions and conclusions expressed or implied are those of the research agency that performed the research, and, while they have been accepted as appropriate by the technical committee, they are not necessarily those of the Transportation Research Board, the National Research Council, the American Association of State Highway and Transportation officials, or the Federal Highway Administration, U.S. Department of Transportation.

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Published reports of the

**NATIONAL COOPERATIVE HIGHWAY RESEARCH PROGRAM**  
are available from:

Transportation Research Board  
National Research Council  
2101 Constitution Avenue, N.W.  
Washington, D.C. 20418

Printed in the United States of America

# FOREWORD

*By Staff  
Transportation  
Research Board*

This report contains findings from research to examine the environmental effects of calcium magnesium acetate (CMA) through laboratory and field experimentation. Previous research by others has suggested CMA as an alternative to the commonly used chloride-bearing highway deicers. With the completion of this study, state highway agencies considering the use of CMA now have access to information on its environmental effects. Specific guidance has been developed, and, in the few instances where research results are not definitive, field monitoring plans are suggested for use when circumstances dictate a conservative approach to the application of CMA. The research report should be of interest to maintenance engineers, environmental researchers, and all other individuals considering the use of CMA.

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Recognizing the corrosive effects and the environmental deficiencies of conventional deicers, sodium and calcium chloride, the Federal Highway Administration initiated research to find a suitable alternative. As a result, calcium magnesium acetate was identified as a possible alternative deicing chemical. To determine potentially undesirable environmental impacts, a chemically pure CMA was evaluated through laboratory investigation by the California Department of Transportation. That research indicated no significant detrimental effects.

Because the manufacture of pure CMA can be an expensive process, more economical methods have subsequently been pursued. However, the environmental effects of CMA produced through methods more oriented toward the economics of large-scale production may not be comparable to the results derived from pure CMA.

Consequently, the University of Washington, Seattle, Washington, was selected to conduct laboratory and controlled field plot studies to evaluate the transport and the environmental fate of CMA manufactured by the fermentation of corn grain sugars under NCHRP Project 4-17, "Environmental Monitoring and Evaluation of Calcium Magnesium Acetate." The results and guidelines on the use of CMA from an environmental perspective are contained herein.

In addition, the University was to develop a study design for large-scale field testing and monitoring purposes. The intent was to conduct this field study under a second phase of NCHRP research. However, research results from the original contract reduced the level of concern for the environmental effects, and a decision was made not to pursue the field program under the NCHRP. In those instances where definitive guidance can not be made based on the findings of NCHRP Project 4-17, field monitoring methods to be conducted by potential users or researchers are suggested.

Readers are reminded that this report addresses only the environmental effects of CMA. Those interested in its capabilities as a deicer or its effects on other materials

must seek the results of additional research, most of which has been sponsored by the Federal Highway Administration.

For many potential applications, the initial cost of CMA seriously impacts the decision to use it. Presently, CMA is considerably more expensive than the commonly used highway deicers. However, if one considers the long-term effects of the common deicers, the cost of CMA may be more reasonable. Going beyond initial costs is a difficult decision for agencies to make, but this report should help.

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## ACKNOWLEDGMENTS

The research on which this report is based was performed under NCHRP Project 4-17 by the Environmental Engineering and Science Program of the Department of Civil Engineering, the Department of Botany, and the College of Forest Resources, University of Washington.

Richard R. Horner, Research Associate Professor of Civil Engineering, was the principal investigator. The other authors of this report are: Richard B. Walker, Professor of Botany, Emeritus; Brian W. Mar, Professor of Civil Engineering; Stuart E. Strand, Research Associate, College of Forest Resources; Dimitris E. Spyridakis, Associate Professor of Civil Engineering; and Sunirmal Banerjee, Associate Professor of Civil Engineering; Mark V. Brenner, graduate student in Civil Engineering; Richard H. Wagner, graduate student in Botany; and Joseph T. Souther, graduate student in Civil Engineering. Dale A. Carlson, Professor of Civil Engineering, Emeritus, served as a reviewer.

The work was done under the general supervision of Professor Horner.

The co-authors, assisted by graduate student research assistants, were responsible for guiding experiments in the various research task areas as follows: (1) soil physical properties—Professor Banerjee, assisted by Mr. Souther; (2) soil chemical properties—Professor Spyridakis, assisted by Mr. Brenner and Mr. Wagner; (3) botany—Professor Walker, assisted by Mr. Wagner; (4) biodegradation—Dr. Strand, assisted by Mr. Wagner; (5) aquatic ecosystems—Professor Horner, assisted by Mr. Brenner.

A major contribution to the success of this project was made by Stanley D. Humann, manager of the Pack Forest research lands, and his crew. Pack Forest, operated by the University of Washington College of Forest Resources, was the site of the controlled plot field experiments that comprised a part of this research effort. Mr. Humann and his associates rendered many invaluable services throughout the construction and operation of the site.



# ENVIRONMENTAL MONITORING AND EVALUATION OF CALCIUM MAGNESIUM ACETATE (CMA)

## SUMMARY

Calcium magnesium acetate (CMA) has been identified as a material that is potentially effective for deicing highways, feasible to manufacture commercially, and environmentally acceptable. Its environmental acceptability required definitive testing prior to widespread application. The first phase of this testing has been completed. It involved laboratory and controlled field plot experiments designed to determine CMA transport characteristics and environmental fate and its impacts on terrestrial and aquatic ecosystems. The ultimate goal of NCHRP Project 4-17 was to develop interim guidelines for using CMA and for assessing its environmental impact. These guidelines are intended to apply to initial CMA use until verified or modified by actual highway experience and full-scale environmental studies.

Laboratory experiments utilized relatively well-standardized procedures to define potential CMA transport, fate, and effects in the environment. The controlled field plots were designed, constructed, and operated to test laboratory-based conclusions in a setting that was natural but allowed a fairly high degree of experimental control. In both situations CMA applications were based on a preliminary modeling exercise conducted to estimate concentrations and mass loadings of the deicer that would enter the environment from actual highway applications. Following are the principal findings in the various areas of inquiry.

### *CMA Transport from Highways*

The modeling predicted that typical CMA concentrations in spray and runoff from highways would be in the order of magnitude 10 to 100 mg/liter and typical annual mass loadings would be approximately 10 tons/linear-mile (5.5 metric tons/km). The maximum concentration expected in any normal circumstances is 5000 ppm, with washoff of a large CMA application by an intense, small storm. Receiving waters would dilute these concentrations.

### *Effects of CMA on Soil Physical Properties*

CMA did not affect the plasticity, moisture-density characteristics, unconfined compression strength, or shear strength of medium-textured soils. CMA did increase the permeability of these soils by up to 20 times over controls, but the final hydraulic conductivity rates of approximately  $10^{-5}$  cm/s are not believed to be a cause for any concern relative to CMA use or environmental impact.

### *Physicochemical Effects of CMA in Soils*

A significant proportion of applied acetate can be captured on the soil surface and not be transported in runoff. However, major cation and trace metals removals from runoff were unreliable. When infiltrating soils, the majority of acetate is potentially mobile rather than bound. Yet, less than 10 percent of applied acetate appeared in soil water or groundwater in the controlled field plots, where water flushing rates were less extreme than in the laboratory.

Most soils have capacities to immobilize years of calcium and magnesium applications in the amount expected from highways. Laboratory experiments demonstrated a potential to release certain trace metals (iron, aluminum, zinc, and copper), preexisting in soils, through ion exchange reactions involving calcium and magnesium. Field evidence (appearance in shallow groundwater) confirmed this tendency for some trace metal mobilization, but the trend was not as consistent or as strong as in the laboratory. The quantities released in these experiments would pose no environmental hazards, but it is unknown whether mobilization from heavy metal-contaminated, actual roadside soils would do so.

#### *Biodegradation of CMA*

At temperatures of 10 and 20 C, a high level of CMA decomposition was achieved within 2 weeks in soils. However, at 2 C up to 4 weeks were required to degrade the material fully. In field plot soils a large proportion of the CMA biodegradation occurred in the top 3 cm. Soil bacteria counts were temporarily elevated after CMA application.

In water, the ultimate biochemical oxygen demand (BOD) was found to be about 75 percent of applied CMA concentration. The majority of this ultimate BOD was exerted within 5 days at 20 C and 10 days at 10 C, but not until 20 days at 2 C. At the latter temperature it was estimated that 100 days would be required for full decomposition.

The demonstration of retarded CMA decomposition in both soils and water at near freezing temperature indicates that, without warming, acetate would be present in both media for a period of weeks after being washed or sprayed from a highway. Oxygen demand would be exerted gradually under these conditions but much more rapidly at higher temperatures.

#### *Effects of CMA on Plants*

Various herbaceous and woody plant species withstood root zone CMA concentrations up to 2500 ppm. The larger concentrations killed seedlings, probably because of osmotic stress, unless planting was delayed for a day or two, while cations were adsorbed in the soil.

Field applications by spray and flood of 3000 ppm did not affect the yield, cover, vigor, or rooting of herbaceous and woody seedlings or Douglas fir and alder saplings. No external damage symptoms appeared in any of the plant specimens subjected to these treatments.

#### *Effects of CMA on Surface Water Quality*

Oxygen depletion due to CMA addition to water was demonstrated in both the laboratory and the field ponds. CMA concentrations of 100 mg/liter and higher fully depleted oxygen within 2 days in BOD tests. Approximately 10 mg CMA/liter temporarily reduced saturated dissolved oxygen levels in field ponds by about half. This ability for relatively low CMA concentrations to reduce dissolved oxygen in water is the leading potential environmental impact of concern identified by the research.

The only other potential direct surface water quality impact of some concern is phosphorus enrichment of surface waters by CMA produced from agricultural waste containing the nutrient. This enrichment raises the possibility of eutrophication, especially in small ponds and lakes.

#### *Effects of CMA on Aquatic Biota*

Under differing nutrient regimes, laboratory experiments demonstrated potential for CMA both to inhibit and enhance algal growth. However, neither occurred in the field ponds. Because of the production of a fungal food supplement, aquatic invertebrates survived and reproduced in the laboratory optimally at 500 ppm CMA, relative to controls and higher concentrations. At higher levels they were harmed by osmotic stress and low oxygen. Fish (rainbow trout and fathead minnows) appeared to be much more resistant to osmotic stress and, if test chambers were aerated, hatching and survival were high up

to concentrations in excess of 1000 ppm. However, when oxygen was allowed to deplete, mortalities were high. Conditions in the field ponds had no detectable negative effects on invertebrate or fish health, reproduction, or survival.

These findings led to the development of several recommendations to guide the use of CMA in the interim before a full-scale highway application study can be performed. The guidelines are intended to control the location and timing of CMA application, chiefly to avoid the realization of potential impact on aquatic dissolved oxygen resources and of trace metal mobilization.

## CHAPTER ONE

# BACKGROUND AND RESEARCH APPROACH

## HIGHWAY DEICING AGENTS AND THEIR PROBLEMS

Evidence has gathered over the years that highway deicing salts, primarily sodium chloride (NaCl), negatively impact the natural environment and constructed facilities in a variety of ways. Roadside trees and other vegetation have been damaged by direct impaction and soil accumulation of chlorides (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9). Soil property changes also have accompanied salt accumulation (10, 11). Considerable effort has been exerted to document the effects of deicing salts on aquatic ecosystems and domestic water supplies receiving roadway drainage. Natural waters may be affected by density stratification, the promotion of nuisance blue-green algal blooms, and ion exchange with toxic mercury sequestered in the sediments (7, 8, 12). A sufficient increase in the ionic concentration can alter the ionic balance in aquatic organisms. High chloride concentration imparts unpleasant taste and odor to drinking water, while sodium (Na) endangers hypertensive human consumers (13, 14, 15). Special problems associated with deicing are additives and contaminants in the agent, prominently including ferrocyanides added to reduce caking (7, 16). Chloride accelerates metal corrosion, causing substantial economic damage to public property and private vehicle owners (17). Bridge decks and underground utilities are especially vulnerable (18, 19). Portland cement also exhibits relatively poor resistance to chloride salts (20, 21).

Recognition of the numerous drawbacks of continued use of sodium and calcium chlorides for highway deicing has stimulated substantial thought and effort to identify alternatives. A deicing agent operates through solvent freezing point depression by the solute molecules or ions. The degree of depression is dependent chiefly on the number of molecules or ions in solution. Therefore, materials of relatively low molecular weight and high solubility are preferred, because they offer the maximum effect on the melting point per unit weight applied.

Dunn and Schenk (22) reported on a comprehensive investigation to identify alternative highway deicing agents. They screened numerous compounds on the basis of properties, cost, availability, toxicity, and potential to affect the natural and man-

made environments. Two candidates, methanol and calcium magnesium acetate (CMA), were selected. The latter has received most of the attention, probably because of its greater persistence. Urea also has been used experimentally and in some regular applications as a highway deicer. While performance reports are generally positive (23, 24, 25), the nitrogen content of urea could promote eutrophication of surface receiving waters.

## PREVIOUS INVESTIGATIONS OF CALCIUM MAGNESIUM ACETATE

With identification of CMA as a promising alternative to chloride salts, the Federal Highway Administration (FHWA) sponsored several research efforts to determine its environmental acceptability, manufacturing technology, and technical and economic feasibility (26). Investigation of CMA manufacture established that the most feasible method is to react acetic acid with dolomitic lime (26). Many ways of producing acetic acid were studied. The selected process employs an anaerobic, thermophilic bacterium, *Clostridium thermoaceticum*, to ferment biomass-derived sugars, with shelled corn being the preferred feedstock. Recently completed was additional research at the University of Georgia to develop a mutant strain of the bacterium that can tolerate low pH and high calcium (Ca), magnesium (Mg), and acetate concentrations, as well as improve productivity, yield, and selectivity (27). The purity of the eventual commercial material depends largely on trace metals in the dolomite. It has not been necessary to use additives to improve CMA handling characteristics (28).

For performance evaluation of CMA in the field, 200 tons (181.8 metric tons) were produced by reacting purchased acetic acid with dolomitic lime. This quantity was distributed in small lots among 24 states and in larger volumes to the States of Michigan and Washington for highway deicing evaluations under actual conditions. Results reported by several states agree that CMA is capable of achieving a degree of bare pavement equal to that realized with sodium chloride but that the latter

acts slightly faster (24, 26). Washington State reported CMA performance equivalent to that of urea but experienced some minor, but manageable, handling problems due to dust and the relatively low density of CMA (24, 25). Other studies of CMA performance are underway in Sweden (29) and Austria (30).

The California Department of Transportation (CalTrans) performed the most extensive investigation of CMA environmental effects prior to this research. The CalTrans work included aquatic bioassays on fish, macroinvertebrates, zooplankton, and phytoplankton; assessment of the effects of irrigation and foliar CMA applications to terrestrial plants; and an evaluation of CMA effects on soils. This research employed CMA produced from reagents in small quantities. The following paragraphs discuss its results.

Both acute static and chronic water renewal fish bioassays were performed, the former on fathead minnow (*Pimephales promelas*) and rainbow trout (*Salmo gairdneri*) and the latter to evaluate the effects of CMA on hatching and development of rainbow trout. Magnesium and calcium acetates separately, an equimolar mixture of the two, and sodium chloride were tested in the short-term static bioassays. The following 96-hr LC<sub>50</sub> values indicate the relative abilities of the compounds to cause mortalities in the test specimens (31):

|                   | Rainbow Trout<br>(mg/liter) | Fathead Minnow<br>(mg/liter) |
|-------------------|-----------------------------|------------------------------|
| Magnesium Acetate | 4,300                       | 9,000                        |
| Calcium Acetate   | 16,200                      | 14,300                       |
| Equimolar Mixture | 18,700                      | —                            |
| Sodium Chloride   | 12,200                      | 11,400                       |

Thus, calcium acetate alone and the mixture of acetates were less lethal to rainbow trout than NaCl.

In the CalTrans chronic tests, a continuously maintained 5000 mg/liter concentration of equimolar CMA slightly delayed hatching and was associated with some hatching difficulty in a small number of cases but did not reduce its ultimate success (31). Bioassays at lower CMA concentrations (1, 50, 100, 500 and 1000 mg/liter) exhibited no significant hatching delays or difficulty.

CalTrans performed similar series of acute and chronic bioassays on the zooplankton *Daphnia magna*. The 96-hr LC<sub>50</sub> values for CMA and NaCl were 445 and 2450 mg/liter, respectively (31). The long-term bioassays reflected the same trend. Together, the results indicate that observable effects on *D. magna* occur between 125 and 250 mg/liter of CMA but not until approximately 2500 mg/liter of NaCl (31). Some CMA test jars developed bacterial blooms and complete mortality, apparently because of oxygen depletion.

The phytoplankters *Selenastrum capricornutum* and *Anabaena flosaquae* were exposed to NaCl (1 to 1000 mg/liter), CMA (1.67 to 1671 mg/liter), Ca acetate (15.07 to 1507 mg/liter), and Mg acetate (18.35 to 1835 mg/liter) in the CalTrans laboratory. Cell volumes were compared with controls over a 14-day growth period. The separate acetates and the equimolar mixture all induced significant inhibition at concentrations above 83.55 mg/liter, whereas NaCl did not significantly reduce growth at any concentration (31).

Macroinvertebrate bioassays on the chironomid *Cheromus plusmosis* were inconclusive (26).

CalTrans exposed 18 species of terrestrial plants to CMA and NaCl in irrigation and foliar applications. In the irrigation ex-

periments, nine species were more severely damaged by NaCl and one by CMA. In the remainder, the differences were not sufficient for comparison. When sprayed on foliage, the NaCl application caused greater damage than CMA to 17 of the 18 species (31).

CalTrans exposed soils from various parts of the United States to a 1 Normal CMA solution in laboratory lysimeters. The principal result of this study was that CMA is capable of extracting substantial amounts of iron, aluminum, sodium, hydrolyzable orthophosphate, and potassium from soil (31).

Several other investigations of some aspect of CMA environmental effects have also been reported. Work sponsored by the Austrian Federal Ministry for Construction and Technology (30) determined that, while acetic acid degrades very rapidly, CMA is more stable when applied in large amounts (5 g CMA/50 g soil or plant material). Experiments were also carried out in pots and in the field to determine the effects of CMA on plants. Pot trials demonstrated favorable effects of CMA relative to NaCl on wheat, rape, and cress. With high applications in the field (60 to 100 g CMA/m<sup>2</sup>), no adverse symptoms appeared in exposed spruce, pine, maple, or salt-tolerant shrubs.

In Central Alaska three small ponds were treated with CMA and compared to three control ponds (32). The ponds were observed for cation concentrations, vascular plant standing crop, algal colonization on artificial substrates, and dissolved oxygen. While calcium levels were substantially elevated in treatment ponds, dissolved oxygen levels and algal growth did not differ significantly between the two sets of ponds. However, there were no data given on the loadings or concentrations of CMA introduced to the ponds.

Elliott and Linn (33) recently reported on laboratory batch and column experiments designed to measure the effect of CMA on copper and zinc mobility. In batch suspensions in the pH range 3 to 7, CMA with a 3:7 Ca/Mg molar ratio in two concentrations (0.01 and 0.1 M) substantially enhanced Cu and Zn desorption. In acidified soil columns CMA initially increased metal efflux. However, within 10 pore volume displacement net suppression of leachate Cu and Zn occurred, apparently because of the neutralizing effect of acetate. The authors speculated that highway deicing with CMA may temporarily increase translocation of metals, but that sustained use would inhibit heavy metal mobility.

A different concern is represented by a study performed in Buffalo, New York, concerning the potential impact of widespread CMA use on sewage treatment plant operations (34). This study concluded that partial or complete substitution of CMA for salt in the Buffalo area, much of which is served by combined storm/sanitary sewers, would significantly increase treatment plant organic loadings and affect operations.

## PREVIOUS INVESTIGATIONS OF CMA COMPONENTS

### General

In addition to the investigations of CMA itself reported above, review of previous work performed on its calcium, magnesium, and acetate components separately was helpful in defining the issues at the outset of this research.

From the general standpoint, CMA might be expected to increase water hardness, which is composed chiefly of calcium and magnesium ions. Acetate has the potential to exert an ox-

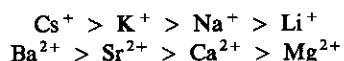
xygen demand on surface receiving water during biodegradation, but the demand would be created during a period of the year when oxygen solubility is maximized by relatively low temperature. Calcium and magnesium have a stronger tendency than sodium to precipitate as carbonates, thereby having less effect on water density and the turnover of lakes (35, 36). No components of pure CMA are toxic, and the material does not present the potential of causing or aggravating hypertension, as do sodium salts. However, impurities introduced in manufacture or handling could be toxic to plants, aquatic life, and humans. CMA appears to have less potential than NaCl to impact soil chemistry and physics negatively. No chloride would affect chemistry, and divalent cations (Ca and Mg) tend to improve soil structure, whereas monovalent Na has a tendency to cause structural breakdown (37, 38, 39). The result of such breakdown is reduced air and water permeability (40).

### Ion Exchange Chemistry

Of special interest is the relative ability of added sodium, versus calcium and magnesium, to exchange with and mobilize other metals already present in the receiving soils. This interest was stimulated by the CalTrans finding of iron and aluminum release from CMA-treated soils. The question is, do Ca and Mg have a substantially greater tendency to cause such metal mobility than does Na, which is already widely distributed in the environment by deicing activities? This question was pursued theoretically by exploring the literature of ion exchange chemistry.

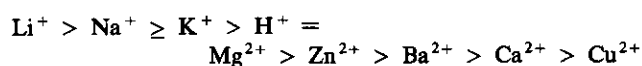
Interactions between soil particles and soil solution metals are primarily electrostatic in nature. They are influenced mainly by the valence of the metal and the charge density of the soil particle involved. Some solid phase-soil solution interactions are specific to the ion involved, and include ion-dipole and ion-induced dipole interaction between the counter ions and water molecules (41).

Although the metal selectivity varies with the type of exchanger, the following exchanger affinity sequence for the monovalent and divalent cations, respectively, has been demonstrated, where hydration predominates over electrostatic interactions:



This sequence means, for example, that Na will be given up to solution in preference to K. However, as electrostatic forces become dominant, the above sequence will tend to be reversed, so that affinity of the exchange increases with decreasing atomic radius of the solute ion (42). These circumstances normally prevail in clay soils, while hydration may be more dominant in sands.

In comparing mono- and divalent metals, the affinity of exchangers for the latter is generally larger than for monovalent ions. However, this selectivity for ions of higher valence decreases as the ionic strength of the solution increases. The order of displacement of metals from a variety of exchangers, such as clays, resins, and undecomposed organic matter, follows, in general terms, the valence laws and lyotropic series (43):



Accordingly, the monovalent ions have a higher tendency toward displacement into solution than the divalent metals, which are more strongly held by the exchanger. Therefore, under conditions of relatively low ionic strength, sodium has less tendency than calcium or magnesium to exchange with and mobilize bound metals.

The tendency expressed by the above sequence can be affected by changing selectivity of ion exchangers with metal concentration. As sodium concentration increases in soil solution relative to concentrations of other metals, there have been observed exchanges of  $\text{Na}^+$  for bound  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$ , and other divalent metals. This phenomenon is very well documented in the literature pertaining to arid land irrigation and wastewater applications on soils (44, 45).

As a result of this investigation of the pertinent literature, it may be concluded that sodium chloride deicers normally would exhibit somewhat less tendency than CMA to displace bound trace metals into soil solution. The only exception to this general tendency should be the unusual case where soil has been heavily amended with sodium through seawater inundation, deposition from irrigation waters, or, perhaps, very heavy past NaCl deicing. Establishing the significance of this tendency in actual cases awaits highway scale trials and soil water monitoring.

### Transport and Fate

The literature offers little on the transport and environmental fate of Ca, Mg, and acetates in general. Some understanding can be inferred, or at least hypotheses can be proposed, on the basis of the well-known Ca and Mg chemistry and some work done on nitrilotriacetate (NTA) performed in the 1970's. NTA was considered as a substitute for phosphate builders in detergents until concerns with toxicity developed.

Calcium concentrations in natural waters are controlled primarily by carbonate precipitation depending on pH. Magnesium carbonate relationships are not as simple as those of calcium because of the presence of various forms having different solubilities. Among the common forms, magnesite,  $\text{MgCO}_3$ ; nesquehonite,  $\text{MgCO}_3 \cdot 3\text{H}_2\text{O}$ ; and lansfordite,  $\text{MgCO}_3 \cdot 5\text{H}_2\text{O}$ ; are more soluble than calcite,  $\text{CaCO}_3$ . However, hydromagnesite,  $\text{Mg}_4\text{CO}_3(\text{OH})_2 \cdot 3\text{H}_2\text{O}$ , is quite insoluble (35). Precipitation of the less soluble species can limit Mg concentrations in water, depending on conditions. In contrast, there are no important precipitation reactions that can maintain low sodium concentrations if a supply exists.

Acetate presents a readily usable carbon source for microorganisms, offering favorable energetics for cell production (46). Tiedje and Mason (47) studied the biodegradation of NTA in soils by measuring the total  $\text{CO}_2$  and  $^{14}\text{CO}_2$  production. The degradation rate increased with increased NTA applied but did not depend on pH, drainage, soil texture, or plant cover. Degradation was essentially complete within 30 days at temperatures of 12.5 and 24 C. It even occurred at 2 C if previously acclimatized at 12.5 C to develop a microbial population. However,  $^{14}\text{CO}_2$  production did not occur anaerobically. One possible intermediate, iminodiacetate, was identified. Other investigators (48) identified glycine and ammonia as intermediates. With CMA being composed of organic molecules simpler than NTA, it is reasonable to hypothesize that its biodegradation would occur more rapidly and without forming intermediates. Reactions could occur in environmental media to form secondary organic products, but these are not considered likely.

Other work with NTA investigated its chemical relationships with the metals. The National Academy of Sciences (49) reported a strong affinity to form salts with iron, calcium, magnesium, and zinc. Gregor (50) and Hamilton (51) observed that NTA can be responsible for solubilizing normally insoluble complexes of lead and other metals. Nilsson (52) found that copper and lead concentrations in biologically treated wastewater after sulfate flocculation were a function of the amount of NTA present. Stumm and Morgan (41) pointed out that, while organic ligands have the potential to form soluble metal chelates in natural waters, the effect of competition by Ca and Mg, present in much larger concentrations, should overcome this tendency. In the case of CMA, these elements would be added along with the organic ligand. Nevertheless, potential mobilization of metals under certain conditions by CMA is an issue that requires in situ highway study.

## RESEARCH QUESTIONS AND GENERAL APPROACH

The results of the limited previous research on CMA environmental effects suggested a number of questions, listed below, to be considered in this research. Answering these questions constituted the basic objectives of the program.

1. *Transport and environmental fate:*
  - a. What is the composition of production-run CMA, including impurities and additives, likely to be used in actual highway application?
  - b. What are the rates and mechanisms of transport of CMA and its impurities through vegetation, soils, surface water, and groundwater to the ultimate sinks?
  - c. What are the rates and mechanisms of accumulation and decay of CMA and its impurities in soil, soil water, terrestrial vegetation, surface water, aquatic biota, and groundwater?
  - d. Do biological or chemical reactions in these environmental media form secondary organic or inorganic products or mobilize potentially toxic substances such that their biological availability increases?
2. *Impacts on soils, water, and groundwater:*
  - a. What effects do CMA and its impurities have on soil chemistry and physics relative to controls?
  - b. What effects do CMA and its impurities have on the quality of soil water and groundwater relative to controls?
3. *Impacts on terrestrial ecosystem:*
  - a. What effects do CMA and its impurities have on typical roadside vegetation relative to controls?
  - b. How do the overall assemblages of plants and animals compare on control sites and those treated with CMA?
4. *Impacts on runoff water and surface receiving water ecosystems:*
  - a. What effects do CMA and its impurities have on the quality of runoff water relative to controls?
  - b. What effects do CMA and its impurities have on fish, macroinvertebrates, zooplankton, and phytoplankton relative to controls?
  - c. What is the biochemical oxygen demand (BOD) of CMA at various concentrations in water?

Answers to these questions were intended to serve as a basis for developing interim guidelines for using CMA in actual high-

way practice and for preparing environmental impact assessments regarding CMA use.

To answer the questions posed, a program of laboratory and controlled field plot studies was outlined. Laboratory studies, for the most part, were assays conducted according to standard procedures. These experiments were designed to determine the properties of CMA in environmental media; the types of effects CMA exposure could create on soils, vegetation, and aquatic biota; and the potential degree of those effects. The philosophy in laboratory assays was to create a range of CMA exposures that would produce effects ranging from none to severe. As a guide for experimental planning, preliminary modeling was used to predict typical and worst-case exposures in actual highway applications (see Appendix C). In some cases, when effects did not appear even with worst-case exposure, higher CMA levels were applied to define fully the responses of the test specimens. All laboratory experiments were conducted with untreated controls, as well as CMA-treated vessels, and were replicated.

Controlled field plots were small terrestrial and aquatic communities established in the natural environment, but with a greater degree of control than would be afforded by full-scale systems at highway locations. One purpose of experimentation at controlled field plots was to verify laboratory findings in natural conditions. The plots also permitted extending the study to investigate environmental transport of CMA on the surface and through the soil, and the interrelationships of organisms living in diverse communities. Controlled plots were operated during two winter application seasons and were monitored before, during, and after those seasons. Only one level of CMA exposure was applied in each of the winters. These levels (differing in mass loading but not in concentration in the two winters) were relatively high in the range expected in actual highway use, in order to estimate maximum effects that might occur in practice. Replicated experiments were conducted in both control and CMA-treatment plot systems. Soil bins and boxes were adjuncts to the controlled field plots used to supplement the vegetation studies and investigate some variation in exposure level in relatively natural conditions.

The following two sections generally describe the laboratory and controlled plot experiments, respectively. Specific methods are presented in the appendices.

## LABORATORY EXPERIMENTS

The laboratory portion of the research comprised the following experiments: analysis of CMA properties, effects of CMA on soil physical properties, effects of CMA on soil chemical properties, effects of CMA on terrestrial vegetation, biodegradation of CMA in soils and water, and bioassays on aquatic organisms.

Initial CMA analysis was performed to characterize it for purposes of planning laboratory and field experiments. This analysis concentrated on establishing the levels of acetate and related compounds and various metals present.

Soil physical and chemical experiments were performed on four soils selected to represent a range of soil properties typical of highway rights-of-way. In particular, the soils selected offered ranges of organic and calcium carbonate content and cation exchange capacity. They ranged over the medium textures from sandy loam and silt loam to loam, although very fine textured soils were not represented.

For laboratory assay of plant responses to CMA exposure,

greenhouse pot tests were run on six species planted in two of the soils used for soil testing. Pots were observed for survival, phenological abnormalities, and shoot and root yields.

Study of CMA biodegradation in soils was performed by measurement of carbon dioxide ( $\text{CO}_2$ ) evolved in the respiration process. Incubations were carried out in the four soils used in soil testing at two temperatures. CMA decomposition in water was investigated through biochemical oxygen demand (BOD) measurement.

Bioassays were performed on four types of aquatic organisms: fish (two species), macroinvertebrates, zooplankton, and algae. In the cases of fish and zooplankton, both acute (4-day) and chronic tests were conducted. Bioassays on macroinvertebrates and algae extended over approximately 2 weeks.

## CONTROLLED PLOT EXPERIMENTS

### Site Selection

Controlled plots were sited according to the following criteria:

1. General winter/spring climate common to a large portion of the continental United States.
2. Soils neither atypically shallow or deep and of generally moderate permeability.
3. General topography and vegetation typical of roadsides.
4. Accessible groundwater without the need for expensive well drilling.
5. Availability of a water supply.
6. Convenience to the University of Washington.
7. Facilities suitable for carrying out experiments and offering security.

The site selected was at Pack Forest, a large tract maintained for research purposes by the University of Washington College of Forest Resources. Pack Forest is located in the Cascade Mountain foothills approximately 90 km southeast of Seattle. There is a resident manager, a crew, and equipment available for the support of research. The tract offered a number of potential locations to establish the plots. The site selected is in a large open area known as Murphy's Ranch that had previously been pasture. At elevation 366 m and receiving cold air drainage from nearby Mount Rainier, the site experiences considerably colder winter conditions than the Puget Sound lowlands. Soils, topography, and vegetation types fit the criteria. Two surface water sources are accessible at this site, and the permanent water table is within 3 m of the surface throughout the year.

### Controlled Plot Configuration

Controlled plot experiments were designed to simulate distribution of CMA from highway surfaces to adjacent soils, vegetation, and water. CMA transport in water solution may be through spray, in surface runoff, or by both processes. Rising slopes immediately adjacent to the pavement would receive spray alone, while contiguous falling slopes would receive both spray and runoff. Spray would not reach areas farther from the pavement; however, surface runoff may do so. Controlled plots were treated to distinguish the effects of spray versus flood (runoff) treatment, as well as those of CMA solution versus water not containing CMA (experimental controls).

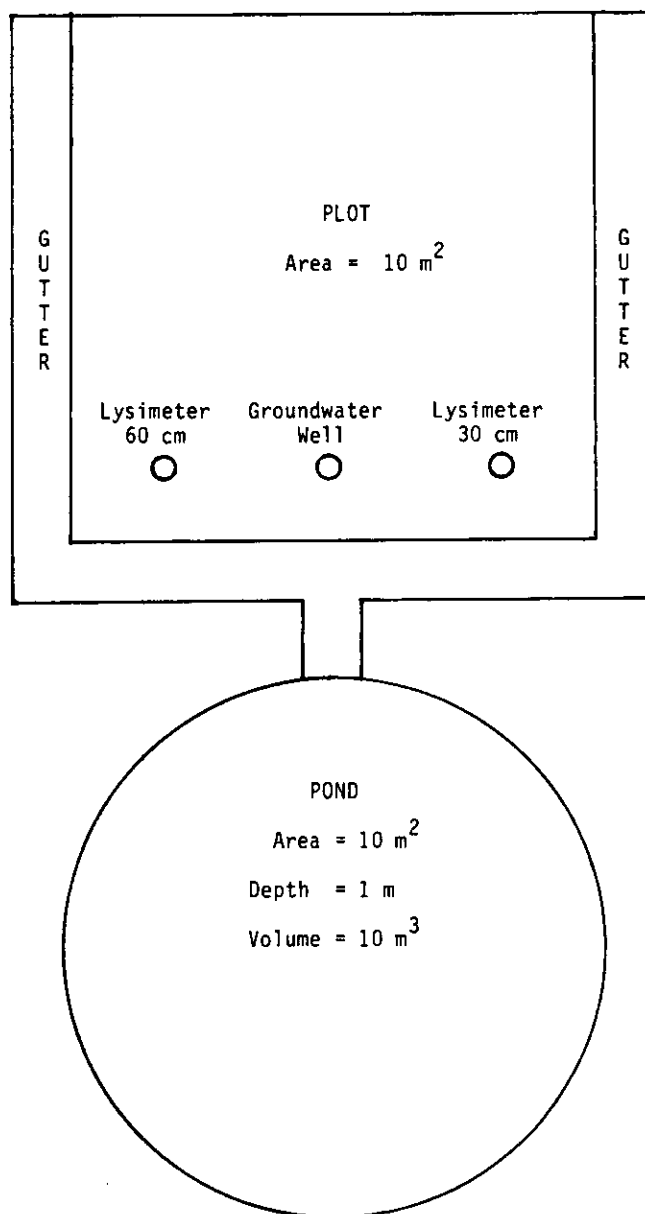


Figure 1. Schematic of a controlled field plot system.

In order to provide duplicate plot systems for CMA-spray, CMA-flood, water-spray, and water-flood treatments, eight areas were prepared as identically as possible. Each area consisted of a plot for terrestrial experiments, a pond for aquatic experiments, and a trench system to collect runoff from the plot and channel it to the pond. The eight systems were constructed along a north-south line and spaced approximately 7 m apart. Figure 1 illustrates a controlled plot schematically, and Figure 2 is a photograph of one system. Figure 3 portrays three of the eight systems together, along with the nearby bins. The terrestrial plots were squares of  $10 \text{ m}^2$  area. They were established on slopes of 8 percent by excavating and filling as necessary. Care was taken to use similar native topsoil obtained immediately adjacent to the plots to fill in all plots when necessary. After shaping, plots were rototilled, raked, and fertilized. They



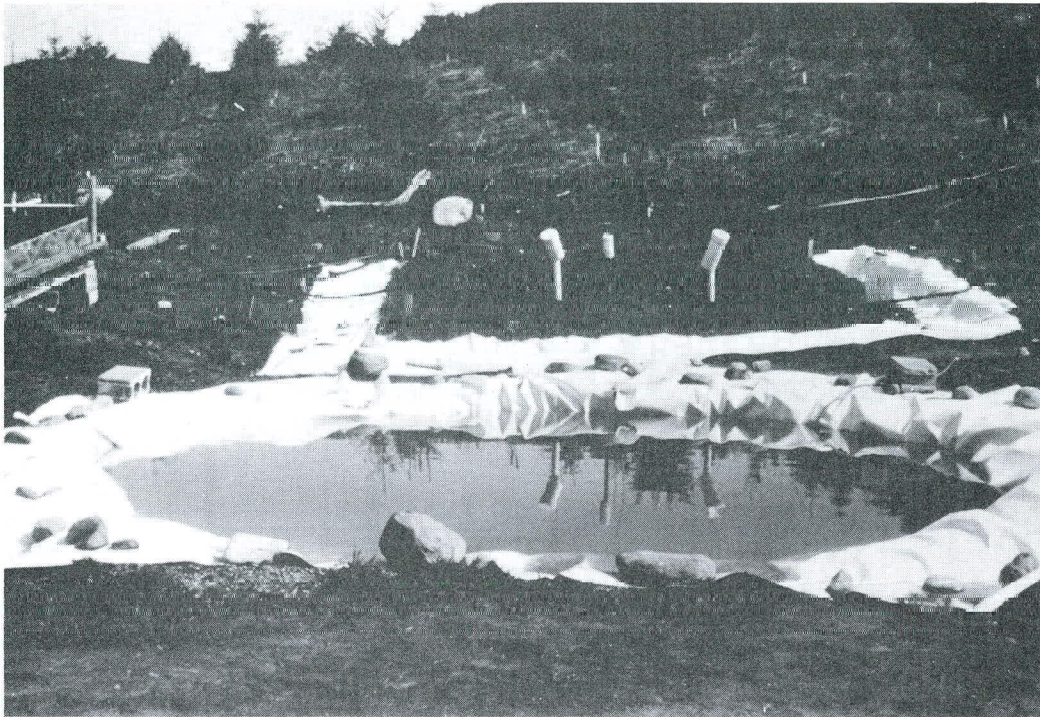


Figure 2. Photograph of a controlled field plot system.

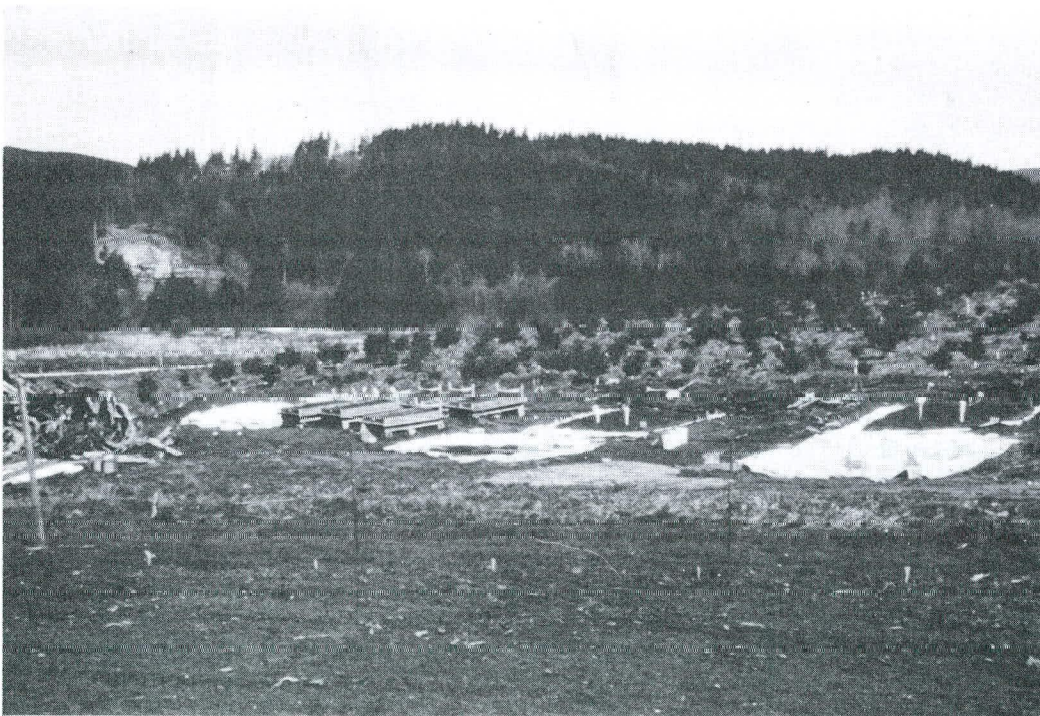


Figure 3. Photograph of field plots and bins.

then were seeded in June of 1985, with 84 g/plot of red fescue (*Festuca rubra*), a grass widely used on roadsides at various elevations, and 16.8 g/plot of Dutch white clover (*Trifolium repens*), a legume also commonly planted along highways in conjunction with grasses. After seeding, each plot received sieved Canadian peat moss. Also planted were seedlings of the following species: buckhorn plantain (*Plantago lanceolata*), common on roadside shoulders in much of the nation; buttercup (*Ranunculus acris*), also widely distributed; Douglas fir (*Pseudotsuga*

*menziesii*), a representative conifer; and red maple (*Acer rubra*), a deciduous woody species representing a genus that occurs near roads in various parts of the country subject to deicing.

Suction lysimeters and a well point were installed in each plot to sample soil water and groundwater, respectively. Lysimeters were placed for sampling at 30 and 60 cm depths, while well points were driven 3 m deep. Appendix C gives additional detail on the lysimeters and well points.

A drainage system was established to prevent surface runoff



or interflow in the unsaturated soil horizons from entering the plots from offsite. This system consisted of an interception trench upslope from the plots, a polyethylene sheeting barrier to the depth of the deeper lysimeter along the upslope edge of each plot, and drainage connections to an existing ditch below the plots.

Ponds were excavated 1 m deep to provide 10 m<sup>3</sup> water volumes. They were approximately round in shape with diameters of about 3.6 m. They were lined with 19-mil thick reinforced polyvinyl chloride (PVC) to provide a seal. Preliminary tests were conducted to ensure that the liner would not be toxic to aquatic life. Then, mixed-size gravel and cobbles were placed on the beds of the ponds to provide a natural substrate for invertebrate and periphyton habitat. The ponds were initially stocked with several species each of algae, zooplankton, and invertebrates obtained from laboratory sources, as well as bluegill sunfish and fathead minnows from hatcheries. Smaller life forms also colonized from the supply pond and the surrounding environment.

Shallow trenches were dug along three sites of each plot to catch side- and end-slope runoff. A trench was then extended to discharge to the pond associated with each plot. Trenches were lined with the same PVC sheeting as used for ponds.

The main water supply for ponds and site treatments came from a former farm pond located 200 m higher in elevation and approximately 1 km from the plot site. Water was conveyed to the site by gravity through 0.75-in. (1.9 cm) internal diameter polyethylene pipe. An auxiliary water supply, available in the winter, was created by damming an intermittent stream just south of the site. At the site, water could be directed to the plots (for irrigation) or ponds or into supply tanks through a valving system. Local distribution was via 1-in. (2.5 cm) internal diameter polyethylene pipe.

Two previously unused supply tanks were placed at a slightly higher elevation than the plots, one for CMA solution and one for control water. These tanks are fiberglass and have capacities of approximately 1350 l. Distribution of CMA or water to the flood plots during treatments was via perforated 0.5-in. (1.3 cm) internal diameter PVC pipes placed parallel to the top of the slope on each flood plot. During the first winter treatments, distribution was by gravity, but a small gasoline engine-driven pump was used in the second winter. Distribution to spray plots was by gravity through garden sprayers (5/plot) during the first winter. Uniformity of coverage was improved the second winter by pumping through a hand-held hose nozzle. The volume of treatments was controlled by measuring with calibrated site glasses on the tanks during the first winter and by metering from calibrated 55-gal drums during the second treatment season. The latter system offered greater convenience and more accurate control.

A secondary, but supplementary, exercise involved CMA and water (control) treatment of six bins containing soil and vegetation located outside the Botany Greenhouse on the University of Washington campus and six identical bins at the higher elevation field site. These bins allowed more variation in the tests than the plots alone would and increased the experimental control on botanical work. Bins were constructed of exterior plywood to dimensions of 1 m width, 2 m length, and 30 cm depth. They were fitted with drains and placed on concrete blocks to lie at the same 8 percent slope as the field plots. Bins were filled with soil from the Pack Forest field site and planted in the same manner as the plots. Bins were fitted for both spray and flood

applications as described above for the plots. Distribution at the Pack Forest bins was the same as to the plots. Campus bins were supplied from calibrated 55-gal drums under air pressure during the first winter and by pumping during the second.

Smaller (0.5 m<sup>2</sup>) duplicate soil boxes near the field plots were planted with Douglas fir, balsam fir (*Abies balsamina*), and red maple seedlings and treated by spray with water (controls) and CMA during the second winter. These boxes were necessitated when poor tree seedling survival resulted in all bins and plots, due to shading and competition by vigorously growing grasses.

To provide observations of CMA effects on larger tree specimens, two sets of four Douglas firs each about 1 m in height were transplanted to the field site. Similar sets of red alder (*Alnus rubra*) about 3 m high growing near the field plots were tagged. These sets were sprayed with CMA solution or water during the second winter.

### Controlled Plot Operation

The preliminary modeling exercise (detailed in Appendix C) and certain laboratory results guided design of the controlled plot treatment protocol. The fundamental basis for CMA treatments was the annual mass loading estimated to result from runoff from a highway segment receiving a typical winter application. By basing loading on 250-lb CMA/highway-mile (69 kg/highway-km) for each storm, 20 annual storms and a plot length of 10.4 ft (3.2 m) on a side imagined to parallel a highway, each plot would receive a 4.5 kg CMA/year loading.

The next question concerned the hydraulic loading. The volume of water that could be supplied from the tanks on any one treatment occasion was somewhat limited by tank size and time. Therefore, it was desirable to apply the selected mass loading at a relatively high concentration that still is within realistic limits and is known not to be lethal to plants that would receive the direct spray or flood. The division between the soil solution conductivity at which very sensitive plants may be affected and where yields of many plants may be affected is approximately 4 mmho/cm (44). The salt concentration equivalent to that conductivity is 45 milliequivalents per liter. To hold salt concentration below that level (at 40 meq/liter) requires that CMA concentrations be limited to no more than 3000 mg/liter. That concentration was shown in pot tests to cause some effects to the most sensitive plants but not widespread mortality (see Appendix H). Therefore, it is feasible from the botanical standpoint.

A concentration of 3000 mg CMA/liter was also shown to cause lethality in *Daphnia* and *Hyallela* in water and to suppress algae (see Appendixes K and L). However, the solution applied to the plots was treated by vegetation before draining to the ponds, where it was then greatly diluted. Therefore, 3000 mg/liter also seemed to be feasible from the aquatic biology standpoint.

Modeling predicted that the worst-case CMA concentration in actual highway runoff would be 5000 mg/liter. Ordinary concentrations should be an order of magnitude or more lower. Therefore, application of 3000 mg/liter represented a relatively severe case. If no effects were to be observed at that concentration, none would occur at lesser concentration.

The dilution water volume needed to supply the selected annual loading at 3000 mg CMA/liter was 300 liters/plot for each of five applications. A total annual hydraulic loading of 1500 liters/plot corresponds to 15 cm/year, or about 6 in., of

runoff over the entire surface. That hydraulic loading, in turn, is equivalent to about 50 to 80 in./winter of snow, depending on density. Such a hydraulic loading appeared to simulate a natural condition rather well and was applied to all CMA and control plots during the first winter.

After the first winter's treatments, it was observed that effects attributable to CMA were few and never very dramatic. This result was extremely useful because it demonstrated that a season's realistic loadings, delivered in relatively concentrated form (3000 mg CMA/liter), did not produce severe environmental impacts on somewhat controlled, but largely natural, terrestrial and aquatic communities. Nevertheless, there were some good reasons to elevate these loadings for the final winter's experimentation. One reason was the desire to determine whether any effect thresholds exist at somewhat higher but still realistic loadings. A second reason was to cover situations where annual loadings could be higher than those estimated as described above. Such situations could result in areas receiving more storms and/or greater snowfall, using more CMA per unit area per storm, or having larger highway surface areas. A third reason for seeking more experimental flexibility was to investigate more completely the aquatic effects of direct runoff to the ponds. The first winter's treatments produced little immediate runoff that entered the ponds (most applied water percolated into the plots).

Accordingly, both the hydraulic and CMA mass loadings to the plots were increased for the second winter. The maximum concentration of 3000 mg CMA/liter was not increased, for reasons discussed above. Without raising concentrations, it was necessary to apply more water in order to achieve higher CMA loadings. The additional water also represented higher hydraulic loadings not considered in the first year's experiments.

A summary of the two winter's treatment protocols follows:

| Test Series                                     | Year 1 | Year 2 |
|---|--------|--------|
| CMA concentration (mg/liter)                    | 3000   | 3000   |
| Annual plot loading (kg CMA)                    | 4.5    | 9.0    |
| Areal plot loading (g CMA/m <sup>2</sup> -year) | 450    | 900    |
| Plot annual hydraulic loading (liters of water) | 1500   | 3000   |
| Plot annual hydraulic loading (cm water/year)   | 15     | 30     |
| Number of treatments                            | 5      | 5      |

The five treatments during the second winter were conducted between December 18, 1986, and February 17, 1987. All of the first year's treatments were in March of 1986, because of finishing work needed at the site before treatments could begin. Treatments were allocated randomly among plots as follows. Plots are numbered from north-to-south.

|                    |                    |
|--------------------|--------------------|
| Plot 1 CMA-Spray   | Plot 5 CMA-Flood   |
| Plot 2 Water-Spray | Plot 6 Water-Flood |
| Plot 3 CMA-Spray   | Plot 7 Water-Flood |
| Plot 4 Water-Spray | Plot 8 CMA-Flood   |

Bins had 20 percent of the surface area of plots. Therefore, treatment of bins equivalent to the first winter's plot treatments required 0.9 kg CMA/year and 60 liters water treatment. It was decided to modify this application regime on bins in order to diversify the experiments as follows:

1. Combined spray and flood treatment at the same CMA and hydraulic loadings and same CMA concentration received by the plots (greenhouse bins 1 [control] and 4 [CMA] and field bins 7 [control] and 10 [CMA]).

2. Flood treatment at the same CMA mass loading but twice the hydraulic loading and half the CMA concentration as received by plots (greenhouse bins 2 [control] and 5 [CMA] and field bins 8 [control] and 11 [CMA]).

3. Flood treatment at 50 percent greater CMA mass and hydraulic loadings and the same CMA concentration as received by plots (greenhouse bins 3 [control] and 6 [CMA] and field bins 9 [control] and 12 [CMA]).

The purpose of the first series was to investigate the effects of receiving both spray and flood applications, in comparison to the single application mode on the plots. The second and third series were intended to reveal any differences caused by higher hydraulic or CMA mass loadings at the same or a reduced concentration.

During the second winter, the same application sequence was applied to the bins, but hydraulic and CMA mass loadings were again doubled, just as on the plots. A summary of the two winters' bin treatments follows:

| Test Series   | Year 1 | Year 2 |
|---|--------|--------|
| A. Combined spray/flood, CMA and hydraulic loadings and CMA concentration = plots:                      |        |        |
| mg CMA/liter  | 3000   | 3000   |
| kg CMA  | 0.9    | 1.8    |
| g CMA/m <sup>2</sup> -yr  | 450    | 900    |
| liters water/yr   | 300    | 600    |
| cm water/yr   | 15     | 30     |
| B. Flood at CMA mass loading = plots but twice the hydraulic loading and one-half the CMA concentration |        |        |
| mg CMA/liter  | 1500   | 1500   |
| kg CMA  | 0.9    | 1.8    |
| g CMA/m <sup>2</sup> -yr  | 450    | 900    |
| liters water/yr   | 600    | 1200   |
| cm water/yr   | 30     | 60     |
| C. Flood at 1.5X CMA mass and hydraulic loadings and CMA concentration = plots                          |        |        |
| mg CMA/liter  | 3000   | 3000   |
| kg CMA  | 1.35   | 2.7    |
| g CMA/m <sup>2</sup> -yr  | 675    | 1350   |
| liters water/yr   | 450    | 900    |
| cm water/yr   | 22.5   | 45     |

During the second winter, the soil boxes containing tree seedlings were spray-treated five times with control water and CMA at 3000 mg/liter concentration to produce total area/CMA mass and hydraulic loadings equal to plots (900 g CMA and 300 liters of water). The larger trees were spray-treated on the same schedule in the same quantities of CMA and water.

## ORGANIZATION OF THE REPORT

This report is organized in two parts. The first part introduces the topic; provides background; describes general approaches taken in designing the research overall and the specific experiments; and summarizes findings, conclusions, and applications of these conclusions to highway practice. The findings (Chapter Two) comprise brief presentations of the many results, concentrating on those with the greatest implications for CMA use and environmental impact assessment.

That chapter is intended for the reader whose interest in the findings is primarily from the management viewpoint. Chapter

Three presents the guidelines for CMA use and environmental impact assessment that represents the ultimate goal of this research.

The second part of the report is composed of 13 appendixes

that present and discuss the various experimental methods and results in full. This portion is intended for readers who are interested in detailed exposition of the scientific and technical aspects of the research.

## CHAPTER TWO

# FINDINGS

## ENVIRONMENTAL TRANSPORT OF CMA

### General

Definition of the environmental transport of CMA involved theoretical considerations and modeling, as well as field plot measurements of various constituents associated with CMA in surface runoff, percolating soil water, and groundwater. Theory and modeling were applied to CMA application and transport from highways in order to project conditions that would affect the surrounding environment. Overland flow and vertical transport in soils were also subjects for the application of theory as a basis for defining potential impacts. (The findings reported in this section are detailed in Appendix C.)

### Theoretical Considerations and Modeling

Modeling of CMA application and transport from highways was based on expected application rates and climatological conditions. The outcome of the exercise was as follows:

- Estimated typical annual CMA loading—10 tons/linear-mile (5.5 metric tons/km).
- Estimated typical CMA concentration in highway spray or runoff—order of magnitude 10 to 100 ppm.
- Estimated normal upper limit concentration—order of magnitude 1000 ppm.
- Worst-case concentration—5000 ppm.

The literature of overland flow treatment processes applied to other waste streams was the principal basis for a preliminary consideration of surface transport of CMA. This literature suggested that vegetation and the soil surface could remove both acetate and cations from runoff streams in significant amounts.

Vertical transport of CMA in soils was initially analyzed by considering cation exchange and prospective acetate biodegradation rate. It was shown that most soils could capture many years' input of calcium and magnesium at the expected highway loading rates, unless flushing was exceptionally rapid. However, the exchange reactions could release other metals preexisting in the soils. Acetate decomposition could be very restricted near freezing, and it was estimated that more than 80 days would be needed for complete decay in soil near 0 C.

### Runoff Characteristics and Surface Transport

Monitoring of runoff from field plots and bins confirmed the expected ability of vegetation and the soil surface to entrain acetate. Acetate reduction was found to be a function of distance flowed and to decline almost 25 percent from the initial amount in just 3 m. Data were insufficient to establish the best model of acetate reduction, but they did fit the expected exponential decline with distance. Substantially greater reductions are thought to be possible with longer slopes.

In contrast, neither the major cations nor trace metals were consistently captured in overload flow, as the literature had suggested they would be. Perhaps, the high Ca and Mg concentrations stimulated complex ion exchanges in the soil that modified the environment for metal retention.

### Vertical Transport in Soil

Acetate was usually detected in soil water at 30- and 60-cm depths 2 to 3 weeks after the initial CMA application of the winter. It then disappeared from the soil water within about the same period after the final treatment. Concentrations were always less than 11 percent of those applied at the surface (maximum 137 mg/l). Figure 4 shows this pattern with the first winter's data.

There was no demonstrable tendency for CMA application to enrich soil water with cations or other anions (chloride, nitrate, sulfate, and phosphate). Therefore, the possibility of trace metals release through cation exchange was not confirmed in the soil water.

However, groundwater at 3 m depth did exhibit higher iron and aluminum in three of four CMA plot wells. Zinc was also enriched in one well. These results offered some field verification of trace metal release by exchange processes demonstrated in the laboratory and suggested as a possibility by theoretical considerations and literature.

Acetate also appeared in groundwater samples, but not exclusively beneath CMA plots. It apparently remained undegreded for the months required for groundwater to flow between plots. Concentrations were never higher than 46 mg/liter (less than 4 percent of applied concentration), except in one sample.

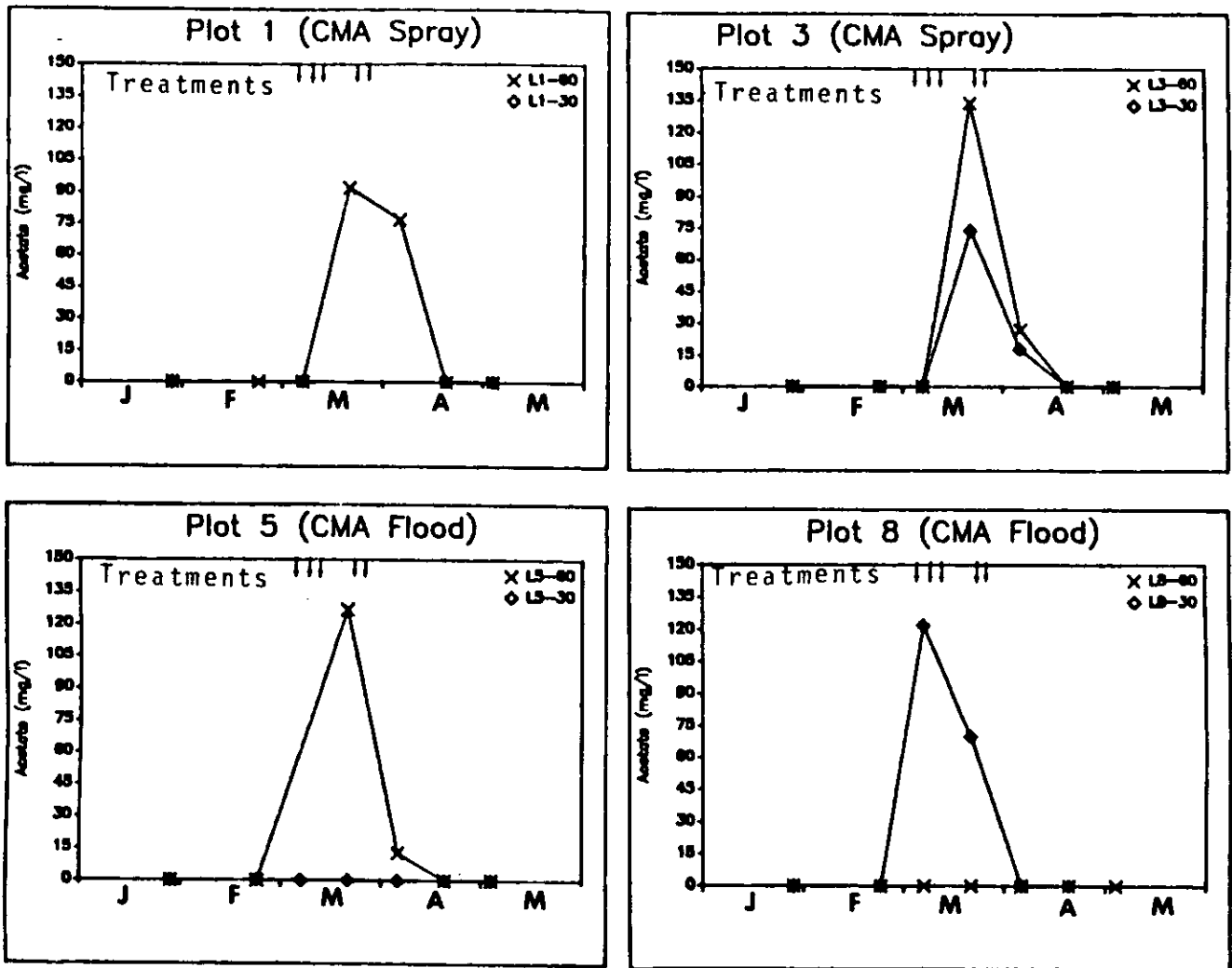


Figure 4. Acetate measured in CMA plot lysimeters (1986 treatments).

## EFFECTS OF CMA ON SOIL PHYSICAL PROPERTIES

### General

Effects of CMA on soil physical properties were investigated through laboratory testing of four soils selected to be representative of roadsides, as well as through tests on the field plot soils. The same four soils were used for testing of CMA effects

on soil chemical properties and plants, and the biodegradation of CMA. These soils (see Table 1) were first subjected to general analyses to characterize their properties. Appendix D details the experiments summarized in this section.

Table 1. General properties of test soils.

| Soil                 | Textural Class | % Organics | Cation Exchange Capacity (meq/100 g) | Exchangeable Cations (ppm) |     |     |
|----------------------|----------------|------------|--------------------------------------|----------------------------|-----|-----|
|                      |                |            |                                      | Ca                         | Mg  | K   |
| Pack Forest          | Loam           | 6.0        | 12.6                                 | 754                        | 125 | 217 |
| Lee Forest           | Silt loam      | 12.3       | 26.5                                 | 1030                       | 94  | 148 |
| Puyallup             | Sandy loam     | 5.2        | 8.8                                  | 899                        | 65  | 155 |
| Vantage <sup>a</sup> | Sandy loam     | 2.3        | 12.8                                 | 1320                       | 365 | 337 |

<sup>a</sup>Calcareous soil with 1.25% calcium carbonate.

### Laboratory Test Results

Laboratory tests included plasticity, compaction, permeability, and unconfined compression strength. Three of the four soils were nonplastic. The liquid limit, plastic limit, and plasticity index of the fourth soil (Pack Forest) was unaffected by treatment with 5000 ppm CMA solution, the highest concentration ever expected in highway runoff. In compaction testing of the four soils, there was negligible variation of their moisture-density characteristics from values determined with water.

Table 2 summarizes permeability test results. Permeability decreased with time in all samples but Vantage at 20 C. There was little or no decrease with time at 2 C, however, pointing out the role of microbial growths in restricting soil permeability. Despite the stimulation of microbial growths by acetate, CMA-treated samples were always higher in permeability than controls at the completion of curing, especially in the calcareous soil. Figure 5 shows the 2 C results in this soil. The apparent reason for the higher permeability is particle flocculation by Ca and Mg, creating more pore space. While CMA does appear to create substantial increases in soil hydraulic conductivity, rates remained of the order  $10^{-5}$  cm/s ( $< 1$  cm/day). Such an increase, if realized in practice, is not expected to make a real difference in the vertical transport of CMA solution in soils.

Unconfined compression tests on the four soils at 20 C and one of them at 2 C revealed negligible differences between CMA-treated samples and controls.

### Field Test Results

Samples from three CMA and two control plots were tested for plasticity properties. Shear vane tests were performed in situ

Table 2. Summary of soil permeability test results.

| Temperature (C)         | Approximate Permeability Change with Time |                    | Approximate CMA/Control Permeability <sup>d</sup> |
|-------------------------|---|--------------------|---|
|                         | Control                                   | CMA                |   |
| <u>Pack Forest Soil</u> |   |                    |   |
| 20                      | -5x                                       | -7x <sup>a</sup>   | 4   |
| 2                       | 0   | 0 <sup>a</sup>     | 4   |
| <u>Lee Forest Soil</u>  |   |                    |   |
| 20                      | -6x                                       | -4x <sup>a</sup>   | 2-3   |
| 2                       | 0   | 0 <sup>a</sup>     | 2-3   |
| <u>Puyallup Soil</u>    |   |                    |   |
| 20                      | -2-3x                                     | -2-3x <sup>a</sup> | 1-2   |
| 2                       | 0   | 0 <sup>a</sup>     | 2   |
| <u>Vantage Soil</u>     |   |                    |   |
| 20                      | 0   | 0 <sup>a</sup>     | 10  |
| 2                       | 0   | +1.5 <sup>a</sup>  | 20  |
| 2                       | 0   | 0 <sup>b</sup>     | 10  |
| 2                       | 0   | 0 <sup>c</sup>     | 5   |

<sup>a</sup>5000 ppm CMA

<sup>b</sup>500 ppm CMA

<sup>c</sup>50 ppm CMA

<sup>d</sup>At conclusion of curing

at three CMA and two control plots. Only negligible differences appeared in the results.

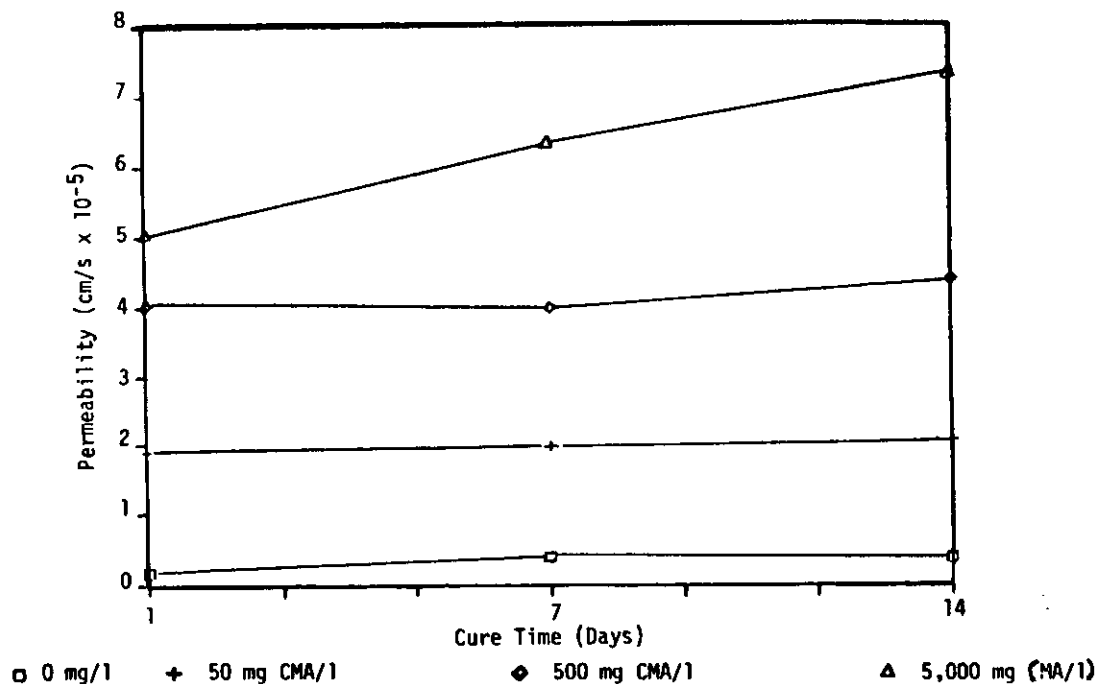


Figure 5. Permeability test results for Vantage soil with 2 C cure.

## PHYSICOCHEMICAL REACTIONS INVOLVING CMA IN SOILS

### General

Physicochemical reactions of CMA in soils were specifically evaluated through two sets of laboratory procedures. In the first, the four test soils described above were batch-treated with CMA solutions of several concentrations (0 to 5000 mg/liter) and incubated at 2 and 20 C for 14 days. A second experiment was conducted to determine the extent of CMA adsorption on three soils and the subsequent desorption when flushed with water.

Appendix E provides full details on these experiments.

### Batch Incubations

Acetate decline during the 14-day incubations was signaled directly by the periodic acetate measurements and indirectly by the pH, alkalinity, and conductivity changes. Acetate removal by biodegradation was usually well advanced by day 7 and complete or nearly complete by day 14 at 20 C, regardless of added concentrations. However, at 2 C, little decomposition was evident in the first week. While removal was nearly complete at some concentrations at that temperature by the end of the experiment, it was retarded with higher additions. The high osmotic pressure seemed to lengthen the lag time for microbial populations to develop. Alkalinity, conductivity, and pH mirrored these changes, with the two former measures tending to decrease over time and the latter to rise somewhat. All such changes were attenuated at 2 C and were slightly less pronounced in the Vantage soil (calcareous, low in organics).

Among the metals, Fe and Al were inversely related to CMA addition in the Vantage soil, indicating their release from the soil. This effect was not apparent in the other soils and did not appear with any other metals. The anions chloride, nitrate, and sulfate were not affected by the experimental treatments.

### Adsorption/Desorption Experiment

Applications up to 5000 mg CMA/liter, the maximum concentration expected in highway runoff, did not saturate the soils because of their much higher cation exchange capacity. However, Cu, Zn, and Al were desorbed rather than adsorbed in all soils at all applied CMA concentrations. This result is an illustration of the ability of Ca and Mg to release native soil metals while undergoing exchange reactions. Together with the batch results and field measurements of groundwater, as well as reports in the literature cited in Chapter One, these findings point out the potential for mobilization of some metals when CMA contacts soils. These releases were not significant in terms of environmental impact in these experiments, but it is unknown whether they would be with trace metal-contaminated soils, such as could be found along heavily trafficked highways.

The three soils with moderate-to-high organic contents were not saturated in acetate by the experimental CMA additions. The Vantage soil probably was saturated. However, a significant proportion of adsorbed acetate was subsequently desorbed. Because of incomplete adsorption and subsequent desorption if undegraded, 70 percent of the applied acetate could be mobile on the average, even in the organic soils. This percentage would

be higher (> 85 percent) in a soil like Vantage. Thus, there is a potential for acetate moving through soils to reach groundwater. In the field experiments, however, only small quantities (usually < 5 percent) were ever detected in groundwater. The difference is probably because of the more extreme flushing conditions in the laboratory and the more effective operation of chemical and biological mechanisms that reduce free acetate in natural soil columns.

## CMA DECOMPOSITION AND MICROBIAL ACTIVITY IN SOILS

### General

CMA decomposition was further investigated by batch-treating the four test soils with a range of CMA concentrations (0 to 5000 ppm) and incubating at 2 and 10 C. The carbon dioxide produced by microorganism respiration during incubation was the measure of CMA decomposition. Soil cores were also collected from the field plots, incubated at 2 C, and observed for CO<sub>2</sub> release over time.

The demonstration of substantial microbial activity in the presence of CMA stimulated an interest in making some direct observations of the microbial community. Accordingly, field plot soil samples were analyzed for the abundance of bacteria, fungi, and soil fauna. Appendixes F and G present the methods and results of these experiments in detail.

### Batch Incubations

Figure 6 shows CO<sub>2</sub> production over time at 2 C in the four test soils. There was no significant difference between controls and 125 ppm CMA treatments in any soil or on any day. Peak respiration rates tended to increase with increasing CMA concentration. A longer lag in reaching peak rate was generally evident in the more concentrated treatments. Development of the microbial community was probably hindered by the osmotic effect of high cation additions.

The Lee Forest soil exhibited the highest peak respiration rates, followed by Puyallup, Pack Forest (Murphy's Ranch), and Vantage. This order is approximately the same for the soil organic content; Puyallup and Pack Forest switch positions in that order, but are close in organic content. To reach peak respiration rate took 13 to 29 days at 2 C and 7 to 9 days at 10 C.

### Soil Microbial Activity

Soil cores were taken in the late summer after a winter of treatments, when CMA biodegradation was expected to be complete, and immediately at the end of the second winter's treatments. In addition to organism counts, soil pH was measured on each occasion.

No statistically significant differences among treatment plots appeared in pH, bacterial counts, or fungal counts in the late-summer samples. Soil fauna were not compared statistically but did not differ qualitatively. In the winter samples, pH differed significantly between control (mean 5.2) and CMA plots (mean 6.0). Bacteria counts were significantly higher on CMA flood plots but were statistically indistinguishable among control-

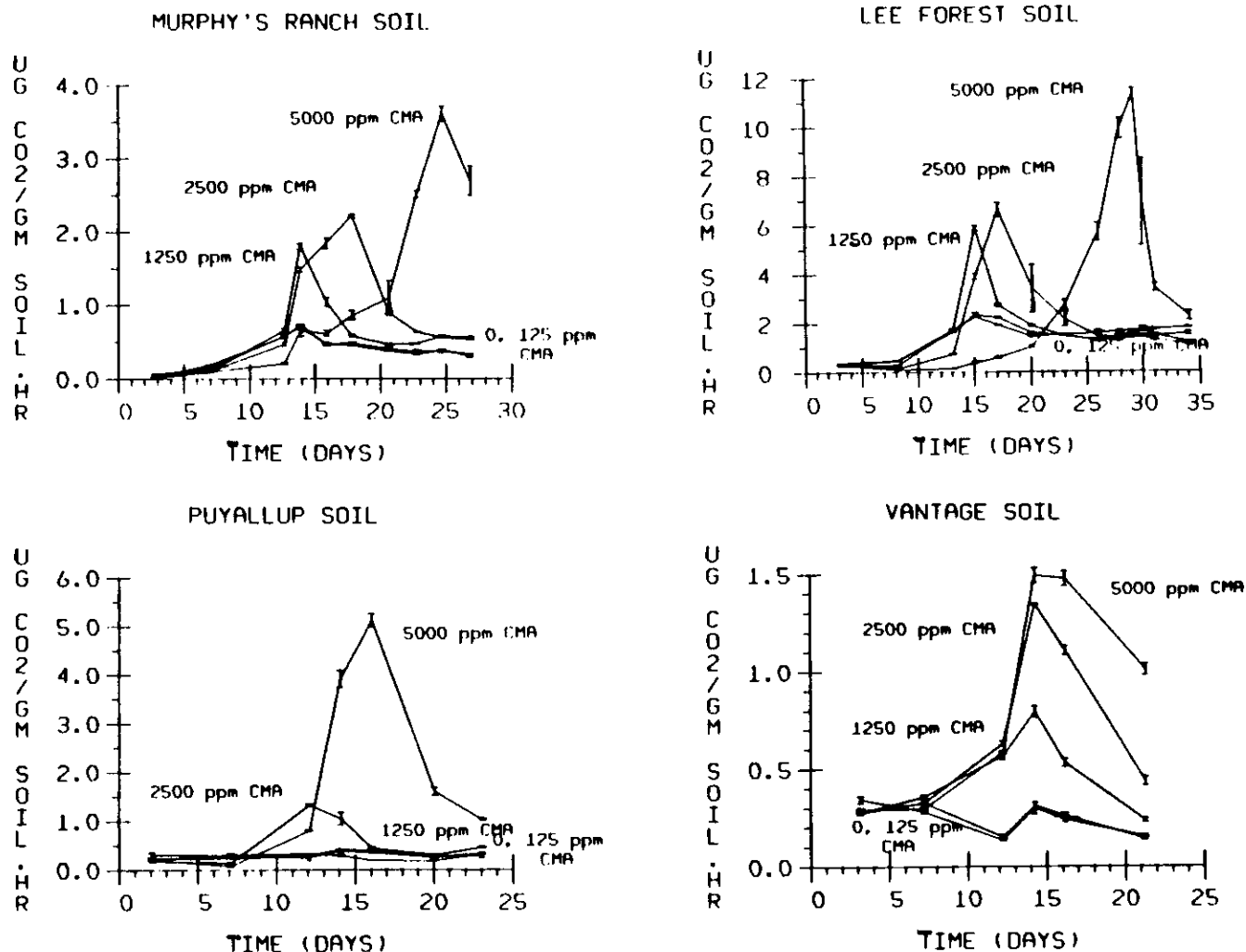


Figure 6. Carbon dioxide production over time at 2 C in four test soils.

spray, control-flood, and CMA-spray plots. No significant differences were noted in fungal counts. Fauna were comparable among plots, except for elevated mite populations on CMA-flood plots.

The results suggest that soil pH and bacterial populations can be temporarily changed by CMA, but that these changes do not persist. Although not observed, it is believed that the bacteria are strictly saprophytes and are not pathogenic. Additional work should verify this hypothesis.

#### EFFECTS OF CMA ON TERRESTRIAL VEGETATION

##### General

Experiments were designed and carried out to: (1) determine through bench-scale pot tests the concentrations of CMA in the root zone that would depress growth and (at higher levels) kill plants, (2) determine the effects on plant vigor and dry matter yield of CMA applications to roadside-type vegetation on field plots and bins, and (3) to ascertain the effects of CMA sprays

on woody plants. Detailed results are presented in Appendix H.

##### Pot Tests

A variety of herbaceous and woody species were grown in soil pot tests using a silt loam and a sandy loam soil to include any influence of soil texture. Most plants grew well with additions of 0.5 or 1.0 g CMA/kg soil, and there was often some stimulation of yield at these levels (Figure 7). Higher amounts of CMA depressed growth, and at 4.0 g CMA/kg soil, all seedlings died if planted immediately. However, most lived and grew fairly well if planting was delayed about 2 days. The response of red maple (Figure 8) illustrates these actions. The ability of the soil to exchange with added CMA in a day or two and the degradation of part of the acetate made the rooting environment more favorable.

Each 0.5 g CMA/kg soil was equivalent to 1250 mg CMA/liter when the soil was moistened to field capacity. Therefore, CMA concentrations in the root zone substantially greater than routinely expected in highway runoff were neutral or beneficial



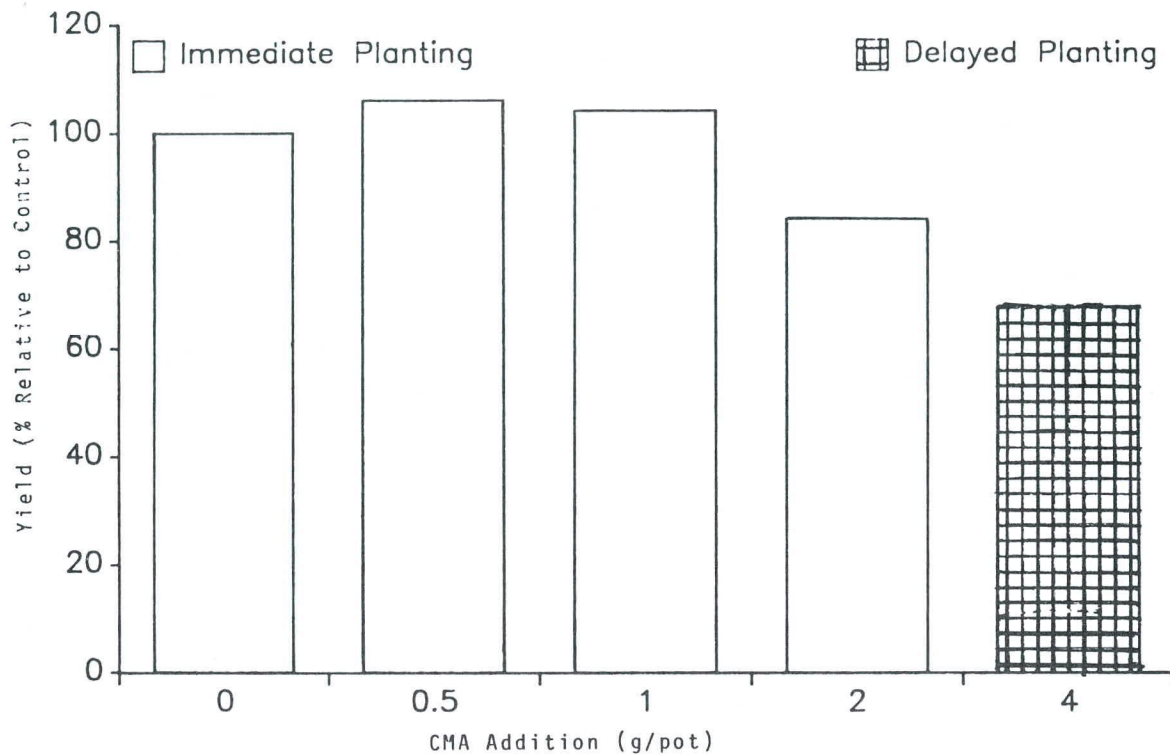


Figure 7. Relative yields of romaine lettuce in Puyallup soil versus CMA addition to pot tests.



Figure 8. Red maple responses to CMA additions in pot tests.

to plant growth. The highest projected concentration (5000 mg/liter = 2 g/kg) was inhibitory to seedlings if they were immediately planted after CMA application. However, this combination of circumstances is unlikely to occur in reality.

#### Field Plot and Bin Observations

The growth of a grass/clover mixture on both field plots and on bins of the same soil at the Pack Forest site and on the campus was vigorous and simulated a typical roadside vegeta-



tion. Applications of CMA to this vegetation resulted in no appreciable influences. Growth was equally vigorous in the controls and the CMA-treated parcels. Small, statistically significant effects in the bins proved to be contradictory, as at Pack Forest the control bins had slightly higher yields, but on the campus the CMA treatments gave marginally better yields. Temperature differences are believed to be responsible for this contradiction. In all cases, vigor of the plant tops and rooting development were favorable.

### Observations of Woody Plants

To study the influence of CMA sprays on woody vegetation that could be expected naturally along roadsides or proximal embankments, several species were treated at the Pack Forest site. Douglas fir and red alder saplings, and Douglas fir, balsam fir, and red maple seedlings, all were sprayed repeatedly with CMA during the winter of 1986–1987. Upon observation in April 1987, the controls and CMA-treated plants were equally vigorous. No symptoms of injury or stress were noted in any case. Thus, such spraying can be said to have no immediate adverse effects. These plants will, however, be observed again in June 1987, to ascertain any influences on length of shoot growth or other aspects.

From the foregoing results, it can be said that CMA applications are benign to terrestrial vegetation, except when concentrations in the root zone are high enough to cause osmotic stress. This effect was demonstrated in pot tests with large additions, but was not induced by any of the field applications and is not expected under actual conditions of CMA use.

## EFFECTS OF CMA ON SURFACE WATER QUALITY

### General

Surface water quality effects associated with CMA were studied in general through field pond monitoring. Laboratory work was performed for the special purpose of investigating CMA decomposition in water and the associated use of dissolved oxygen. This work involved standard biochemical oxygen demand (BOD) testing. Appendix I details both laboratory and field results related to dissolved oxygen (DO), and Appendix J presents all other water quality observations.

### Dissolved Oxygen Depletion

The initial BOD experiment used four CMA concentrations (10 to 5000 mg/liter) and controls in 20 C, 20-day incubations. Only the lowest concentration preserved any DO past the first 2 days of the experiment. This quantity degraded over the days at a rate constant of 0.107/day. The ultimate BOD was estimated to be 72 percent of applied CMA, a value very close to theoretical expectations. Exertion of this ultimate BOD was 69 percent complete in 5 days, 83 percent in 10 days, 88 percent in 15 days, and 92 percent in 20 days at 20 C. Complete destruction would take 30 to 40 days.

A second BOD experiment was run with 10 mg CMA/liter incubated for 20 days at 10 and 20 C and for 40 days at 2 C. A 3-day lag occurred at 10 C, and it took 12 days for those samples to equal the 20 C samples in oxygen depletion, at which

point decay proceeded similarly at the two temperatures. At 2 C the lag was 9 days, and it was predicted that 100 days would be required for complete removal of the ultimate BOD. Overall rate constants at 20, 10, and 2 C were 0.130, 0.064, and 0.020 per day, respectively.

Most field ponds draining CMA-treatment plots received roughly 10 mg CMA/liter after each treatment. These ponds all exhibited dissolved oxygen depletion to levels as low as 5 mg/liter. However, control ponds and the one CMA pond that received little runoff had DO > 10 mg/liter throughout the period. Typical highway runoff is predicted to have CMA concentration closer to 100 than to 10 mg/liter. Therefore, substantial acetate reduction before discharge to a receiving water or dilution in the water would be necessary to prevent oxygen depletion. Most receiving waters would probably provide such a level of dilution.

### Other Water Quality Conditions

Field ponds responded to CMA additions through runoff with conductivity, alkalinity, and total phosphorus increases. Metals did not follow a clear trend, and acetate was rarely detectable in ponds.

Ponds receiving substantial CMA runoff experienced an approximate doubling in total phosphorus concentration. Control ponds also had smaller increases, probably from fertilizer and decaying plants. If spring snow melt occurs near the start of the growing season, CMA manufactured from agricultural wastes and high in phosphorus could cause algal blooms in receiving waters.

## EFFECTS OF CMA ON AQUATIC BIOTA

### General

Responses of biota at several trophic levels to CMA were studied in laboratory bioassays and in the field ponds. Acute (4-day) bioassays were performed on a test alga (*Selenastrum capricornutum*), a species of zooplankton (*Daphnia magna*), and two fish species (rainbow trout, *Salmo gairdneri*; and fathead minnow, *Pimephales promelas*). Extended bioassays were performed on *D. magna*, an aquatic amphipod invertebrate (*Hyalella azteca*), and rainbow trout eggs and the subsequent fry. In addition, the algal bioassay and invertebrate experiments provided some data on bacteria and periphyton growth, respectively.

Field ponds were stocked with various algae, zooplankton, and fish and colonized by other forms from the supply pond and the atmosphere. These ponds were regularly monitored for phytoplankton and periphyton biomass, and abundances of zooplankton, macroinvertebrates, and fish.

Detailed descriptions of the experiments and observations on algae and other microorganisms are presented in Appendix K. Appendix L covers the animals in detail.

### Phytoplankton, Periphyton, and Bacteria

In the *Selenastrum* bioassay biomass was significantly inhibited relative to controls by 1 mg CMA/liter. Application of 10 and 50 mg/liter led to additional significant inhibitions, while

the minimal growth in all higher concentrations could not be distinguished from that at 50 mg/liter. In a parallel experiment sodium chloride did not significantly inhibit until 4000 mg/liter was applied. These experiments demonstrated trends very much like those observed previously by CalTrans in testing reagent-grade CMA. Bacterial counts in CMA test vessels were significantly above controls with all concentrations 100 mg/liter and higher. It is believed that algal inhibition was primarily the result of losing the competition for nutrients to bacteria.

In contrast to inhibition of planktonic *Selenastrum*, attached blue-green algae were stimulated in the invertebrate test channels by moderate (100 and 500 mg/liter) CMA concentrations. These algae developed in low-nutrient water, whereas the *Selenastrum* bioassays were carried out in nutrient-rich medium. This distinction may hold the key to the different responses. Thus, CMA may be stimulatory to at least some algae in nutrient-poor environments, but advance bacteria to algal inhibiting-levels in richer waters. This is only a hypothesis, however, that needs further testing for acceptance.

Pond algal and periphyton biomass, measured in terms of chlorophyll *a*, showed no significant and conclusive responses to CMA in either direction. While differences may have been masked by variability much higher than in the laboratory, the lack of effects suggests that the responses generated in tightly controlled laboratory experiments are rather unlikely to develop in natural systems.

#### Aquatic Animals

From the acute *Daphnia* bioassay, 96-hour  $LC_{50}$  (concentration lethal to 50 percent of the organisms) was established to be 2000 mg CMA/liter. Animals in 500 mg/liter solutions survived better than controls. In the chronic test, the longest survival, greatest number of broods, and largest total number

of offspring all occurred in this treatment. This level apparently stimulated production of fungal food sources not available in lower CMA concentrations. Higher amounts inhibited survival and reproduction, with both oxygen and osmotic stresses possibly being involved.

$LC_{50}$  concentrations were very high for the fish (17,500 mg/liter for rainbow trout and 12,500 mg/liter for fathead minnow). These concentrations are well in excess of any expected in highway runoff. Chronic rainbow trout bioassays were performed with concentrations ranging up to 5000 mg CMA/liter. Hatching was successful at all concentrations, but those above 1000 mg/liter eventually killed the fry. Oxygen was not a factor because these bioassays were aerated, except for a special extended chronic test.

In comparison to the CalTrans results in very similar tests, there was no indication that potential production-run CMA was more harmful to the survival and reproductive success of aquatic animals. The results for fish were very similar in the two cases, while zooplankton registered survival and successful reproduction to higher CMA concentrations in the present tests than in the CalTrans set using reagent-grade material.

Like the *Daphnia*, *Hyallela* registered the greatest survival at 500 mg CMA/liter, also probably because of fungal food supplement. These organisms were quite tolerant of low oxygen, although rapid depletion, probably in conjunction with osmotic stress, was fatal to all specimens at > 2500 mg CMA/liter.

As with the phytoplankton and periphyton, pond animals exhibited no conclusive responses to CMA addition in runoff. All stocked bluegills survived throughout the experiments, and fathead minnows reproduced prolifically. The ponds did not contain salmonids, which are more sensitive to oxygen depletion. Still, DO was not depressed long and was always above 4 mg/liter. These conditions would not likely affect salmonids negatively, but further drop may.

#### CHAPTER THREE

## CONCLUSIONS AND APPLICATIONS

#### INTERIM GUIDELINES FOR CMA USE

One of the general goals of this research program was to draft interim guidelines for CMA use. These guidelines were to be based on the findings, properly qualified in view of the small-scale tests performed. They were intended to apply until a full-scale demonstration could be carried out that would either verify their appropriateness or suggest modification.

Such guidelines have been considered in interpreting the results of the various experiments. They are presented in the Conclusion sections of several appendixes and are listed below. In some cases the guidelines should be applied especially in certain circumstances that make a negative environmental im-

pact more likely. These special circumstances are noted with each guideline, as appropriate. One or more reasons, rooted in the research results, exist for each guideline. These reasons are noted generally in parentheses following the guideline statement.

1. When: (a) receiving water is inhabited by protected aquatic species, especially salmonid fishes; or (b) snowstorms occur late in the season, when receiving water may already have warmed; then: Do not apply CMA in catchments where highway runoff can directly reach receiving waters that will have less than 100:1 dilution available in the runoff season. (*Protection against dissolved oxygen depletion*)
2. Provide vegetated drainage courses between highways and

receiving waters to the extent possible. (*For removal of acetate and other constituents from runoff*)

3. Avoid CMA use when a high rate of runoff will pass over very coarse soil that overlies a sensitive aquifer or is adjacent to a receiving water that should be protected from dissolved oxygen depletion. (*To avoid high acetate mobility in soil with low anion exchange capacity*)

4. Tentatively, withhold use of CMA along highway segments that may have relatively high heavy metal concentrations in roadside soils (from high traffic volumes or atmospheric sources), and that are above important aquifers or adjacent to surface water that has a protected beneficial use (e.g., drinking water source or protected aquatic life habitat). (*To avoid possibility of heavy metal mobilization through cation exchange reactions, until that question can be considered in additional research*)

5. Avoid CMA use when a large highway runoff volume can directly reach a small, poorly flushed pond or lake. (*To guard against phosphorus enrichment and consequent eutrophication, as well as oxygen depletion*)

6. Take care to avoid spills and runoff from CMA stock piles in the vicinity of receiving waters at all times. Storage under cover is preferred in such locations. (*To avoid acute oxygen depletion, osmotic, and nutrient-enrichment effects*)

#### A TENTATIVE MODEL FOR PREDICTION OF CMA ACTIVITY IN THE ENVIRONMENT AND IMPACT ASSESSMENT

A second general goal of the research program was to develop means of assessing its environmental impact. These provisions were also to be based on findings, again properly qualified pending full-scale verification, or alteration as a result of new findings in highway-scale tests.

Such means have been considered in interpreting the various experimental results. They are detailed in the appendix discussion and conclusion sections and are listed below. These provisions are presented as a tentative model for making environmental assessments. In many cases quantitative statements can be made on the basis of the research results. In others, a qualitative or conceptual statement is all that can be made at this time. The recommended assessment procedures follow:

##### A. Toxicity

A.1. CMA of the type tested in these experiments has low toxicity. No more than four-fold dilution of the pure material is required to avoid violation of water quality standards designed to protect human consumers and aquatic life against toxicity (although higher dilution is typically needed to protect against oxygen depletion).

##### B. Overland Transport

B.1. At least 25 percent of volatile fatty acid salts (acetate and butyrate) can be removed from runoff during flow over vegetation in less than 5 m. Further reductions with greater lengths are presently unknown.

B.2. Cation removal cannot be assumed in 3 m of overland flow through vegetation.

##### C. Soil Physical Properties

C.1. Medium-textured soil plasticity, moisture-density characteristics, unconfined compression strength, and shear strength are unaffected by CMA at any concentration expected in highway runoff.

C.2. CMA increases permeability by 2 to 4 times in noncalcareous, medium-textured soils and by 5 to 20 times with additions of 50 to 5000 ppm CMA to calcareous sandy loam. Final hydraulic conductivity rates are approximately  $10^{-5}$  cm/s.

##### D. Ion Mobility

D.1. Certain soil trace metals (Fe, Al, Zn, Cu) are potentially mobilizable by CMA contact. Based on laboratory results, amounts that can be released are approximately: Fe, 1–4; Al, 1–7; Zn, 0.1–0.6; and Cu, 0.01–0.1  $\mu\text{g/g}$  soil. Actual releases in natural soils appear to be less, but cannot be quantified specifically.

D.2. Native soil anions are not mobilized by CMA contact.

D.3. Through lack of initial adsorption and subsequent desorption of sorbed material, 70 percent of applied acetate can potentially be mobile in organic, medium-textured soils. The potential mobility is > 85 percent in a low-organic, calcareous sandy loam. However, less than 10 percent mobility was actually observed in an organic loam in the field.

D.4. The pH of an acid ( $\sim$  pH 5) loam can be raised temporarily about one pH unit by CMA in simulated highway spray and runoff.

##### E. CMA Biodegradation in Soils

E.1. Acetate degrades essentially completely in soils within two weeks at 10 and 20 C, but not until 2 to 4 weeks at 2 C.

E.2. A lag of about 1 week preceding much decomposition occurs with high (5000 ppm) CMA in noncalcareous soils.

E.3. Acetate decomposition in soils approximately follows the Arrhenius ( $Q_{10}$ ) Law in the range tested; i.e., a doubling of rate with 10 C temperature rise.

E.4. A soil column of 3 cm depth is sufficient to decompose much of the acetate added in simulated highway runoff or spray.

E.5. Soil bacteria populations can be raised temporarily about an order of magnitude (to about  $10^8$  colonies/g dry soil) by CMA runoff.

##### F. CMA Effects on Plants

F.1. Plants can withstand or be slightly enhanced by root-zone soil solution concentrations up to 2500 ppm CMA. Because of osmotic stress, higher concentrations are lethal if seedlings are exposed immediately, but not if planting is delayed 1 to 2 days (although growth may still be inhibited to a degree that is species-dependent).

F.2. Spraying or flooding plants with 3000 ppm CMA produces no effects on yield, cover, rooting, vigor, or external damage symptoms.

##### G. Effects of CMA on Water Quality

G.1. Overall decomposition rates of CMA in water are approximately 0.11–0.13/day at 20 C, 0.06/day at 10 C, and 0.02 at 2 C.

G.2. The ultimate BOD is about 75 percent of added CMA concentration. Approximate exertion of that BOD is as follows:

| Temperature<br>(C) | Percent Exerted |            | Days to<br>Complete<br>Decomposition |
|--------------------|-----------------|------------|--------------------------------------|
|                    | In 5 Days       | In 20 Days |                                      |
| 20                 | 70              | 90         | 30–40                                |
| 10                 | 55              | 90         | 30–40                                |
| 2                  | 0               | 80         | 100                                  |

- G.3. 10 mg CMA/liter can temporarily reduce saturated dissolved oxygen by about half at approximately 5 C. CMA > 100 mg/liter can reduce DO to zero within 2 days.
- G.4. Surface water conductivity, alkalinity, and total phosphorus increase in proportion to CMA runoff. Acetate and metals do not, however. Total phosphorus concentration in runoff is approximately 0.02 percent of the total applied CMA concentration.
- H. *Effects of CMA on Aquatic Biota*
  - H.1. Any CMA can potentially inhibit phytoplankton biomass in high-nutrient conditions, probably due to a nutrient-competition advantage afforded bacteria (although not verified in the field). CMA > 50 mg/liter almost entirely inhibited phytoplankton in laboratory bioassays.
  - H.2. 100 to 500 mg CMA/liter can potentially enhance blue-green periphyton in low-nutrient conditions (although not verified in the field).
  - H.3. 500 mg CMA/liter is optimum for survival and reproduction of aquatic invertebrates, but higher concentrations are lethal due to osmotic stress and low oxygen.
  - H.4. 96-hour  $LC_{50}$  = 2000 mg CMA/liter for *Daphnia magna*.
  - H.5. Rainbow trout can hatch in CMA equal to the maximum concentration ever expected in highway runoff (5000 mg/liter), but this concentration is subsequently fatal to the young fry. At 1000 mg/liter, though, > 90 percent can survive.
  - H.6. More mature rainbow trout fry and fathead minnows can survive 5000 mg/liter at 90 percent rates.
  - H.7. 96-hour  $LC_{50}$  concentrations for rainbow trout and fathead minnows are 17,500 and 12,500 mg CMA/liter, respectively.
  - H.8. CMA at approximately 10 mg/liter after mixing did not affect the health, reproduction, or survival of aquatic animals in field ponds.

## FURTHER RESEARCH NEEDS

### Priorities

The laboratory and controlled field plot experiments resulted in a number of clear demonstrations of CMA transport and fate in the environment and its potential effects of biotic and abiotic components of ecosystems. If CMA is to be seriously considered for widespread highway application, full-scale, general experiments with actual highway applications should be undertaken for verification purposes. Of particular interest in these experiments are the following issues, listed in a rough priority order:

1. Actual CMA concentrations and mass loadings that enter surface receiving waters with highway runoff and the extent of dissolved oxygen depletion that results.
2. The extent of trace metal mobilization in actual roadside soils; concentrations of these metals occurring in soil water, groundwater, and surface runoff.
3. Acetate mobility in soils with actual highway application rates; especially its extent and consequences in groundwater.
4. Composition of the soil microbial community that develops with actual CMA additions.
5. The rate at which roadside soils become saturated with major cations.

The remainder of this chapter provides an experimental design to perform the needed research. Detail is presented on study site selection, installation, and monitoring, as well as laboratory procedures.

## Study Site Selection

### General

This section provides specific study-site selection criteria and the general locations of some potential sites that could meet these criteria. Criteria are divided into primary and secondary. Primary criteria must be met to achieve the objectives represented by the priorities defined previously. Secondary criteria represent features that are not obligatory but are desirable from the standpoint of offering better definition of site conditions.

It is intended that the bulk of the experimental work be performed at two locations, one characterized by calcareous, neutral soils; and the other, by noncalcareous, relatively acid soils. The two locations should offer some differences in roadside vegetation types. At each location three similar watersheds will be selected, one to receive CMA on a highway segment in the watershed, another to receive alternative deicing treatment on a similar highway segment, and a third that will not have any deicing activity.

The criteria emphasize selecting two locations that have relatively severe winters and high deicing agent use. Experiments to date have found few potential environmental impacts resulting from CMA in the concentrations and mass loadings routinely expected. The highest concentrations and loadings are expected where CMA accumulates in a persistent snow pack that melts relatively rapidly at the end of the winter. Therefore, experimenting at locations offering this pattern would provide a "worst-case" test of environmental impact.

### Selection Criteria

#### Primary Criteria:

1. Locations that receive relatively heavy snowfalls and have snow packs that persist at least 2 months and then generally melt fairly rapidly.
2. Locations that have calcareous, neutral soils and noncalcareous, relatively acidic soils.
3. Ability to monitor without disturbance for two winters preceded and followed by full growing seasons.
4. Availability of three watersheds in close proximity that are similar physically, chemically, biologically, and socioeconomically, two of which can receive different deicing treatments on highway segments (CMA and the regular deicer) and the third of which can receive no deicer (it may have no highway or a highway that is not deiced).
5. Similar highway maintenance procedures among watersheds, other than deicing.
6. Relatively small watersheds for uniformity and manageable monitoring.
7. Presence in each watershed of a stream that receives highway drainage and has resident fish. Also, the presence of a downstream lake or pond to represent a standing water community.
8. Presence of relatively homogeneous groundwater aquifers of known hydrologic properties.

9. Availability of some groundwater wells in the 2-year plume of groundwater recharge from the experimental areas.
10. Accessibility for regular monitoring functions.

*Secondary criteria:*

1. Availability of data characterizing soils well.
2. Stream flow gaging station(s) in place.
3. Availability of long-term hydrologic and water quality data sets.

*Potential Locations*

Potential locations meeting these criteria are principally in the northern tier of states. While examples of the two major soil types that should be represented in the study can be found nearly throughout the nation, one or the other type tends to predominate in certain areas. The Northcentral states generally have calcareous, neutral soils, along with quite heavy snowfalls and long freezing periods. The Cascade and Sierra Mountains of the Western states have desirable climatological conditions and, almost exclusively, acidic soils. Such soils also occur in the Northeast, especially in the Adirondack Mountains. Arid areas of the West often have calcareous soils, but frequently do not have sufficient snowfall to produce large snow packs in accessible areas.

One consideration in site selection is the degree of interest in deicing alternatives. Previous or ongoing highway application studies (involving CMA or another alternative) have been conducted by the states of Michigan, Wisconsin, South Dakota, and Washington, among the northern tier of states that may have sites that meet the stated criteria. In addition, California and Alaska have investigated the environmental impacts of CMA.

**Study Site Installations**

*General*

Few installations will be required at the study sites. The only pieces of equipment that must be put in place for the duration of the study are meteorological instrumentation, lysimeters for collection of soil water, groundwater wells, and runoff composite sampling systems. Specifications for each are presented in the following sections.

*Meteorological Instrumentation*

Install appropriate, secure housing for meteorological instrumentation at each study site. Equip each station with a precipitation collector, precipitation event recorder, and an air temperature recorder. Place thermocouples in the soil nearby at depths of 2, 30, and 60 cm. These thermocouples must be appropriate to allow soil temperature reading on a calibrated millivolt meter.

*Lysimeters*

Lysimeters are devices to collect soil water under vacuum over time. It is recommended that tube-type lysimeters be placed at 30 and 60 cm depths (Soil Moisture Equipment Corporation,

Santa Barbara, Calif., catalog numbers 1900L12 and 1900L24, respectively, or equivalent) in a major highway drainage path. Each should be duplicated to provide duplicate samples for analysis or, if necessary, to furnish a spare if one becomes clogged or loses vacuum.

To install lysimeters, core or auger holes of the required diameter and depth. Place pea gravel around the porous ceramic collection cups and seal on top of the gravel with moist bentonite to prevent channeling of water from the surface. Backfill the holes with soil and seal around the tubes at the surface with bentonite.

*Groundwater Wells*

Because of the high cost of installing groundwater wells, it is proposed that most groundwater samples be obtained from existing wells drawing from aquifers recharged by highway runoff. These wells will be used exclusively in the two watersheds at each location that receive alternative or no deicing treatment. In the watershed where CMA is used it is recommended that a special well equipped for groundwater sampling at several depths be drilled. This well must be located in the plume of recharge, and it should be relatively close to the recharge zone in order to provide the maximum length of time during the study to sample groundwater that may be affected by CMA.

Existing wells in each of the three watersheds at each location should be located using well logs. They must be within the 2-year plume of groundwater recharge from the treated highway segment and should be at about the same depth and in comparable strata.

*Runoff Composite Sampling Systems*

It is recommended that surface runoff be sampled with the composite sampling system developed by Clark and Mar (53, 54). This system, consisting of a flow splitter, collection tank, and connecting piping, is illustrated schematically in Figure 9. Specifically designed for the characteristics of the site where it will be used, the flow splitter captures a set fraction of the total runoff and channels it to the collection tank, while the remainder discharges to the ordinary flow path. Calibration permits estimation of the total runoff volume. After the runoff event ceases, a composite sample can be drawn from the tank. The flow splitter and tank are constructed with marine plywood and exterior plywood, respectively, and are protected with several coats of marine resin. Connecting piping can be domestic rain spout. Appendix M (section M-1) presents a design procedure and further specifications for this equipment.

The number and placement of composite sampling systems will depend on the configuration of each watershed. In general, it would be desirable to sample drainage directly from the highway in order to determine pollutants in unaltered runoff. From the highway, the runoff would in most cases probably drain over a vegetated surface. Some would percolate into the soil, and the plant and soil contact would alter the quality of the remaining surface runoff. Therefore, it would be desirable to sample the runoff water again just before it enters the receiving water.

While the pattern described would be best experimentally, it may not be feasible in all situations, however. To use a quan-

Approximate Scale 1" = 2.5'

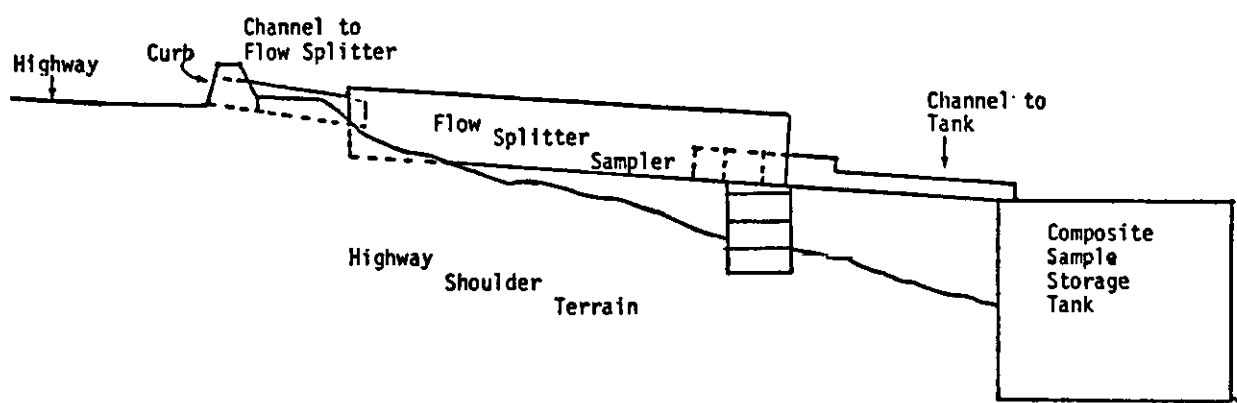


Figure 9. Runoff composite sampling system.

titative sampling device, all runoff from a defined area must be routed to a single point, which may not occur or be possible to engineer conveniently. Therefore, the exact configuration of runoff sampling must be decided after sites are selected.

### Study Site Monitoring

#### General

This section outlines the monitoring tasks proposed to serve the priorities listed earlier. It is organized on the basis of the environmental medium being monitored. Depending on the medium, samples collected according to these procedures may apply to more than one purpose. The following section presents analytical procedures to be performed on the samples in the laboratory. Appendix M contains several detailed procedures referenced in this section.

#### Meteorological Conditions

Monitor precipitation and air temperature using the recording equipment installed. On a regular basis, but at least biweekly during the snow and snow-melt seasons, measure soil temperature with the installed thermocouples and a millivolt meter. Measure the snow pack on the same schedule.

#### Surface Runoff

Surface runoff will be monitored using the compositing systems detailed in the previous section. Following are the instructions for operating these systems. Appendix M (section M-2) includes a sample field data sheet for this procedure.

1. Prior to the beginning of runoff, install a polyethylene bag (two if relatively thin gage) in the collection tank.
2. Time a trip to reach the site before the tank overflows.
3. Gage the tank to determine the volume of runoff collected (take quadruplicate readings on each side and average).

4. Use the calibration factor to estimate the total volume of runoff.

5. Stir the tank vigorously; then take a 2-liter sample by dipping a plastic sample bottle below the water surface.

6. Measure pH in the field.

7. Place the sample on ice for transport to the laboratory.

8. Puncture the polyethylene bag if the tank can drain by gravity, or pump it dry. Replace the bag in preparation for the next event.

#### Soil and Soil Water Chemical Properties

It is proposed that soil and soil water be sampled and analyzed for determination of cation saturation, metals and acetate mobility, and microbial activity. The following procedures should be performed in the field:

1. *Soil sampling along a transect for cation analysis*—This procedure should be performed once before the 2-year deicing treatment sequence begins and once each spring following treatments and completion of snow melt. Establish transects perpendicular to highways receiving CMA or alternative deicing agent, as well as in a similar area in the watershed receiving no deicing treatment. Randomly select approximately 10 sampling spots in a 50 m distance along the transects. At these spots collect 30-cm long soil cores with a corer that permits retaining intact cores. Transport the cores intact in labeled containers to the laboratory.

2. *Soil water collection from lysimeters*—Prepare lysimeters for soil water collection by placing 1.5 ml of chloroform in the ceramic cups to preserve organics such as acetate. After a period of collection, use a hand vacuum pump to apply a vacuum of 0.5 atmosphere. Generally, sample lysimeters monthly before, during, and after treatments, but reduce sampling interval to weekly or biweekly while snow melt is occurring. To draw samples use a vacuum test hand pump (Soil Moisture Equipment Corporation Model 200562, or equivalent). Measure pH in the field. Place samples in labeled bottles and on ice for transport to the laboratory.

3. *Soil sampling for analysis of microbial activity*—This procedure should be performed before the end of the summer preceding treatments, shortly after snow melt is complete following the two treatment winters, and 1 and 2 months later. Randomly select a sampling location in the vicinity of the transect described above. Near this spot perform the soil respiration experiment described in Appendix M (section M-3). Take a 5-cm deep soil core at the spot and at four other points around a circle of 50 cm radius surrounding the spot. Place the five samples in separate, labeled plastic bags, and return the samples on ice to the laboratory.

### *Groundwater Chemical Properties*

Existing and new groundwater wells should be sampled quarterly before, during, and after treatments throughout the duration of the project. New wells in the watershed receiving CMA will be equipped to sample at several depths. Devise a method to determine dissolved oxygen in situ by meter and measure pH in the field. Samples should be labeled and transported on ice to the laboratory.

### *Surface Water Quality and Aquatic Biota*

As noted in the site selection criteria, it is desired that each study watershed have a receiving stream and downstream lake or pond, so that effects on both running and standing water communities can be studied. Procedures for sampling these two communities follow, and the next section presents analytical procedures:

#### 1. *Streams*—

- Measure dissolved oxygen and temperature daily in the field during snow-melt runoff. Continue until oxygen concentration becomes saturated, if it has been depressed.
- Collect a sample for biochemical oxygen demand (BOD) and specific conductivity determinations daily during snow melt runoff and until oxygen returns to saturation. Transport on ice to the laboratory.
- Collect a sample for routine analyses as specified in the following section weekly during snow-melt runoff. Measure pH in the field. Transport the remaining sample on ice to the laboratory.
- Collect a sample for routine analysis of the constituents specified in the next section before, during, and after treatments throughout the duration of the project, except during snow-melt runoff (see steps a–c). Perform dissolved oxygen, temperature, and pH analyses in the field on these occasions.
- On one occasion during the summer before treatments and once during each summer following treatments (on approximately the same date), perform an electrofishing survey of the stream. Use the method in Appendix M (section M-4). Also, perform benthic macroinvertebrate surveys on this schedule using a Surber or another appropriate sampler at randomly selected locations in a stream riffle. Preserve samples in ethanol until identified and enumerated in the laboratory.

2. *Stream sediments*—Before treatments and in the spring following snow-melt runoff period, collect and composite a num-

ber of stream sediment samples representing each sediment size class. Transport to the laboratory for extraction and metals analysis.

#### 3. *Lakes or ponds*—

- Perform procedures a–d outlined for streams above.
- On weekly sampling occasions during snow-melt runoff, also collect samples for chlorophyll *a* measurement, algal cell count, and bacterial plate count. Preserve samples as indicated in Table 3.
- On routine sampling occasions, also collect samples for chlorophyll *a* measurement, algal cell concentration, bacterial plate count, total phosphorus analysis, and zooplankton identification.

## **Laboratory Procedures**

### *General*

The study site monitoring program outlined above includes collection of water samples from several media (runoff, the unsaturated soil groundwater, streams, and lakes or ponds). These various samples are scheduled for a number of analyses, some common to all and others depending on the medium sampled. In addition, soils and stream sediments are designated to be extracted for metals analysis. Table 3 presents the methods recommended for use in all of these analyses. The following sections in this chapter list the specific analyses to be performed on each sample type and discuss any special considerations applying to these analyses. Appendix M contains several detailed procedures referenced in this chapter.

### *Surface Runoff*

Composite runoff samples should be analyzed for specific conductivity, total alkalinity, total suspended solids, biochemical oxygen demand (BOD), volatile fatty acids (VFA), dissolved (filtered) and total extractable metals, and total phosphorus as specified in Table 3. If sodium chloride is the alternative deicing agent used in the catchment near the watershed receiving CMA, the metals analysis of all samples should include sodium. Also, all samples should be analyzed for the chloride ion in this case. If urea is the alternative deicing agent, all samples should receive analysis for ammonia-nitrogen, nitrate + nitrite-nitrogen, and total Kjeldahl nitrogen. If another alternative deicer is selected, other analyses may have to be specified.

### *Soil and Soil Water Chemical Properties*

Soil cores 30 cm in length should be cut into 0–2, 2–5, 5–10, 10–15, 15–20, and 20–30 cm sections. Extract each section with DTPA according to the method of Lindsay and Nowell (57). Analyze extracts for metals as specified in Table 3. If the alternative deicing agent is NaCl, the metals analysis should include sodium. In addition, determine the cation exchange capacity of all samples according to the procedure in Appendix M (section M-6).

Soil water obtained from lysimeters should be analyzed for specific conductivity, total alkalinity, VFA, and total extractable metals as specified in Table 3. If NaCl is the alternative deicing agent in use, analyze all samples for sodium and chloride. If

Table 3. Laboratory procedure specifications.

| Constituent                                | Method                                       | References                              |   | Preservation  | Maximum Holding Period |
|--|--|---|---|---|------------------------|
|  |  | American Public Health Association (55) | U.S. Environmental Protection Agency (56) |   |                        |
| pH   | Potentiometric                               | 423                                     | 150.1                                     | None  | Field                  |
| Specific Conductivity                      | Wheatstone bridge-type meter                 | 205                                     | 120.1                                     | 4°C refrigeration   | 24 hours               |
| Dissolved Oxygen                           | Azide modification of Winkler Method         | 421B                                    | 360.2                                     | None  | Field                  |
| Total Alkalinity                           | Titrimetric                                  | 403                                     | 310.1                                     | 4°C refrigeration   | 24 hours               |
| Total Suspended Solids (TSS)               | Gravimetric                                  | 209C                                    | 160.2                                     | 4°C refrigeration   | 7 days                 |
| Biochemical Oxygen Demand (BOD)            | 5 day, 20°C Respirometric                    | 507                                     | 405.1                                     | 4°C refrigeration   | 24 hours               |
| Volatile fatty acids (VFA)                 | Gas chromatographic                          | 504A                                    | --  | H <sub>2</sub> SO <sub>4</sub> to pH < 2                      | 24 hours               |
| Metals (Al, Ca, Cu, Fe, K, Mg, Pb, Se, Zn) | Inductively coupled plasma (ICP)             | 305                                     | --  | HNO <sub>3</sub> to pH < 2                                    | 6 months               |
| Sodium (Na)                                | Atomic absorption <sup>b</sup>               | 303A                                    | Section 200                               | HNO <sub>3</sub> to pH < 2                                    | 6 months               |
| Chloride                                   | Argentometric                                | 407A                                    | --  | None  | 7 days                 |
| Total Phosphorus                           | Ascorbic acid following persulfate digestion | 424F                                    | 365.2                                     | H <sub>2</sub> SO <sub>4</sub> to pH < 2<br>4°C refrigeration | 4 weeks                |
| Ammonia-Nitrogen                           | Phenate                                      | 417C                                    | --  | H <sub>2</sub> SO <sub>4</sub> to pH < 2<br>4°C refrigeration | 24 hours               |
| Nitrate + Nitrite-Nitrogen                 | Automated cadmium reduction                  | 418F                                    | 353.2                                     | H <sub>2</sub> SO <sub>4</sub> to pH < 2<br>4°C refrigeration | 24 hours               |
| Total Kjeldahl Nitrogen                    | Macro-Kjeldahl                               | 420A                                    | --  | H <sub>2</sub> SO <sub>4</sub> to pH < 2<br>4°C refrigeration | 4 weeks                |
| Chlorophyll <i>a</i>                       | Fluorometric                                 | 1002G                                   | --  | Filter same day, freeze filter                                | --                     |
| Algal cell concentration                   | Counting                                     | 1002F                                   | --  | Lugol's solution  | --                     |
| Zooplankton                                | Counting                                     | --                                      | --  | Formalin  | --                     |
| Bacteria Plate Count                       | Pour plate                                   | 907A                                    | --  | None  | Same day               |

Notes: <sup>a</sup>Acetate and other water-soluble fatty acids. See Appendix M-5 for additional details.

<sup>b</sup>Significant Ca concentration produces excessive interference in Na analysis by ICP and requires analyzing Na by atomic absorption.

urea is the alternative deicing agent, analyze ammonia-nitrogen, nitrate + nitrite-nitrogen, and total Kjeldahl nitrogen in all samples.

Samples for analyses of soil microbial activity should be held at 5 C until analyses are performed. Within 24 hours of collection, extract samples for adenosine triphosphate (ATP) determination according to the method of Dople and Hanks (58). As soon as possible, but always within 2 days, begin procedures for pH measurement and counts of microfauna, microfloral colonies (fungi and bacteria), and culturing of specific fungal and bacterial species of interest. Procedures for pH and microfauna and -flora counts are contained in Appendix M (section M-7).

#### Groundwater Chemical Properties

Groundwater samples should be analyzed in the laboratory for specific conductivity, total alkalinity, VFA, and dissolved and total extractable metals as specified in Table 3. If NaCl is

the alternative deicer, all samples should be analyzed for sodium and chloride. If urea is the alternative, all should be analyzed for the nitrogen species listed in the preceding section.

#### Surface Water Quality and Aquatic Biota

All stream and lake or pond samples returned to the laboratory should be analyzed for specific conductivity, total alkalinity, total phosphorus, BOD, VFA, and dissolved and total extractable metals. In addition, total suspended solids should be measured in stream samples. As with other water samples, the analysis should include sodium and chloride if NaCl is the alternative deicer, or nitrogen species if urea is the alternative.

Stream invertebrates should be identified to genera in the case of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies). Identification to order or family will be sufficient for other organisms. Enumerate each taxon and express results for each stream reach in numbers/m<sup>2</sup>.



Stream sediments should be extracted with DTPA as detailed by Lindsay and Nowell (57) and analyzed for metals, including sodium if NaCl is the alternative deicer.

In addition to the chemical analyses specified above, lake or pond water samples should be analyzed for chlorophyll *a*, algal cell concentrations, bacterial plate counts, and zooplankton concentrations. General specifications for these procedures are in Table 3. Algal cells and zooplankton should be identified to the genus level and enumerated for presentation of results in terms of numbers per unit water volume.

#### Quality Assurance/Quality Control Plan

The quality assurance/quality control plan to be followed in the research should be guided generally by the U.S. Environmental Protection Agency's "Handbook for Analytical Quality Control in Water and Wastewater Laboratories" (59). The elements of this plan follow:

1. *Sample labeling and handling*—All samples should be labeled with the station, date, time, and preservative added (if any). A custody transfer tag should accompany each sample to register those handling it. Preservation and holding times recommended by the EPA should be observed, as indicated in Table 3.
2. *Equipment calibration*—All calibration procedures recommended by equipment manufacturers and the analytical methods should be observed. Records of calibrations should be maintained.
3. *Replicate samples*—At least 5 percent of the samples of each type should be collected in duplicate on the basis of a random selection.
4. *Replicate measurements*—At least 5 percent of the analyses should be randomly selected for duplication.
5. *Split samples*—Some samples should be sent to another laboratory for comparative analyses of each constituent.
6. *Spiked samples*—Known amounts of each constituent should be added periodically to actual samples or blanks to check accuracy.
7. *Sample preservation blanks*—Preservative contamination should be checked periodically by adding to distilled water in the specified amounts and analyzing for each constituent.
8. *Reference samples*—EPA reference samples for each constituent should be run every 6 months.

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## APPENDIX A

### GENERAL ANALYTICAL PROCEDURES

#### INTRODUCTION

Various experiments reported in the succeeding appendices required the same analyses of laboratory or controlled field plot samples. These samples included water from laboratory test vessels, surface runoff from the plots and bins, soil water from the unsaturated zone, groundwater, and field ponds. In addition, CMA itself and soil extracts from laboratory experiments and field plots were subjected to some of these same analyses. This appendix serves to specify the analytical procedures common to a number of experiments. Specialized procedures are presented as appropriate in the following appendices.

This appendix also reports data that were obtained on meteorological conditions at the controlled field plots through onsite measurements. These data define conditions affecting a number of the experiments discussed in subsequent appendices.

#### EXPERIMENTAL METHODS

##### General Laboratory Methods

Table A-1 lists the general constituents measured in this research program, along with the methods and equipment used in the analyses and the techniques used to preserve the samples until analysis. Analysis of volatile fatty acid salts is not as highly standardized as other procedures. Therefore, the methods used for this analysis will be explained in detail.

Table A-1. Laboratory Analysis Specifications

| Constituent                                | Method                                       | References                             |  | Equipment  | Preservation  |
|--|--|--|--|--|---|
|  |  | American Public Health Association (1) | U.S. Environmental Protection Agency (2) |  |   |
| pH   | Potentiometric                               | 423                                    | 150.1                                    | Cole-Parmer Model 5985-80 meter                                | None  |
| Specific Conductivity                      | Wheatstone bridge-type meter                 | 205                                    | 120.1                                    | Barnstead Meter  | 4 C refrigeration   |
| Dissolved Oxygen                           | Azide modification of Winkler Method         | 421B                                   | 360.2                                    | --   | None  |
| Total Alkalinity                           | Titrimetric                                  | 403                                    | 310.1                                    | --   | 4 C refrigeration   |
| Total Suspended Solids (TSS)               | Gravimetric                                  | 209C                                   | 160.2                                    | Mettler Type H15 analytical balance                            | 4 C refrigeration   |
| Biochemical Oxygen Demand (BOD)            | Azide modification of Winkler Method         | 507                                    | 405.1                                    | --   | 4 C refrigeration   |
| Volatile fatty acid salts (VFA)            | Gas chromatographic                          | 504A                                   | --                                       | Hewlett-Packard Model 5840A gas chromatograph                  | H <sub>2</sub> SO <sub>4</sub> to pH                          |
| Metals (Al, Ca, Cu, Fe, K, Mg, Pb, Se, Zn) | Inductively coupled plasma (ICP)             | 305                                    | --                                       | Jarrell-Ash ICP  | HNO <sub>3</sub> (Ultrex) to pH < 2                           |
| Total Phosphorus                           | Ascorbic acid following persulfate digestion | 424F                                   | 365.2                                    | Perkin-Elmer Lambda 3 spectrophotometer<br>Barnstead autoclave | H <sub>2</sub> SO <sub>4</sub> to pH < 2<br>4 C refrigeration |
| Chloride                                   | Ion chromatographic                          | --                                     | --                                       | Dionex ion chromatograph                                       | 4 C refrigeration   |
| Nitrate-Nitrogen                           | Ion chromatographic                          | --                                     | --                                       | Dionex ion chromatograph                                       | Freezing  |
| Sulfate                                    | Ion chromatographic                          | --                                     | --                                       | Dionex ion chromatograph                                       | Freezing  |

<sup>a</sup>Acetate and other salts of water-soluble fatty acids. See text for additional details.

Volatile fatty acids (VFA) include acetic ( $\text{CH}_3\text{COOH}$ ), propionic ( $\text{C}_3\text{H}_5\text{COOH}$ ), butyric ( $\text{C}_4\text{H}_7\text{COOH}$ ), and other acids of higher carbon number. VFA were determined by direct aqueous injection of acidified samples into the Hewlett-Packard gas chromatograph with flame ionization (FID) detector. With each analysis an acidified blank and compound standards containing five concentrations of acetic, propionic, and butyric acids were run to develop a standard curve. Acidification with formic acid to  $\text{pH} < 2$  drives the chemical equilibrium toward the acid species of the acetate, propionate, and butyrate salts actually present in samples for detection by gas chromatograph. It was determined through initial work with liquid, undiluted CMA that acetate and butyrate were present in the material, but that propionate and other VFA were not. Nevertheless, propionate was routinely measured and was detected in a small number of samples.

Following are specifications for the equipment and settings used in VFA analysis:

Gas chromatograph column--6 ft x 2 mm ID glass column packed with 0.3 percent Carbowax 20M/0.1%  $\text{H}_3\text{PO}_4$  on 60/80 Carbo-pack C (Supelco, Inc., Bellefonte, PA)

Oven temperature--120 C

FID temperature--250 C

Injection port temperature--200 C

Sample volume--1 l

Carrier gas--Nitrogen at 50 ml/min

To analyze VFA in soils for some experiments, a water-extraction procedure was employed. Following are the steps in this special procedure:

1. Save a soil sample for determination of the moisture content relative to air-dry soil.
2. Place 5 grams of soil in a 50 ml Erlenmeyer flask. Add 7.5 ml of distilled water. Break soil apart with a glass stirring rod. Cover with Parafilm.
3. Put flask on a mechanical shaker for 30 minutes.
4. Set small test tube inside a suction flask. Place Millipore filter on flask. Use a 0.45 micron filter underneath a glass fiber filter (to prevent clogging of the 0.45 micron filter).
5. Pour Erlenmeyer flask contents into the Millipore filter, using the glass stirring rod.
6. Rinse the flask three times with 2.5 ml aliquots of distilled water, pouring through the cake on the Millipore filter.
7. Add concentrated formic acid dropwise to the filtrate, until the filtrate is acidified to pH 2. Test pH by placing a drop of the filtrate onto pH paper.
8. Cover tube with Parafilm until gas chromatographic analysis.

#### Laboratory Quality Assurance/Quality Control

The quality assurance/quality control plan followed in the research program was generally guided by the provisions of the U.S. Environmental Protection Agency (3). These provisions specify sample labeling and handling, equipment calibration, replication, and analysis of check samples.

All samples were labeled completely with type, station, date, and time (if appropriate) and preserved promptly as specified in Table A-1. Calibration procedures recommended by equipment manufacturers and the analytical methods were applied.

Duplicate analyses were run routinely for VFA, metals, and total phosphorus. Other, more routine analyses were duplicated a sufficient number of times to gain assurance of the precision of the methods and equipment, or to check when a problem occurred or was suspected. In these cases duplicates totaled approximately 10 percent of all samples run.

Reference samples with known concentrations were obtained from the U.S. Environmental Protection Agency (USEPA) for the following analyses: pH, total alkalinity, total phosphorus, chloride, nitrate-nitrogen, sulfate, and metals. These reference samples were given to analytical technicians to run as unknowns, and their results were compared with the reported known concentrations.

#### **Meteorological Measurements**

Data were collected on air temperature, precipitation, and soil temperature using equipment installed at the field site. Air temperature was measured with a maximum/minimum mercury thermometer read during each field visit. Total precipitation quantity was measured using a rainfall bucket, also read during each visit. Soil temperature was measured during treatment periods with the use of copper-constantin thermocouples placed at 6, 30, and 60 cm depths and a Wescor millivolt psychrometer.

## **RESULTS**

### **Laboratory Quality Assurance/Quality Control**

The precision of analysis is reported, as appropriate, with the results of specific experiments in the following appendices. Table A-2 presents the results of the analysis of USEPA reference samples. Measurements usually agreed with true values within 10 percent. Exceptions were five metals samples, which deviated from 14.7 to 36.7 percent. Only K exhibited two results more than 10 percent off (both low).

### **Meteorological Measurements**

Figures A-1, 2, and 3 present field site air temperature, precipitation, and soil temperature, respectively, over the course of the controlled plot experiments. During and for a time after treatments minimum air temperatures were generally below 35 F (2 C). Maximum temperatures were usually between 40 and 50 F (4-10 C) in the second treatment season, although somewhat higher in the first, which occurred later (March). During the first set of treatments (March, 1986) surface soil temperatures were more than 8 C, and temperatures at depth were 5-8 C. The soil temperature regime was quite different during the second series (December, 1986 - February, 1987), and temperatures were lower. Despite placement of the controlled plots at some elevation, these temperatures were higher than experienced in winter in many locations that receive deicing materials. Nonetheless, they were in the range shown in laboratory experiments to retard CMA decomposition (see Appendices F and I).

Table A-2. Results of USEPA Reference Sample Analyses

| <u>Constituent</u> | <u>Sample</u>    | <u>Dilution</u> | <u>Unit</u>       | <u>True Value</u> | <u>No. Replicates</u> | <u>Mean of Measurements</u> | <u>Standard Deviation</u> | <u>Difference From True (%)</u> |
|--------------------|------------------|-----------------|-------------------|-------------------|-----------------------|-----------------------------|---------------------------|---------------------------------|
| pH                 | EPA-WP 882 No. 1 | 1:1             | pH                | 5.6               | 1                     | 5.7                         | --                        | + 1.8                           |
|                    | EPA-WP 882 No. 2 | 1:1             |                   | 7.8               | 1                     | 7.7                         | --                        | - 1.3                           |
| Total Alkalinity   | EPA-WP 882 No. 1 | 1:1             | mg/l as           | 34.4              | 3                     | 34.8                        | 0.60                      | + 1.2                           |
|                    | EPA-WP 882 No. 2 | 1:1             | CaCO <sub>3</sub> | 17.5              | 3                     | 19.4                        | 0.36                      | +10.9                           |
| Total Phosphorus   | EPA-WP 284 No. 5 | 1:1             | µg/l              | 130               | 2                     | 123                         | 5.7                       | - 5.4                           |
| Chloride           | EPA-WP 384 No. 2 | 1:10            | µeq/l             | 32.4              | 3                     | 31.6                        | 0.84                      | - 2.5                           |
| Nitrate-Nitrogen   | EPA-WP 284 No. 3 | 1:1             | µeq/l             | 9.99              | 3                     | 9.21                        | 0.37                      | - 7.8                           |
| Sulfate            | EPA-WP 384 No. 2 | 1:10            | µeq/l             | 14.9              | 3                     | 14.6                        | 0.19                      | - 2.0                           |

Continued

Table A-2 Continued

| <u>Constituent</u> | <u>Sample</u>      | <u>Dilution</u> | <u>Unit</u> | <u>True Value</u> | <u>No. Replicates</u> | <u>Mean of Measurements</u> | <u>Standard Deviation</u> | <u>Difference From True (%)</u> |
|--------------------|--------------------|-----------------|-------------|-------------------|-----------------------|-----------------------------|---------------------------|---------------------------------|
| Metals: Al         | Trace Metals I-1   | 1:1             | µg/l        | 107               | 3                     | 131                         | 4                         | +22.4                           |
|                    | Trace Metals I-2   | 1:1             | µg/l        | 729               | 3                     | 766                         | 2                         | + 5.1                           |
| Cu                 | Trace Metals I-1   | 1:1             | µg/l        | 8.9               | 3                     | 9.2                         | 1.3                       | + 3.4                           |
|                    | Trace Metals I-2   | 1:1             | µg/l        | 339               | 3                     | 335                         | 0                         | - 1.2                           |
| Fe                 | Trace Metals I-1   | 1:1             | µg/l        | 20.9              | 3                     | 21.0                        | 0.4                       | + 0.5                           |
|                    | Trace Metals I-2   | 1:1             | µg/l        | 797               | 3                     | 779                         | 2                         | - 2.3                           |
| Pb                 | Trace Metals I-1   | 1:1             | µg/l        | 42.7              | 3                     | 34.4                        | 7.6                       | -19.4                           |
|                    | Trace Metals I-1   | 1:1             | µg/l        | 435               | 3                     | 391                         | 5                         | -10.1                           |
| Se                 | Trace Metals I-1   | 1:1             | µg/l        | 10.9              | 3                     | 14.9                        | 11.7                      | +36.7                           |
|                    | Trace Metals I-2   | 1:1             | µg/l        | 50.2              | 3                     | 49.0                        | 0.2                       | - 2.4                           |
| Zn                 | Trace Metals I-1   | 1:1             | µg/l        | 9.8               | 3                     | 9.5                         | 0.4                       | - 3.1                           |
|                    | Trace Metals I-2   | 1:1             | µg/l        | 418               | 3                     | 412                         | 0.6                       | - 1.4                           |
| Ca                 | Trace Metals III-1 | 1:1             | µg/l        | 500               | 3                     | 500                         | 5                         | 0                               |
|                    | Trace Metals III-2 | 1:1             | µg/l        | 7500              | 3                     | 7620                        | 90                        | + 1.6                           |
| K                  | Trace Metals II-1  | 1:1             | µg/l        | 200               | 3                     | 148                         | 14                        | -26.0                           |
|                    | Trace Metals III-2 | 1:1             | µg/l        | 1500              | 3                     | 1280                        | 30                        | -14.7                           |
| Mg                 | Trace Metals III-1 | 1:1             | µg/l        | 100               | 3                     | 101                         | 1                         | + 1.0                           |
|                    | Trace Metals III-2 | 1:1             | µg/l        | 500               | 3                     | 514                         | 5                         | + 2.8                           |



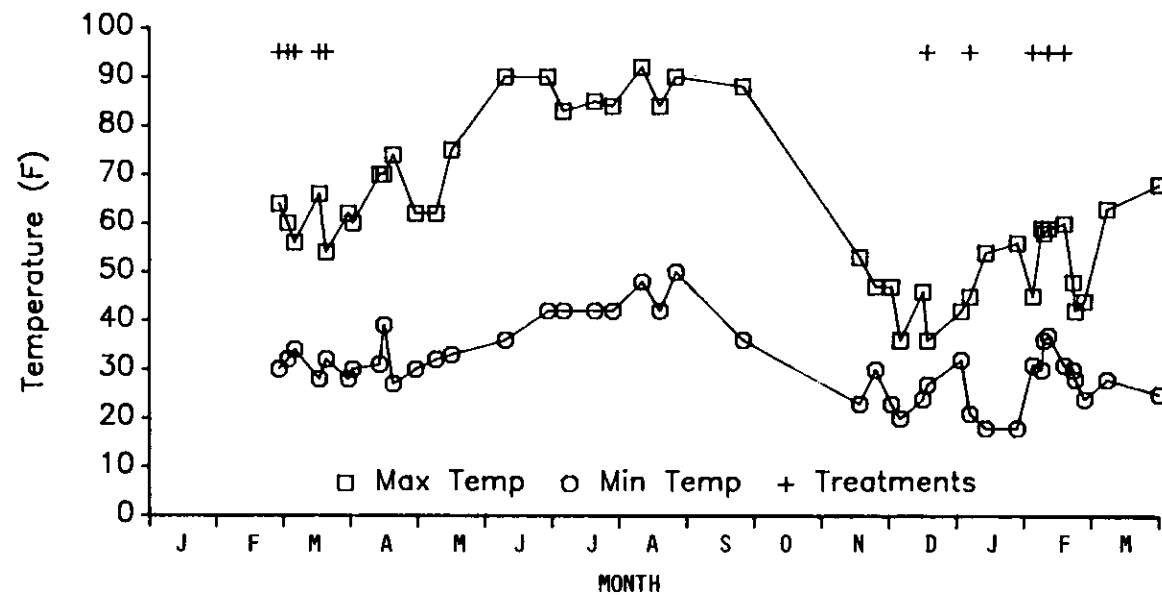


Figure A-1. Maximum and Minimum Air Temperatures Over Period of Field Plot Experiments

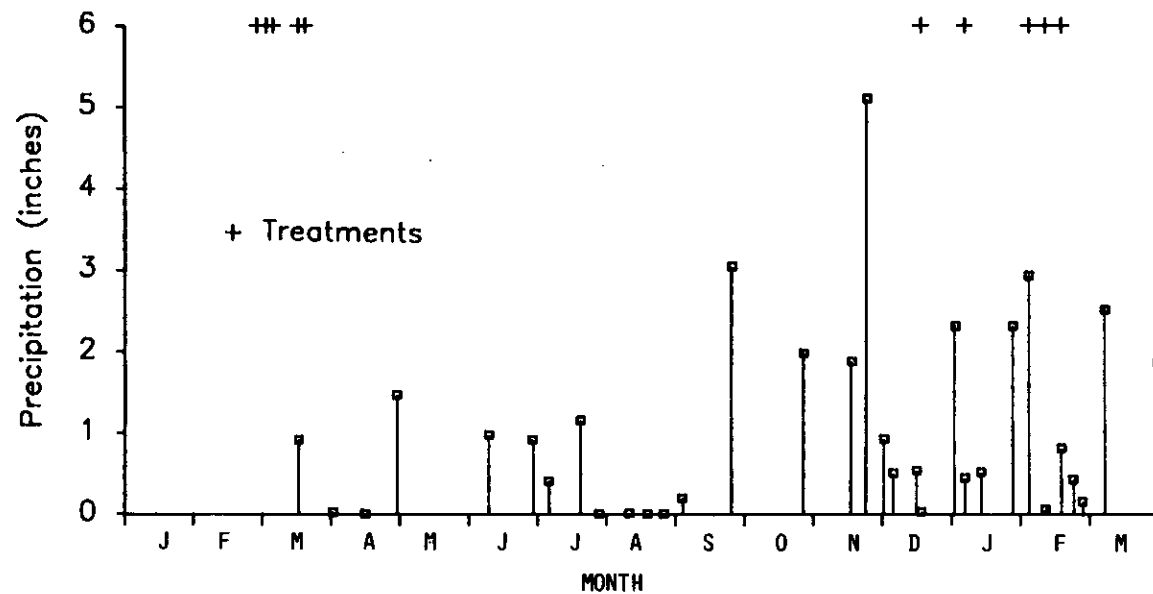


Figure A-2. Precipitation Over Period of Field Plot Experiments

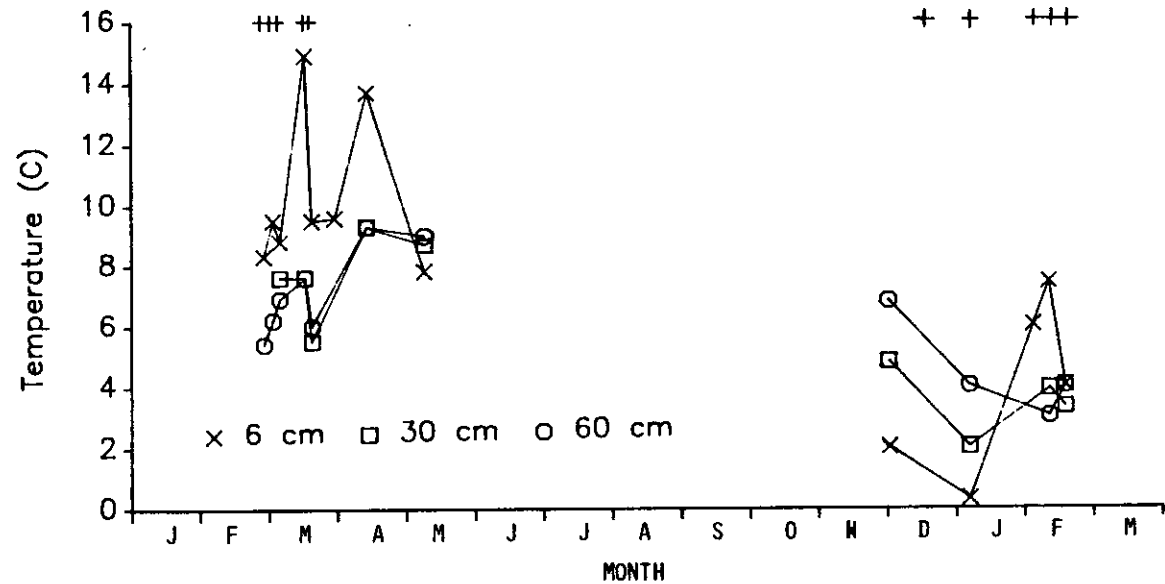


Figure A-3. Soil Temperatures at Three Depths During Two Winter Treatment Periods

The precipitation graph shows the cumulative quantity collected in a rainfall bucket between readings. It reflects the typical precipitation pattern in the Pacific Northwest: high in the fall and winter, tapering off in the spring, and very low in the summer. Precipitation was heavier during the second winter than the first. Most fell as rain rather than snow in both years.

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## APPENDIX B

### CHARACTERISTICS OF CALCIUM MAGNESIUM ACETATE

#### INTRODUCTION

The CMA used in this research program was produced by the Center for Biological Resource Recovery at the University of Georgia under a Federal Highway Administration contract. It was made from corn starch hydrolysate by fermentation using Clostridium thermoaceticum, with the addition of a dolomitic limestone (dolime) slurry to the fermentor (1). During fermentation, pH was maintained above 6 by controlled dolime addition, and glucose (starch hydrolysate) was maintained at about 0.5 percent through addition as needed. At the end of fermentation, the glucose was allowed to decrease to a value as low as possible, while more dolime was added to bring pH to approximately 8 (1).

CMA was received from the University of Georgia in two shipments in March and June, 1985. Transport was in liquid form in new, plastic 55-gallon drums. The first shipment was unrefrigerated, although the air temperatures were relatively low at the time. The drums were stored outside for several weeks upon arrival, after which they were moved to a 2 C cold room. The second shipment came by refrigerated truck and went immediately into cold storage until use. These precautions were taken to minimize acetate decomposition.

Between shipments, the Center for Biological Resource Recovery staff continued to experiment with the fermentation process, particularly to adjust the Ca:Mg ratio. As a result the contents of the two shipments differed somewhat, although not to a substantial degree.

Furthermore, most procedures were conducted with CMA composited from a number of drums. Since the makeup of a commercial grade has not yet been established, this procedure was deemed most appropriate.

Upon receipt of CMA, the first tasks were to characterize its properties and to experiment with handling it. Their results are reported below. One consideration in defining properties was whether contaminants of environmental concern could be expected to be added incidently or to improve handling or performance characteristics during actual highway use. The Washington State Department of Transportation (WSDOT) had previously tested CMA in highway use. Discussions with the WSDOT principal investigator established that no such impurities are likely. A problem with dustiness could be corrected by hydration of the dry compound, without the need for any additives (2).

The first investigation of handling CMA was concerned with the ability to draw homogenous samples from the drums. Contents were stirred with an electrically driven agitator and pumped. However, analyses (see below) indicated that carefully drawn samples were quite variable, apparently due to the large quantities of organic and inorganic settleable solids present. This result also indicated the advisability of compositing CMA lots.

Subsequently, the idea of evaporating the liquid to produce dry CMA arose for several reasons. The dry material could be used directly for certain purposes or redissolved in water for most experiments. One reason to consider working with dry CMA was the belief that an eventual commercial grade almost certainly would be a solid. It was posited that a number of attractive experimental advantages could also be derived by

using the solid material. The greatly reduced volume would permit convenient storage, transport to the field, and compositing. It was thought that dessication would effectively prevent biodegradation of the acetate. Moreover, properties of a CMA batch could be accurately determined and regulated for the various experimental purposes. With these advantages in mind, tests were conducted to develop a workable drying technique and to establish whether drying would affect the characteristics of the material in a manner harmful to the objectives of the program (see below). After a convenient technique was developed and it was demonstrated that essential properties of the material were maintained in drying, a large-scale drying operation was instituted to serve the needs of the laboratory and field experiments.

## EXPERIMENTAL METHODS

### Investigation of Drawing Homogenous Liquid Samples

In order to investigate the ability to draw homogenous samples of liquid CMA from the drums in which it was shipped, two samples were drawn from each of seven drums in the first shipment. Care was taken to mix these drums equally and to employ the same sampling technique in each case. The samples were analyzed for conductivity and pH by the procedures given in Table A-1 and for turbidity with a nephelometric turbidimeter.

### Analysis of CMA Drum Contents

CMA drawn as a liquid from the drums in which it was shipped was analyzed principally for volatile fatty acid salts and metals. The procedures for these analyses are specified in Appendix A.

A concern early in the project was whether alcohols are present in CMA and should be analyzed routinely in experimental samples. Six alcohols (ethanol, propanol, isobutanol, butanol, isopentanol, and pentanol) were analyzed in a CMA drum sample using an aqueous injection in the Hewlett-Packard gas chromatograph fitted with an alcohol column (GP 80/100 Carbopack C/0.2% Carbowax 1500, 61 X 2 mm).

A similar concern was whether sufficient glucose remained after fermentation to warrant regular analysis during experimental work. Data were received from the University of Georgia showing that no glucose remained in some drums and that molar ratios of glucose:acetate were always less than 0.005. Therefore, no effort was made to develop this analysis and run it routinely.

### Investigation of Drying CMA

A procedure was tested to evaporate the liquid from CMA drum contents at a relatively low temperature in order to avoid any volatilization and loss of the acetate. This procedure involved holding CMA solution in a shallow, open pan on a 55 C hot plate in a greenhouse for a number of days. This operation was carried out primarily in the late spring through early fall period, when relatively high air temperature reduced the need for auxiliary heating and relatively low humidity countered the strong hygroscopic property of CMA. After a nearly dry product was obtained, it was placed in a 70 C oven for one hour to drive off any remnant moisture. The material was then immediately placed in closed, clean, dry containers for storage.

In order to investigate the effect of drying on properties, the dry CMA produced as described was redissolved in water and analyzed in

the same manner as liquid material drawn from the drums in which it was shipped. Analyses included VFA and metals, performed as indicated in Table A-1.

## RESULTS

### Investigation of Drawing Homogenous Liquid Samples

Table B-1 presents the results of analyzing paired liquid CMA samples from seven drums. Conductivities ranged from 14.1 to 16.6 millimhos in individual samples but were within five percent in samples drawn from the same drum. Similarly, pH varied over about two units in different drums but never more than 0.4 unit in samples from a single drum. Turbidity was very variable among drums and differed by as much as 48 percent between paired samples.

### Analysis of CMA Drum Contents

Table B-2 presents our analysis of the contents of seven CMA drums making up the first shipment received from the University of Georgia. These data indicate that the average drum was approximately 3.3 percent CMA (the sum of acetate, calcium, magnesium, and the associated water molecules of hydration; one per calcium acetate molecule and four per magnesium acetate molecule). With the exception of potassium, concentrations of other metallic constituents were relatively small. Despite the difficulties posed by drawing homogenous samples, variation among drums was generally minor.

Analysis of alcohols in a sample of one drum produced a small ethanol peak on the chromatogram, but the concentration was below the detection limit. No other alcohol peaks appeared. With the lack of

Table B-1. Comparisons of Three Constituents in Duplicate Samples Drawn from Seven CMA Drums in the First Shipment

| <u>Drum No.</u> | <u>Sample</u> | <u>Conductivity<br/>(millimhos)</u> | <u>pH</u> | <u>Turbidity<br/>(NTU)</u> |
|-----------------|---------------|-------------------------------------|-----------|----------------------------|
| 2A              | 1             | 14.9                                | 8.2       | 69                         |
|                 | 2             | 14.9                                | 8.4       | 40                         |
| 2B              | 1             | 14.1                                | 7.8       | 81                         |
|                 | 2             | 14.8                                | 7.4       | 85                         |
| 3A              | 1             | 16.4                                | 6.4       | 510                        |
|                 | 2             | 16.6                                | 6.4       | 490                        |
| 3B              | 1             | 16.6                                | 6.5       | 500                        |
|                 | 2             | 16.6                                | 6.5       | 480                        |
| 4B              | 1             | 15.5                                | 6.4       | 420                        |
|                 | 2             | 15.6                                | 6.4       | 370                        |
| 5A              | 1             | 15.2                                | 6.4       | 1110                       |
|                 | 2             | 15.2                                | 6.3       | 750                        |
| 5B              | 1             | 15.2                                | 6.4       | 1240                       |
|                 | 2             | 15.1                                | 6.4       | 900                        |

Table B-2. Analyses of Acetate and Metals in CMA Drums in the First Shipment<sup>a</sup>

| Drum No.           | Acetate <sup>b</sup> | Ca   | Mg   | K    | Al   | Se   | Fe   | Cu    | Pb   | Zn   |
|--------------------|----------------------|------|------|------|------|------|------|-------|------|------|
| 2A                 | N.A.                 | 2650 | 2800 | 1370 | 0.58 | 1.17 | 2.04 | 0.03  | 0.58 | 0.18 |
| 2B                 | 18980                | 3130 | 2660 | 1360 | 0.68 | 1.31 | 2.63 | 0.02  | 0.62 | 0.14 |
| 3A                 | 20480                | 2730 | 3630 | 1330 | 0.62 | 1.76 | 12.4 | 0.02  | 0.77 | 0.13 |
| 3B                 | 18540                | 2670 | 3660 | 1350 | 0.62 | 1.65 | 13.0 | 0.02  | 0.85 | 0.14 |
| 4B                 | 20470                | 2390 | 3110 | 1260 | 0.50 | 1.55 | 7.50 | 0.01  | 0.63 | 0.11 |
| 5A                 | N.A.                 | 2980 | 2940 | 1310 | 0.51 | 1.52 | 16.6 | 0.02  | 0.62 | 0.11 |
| 5B                 | 19060                | 2970 | 2910 | 1300 | 0.55 | 1.46 | 13.0 | 0.02  | 0.67 | 0.12 |
| Mean               | 19506                | 2790 | 3101 | 1330 | 0.58 | 1.49 | 9.03 | 0.02  | 0.68 | 0.13 |
| Standard Deviation | 906                  | 250  | 400  | 40   | 0.07 | 0.20 | 5.94 | 0.006 | 0.10 | 0.02 |

<sup>a</sup>All values are in mg/l.<sup>b</sup>N.A.--not available.

significant alcohol concentrations in this concentrated sample, it was decided that alcohols need not be analyzed in experimental samples, which were more dilute in all cases.

Table B-3 presents an analysis of two drums in the second shipment. As may be seen, these drums were more concentrated (average 5.3 percent CMA). In these samples Ca:Mg ratio averaged 1.37:1, whereas it had been 0.9:1 in the first shipment. With the exception of higher Fe and lower Pb, these drums were similar to the first shipment in other metals. Variability between drums again was generally slight.

#### Investigation of Drying CMA

CMA from drums in the first shipment was dried in three independent lots according to the procedure outlined above. Dry material was redissolved in distilled water and analyzed for VFA and metals. Table B-4 presents the results. Properties of the three lots

differed very little. With the exception of iron content, they also differed little from the original aqueous material. There was no apparent tendency for drying either to concentrate or dilute metals.

Of specific interest was the phosphorus (P) content of the CMA, because of its possible role in eutrophication of surface waters receiving CMA runoff. The mean of three determinations of P in dry CMA was 0.020 percent (standard deviation = 0.005 percent), which agreed exactly with previous measurement at the University of Georgia. Therefore, each 100 mg/l of CMA dissolved in runoff water would deliver 20  $\mu$ g P/l to the receiving water. This P transport would have eutrophication potential depending on exactly what CMA concentration drains off and to what extent it is diluted in the receiving body.

Table B-3. Analyses of Acetate and Metals in Two CMA Drums in Second Shipment<sup>a</sup>

| <u>Drum No.</u>    | <u>Acetate</u> | <u>Ca</u> | <u>Mg</u> | <u>K</u> | <u>Al</u> | <u>Se</u> | <u>Fe</u> | <u>Cu</u> | <u>Pb</u> | <u>Zn</u> |
|--------------------|----------------|-----------|-----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|
| 1                  | 30250          | 5630      | 4080      | 1620     | 0.06      | 1.17      | 38.5      | 0.00      | 0.008     | 0.19      |
| 2                  | 32200          | 5670      | 4140      | 1660     | 0.71      | 0.94      | 40.4      | 0.00      | 0.010     | 0.23      |
| Mean               | 31225          | 5650      | 4110      | 1640     | 0.39      | 1.06      | 39.5      | 0.00      | 0.009     | 0.21      |
| Standard Deviation | 1380           | 28        | 42        | 28       | 0.46      | 0.16      | 1.3       | 0.00      | 0.001     | 0.03      |

<sup>a</sup>All values are in mg/l.Table B-4. Comparisons of Properties of Redissolved Dry CMA and Original Drum Contents<sup>a</sup>

| <u>Sample</u>                      | <u>Acetate</u> | <u>Ca</u> | <u>Mg</u> | <u>K</u> | <u>Al</u> | <u>Se</u> | <u>Fe</u> | <u>Cu</u> | <u>Pb</u> | <u>Zn</u> |
|------------------------------------|----------------|-----------|-----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Dried Lot 1                        | 21180          | 2480      | 2920      | 1290     | 0.46      | 1.04      | 1.38      | 0.04      | 0.23      | 0.16      |
| Dried Lot 2                        | 21150          | 2500      | 3030      | 1260     | 0.49      | 1.08      | 0.88      | 0.00      | 0.25      | 0.30      |
| Dried Lot 3                        | 20620          | 2600      | 3060      | 1300     | 0.52      | 1.10      | 0.98      | 0.01      | 0.28      | 0.19      |
| Mean of Dried Lots                 | 20980          | 2530      | 3000      | 1280     | 0.49      | 1.07      | 1.08      | 0.02      | 0.25      | 0.22      |
| Standard Deviation of Dried Lots   | 315            | 64        | 74        | 21       | 0.03      | 0.03      | 0.26      | 0.02      | 0.03      | 0.07      |
| Mean of Drum Contents <sup>b</sup> | 19506          | 2790      | 3101      | 1330     | 0.58      | 1.49      | 9.03      | 0.02      | 0.68      | 0.13      |

<sup>a</sup>All values are in mg/l.<sup>b</sup>From Table B-2.

## DISCUSSION

Additional lots of dry CMA were produced during the two summers over which the project extended. All products of the first summer's effort was composited to form a single batch, which was used in laboratory experiments and the first winter field season. Remains from that batch were then composited with all of the second summer's product to make a uniform batch for the second winter's treatments. Compositing was considered to be the best procedure in light of some variability between CMA in the two shipments.

The investigation of drawing homogenous liquid samples from drums showed that it was possible to obtain a relatively high degree of uniformity in dissolved components but little homogeneity in particulates. Even with compositing of drums, uniformity in CMA stock between experiments could not have been ensured, and compositing of the large volumes of liquid would have been very difficult. This finding, coupled with the success of drying and the advantages of working with the dry solid, led to a decision to dry sufficient CMA for all laboratory and controlled plot field experiments. The similarity in properties between redissolved dry CMA and the original aqueous product supported a further decision not to duplicate experiments using both forms.

The measurement of acid pH in several drums in the initial shipment, when they had been adjusted to pH 8 after fermentation, initially caused fear that saprophytes were degrading the acetate. Observation of growing cell masses in samples aggravated that fear. However, as the acetate analyses show, low and high pH drums had

comparable acetate. Organisms apparently were utilizing another substrate. Nevertheless, drums were quickly moved to cold storage before much acetate destruction could occur.

With the finding that low drum pH did not signal the destruction of acetate, there was no further concern with variable pH. There was no attempt to control or adjust pH beyond the procedure routinely employed by the University of Georgia.

The CMA received from the University of Georgia was relatively high in potassium. Comparisons of measurements taken after receipt of the drums with those made in Georgia before shipment (3) showed agreement in metals concentrations in general and K in particular. The CMA manufacturers did not discuss this point or possible sources of the potassium. One possible source is the fermentation medium, which contained 1250 grams of potassium phosphates per each 400 liter lot (3).

Since the research involved an environmental evaluation of CMA, an initial prediction of its potential impact was useful to help plan experiments. This prediction was developed by determining how much the dry material would have to be diluted to avoid violating water quality standards. The major constituents, calcium, magnesium, potassium, and acetate, can effect aquatic life osmotically, but no standards apply to them. Drinking water and aquatic life protection standards do exist for various heavy metals. Table B-5 illustrates a comparison between CMA content of Cu, Pb, and Zn and the standards for these elements. No dilution would be required to meet four of the six standards, while no more than 1:4 dilution by weight would be needed to avoid a violation of the others. Because CMA in actual use would normally be diluted in



Table B-5. CMA Content of Three Heavy Metals Compared to Drinking Water and Aquatic Life Protection Standards<sup>a</sup>

|   | <u>Cu</u> | <u>Pb</u> | <u>Zn</u> |
|---|-----------|-----------|-----------|
| CMA Content <sup>b</sup>  | 0.02      | 0.25      | 0.22      |
| Drinking Water Standard   | 1         | 0.05      | 5         |
| CMA Dilution Needed to Achieve Drinking Water Standard <sup>c</sup> | None      | 1:4       | None      |
| Maximum for Aquatic Life <sup>d</sup>                               | 0.022     | 0.17      | 0.32      |
| CMA Dilution Needed to Achieve Aquatic Life Standard <sup>c</sup>   | None      | 1:5       | None      |

<sup>a</sup>All values are in parts per million (= mg/l in water solution).

<sup>b</sup>Mean of dried lots from Table B-4.

<sup>c</sup>Dilution is expressed in terms of parts CMA:parts water diluent by weight.

<sup>d</sup>Maximum exposure to aquatic life in waters with total hardness of 100 mg/l as CaCO<sub>3</sub> (4). Because of the antagonism of hardness-producing elements to heavy metal toxicity, standards are lower in softer waters and higher in harder waters.

large meltwater runoff volumes, there was early evidence that heavy metal toxicity impacts associated with CMA should be minimal.

#### REFERENCES

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## APPENDIX C

### ENVIRONMENTAL TRANSPORT OF CMA

#### INTRODUCTION

##### General

Attention to environmental transport of CMA in this research program involved both theoretical considerations and experiments at the controlled field plots and in the bins. These studies concerned CMA application to and transport from highways, CMA runoff characteristics, surface transport of runoff (overload flow), and vertical transport of CMA solution in soils. This appendix presents the results of these studies. Theoretical considerations are covered in the Introduction, and experiments and their results are summarized in the remainder of Appendix C.

The consideration of highway application and the subsequent removal of CMA from highways was theoretical. Performing this exercise was necessary to obtain estimates of concentrations and mass loadings of CMA constituents in actual highway runoff. These estimates, in turn, were used to hypothesize potential impacts on various receptors and to plan experiments to test for these impacts. In particular, typical and worst-case treatments had to be established for both laboratory and controlled plot experiments. The consideration of highway application and removal was based on typical CMA application rates used in previous highway performance trials, and on scenarios representing a range of meteorological events.

After preliminary theoretical analysis, determinations of runoff characteristics and surface and vertical transport of CMA solution were based on measurements at the controlled field plots and in the bins that had been installed principally for observation of CMA effects on plants. Plot and bin runoff was directly analyzed for physical and chemical constituents. In the case of bins, samples were collected at intermediate points along the flow path to determine changes brought about by contact with vegetation and the soil surface. Vertical CMA transport was measured in the unsaturated zone of controlled plot soils with the use of lysimeters and in the saturated zone with well points.

Measurements taken in lysimeter and groundwater samples also supplemented laboratory findings on physicochemical reactions involving CMA in soils. These results are discussed in Appendix E.

##### CMA Application and Transport from Highways

Washington State Department of Transportation tested CMA effectiveness in highway conditions prior to the start of this research. The typical application required was 250-300 lb per lane-mile (69-83 kg/lane-km) per storm (1). A more recent study in Ontario determined that an average quantity in the same range was required over a full winter of testing (2). As a basis for modeling typical annual CMA application to a multi-lane road, the following conditions were assumed:

250 lb/lane-mile/storm

20 storms/year

4 lanes (in one or two travel directions)

draining to the same point

The annual loading equivalent to these conditions would be 10 tons per linear mile of highway (5.5 metric tons/km), or about 4 lb per linear foot (6 kg/m). It was assumed that this entire loading would be removed from the highway and could affect terrestrial and aquatic organisms in the transfer path.

In addition to cumulative annual mass loading, there was interest in modeling CMA concentration (mass per unit volume) in highway spray or runoff, in order to represent conditions that may affect receptor organisms at any given time in a typical winter or early spring period. Modeling concentration required the assumption of a total annual water volume in which CMA would be diluted and some pattern of melting. The dilution water volume would include runoff from the road surface plus the precipitation on the runoff path. The basic volume used for estimates assumed precipitation on a 50-foot (15.2 m) wide zone, including traffic lanes and a shoulder between the highway and surface water that receives highway runoff. If it is further assumed that minimum winter precipitation is 12 inches (30.5 cm), water equivalent, then the available dilution water would be 50 ft<sup>3</sup> per linear foot of highway (4.6 m<sup>3</sup>/m). Dilution of 4 lb of CMA in 50 ft<sup>3</sup> of water would result in a concentration of 1282 mg/l (= 1282 ppm in water solution).

This CMA concentration estimate implicitly assumes that the maximum annual application in a relatively arid area is washed off in a single melting event. Lower concentrations would result from different assumptions about dilution volume and melting pattern. More typical concentrations likely would be at least an order of magnitude less than the estimate above. On the other hand, washoff of a large CMA

application by a very intense, small storm could result in a concentration of perhaps 5000 ppm. Therefore, it was concluded that typical CMA concentrations to expect in typical actual highway runoff or spray are order of magnitude 100 mg/l, while a normal upper limit would be order of magnitude 1000 mg/l and a worst case condition would be 5000 mg/l.

#### Overland Flow

Previous research has demonstrated that draining contaminated wastewater streams over vegetated soil can reduce pollutant concentrations appreciably. Mar et al. (3) established that 60 m of vegetated ditch is capable of reducing total suspended solids and lead in freeway runoff by 80 percent and copper and zinc by 60 percent. Most previous applications of overland flow in wastewater treatment have used sheet flow over broad, grassed slopes to polish pretreated municipal sewage and food processing wastewater. These systems have been reported to remove the majority of the solids, biochemical oxygen demand (BOD), and metals from the waste streams, but less of the nutrients, over an annual cycle (4). A number of mechanisms operate to capture pollutants, including sedimentation, filtration, adsorption, precipitation, ion exchange, plant uptake, and biodegradation. The relative importance of these mechanisms depends on the particular pollutant (4).

These reports of pollutant removal from highway runoff and wastewater during transport through a vegetated medium suggested that CMA, which is composed principally of two metals and a degradable organic, would be reduced in runoff in a similar manner. This supposition was a subject for investigation during the research.

The results of two long-term investigations of overland flow treatment of municipal wastewater have been applied to develop models of BOD reduction for design purposes (5, 6). Both models proposed an exponential BOD removal rate with distance downslope. There was specific interest in determining whether acetate in CMA would follow such a pattern and, if so, in establishing appropriate model parameters for acetate.

#### Vertical Transport

In order to gain some preliminary feeling for the vertical transport and fate of CMA in soil, predictions of cation exchange and acetate decomposition were based on the highway application and removal estimates discussed above. The following assumptions were made for the analysis of cation exchange:

Soil bulk density = 125 lb/ft<sup>3</sup> (2 g/cm<sup>3</sup>)

Soil cation exchange capacity = 10 meq/100 g

(Therefore, 1 ft<sup>3</sup> of soil would have 5.7 equivalents of exchange capacity.)

Based on formulas for calcium and magnesium acetates, respectively, of Ca (CH<sub>3</sub>COOH)<sub>2</sub>·H<sub>2</sub>O and Mg (CH<sub>3</sub>COOH)<sub>2</sub>·4H<sub>2</sub>O, CMA would contain 16.4 percent Ca plus Mg by weight, if a 1:1 ratio of calcium and magnesium acetates is assumed. Therefore, an annual application of 4 lb CMA/linear foot of highway would contain 0.7 lb., or 0.35 equivalents, of cations. Just 1 ft<sup>3</sup> of soil adjoining the highway would have sufficient exchange capacity to assimilate approximately 16 years of cation loading. Therefore, the ability of the soil to retain cations was not regarded to be an issue, although leakage through the soil due

to excessive application rates could be. Also, ion exchange that sequesters Ca and Mg could mobilize other cations originally present in the soil.

At 20 C an estimated soil respiration rate for a labile substrate such as acetate is on the order of 10 g CO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup> (7). At soil temperatures of 0-5 C, such as would be the case during snowmelt runoff, the respiration rate may be approximately 1 g CO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup>. Based on stoichiometry, about 1.5 g CO<sub>2</sub> would be produced per g CMA oxidized; thus, an estimate of the rate of biological oxidation of CMA at cold temperatures is 0.7 g CMA m<sup>-2</sup> day<sup>-1</sup>. If a roadside strip of soil 10 m wide received only 10 percent of the maximum annual loading estimated above, or 0.6 kg/linear m of highway, it would take more than 80 days to degrade all of the CMA near 0 C. This calculation suggested that there is a potential for accumulation of CMA, perhaps resulting in stress on oxygen resources in receiving waters when major runoff occurs.

#### EXPERIMENTAL METHODS

##### Runoff Characteristics and Surface Transport

Runoff from the controlled field plots was channeled to a single point for discharge to the ponds. Prior to discharge, it was collected in 20 l buckets for the determination of volume and sampling. Samples were analyzed for pH, specific conductivity, total suspended solids (TSS), volatile fatty acid salts (VFA), total phosphorus, and metals as specified in Table A-1.

Runoff from the bins was also collected for measurement of volume and determination of VFA and metals. In addition, duplicate vials were

implanted in the campus bin soils (mouths flush with the soil surface) at about one-third and two-thirds of the 2 m lengths to collect water for the same analyses. The objective of this experiment was to determine the changes in metals and VFA concentrations with length during overland flow. This small-scale experiment was performed to give an indication of these changes that could be explored further in full-scale research at highway sites. It was hoped that a larger experiment could be conducted during this research. However, high rates of water percolation in the field soils and insufficient CMA supplies prevented undertaking this experiment.

As a basis for comparison, CMA and control water feed tank contents were sampled and analyzed for the same constituents as the runoff.

#### Soil Water Characteristics

Characteristics of the unsaturated zone soil water in controlled field plots were determined with the aid of tube-type lysimeters placed to sample at 30 and 60 cm depths (Soil Moisture Equipment Corporation catalog numbers 1900L12 and 1900L24, respectively). Prior to installation, lysimeters were soaked in 10 percent nitric acid and rinsed several times with distilled-deionized water to remove any metals present. When the lysimeters were originally installed in the field plots, pea gravel was placed around the porous ceramic collection cups. Moist bentonite seals were placed on top of the gravel and at the surface to prevent direct channeling of surface water.

Lysimeters were prepared for collection by injecting 1.5 ml of chloroform to preserve organics such as acetate. A vacuum of 0.5

atmosphere was then applied with a hand pump to cause soil water to be drawn into the ceramic cups. Samples were drawn with a Soil Moisture Equipment Corporation Model 200562 hand pump. Volume was measured and samples were analyzed for pH, specific conductivity, total alkalinity, VFA, metals, and total phosphorus and, on some occasions, the anions chloride, nitrate, and sulfate. Procedures were as specified in Table A-1. The sampling interval varied during the year from approximately biweekly during and for a time after winter treatments to bimonthly or quarterly after acetate disappeared.

#### Groundwater Characteristics

Characteristics of water in the saturated zone underlying the controlled field plots were determined with the aid of well points driven to 3 m depth. Well points were fashioned from 3/4-inch cast iron pipe. Pipe sections were closed at one end using a vice and mallet, and the closure was sealed with epoxy adhesive to prevent entrance of soil. A series of 3/16-inch diameter holes was drilled at 90° around the circumference of each section over a length of about 18 inches near the closed tip.

Water infiltrating through the drilled holes was sampled by a hand pump attached to an aluminum tube lowered through the well point pipe to the saturated zone. Metal contact in sampling was controlled by using the same materials at both control and treatment plots. Volume was measured and samples were analyzed for pH, specific conductivity, VFA, and metals. When there was sufficient sample, total alkalinity and total phosphorus were also measured. Analyses were according to the methods in Table A-1. The sampling interval was the same as for the

lysimeters during treatment periods but was more frequent otherwise (approximately monthly), because of the slower appearance, and then slower disappearance, of CMA components in the groundwater.

## RESULTS

### Runoff Characteristics and Surface Transport

Most CMA solution and control water applied to the control plots percolated into the soil. During the second winter of treatments each plot received 600 l of CMA solution or water in each of five treatments. Table C-1 presents the means and ranges of runoff volumes measured on the respective plots during those treatments. Runoff rarely exceeded 10 percent of the applied water volume. Plot 8 experienced the most surface runoff, because of a tendency for soil interflow to enter the runoff channel at one point. Even in this case runoff never reached one percent of the volume of the pond into which it discharged.

Runoff volumes were quite variable among plots and among treatments on each plot. Variability was too great for meaningful statistical comparisons, but no trends associated with treatments were apparent.

Tables C-2 and C-3 give means and standard deviations of metals and other constituents, respectively, measured in supply tanks and runoff. The major cations (Ca, Mg, and K) decreased from supply tank concentrations during flow over some plots and increased in other cases. The metals Fe, Cu, Zn, and Al were actually higher in the control water than in the CMA tank. Again, there was no clear pattern of change in the runoff during overland flow. The remaining metals (Pb and Se) were

Table C-1. Means and Ranges of Controlled Plot Runoff Volumes During the 1986-87 Treatments

of

| Plot Number | Treatment   | Runoff Volume (l) <sup>a</sup> |           |
|-------------|-------------|--------------------------------|-----------|
|             |             | Mean                           | Range     |
| 1           | CMA-Spray   | >33.0                          | 0-56.4    |
| 2           | Water-Spray | >38.4                          | 27.2-58.0 |
| 3           | CMA-Spray   | >11.8                          | 0->30     |
| 4           | Water-Spray | 18.3                           | 10.0-41.4 |
| 5           | CMA-Flood   | 1.4                            | 0-4.7     |
| 6           | Water-Flood | >44.7                          | 20.1-90.1 |
| 7           | Water-Flood | >7.7                           | 0->16     |
| 8           | CMA-Flood   | 45.4                           | >3.4-87.1 |

<sup>a</sup>> signifies that the actual volume was slightly greater. Small losses that were not measured sometimes occurred.

Table C-2. Means and Standard Deviations of Metals in Plot Runoff and Supply Tanks

|            |             | Concentration (mg/l) |       |      |       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |  |
|------------|-------------|----------------------|-------|------|-------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|--|
| Plot       |             | Ca                   |       | Mg   |       | K    |      | Fe   |      | Cu   |      | Zn   |      | Se   |      | Pb   |      | Al   |      |      |  |
| No.        | Treatment   | Year                 | Mean  | S.D. | Mean  | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. |  |
| 1          | CMA-Spray   | 1                    | 130.3 | 3.1  | 132.0 | 10.5 | 65.4 | 5.3  | 1.4  | 1.6  | 0.03 | 0.02 | 0.11 | 0.08 | 0.06 | 0.01 | 0.04 | 0.01 | 1.30 | 1.80 |  |
|            |             | 2                    | NA    |      |       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |  |
|            |             | 1 + 2                | 130.3 | 3.1  | 130.5 | 10.5 | 65.4 | 5.3  | 1.4  | 1.6  | 0.03 | 0.02 | 0.11 | 0.08 | 0.06 | 0.01 | 0.04 | 0.01 | 1.30 | 1.80 |  |
| 2          | Water-Spray | 1                    | 3.1   | 0.6  | 1.3   | 0.2  | 1.6  | 0.6  | 1.0  | 0.8  | 0.01 | 0.00 | 0.05 | 0.03 | 0.03 | 0.01 | 0.03 | 0.01 | 1.06 | 0.86 |  |
|            |             | 2                    | 4.0   | 0.5  | 1.6   | 0.3  | 2.5  | 0.9  | 0.6  | 0.8  | 0.00 | 0.00 | 0.03 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.59 | 0.75 |  |
|            |             | 1 + 2                | 3.7   | 0.5  | 1.5   | 0.3  | 2.2  | 0.8  | 0.7  | 0.8  | 0.00 | 0.00 | 0.04 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.75 | 0.79 |  |
| 3          | CMA-Spray   | 1                    | 114.9 | 48.7 | 138.0 | 8.7  | 52.4 | 10.8 | 0.3  | 0.5  | 0.02 | 0.02 | 0.10 | 0.15 | 0.09 | 0.02 | 0.05 | 0.02 | 0.25 | 0.33 |  |
|            |             | 2                    | NA    |      |       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |  |
|            |             | 1 + 2                | 114.9 | 48.7 | 138.0 | 8.7  | 52.4 | 10.8 | 0.3  | 0.5  | 0.02 | 0.02 | 0.10 | 0.15 | 0.09 | 0.02 | 0.05 | 0.02 | 0.25 | 0.33 |  |
| 4          | Water-Spray | 1                    | NA    |      |       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |  |
|            |             | 2                    | 3.3   | 0.8  | 1.1   | 0.3  | 2.2  | 0.7  | 0.4  | 0.4  | 0.01 | 0.01 | 0.03 | 0.02 | 0.02 | 0.01 | 0.05 | 0.06 | 0.32 | 0.28 |  |
|            |             | 1 + 2                | 3.3   | 0.8  | 1.1   | 0.3  | 2.2  | 0.7  | 0.4  | 0.4  | 0.01 | 0.01 | 0.03 | 0.02 | 0.02 | 0.01 | 0.05 | 0.06 | 0.32 | 0.28 |  |
| 5          | CMA-Flood   | 1                    | NA    |      |       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |  |
|            |             | 2                    | 159.3 | 9.1  | 174.0 | 18.9 | 78.6 | 3.5  | 0.8  | 0.0  | 0.00 | 0.01 | 0.03 | 0.00 | 0.02 | 0.07 | 0.00 | 0.25 | 0.09 | 0.10 |  |
|            |             | 1 + 2                | 159.3 | 9.1  | 174.0 | 18.9 | 78.6 | 3.5  | 0.8  | 0.0  | 0.00 | 0.01 | 0.03 | 0.00 | 0.02 | 0.07 | 0.00 | 0.25 | 0.09 | 0.10 |  |
| 6          | Water-Flood | 1                    | 3.0   | 0.9  | 1.2   | 0.4  | 2.0  | 0.9  | 0.9  | 0.7  | 0.01 | 0.00 | 0.08 | 0.06 | 0.03 | 0.01 | 0.04 | 0.01 | 0.99 | 0.88 |  |
|            |             | 2                    | 4.5   | 1.7  | 1.5   | 0.5  | 1.8  | 0.6  | 0.4  | 0.4  | 0.01 | 0.02 | 0.03 | 0.03 | 0.04 | 0.06 | 0.06 | 0.08 | 0.35 | 0.25 |  |
|            |             | 1 + 2                | 4.1   | 1.5  | 1.4   | 0.5  | 1.9  | 0.7  | 0.5  | 0.5  | 0.01 | 0.01 | 0.04 | 0.04 | 0.04 | 0.05 | 0.06 | 0.06 | 0.53 | 0.43 |  |
| 7          | Water-Flood | 1                    | NA    |      |       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |  |
|            |             | 2                    | 4.6   | 0.6  | 1.5   | 0.1  | 1.5  | 0.4  | 0.3  | 0.3  | 0.00 | 0.01 | 0.04 | 0.03 | 0.02 | 0.02 | 0.03 | 0.04 | 0.36 | 0.60 |  |
|            |             | 1 + 2                | 4.6   | 0.6  | 1.5   | 0.1  | 1.5  | 0.4  | 0.3  | 0.3  | 0.00 | 0.01 | 0.04 | 0.03 | 0.02 | 0.02 | 0.03 | 0.04 | 0.36 | 0.60 |  |
| 8          | CMA-Flood   | 1                    | 139.5 | 6.9  | 136.1 | 4.9  | 54.6 | 8.1  | 0.5  | 0.5  | 0.01 | 0.01 | 0.17 | 0.43 | 0.07 | 0.02 | 0.05 | 0.01 | 0.21 | 0.21 |  |
|            |             | 2                    | 155.3 | 17.3 | 166.5 | 17.7 | 73.2 | 10.0 | 0.8  | 0.4  | 0.03 | 0.04 | 0.09 | 0.01 | 0.08 | 0.06 | 0.09 | 0.15 | 0.14 | 0.11 |  |
|            |             | 1 + 2                | 147.4 | 12.1 | 151.3 | 11.3 | 63.9 | 9.1  | 0.7  | 0.5  | 0.02 | 0.03 | 0.13 | 0.22 | 0.08 | 0.04 | 0.07 | 0.08 | 0.18 | 0.16 |  |
| CMA Tank   |             | 1                    | 135.3 | 32.2 | 160.5 | 9.2  | 63.9 | 12.5 | 0.2  | 0.2  | 0.03 | 0.04 | 0.08 | 0.10 | 0.10 | 0.01 | 0.06 | 0.01 | 0.05 | 0.02 |  |
|            |             | 2                    | NA    |      |       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |  |
|            |             | 1 + 2                | 135.3 | 32.2 | 160.5 | 9.2  | 63.9 | 12.5 | 0.2  | 0.2  | 0.03 | 0.04 | 0.08 | 0.10 | 0.10 | 0.01 | 0.06 | 0.01 | 0.05 | 0.02 |  |
| Water Tank |             | 1                    | 4.8   | 1.1  | 1.5   | 0.1  | 0.6  | 0.3  | 0.4  | 0.1  | 0.04 | 0.03 | 0.29 | 0.42 | 0.02 | 0.01 | 0.03 | 0.02 | 0.27 | 0.22 |  |
|            |             | 2                    | 5.3   | 0.8  | 1.5   | 0.1  | 0.7  | 0.1  | 0.4  | 0.2  | 0.03 | 0.04 | 0.05 | 0.06 | 0.03 | 0.02 | 0.03 | 0.02 | 0.21 | 0.28 |  |
|            |             | 1 + 2                | 5.1   | 0.9  | 1.5   | 0.1  | 0.7  | 0.2  | 0.4  | 0.2  | 0.03 | 0.04 | 0.12 | 0.17 | 0.03 | 0.02 | 0.03 | 0.02 | 0.23 | 0.26 |  |

NA--Not available

Table C-3. Means and Standard Deviations of Six Constituents in Plot Runoff and Supply Tanks During the Second Winter of Treatments

| Plot<br>No. | Treatment   | pH   |      | Spec. Cond.<br>( $\mu$ mho/cm) |      | TSS<br>(mg/l) |      | Acetate<br>(mg/l) |      | Butyrate<br>(mg/l) |      | Total P<br>(mg/l) |      |
|-------------|-------------|------|------|--------------------------------|------|---------------|------|-------------------|------|--------------------|------|-------------------|------|
|             |             | Mean | S.D. | Mean                           | S.D. | Mean          | S.D. | Mean              | S.D. | Mean               | S.D. | Mean              | S.D. |
| 1           | CMA-Spray   | 6.6  | 0.2  | 1579                           | 229  | 323           | 267  | 1096              | 83   | 248                | 17   | 684               | 398  |
| 2           | Water-Spray | 6.9  | 0.2  | 68                             | 23   | 127           | 68   | 0                 | 0    | 0                  | 0    | 238               | 144  |
| 3           | CMA-Spray   | 6.7  | 0.2  | 1601                           | 74   | 113           | 65   | 1031              | 158  | 232                | 46   | 538               | 134  |
| 4           | Water-Spray | 6.4  | 0.2  | 54                             | 21   | 61            | 26   | 0                 | 0    | 0                  | 0    | 193               | 58   |
| 5           | CMA-Flood   | 6.8  | 0.1  | 1349                           | 173  | 62            | 39   | 959               | 62   | 231                | 21   | 475               | 98   |
| 6           | Water-Flood | 6.6  | 0.1  | 68                             | 19   | 100           | 93   | 0                 | 0    | 0                  | 0    | 144               | 30   |
| 7           | Water-Flood | 6.7  | 0.1  | 65                             | 15   | 85            | 47   | 0                 | 0    | 0                  | 0    | 127               | 29   |
| 8           | CMA-Flood   | 6.9  | 0.1  | 1711                           | 144  | 106           | 55   | 1198              | 82   | 278                | 18   | 560               | 133  |
|             | CMA Tank    | 6.8  | 0.2  | 1816                           | 121  | 49            | 18   | 1248              | 70   | 285                | 17   | 598               | 184  |
|             | Water Tank  | 6.8  | 0.5  | 73                             | 20   | 7             | 6    | 0                 | 0    | 0                  | 0    | 56                | 22   |

higher in the applied CMA than the control water, but were still very low and usually declined marginally during travel over the plots. None of these results show a pattern of metal removal through contact with vegetation and the soil surface.

In Table C-3 it may be seen that mean pH was relatively constant over the period and was changed little by flow over the plots. Mean specific conductivity did decline somewhat from values in the supply water and CMA tanks (mean decrease of 13.5 percent for CMA plots). Runoff gained total suspended solids during passage over the plots, but there was no association with the mode of treatment. Both mean acetate and butyrate registered a decline in overland flow (mean 14.2 percent for acetate and 13.2 percent for butyrate). However, mean total phosphorus always increased from the supply water concentration in flow over the control plots, and either increased or dropped a relatively small amount on the CMA plots. This subject and its possible significance is discussed further in Appendix J.

In every case in which a water quality constituent declined in overland flow on CMA plots, the drop was much less on Plot 8 than on other plots. The likely reason was the tendency of Plot 8 soil interflow to enter the runoff channel after brief soil and vegetation contact. If that anomolous plot is omitted, average reductions from CMA supply tank concentrations during passage over the plots would be as follows:



|                       | <u>Spray Plots (1,3)</u> | <u>Flood Plot (5)</u> |
|-----------------------|--------------------------|-----------------------|
| Specific conductivity | 12.4%                    | 23.2%                 |
| Acetate               | 14.8%                    | 23.2%                 |
| Butyrate              | 15.8%                    | 18.9%                 |

Flood water had a full 3 m of contact with vegetation and soil before flowing off the plots, while spray was uniformly applied over the whole slope and, overall, had less vegetation and soil contact.

Figure C-1 summarizes the results of the bin experiments designed to determine CMA removal from runoff in overland flow. An acetate profile is presented only for Bin 6, which received hydraulic and CMA mass loadings 50 percent higher than the plots. A similar trend was reproduced on Bins 4 and 5, which received different hydraulic loadings, CMA concentrations, and/or CMA mass loadings. If the data from the

three campus CMA bins and five treatments in the 1986-87 winter are taken together, mean acetate removals were:

At 0.67 m from application point--11.8%

At 1.33 m from application point--14.6%

At 2 m from application point--18.8%

If the mean 23.2% acetate decrease measured after flow over approximately 3 m of the field flood plot is also included, a rough, first approximation of an overload flow acetate removal model emerges in the form % removal = f(length of overland flow). These data fit linear, exponential, logarithmic, and power regressions almost equally well, as follows (with L = length in meters):

$$\text{Linear: \% removal} = 5.0 L + 8.4 \quad r^2 = 0.995$$

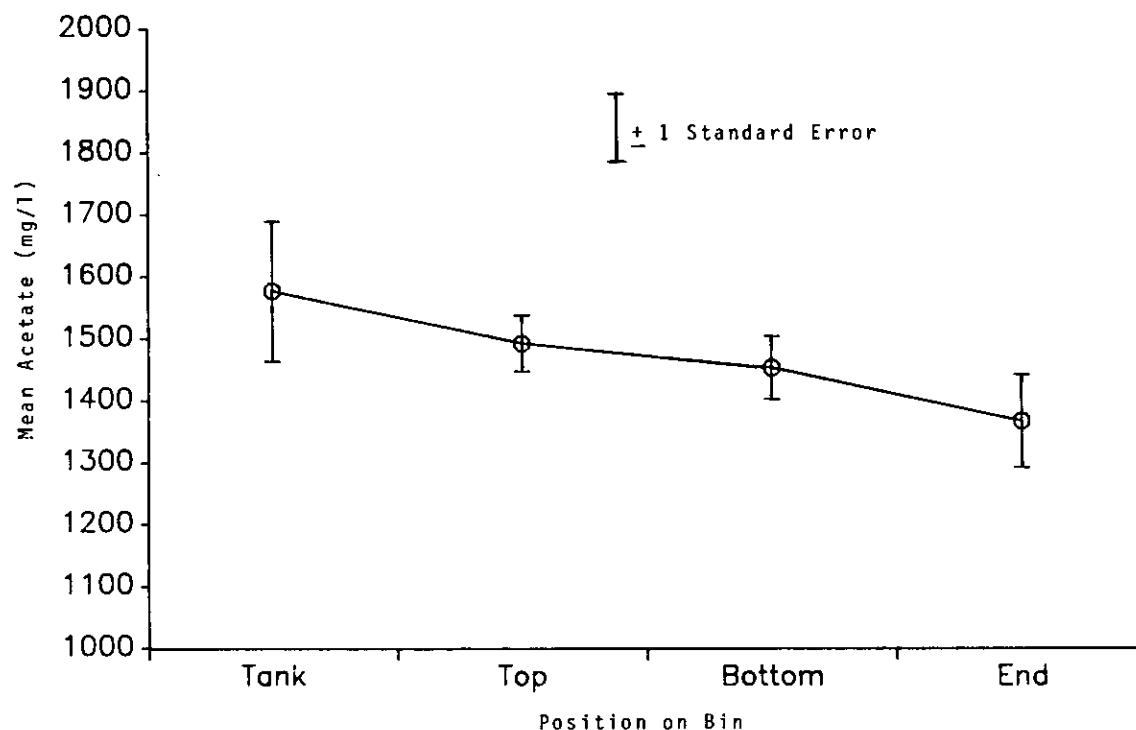


Figure C-1. Acetate Transport in Bin 6

Exponential: % removal =  $9.9 e^{0.29 L}$   $r^2 = 0.984$

Logarithmic: % removal =  $7.6 \ln L + 13.9$   $r^2 = 0.945$

Power: % removal =  $13.7 L^{0.46}$   $r^2 = 0.976$

It must be recalled that the data were limited to 3 m of travel. On longer paths it is unlikely that removal will continue to increase linearly, and an exponential model is more likely to provide a better fit.

As on the field plots, changes in bin runoff metals during overland flow were inconclusive. In any event, there was no identifiable trend toward reductions of either the major or the trace cations.

#### Soil Water Characteristics

Tables C-4 and C-5 give means and standard deviations of metals and other constituents, respectively, measured in lysimeters at 30 and 60 cm depths in controlled field plots. Statistics are reported for four periods: (1) prior to any treatments, (2) the first winter treatment period and one month afterward, (3) the interval between the second and fourth periods, and (4) the second winter treatment period and one month afterward. Figures C-2, C-3, and C-4 illustrate variations in specific conductivity in CMA and water plots and acetate in CMA plots, respectively, over time (no acetate was measured in water plots). Results from the CMA plots demonstrate that treatment effects were most evident in soil water for about one month after treatments ceased and account for the organization of the data in Tables C-4 and C-5.

Table C-4. Means and Standard Deviations of Metals in Lysimeters Over Four Periods During Field Plot Experiments

|      |             |                         | Concentration (mg/l) |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|------|-------------|-------------------------|----------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Plot | Depth       |                         | Ca                   |      | Mg   |      | K    |      | Fe   |      | Cu   |      | Zn   |      | Se   |      | Pb   |      | Al   |      |      |
| No.  | Treatment   | (cm)Period <sup>a</sup> | Mean                 | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. |      |
| 1    | CMA-Spray   | 30                      | P                    | 3.6  | 0.0  | 0.7  | 0.0  | 2.6  | 0.0  | 2.98 | 0.00 | 0.03 | 0.00 | 0.10 | 0.00 | 0.06 | 0.00 | 0.00 | 0.00 | 2.64 | 0.00 |
|      |             | T1                      | 3.6                  | 1.2  | 0.9  | 0.6  | 3.5  | 1.0  | 1.15 | 0.04 | 0.02 | 0.00 | 0.17 | 0.05 | 0.08 | 0.05 | 0.00 | 0.02 | 1.17 | 0.27 |      |
|      |             | I                       | 3.1                  | 2.1  | 0.4  | 0.2  | 2.2  | 0.6  | 3.05 | 2.95 | 0.01 | 0.01 | 0.16 | 0.11 | 0.01 | 0.01 | 0.00 | 0.00 | 1.55 | 1.06 |      |
|      |             | T2                      | 1.9                  | 0.4  | 0.5  | 0.2  | 2.9  | 0.6  | 0.20 | 0.15 | 0.01 | 0.00 | 0.04 | 0.01 | 0.02 | 0.02 | 0.01 | 0.02 | 0.34 | 0.22 |      |
|      |             | 60                      | P                    | 6.6  | 5.9  | 1.8  | 0.6  | 1.0  | 0.5  | 0.08 | 0.00 | 0.01 | 0.01 | 0.11 | 0.14 | 0.01 | 0.00 | 0.00 | 0.01 | 0.12 | 0.02 |
|      |             | T1                      | 9.9                  | 7.8  | 5.9  | 5.0  | 2.4  | 1.2  | 0.03 | 0.02 | 0.00 | 0.00 | 0.04 | 0.03 | 0.02 | 0.01 | 0.00 | 0.00 | 0.07 | 0.04 |      |
|      |             | I                       | 3.3                  | 0.0  | 1.5  | 0.0  | 1.0  | 0.0  | 0.04 | 0.00 | 0.00 | 0.00 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.10 | 0.00 |      |
|      |             | T2                      | 11.3                 | 10.5 | 6.2  | 5.2  | 2.1  | 1.3  | 0.03 | 0.02 | 0.00 | 0.00 | 0.14 | 0.29 | 0.02 | 0.01 | 0.01 | 0.02 | 0.05 | 0.02 |      |
| 2    | Water-Spray | 30                      | P                    | 1.9  | 0.1  | 0.9  | 0.0  | 0.6  | 0.1  | 0.66 | 0.09 | 0.01 | 0.00 | 0.08 | 0.05 | 0.04 | 0.01 | 0.00 | 0.02 | 0.48 | 0.02 |
|      |             | T1                      | 2.2                  | 0.3  | 1.0  | 0.2  | 0.8  | 0.1  | 0.24 | 0.04 | 0.01 | 0.00 | 0.07 | 0.04 | 0.05 | 0.02 | 0.00 | 0.00 | 0.22 | 0.05 |      |
|      |             | I                       | 0.6                  | 0.1  | 0.2  | 0.1  | 0.2  | 0.0  | 0.19 | 0.11 | 0.00 | 0.00 | 0.03 | 0.00 | 0.01 | 0.02 | 0.01 | 0.01 | 0.22 | 0.06 |      |
|      |             | T2                      | 12.2                 | 24.8 | 0.8  | 1.2  | 0.4  | 0.4  | 0.19 | 0.16 | 0.00 | 0.00 | 1.08 | 2.29 | 0.02 | 0.01 | 0.03 | 0.02 | 0.34 | 0.39 |      |
|      |             | 60                      | P                    | 2.2  | 0.1  | 1.4  | 0.0  | 0.3  | 0.1  | 0.09 | 0.00 | 0.00 | 0.00 | 0.07 | 0.05 | 0.02 | 0.00 | 0.00 | 0.00 | 0.07 | 0.01 |
|      |             | T1                      | 28.5                 | 36.8 | 2.0  | 1.0  | 1.6  | 1.5  | 0.16 | 0.20 | 0.02 | 0.02 | 3.15 | 4.80 | 0.02 | 0.00 | 0.00 | 0.01 | 0.19 | 0.22 |      |
|      |             | I                       | 1.0                  | 0.0  | 0.6  | 0.0  | 0.2  | 0.0  | 0.01 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.01 | 0.00 | 0.02 | 0.00 | 0.04 | 0.00 |      |
|      |             | T2                      | 1.7                  | 1.5  | 0.6  | 0.2  | 0.3  | 0.0  | 0.04 | 0.03 | 0.00 | 0.00 | 0.03 | 0.02 | 0.02 | 0.01 | 0.03 | 0.02 | 0.08 | 0.07 |      |
| 3    | CMA-Spray   | 30                      | P                    | 2.5  | 0.1  | 0.6  | 0.0  | 1.1  | 0.2  | 1.28 | 0.06 | 0.01 | 0.00 | 0.02 | 0.01 | 0.03 | 0.01 | 0.00 | 0.00 | 1.74 | 0.08 |
|      |             | T1                      | 5.3                  | 3.4  | 2.3  | 2.0  | 1.3  | 0.4  | 0.87 | 0.45 | 0.01 | 0.00 | 0.08 | 0.07 | 0.02 | 0.00 | 0.00 | 0.00 | 0.91 | 0.21 |      |
|      |             | I                       | 2.3                  | 0.4  | 0.5  | 0.1  | 0.8  | 0.2  | 1.80 | 0.66 | 0.01 | 0.00 | 0.05 | 0.03 | 0.01 | 0.00 | 0.00 | 0.01 | 1.41 | 0.24 |      |
|      |             | T2                      | 2.7                  | 1.0  | 1.1  | 0.5  | 1.0  | 0.2  | 0.32 | 0.29 | 0.01 | 0.00 | 0.04 | 0.02 | 0.01 | 0.01 | 0.00 | 0.01 | 0.58 | 0.44 |      |
|      |             | 60                      | P                    | 3.0  | 0.3  | 0.8  | 0.2  | 1.0  | 0.0  | 0.48 | 0.13 | 0.01 | 0.00 | 0.05 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.53 | 0.11 |
|      |             | T1                      | 8.3                  | 6.4  | 3.8  | 3.3  | 1.7  | 0.7  | 0.26 | 0.14 | 0.01 | 0.00 | 0.05 | 0.05 | 0.02 | 0.01 | 0.00 | 0.00 | 0.27 | 0.08 |      |
|      |             | I                       | 1.6                  | 0.0  | 0.6  | 0.0  | 0.5  | 0.0  | 0.04 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.06 | 0.00 |      |
|      |             | T2                      | 8.9                  | 7.7  | 6.3  | 6.6  | 2.1  | 1.5  | 0.06 | 0.03 | 0.00 | 0.00 | 0.03 | 0.02 | 0.02 | 0.01 | 0.01 | 0.02 | 0.09 | 0.03 |      |
| 4    | Water-Spray | 30                      | P                    | 4.9  | 1.7  | 1.1  | 0.5  | 0.8  | 0.2  | 0.84 | 0.33 | 0.02 | 0.00 | 0.03 | 0.00 | 0.03 | 0.01 | 0.00 | 0.00 | 1.42 | 0.34 |
|      |             | T1                      | 5.9                  | 6.7  | 0.8  | 0.4  | 0.7  | 0.3  | 0.68 | 0.26 | 0.02 | 0.01 | 0.18 | 0.34 | 0.02 | 0.01 | 0.00 | 0.01 | 1.18 | 0.50 |      |
|      |             | I                       | NA                   |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|      |             | T2                      | 2.2                  | 1.2  | 0.2  | 0.1  | 0.5  | 0.1  | 0.46 | 0.14 | 0.01 | 0.00 | 0.17 | 0.20 | 0.01 | 0.01 | 0.02 | 0.02 | 0.97 | 0.22 |      |
|      |             | 60                      | P                    | 9.8  | 2.5  | 1.8  | 0.5  | 2.2  | 0.4  | 0.23 | 0.00 | 0.01 | 0.00 | 0.24 | 0.28 | 0.01 | 0.00 | 0.00 | 0.00 | 0.34 | 0.09 |
|      |             | T1                      | 4.9                  | 3.3  | 0.6  | 0.1  | 1.7  | 0.3  | 0.13 | 0.02 | 0.01 | 0.00 | 0.15 | 0.16 | 0.02 | 0.01 | 0.00 | 0.01 | 0.20 | 0.02 |      |
|      |             | I                       | 2.6                  | 0.0  | 0.6  | 0.1  | 1.7  | 0.0  | 0.40 | 0.00 | 0.01 | 0.00 | 0.07 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.21 | 0.00 |      |
|      |             | T2                      | 2.1                  | 0.5  | 0.5  | 0.1  | 1.3  | 0.1  | 0.18 | 0.08 | 0.00 | 0.00 | 0.03 | 0.01 | 0.02 | 0.01 | 0.03 | 0.03 | 0.16 | 0.05 |      |
| 5    | CMA-Flood   | 30                      | P                    | 5.3  | 0.8  | 1.4  | 0.0  | 1.8  | 0.0  | 0.20 | 0.00 | 0.01 | 0.00 | 0.01 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.44 | 0.00 |
|      |             | T1                      | 5.9                  | 3.6  | 1.4  | 0.4  | 2.2  | 0.3  | 0.18 | 0.07 | 0.01 | 0.00 | 0.19 | 0.34 | 0.02 | 0.01 | 0.00 | 0.00 | 0.44 | 0.12 |      |
|      |             | I                       | 1.8                  | 0.6  | 0.4  | 0.2  | 1.3  | 0.1  | 0.32 | 0.02 | 0.00 | 0.00 | 0.04 | 0.01 | 0.00 | 0.01 | 0.00 | 0.00 | 0.48 | 0.07 |      |
|      |             | T2                      | 5.6                  | 4.2  | 2.3  | 1.5  | 1.6  | 0.5  | 0.17 | 0.07 | 0.00 | 0.00 | 0.10 | 0.17 | 0.01 | 0.01 | 0.03 | 0.05 | 0.39 | 0.13 |      |
|      |             | 60                      | P                    | 2.1  | 0.1  | 1.1  | 0.0  | 0.6  | 0.1  | 0.13 | 0.09 | 0.01 | 0.01 | 0.03 | 0.01 | 0.02 | 0.00 | 0.00 | 0.01 | 0.13 | 0.07 |
|      |             | T1                      | 12.7                 | 12.1 | 8.2  | 7.5  | 2.5  | 1.6  | 0.05 | 0.03 | 0.01 | 0.01 | 0.09 | 0.12 | 0.02 | 0.00 | 0.00 | 0.00 | 0.08 | 0.05 |      |
|      |             | I                       | 1.6                  | 0.0  | 0.9  | 0.0  | 0.5  | 0.0  | 0.03 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.01 | 0.00 | 0.01 | 0.00 | 0.06 | 0.00 |      |
|      |             | T2                      | 4.6                  | 4.4  | 3.3  | 3.5  | 1.6  | 1.3  | 0.13 | 0.16 | 0.00 | 0.00 | 0.12 | 0.22 | 0.02 | 0.01 | 0.05 | 0.07 | 0.13 | 0.07 |      |
| 6    | Water-Flood | 30                      | P                    | 7.2  | 0.0  | 2.5  | 0.0  | 6.3  | 0.0  | 0.72 | 0.00 | 0.01 | 0.00 | 0.06 | 0.00 | 0.02 | 0.02 | 0.00 | 0.00 | 0.37 | 0.00 |
|      |             | T1                      | NA                   |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|      |             | I                       | 12.8                 | 13.8 | 4.3  | 4.9  | 6.6  | 3.0  | 0.39 | 0.08 | 0.01 | 0.01 | 0.22 | 0.37 | 0.02 | 0.01 | 0.00 | 0.01 | 0.42 | 0.51 |      |
|      |             | T2                      | 7.1                  | 0.6  | 1.8  | 0.1  | 3.9  | 0.1  | 0.12 | 0.06 | 0.01 | 0.00 | 0.05 | 0.03 | 0.01 | 0.01 | 0.00 | 0.02 | 0.08 | 0.03 |      |
|      |             | 60                      | P                    | 12.7 | 11.9 | 2.4  | 1.5  | 4.4  | 0.5  | 1.73 | 0.30 | 0.01 | 0.00 | 0.56 | 0.74 | 0.03 | 0.01 | 0.20 | 0.30 | 1.06 | 0.36 |
|      |             | T1                      | 4.0                  | 0.5  | 1.2  | 0.1  | 3.9  | 0.3  | 1.86 | 0.38 | 0.01 | 0.00 | 0.06 | 0.08 | 0.02 | 0.01 | 0.00 | 0.00 | 0.87 | 0.07 |      |
|      |             | I                       | 3.5                  | 0.0  | 1.0  | 0.0  | 2.2  | 0.0  | 0.26 | 0.00 | 0.01 | 0.00 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 |      |
|      |             | T2                      | 5.2                  | 2.8  | 1.3  | 0.4  | 2.8  | 0.5  | 0.19 | 0.11 | 0.01 | 0.01 | 0.17 | 0.29 | 0.01 | 0.01 | 0.01 | 0.03 | 0.23 | 0.13 |      |

Table C-4 Continued

| Plot<br>No. | Treatment   | Depth<br>(cm) | Period <sup>a</sup> | Concentration (mg/l) |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|-------------|-------------|---------------|---------------------|----------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
|             |             |               |                     | Ca                   |      | Mg   |      | K    |      | Fe   |      | Cu   |      | Zn   |      | Se   |      | Pb   |      | Al   |      |
|             |             |               |                     | Mean                 | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| 7           | Water-Flood | 30            | P                   | 6.5                  | 0.0  | 2.1  | 0.0  | 1.9  | 0.0  | 0.23 | 0.00 | 0.01 | 0.00 | 0.04 | 0.00 | 0.02 | 0.00 | 0.01 | 0.00 | 0.24 | 0.00 |
|             |             |               | T1                  | NA                   |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|             |             |               | I                   | 11.8                 | 21.1 | 1.4  | 1.2  | 2.1  | 1.0  | 0.12 | 0.05 | 0.01 | 0.00 | 0.33 | 0.63 | 0.01 | 0.01 | 0.00 | 0.01 | 0.21 | 0.03 |
|             |             |               | T2                  | 2.9                  | 1.7  | 0.5  | 0.1  | 1.3  | 0.1  | 0.14 | 0.02 | 0.01 | 0.00 | 0.09 | 0.08 | 0.02 | 0.02 | 0.01 | 0.02 | 0.25 | 0.06 |
|             |             | 60            | P                   | 8.4                  | 4.8  | 3.3  | 2.1  | 2.4  | 0.8  | 0.19 | 0.13 | 0.01 | 0.00 | 0.05 | 0.01 | 0.02 | 0.00 | 0.00 | 0.00 | 0.23 | 0.13 |
|             |             |               | T1                  | 3.6                  | 0.8  | 1.3  | 0.3  | 1.6  | 0.2  | 0.05 | 0.01 | 0.00 | 0.00 | 0.03 | 0.03 | 0.02 | 0.00 | 0.00 | 0.01 | 0.17 | 0.03 |
|             |             |               | I                   | 2.9                  | 0.0  | 0.9  | 0.0  | 1.2  | 0.0  | 0.23 | 0.00 | 0.01 | 0.00 | 0.18 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.29 | 0.00 |
|             |             |               | T2                  | 2.7                  | 0.7  | 0.8  | 0.2  | 1.0  | 0.1  | 0.13 | 0.08 | 0.00 | 0.00 | 0.04 | 0.04 | 0.02 | 0.01 | 0.02 | 0.02 | 0.27 | 0.02 |
| 8           | CMA-Flood   | 30            | P                   | 3.9                  | 0.0  | 0.9  | 0.0  | 2.3  | 0.0  | 1.91 | 1.07 | 0.02 | 0.00 | 0.04 | 0.00 | 0.03 | 0.00 | 0.00 | 0.00 | 2.75 | 0.00 |
|             |             |               | T1                  | NA                   |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|             |             |               | I                   | 6.0                  | 4.2  | 3.4  | 3.6  | 3.4  | 1.3  | 1.07 | 0.52 | 0.02 | 0.01 | 0.06 | 0.04 | 0.02 | 0.01 | 0.00 | 0.01 | 1.39 | 0.54 |
|             |             |               | T2                  | 7.9                  | 7.8  | 0.9  | 0.3  | 3.3  | 0.4  | 1.85 | 0.15 | 0.02 | 0.01 | 0.10 | 0.04 | 0.01 | 0.01 | 0.01 | 0.01 | 1.99 | 0.19 |
|             |             | 60            | P                   | 19.9                 | 14.2 | 3.3  | 0.7  | 3.0  | 0.7  | 0.11 | 0.06 | 0.01 | 0.00 | 0.57 | 0.45 | 0.02 | 0.00 | 0.00 | 0.00 | 0.11 | 0.03 |
|             |             |               | T1                  | 12.0                 | 10.7 | 3.0  | 0.4  | 2.5  | 0.2  | 0.04 | 0.01 | 0.00 | 0.00 | 0.32 | 0.61 | 0.02 | 0.00 | 0.10 | 0.23 | 0.06 | 0.02 |
|             |             |               | I                   | 9.4                  | 6.9  | 2.6  | 1.4  | 2.9  | 1.4  | 0.16 | 0.17 | 0.00 | 0.00 | 0.09 | 0.07 | 0.02 | 0.02 | 0.04 | 0.04 | 0.10 | 0.08 |
|             |             |               | T2                  | 3.2                  | 0.5  | 1.3  | 0.2  | 1.7  | 0.2  | 0.03 | 0.01 | 0.00 | 0.00 | 0.01 | 0.00 | 0.02 | 0.01 | 0.02 | 0.01 | 0.07 | 0.02 |

<sup>a</sup> P--Prior to first treatment (1/86-2/86)T1--First treatment period and one month afterward  
(3/4/86-4/25/86)I--Intermediate period between T1 and T2  
(4/26/86-12/17/86)T2--Second treatment period and one month afterward  
(12/18/86-3/17/87)

NA--Not available

Table C-5. Means and Standard Deviations of Six Constituents in Lysimeters Over Four Periods  
During Field Plot Experiments

| Plot<br>No. | Treatment   | Depth<br>(cm) | Period <sup>a</sup> | pH   |      | Spec. Cond.<br>( $\mu$ mho/cm) |      | Alkalinity<br>(mg/l CaCO <sub>3</sub> ) |      | Acetate<br>(mg/l) |      | Butyrate<br>(mg/l) |      | Total P<br>(mg/l) |      |
|-------------|-------------|---------------|---------------------|------|------|--------------------------------|------|---|------|-------------------|------|--------------------|------|-------------------|------|
|             |             |               |                     | Mean | S.D. | Mean                           | S.D. | Mean                                    | S.D. | Mean              | S.D. | Mean               | S.D. | Mean              | S.D. |
|             |             |               |                     |      |      |                                |      |   |      |                   |      |                    |      |                   |      |
| 1           | CMA-Spray   | 30            | P                   | NA   |      | 329                            | 0    | 138                                     | 0    | 0                 | 0    | 0                  | 0    | 346               | 0    |
|             |             |               | T1                  | 7.0  | 0.2  | 418                            | 98   | 217                                     | 72   | 0                 | 0    | 0                  | 0    | 166               | 78   |
|             |             |               | I                   | 6.8  | 0.3  | 318                            | 129  | 210                                     | 25   | 1                 | 1    | 0                  | 0    | 167               | 77   |
|             |             |               | T2                  | 7.2  | 0.6  | 309                            | 75   | 123                                     | 47   | 0                 | 0    | 0                  | 0    | NA                |      |
|             |             | 60            | P                   | 5.7  | 0.0  | 52                             | 6    | 18                                      | 4    | 0                 | 0    | 0                  | 0    | 31                | 5    |
|             |             |               | T1                  | 5.7  | 0.3  | 137                            | 83   | 39                                      | 22   | 38                | 51   | 4                  | 5    | 19                | 24   |
|             |             |               | I                   | 5.7  | 0.1  | 55                             | 10   | 24                                      | 5    | 0                 | 0    | 0                  | 0    | 35                | 18   |
|             |             |               | T2                  | 5.8  | 0.4  | 127                            | 73   | 33                                      | 32   | 37                | 51   | 8                  | 11   | NA                |      |
| 2           | Water-Spray | 30            | P                   | 6.4  | 0.0  | 274                            | 40   | 92                                      | 18   | 0                 | 0    | 0                  | 0    | 170               | 43   |
|             |             |               | T1                  | 6.6  | 0.2  | 277                            | 44   | 93                                      | 9    | 0                 | 0    | 0                  | 0    | 79                | 45   |
|             |             |               | I                   | 6.3  | 0.3  | 159                            | 51   | 92                                      | 0    | 0                 | 0    | 0                  | 0    | 83                | 43   |
|             |             |               | T2                  | 6.5  | 0.3  | 105                            | 10   | 43                                      | 5    | 0                 | 0    | 0                  | 0    | NA                |      |
|             |             | 60            | P                   | 5.7  | 0.0  | 56                             | 6    | 17                                      | 1    | 0                 | 0    | 0                  | 0    | 18                | 16   |
|             |             |               | T1                  | 5.9  | 0.1  | 57                             | 2    | 19                                      | 5    | 0                 | 0    | 0                  | 0    | 25                | 23   |
|             |             |               | I                   | 5.8  | 0.1  | 41                             | 12   | 18                                      | 9    | 0                 | 0    | 0                  | 0    | 16                | 7    |
|             |             |               | T2                  | 6.0  | 0.3  | 34                             | 18   | 13                                      | 2    | 0                 | 0    | 0                  | 0    | NA                |      |
| 3           | CMA-Spray   | 30            | P                   | 6.9  | 0.0  | 478                            | 75   | 233                                     | 41   | 0                 | 0    | 0                  | 0    | 77                | 19   |
|             |             |               | T1                  | 6.9  | 0.2  | 437                            | 129  | 181                                     | 71   | 24                | 48   | 1                  | 3    | 96                | 75   |
|             |             |               | I                   | 6.9  | 0.1  | 391                            | 100  | 275                                     | 24   | 1                 | 2    | 0                  | 0    | 56                | 3    |
|             |             |               | T2                  | 7.1  | 0.4  | 366                            | 71   | 202                                     | 51   | 7                 | 7    | 0                  | 0    | NA                |      |
|             |             | 60            | P                   | 6.2  | 0.0  | 213                            | 30   | 56                                      | 22   | 0                 | 0    | 0                  | 0    | 34                | 22   |
|             |             |               | T1                  | 6.4  | 0.2  | 265                            | 100  | 111                                     | 55   | 38                | 60   | 1                  | 3    | 44                | 48   |
|             |             |               | I                   | 6.4  | 0.2  | 219                            | 109  | 130                                     | 36   | 0                 | 0    | 0                  | 0    | 36                | 7    |
|             |             |               | T2                  | 6.7  | 0.4  | 287                            | 152  | 138                                     | 93   | 39                | 54   | 9                  | 9    | NA                |      |
| 4           | Water-Spray | 30            | P                   | 6.3  | 0.0  | 316                            | 69   | 27                                      | 5    | 0                 | 0    | 0                  | 0    | 61                | 59   |
|             |             |               | T1                  | 6.7  | 0.2  | 277                            | 34   | 68                                      | 32   | 0                 | 0    | 0                  | 0    | 99                | 32   |
|             |             |               | I                   | 6.8  | 0.2  | 278                            | 51   | 101                                     | 8    | 0                 | 0    | 0                  | 0    | 99                | 35   |
|             |             |               | T2                  | 7.1  | 0.3  | 197                            | 27   | 84                                      | 10   | 0                 | 0    | 0                  | 0    | NA                |      |
|             |             | 60            | P                   | 5.9  | 0.0  | 228                            | 46   | 27                                      | 5    | 0                 | 0    | 0                  | 0    | 23                | 33   |
|             |             |               | T1                  | 6.5  | 0.2  | 203                            | 17   | 69                                      | 24   | 0                 | 0    | 0                  | 0    | 44                | 51   |
|             |             |               | I                   | 6.5  | 0.2  | 232                            | 28   | 105                                     | 27   | 0                 | 0    | 0                  | 0    | 27                | 4    |
|             |             |               | T2                  | 6.8  | 0.6  | 259                            | 2    | 88                                      | 49   | 0                 | 0    | 0                  | 0    | NA                |      |

Continued

Table C-5 Continued

| Plot No. | Treatment   | Depth (cm) | Period <sup>a</sup> | pH   |      | Spec. Cond. ( $\mu\text{mho/cm}$ ) |      | Alkalinity (mg/l $\text{CaCO}_3$ ) |      | Acetate (mg/l) |      | Butyrate (mg/l) |      | Total P (mg/l) |      |
|----------|-------------|------------|---------------------|------|------|------------------------------------|------|------------------------------------|------|----------------|------|-----------------|------|----------------|------|
|          |             |            |                     | Mean | S.D. | Mean                               | S.D. | Mean                               | S.D. | Mean           | S.D. | Mean            | S.D. | Mean           | S.D. |
| 5        | CMA-Flood   | 30         | P                   | 6.1  | 0.0  | 195                                | 11   | 174                                | 152  | 0              | 0    | 0               | 0    | 43             | 43   |
|          |             |            | T1                  | 6.4  | 0.1  | 184                                | 8    | 74                                 | 44   | 4              | 9    | 0               | 0    | 112            | 57   |
|          |             |            | I                   | 6.4  | 0.2  | 172                                | 33   | 98                                 | 29   | 1              | 2    | 0               | 0    | 235            | 106  |
|          |             |            | T2                  | 6.8  | 0.4  | 215                                | 40   | 99                                 | 16   | 16             | 15   | 4               | 5    | NA             |      |
|          |             | 60         | P                   | 5.8  | 0.0  | 54                                 | 1    | 79                                 | 0    | 0              | 0    | 0               | 0    | 13             | 17   |
|          |             |            | T1                  | 5.5  | 0.4  | 113                                | 0    | 28                                 | 10   | 47             | 70   | 0               | 0    | 124            | 175  |
|          |             |            | I                   | 5.9  | 0.1  | 73                                 | 18   | 39                                 | 0    | 0              | 0    | 0               | 0    | 30             | 0    |
|          |             |            | T2                  | 6.1  | 0.3  | 65                                 | 51   | 26                                 | 25   | 11             | 22   | 4               | 5    | NA             |      |
| 6        | Water-Flood | 30         | P                   | 7.8  | 0.0  | 1559                               | 0    | 806                                | 0    | 0              | 0    | 0               | 0    | 40             | 0    |
|          |             |            | T1                  | 7.9  | 0.1  | 1613                               | 99   | 703                                | 205  | 0              | 0    | 0               | 0    | 236            | 133  |
|          |             |            | I                   | 7.8  | 0.1  | 1264                               | 192  | 720                                | 0    | 0              | 0    | 0               | 0    | 161            | 4    |
|          |             |            | T2                  | 7.9  | 0.5  | 964                                | 50   | NA                                 |      | 0              | 0    | 0               | 0    | NA             |      |
|          |             | 60         | P                   | 6.9  | 0.0  | 1031                               | 136  | 523                                | 109  | 0              | 0    | 0               | 0    | 96             | 45   |
|          |             |            | T1                  | 7.2  | 0.1  | 839                                | 12   | 438                                | 24   | 0              | 0    | 0               | 0    | 113            | 38   |
|          |             |            | I                   | 7.0  | 0.2  | 834                                | 166  | 603                                | 72   | 0              | 0    | 0               | 0    | 690            | 788  |
|          |             |            | T2                  | 7.3  | 0.5  | 650                                | 67   | 311                                | 44   | 0              | 0    | 0               | 0    | NA             |      |
| 7        | Water-Flood | 30         | P                   | 5.9  | 0.0  | 67                                 | 95   | 50                                 | 0    | 0              | 0    | 0               | 0    | 12             | 0    |
|          |             |            | T1                  | 6.1  | 0.1  | 121                                | 20   | 30                                 | 13   | 0              | 0    | 0               | 0    | 109            | 68   |
|          |             |            | I                   | 6.3  | 0.2  | 152                                | 35   | NA                                 |      | 0              | 0    | 0               | 0    | 390            | 381  |
|          |             |            | T2                  | 6.9  | 0.5  | 172                                | 18   | NA                                 |      | 0              | 0    | 0               | 0    | NA             |      |
|          |             | 60         | P                   | 5.8  | 0.0  | 167                                | 52   | 45                                 | 0    | 0              | 0    | 0               | 0    | 77             | 59   |
|          |             |            | T1                  | 6.0  | 0.1  | 91                                 | 9    | 49                                 | 15   | 0              | 0    | 0               | 0    | 31             | 33   |
|          |             |            | I                   | 5.9  | 0.2  | 118                                | 21   | 65                                 | 5    | 0              | 0    | 0               | 0    | 23             | 5    |
|          |             |            | T2                  | 6.3  | 0.6  | 120                                | 6    | 54                                 | 10   | 0              | 0    | 0               | 0    | NA             |      |
| 8        | CMA-Flood   | 30         | P                   | 6.8  | 0.0  | 386                                | 0    | 70                                 | 0    | 0              | 0    | 0               | 0    | 163            | 0    |
|          |             |            | T1                  | 7.1  | 0.1  | 508                                | 112  | 245                                | 317  | 61             | 71   | 8               | 11   | 127            | 68   |
|          |             |            | I                   | 7.1  | 0.1  | 328                                | 54   | NA                                 |      | 1              | 2    | 0               | 0    | 348            | 118  |
|          |             |            | T2                  | 7.4  | 0.6  | 424                                | 33   | NA                                 |      | 0              | 0    | 0               | 0    | NA             |      |
|          |             | 60         | P                   | 5.6  | 0.0  | 97                                 | 18   | 43                                 | 21   | 0              | 0    | 0               | 0    | 17             | 21   |
|          |             |            | T1                  | 5.7  | 0.1  | 103                                | 9    | 50                                 | 9    | 0              | 0    | 0               | 0    | 28             | 37   |
|          |             |            | I                   | 5.7  | 0.3  | 112                                | 16   | 60                                 | 13   | 0              | 0    | 0               | 0    | 12             | 4    |
|          |             |            | T2                  | 6.0  | 0.4  | 75                                 | 6    | 31                                 | 5    | 1              | 2    | 0               | 0    | NA             |      |

<sup>a</sup> P--Prior to first treatment (1/86-2/86)

T1--First treatment period and one month afterward (3/4/86-4/25/86)

I--Intermediate period between

T1 and T2 (4/26/86-12/17/86)

T2--Second treatment period and one month afterward (12/18/86-3/17/87)

NA--Not available

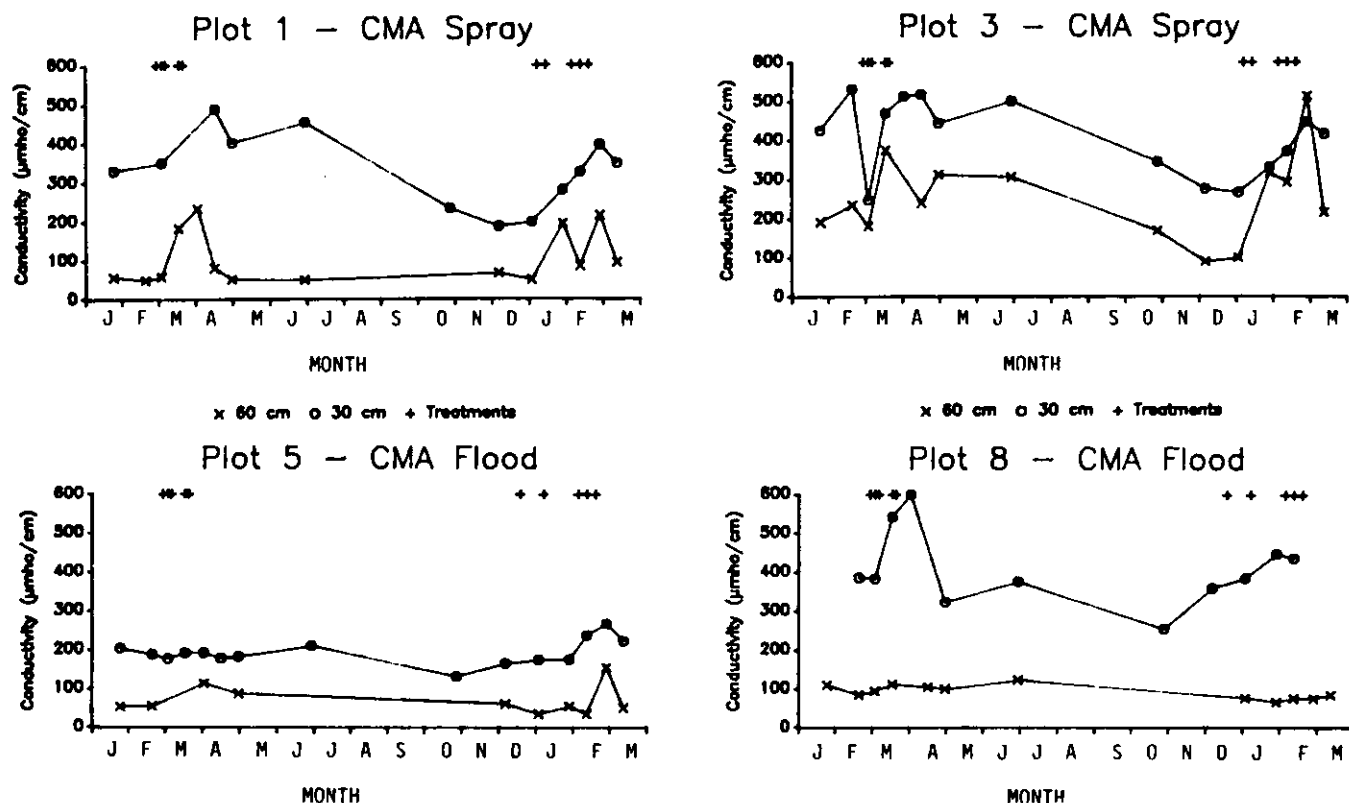


Figure C-2. Specific Conductivity in CMA Plot Lysimeters

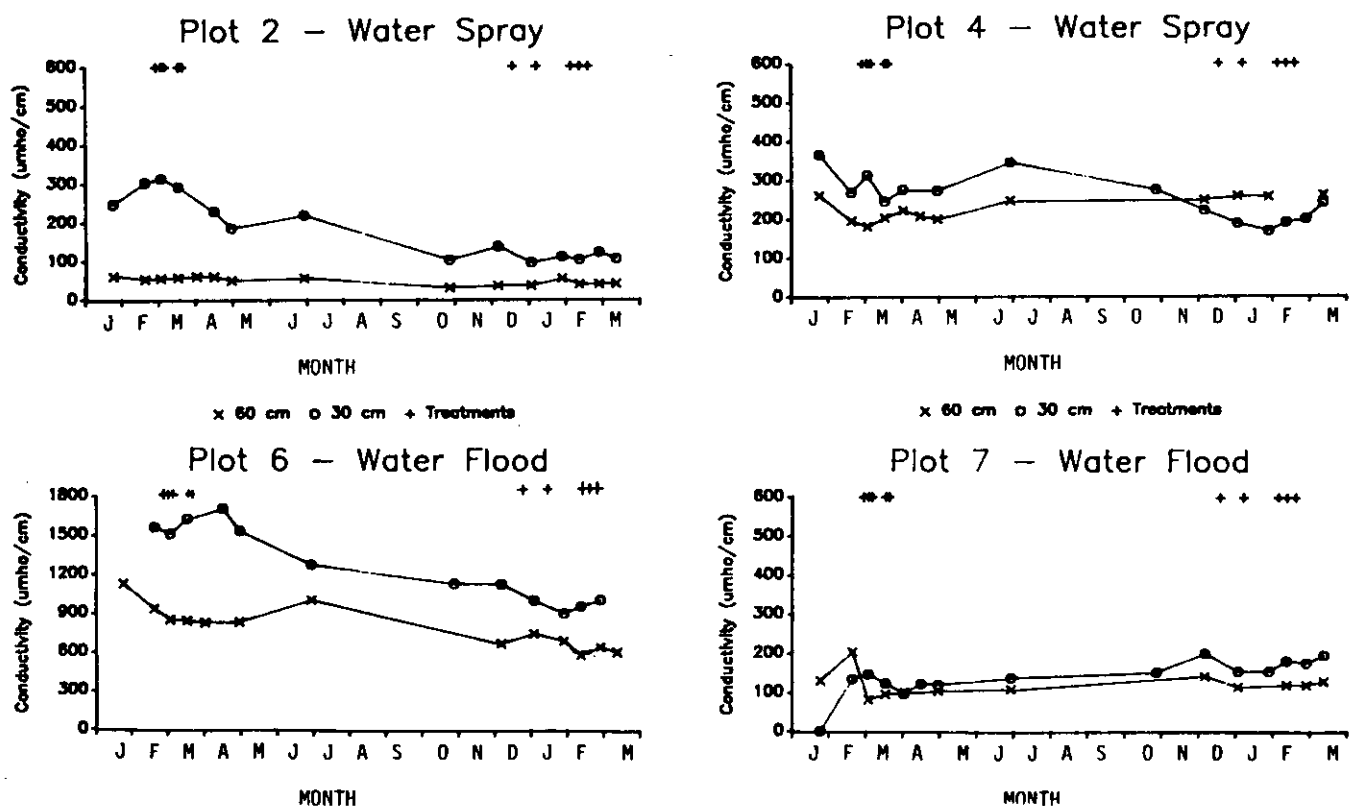


Figure C-3. Specific Conductivity in Water Plot Lysimeters

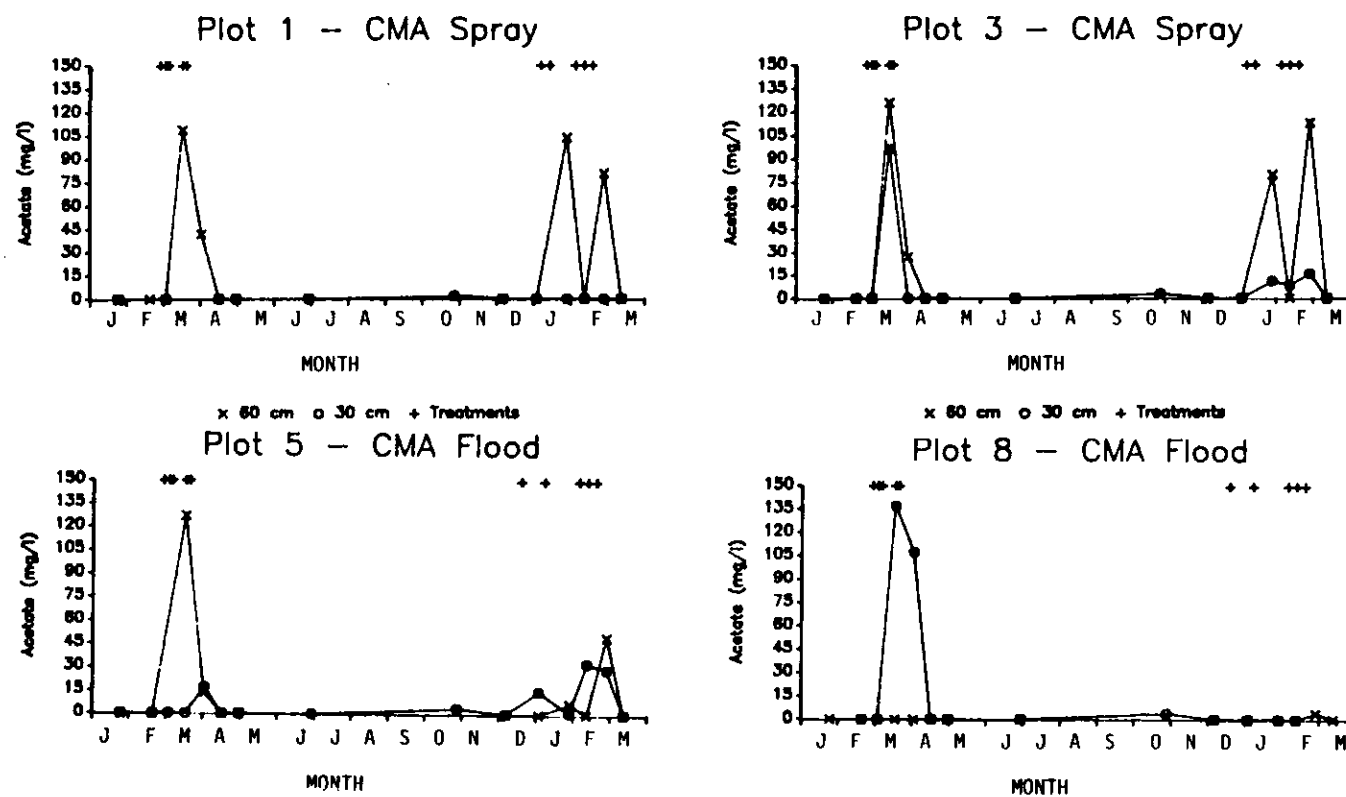


Figure C-4. Acetate in CMA Plot Lysimeters



There was no clear and consistent pattern of Ca, Mg, or K increase in the soil water at either depth during or after treatments. A trend was lacking despite Ca and Mg increases during treatments at 60 cm in some CMA plots. Control plot soil water was just as likely to experience increases in the major cations during the winter, probably because of increased infiltration. The same general statements apply to the trace metal ions; i.e., there was no explicit tendency overall despite some isolated readings that suggested trace metal elevation below CMA plots.

The specific conductivity plots (Figures C-2 and C-3) represent inorganic substances in the soil water. Conductivity tended to be more elevated at 30 than at 60 cm in both CMA and water plots. However, acetate (Figure C-4) was often higher at 60 than at 30 cm. Acetate peaks both rose and declined more rapidly than conductivity peaks. It generally took two-to-three weeks after the initial CMA treatment of the season for acetate to become detectable at depth. It then disappeared from the soil water within about the same period after the final treatment. During the first treatment season, little difference between spray and flood plots in soil water acetate was evident. In the second season, however, acetate did not rise as high in flood plot as in spray plot lysimeters.

In addition to the constituents reported in Table C-5, three anions (chloride, nitrate, and sulphate) were measured in soil water on two occasions near the end of the first treatment season. Table C-6 summarizes results. Data from duplicate plots are grouped in this

Table C-6. Means and Standard Deviations of Three Anions in Controlled Field Plot Soil Water in March and April, 1986

| Treatment   | Depth<br>(cm) | Concentration (mg/l) |             |                  |             |          |             |
|-------------|---------------|----------------------|-------------|------------------|-------------|----------|-------------|
|             |               | Chloride             |             | Nitrate-Nitrogen |             | Sulfate  |             |
|             |               | <u>Y</u>             | <u>S.D.</u> | <u>Y</u>         | <u>S.D.</u> | <u>Y</u> | <u>S.D.</u> |
| CMA-Spray   | 30            | 0.3                  | 0.1         | 0.2              | 0.1         | 8.8      | 1.8         |
|             | 60            | 1.2                  | 0.4         | 0.3              | 0.0         | 12.3     | 5.3         |
| Water-Spray | 30            | 0.7                  | 0.3         | 21.2             | 18.0        | 16.4     | 11.2        |
|             | 60            | 0.9                  | 0.7         | 1.4              | 0.1         | 13.0     | 10.1        |
| CMA-Flood   | 30            | 0.3                  | 0.1         | 0.9              | 0.3         | 16.3     | 8.3         |
|             | 60            | 1.4                  | 0.8         | 0.2              | 0.0         | 3.8      | 0.2         |
| Water-Flood | 30            | 0.9                  | 1.0         | 2.0              | 1.2         | 16.8     | 8.1         |
|             | 60            | 0.2                  | 0.1         | 0.5              | 0.4         | 14.2     | 13.5        |

summary. There was no tendency toward elevation of these anions in plots receiving CMA.

#### Groundwater Characteristics

Tables C-7 and C-8 give means and standard deviations of metals and other constituents, respectively, measured in groundwater drawn from beneath controlled field plots. Statistics are again reported for four periods. These periods are the same as those for which lysimeter data were averaged, except that the treatment period extends two months after the end of applications. Figures C-5 and C-6 illustrate variations in specific conductivity and acetate in CMA and water plots, respectively, over time. Acetate results demonstrate that there was approximately a two-month lag between treatments and when acetate appeared in the saturated zone, where it persisted until close to the next treatment period. This pattern accounts for the organization of the data in Tables C-7 and C-8.

Metals exhibited greater changes and some stronger trends in groundwater over the period of observation than they did in soil water. Still, the trends were not without exception. Ca increased notably below CMA Plots 3 and 8, but not 1 and 5, in the second winter. Elevation in Fe and Al was seen at three of the four CMA plots (excluding 5). Zn was substantially higher in the second winter at Plot 3 but not others. The remaining metals were relatively stable.

All plots exhibited a rise in pH several months after the first treatment season, which extended into the second winter. This increase was very large in Plot 8 groundwater, which also experienced great increases in specific conductivity and calcium. It is doubtful,

Table C-7. Means and Standard Deviations of Metals in Groundwater Over Four Periods During Field Plot Experiments

| Plot |             |                     | Concentration (mg/l) |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|------|-------------|---------------------|----------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
|      |             |                     | Ca                   |      | Mg   |      | K    |      | Fe   |      | Cu   |      | Zn   |      | Se   |      | Pb   |      | Al   |      |
| No.  | Treatment   | Period <sup>a</sup> | Mean                 | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| 1    | CMA-Spray   | P                   | 5.7                  | 0.0  | 2.2  | 0.0  | 1.8  | 0.0  | 9.5  | 0.0  | 0.05 | 0.00 | 0.41 | 0.00 | 0.07 | 0.00 | 0.06 | 0.00 | 10.1 | 0    |
|      |             | T1                  | 7.3                  | 0.0  | 1.1  | 0.0  | 1.4  | 0.0  | 18.2 | 0.0  | 0.06 | 0.00 | 0.08 | 0.00 | 0.10 | 0.00 | 0.06 | 0.00 | 14.4 | 0    |
|      |             | I                   | 6.7                  | 5.5  | 2.8  | 2.2  | 1.7  | 0.6  | 36.1 | 44.8 | 0.07 | 0.09 | 0.09 | 0.09 | 0.13 | 0.16 | 0.12 | 0.15 | 20.1 | 24   |
|      |             | T2                  | 5.1                  | 1.3  | 1.2  | 1.0  | 2.0  | 0.0  | 16.9 | 20.7 | 0.08 | 0.03 | 0.08 | 0.03 | 0.06 | 0.04 | 0.03 | 0.02 | 9.5  | 11   |
| 2    | Water-Spray | P                   | 15.3                 | 0.0  | 9.0  | 0.0  | 1.3  | 0.0  | 1.1  | 0.0  | 0.05 | 0.00 | 0.14 | 0.00 | 0.01 | 0.00 | 0.02 | 0.00 | 0.0  | 0    |
|      |             | T1                  | 9.3                  | 1.1  | 5.5  | 0.8  | 0.9  | 0.1  | 0.1  | 0.1  | 0.00 | 0.00 | 0.05 | 0.09 | 0.02 | 0.01 | 0.02 | 0.01 | 0.1  | 0    |
|      |             | I                   | 6.9                  | 1.1  | 4.5  | 1.2  | 1.0  | 0.2  | 0.2  | 0.1  | 0.01 | 0.01 | 0.03 | 0.04 | 0.02 | 0.02 | 0.06 | 0.08 | 0.1  | 0    |
|      |             | T2                  | 9.4                  | 1.3  | 6.8  | 0.4  | 1.2  | 0.1  | 0.1  | 0.2  | 0.00 | 0.00 | 0.01 | 0.00 | 0.02 | 0.01 | 0.02 | 0.01 | 0.1  | 0    |
| 3    | CMA-Spray   | P                   | 5.0                  | 0.0  | 2.9  | 0.0  | 0.6  | 0.0  | 2.3  | 0.0  | 0.01 | 0.00 | 0.02 | 0.00 | 0.02 | 0.00 | 0.02 | 0.00 | 2.1  | 0    |
|      |             | T1                  | 3.9                  | 0.3  | 2.0  | 0.2  | 0.7  | 0.2  | 8.2  | 6.0  | 0.03 | 0.02 | 0.05 | 0.04 | 0.05 | 0.03 | 0.04 | 0.02 | 6.6  | 6    |
|      |             | I                   | 6.0                  | 2.0  | 2.4  | 1.6  | 1.3  | 0.5  | 7.9  | 8.3  | 0.03 | 0.03 | 0.10 | 0.10 | 0.05 | 0.05 | 0.04 | 0.03 | 7.4  | 9    |
|      |             | T2                  | 17.9                 | 26.5 | 2.2  | 2.0  | 1.4  | 0.8  | 13.3 | 27.2 | 0.02 | 0.03 | 1.27 | 2.72 | 0.05 | 0.05 | 0.06 | 0.08 | 5.2  | 10   |
| 4    | Water-Spray | P                   | NA                   |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|      |             | T1                  | 6.3                  | 1.8  | 2.7  | 0.8  | 1.1  | 0.3  | 1.4  | 1.5  | 0.01 | 0.00 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | 0.01 | 0.7  | 0    |
|      |             | I                   | 6.0                  | 5.6  | 1.9  | 1.7  | 1.7  | 0.8  | 11.2 | 12.1 | 0.02 | 0.02 | 0.05 | 0.03 | 0.04 | 0.03 | 0.04 | 0.04 | 6.2  | 7    |
|      |             | T2                  | 2.7                  | 0.4  | 1.4  | 0.6  | 2.5  | 1.0  | 11.3 | 12.6 | 0.01 | 0.01 | 0.03 | 0.01 | 0.04 | 0.02 | 0.03 | 0.02 | 4.1  | 4    |
| 5    | CMA-Flood   | P                   | NA                   |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|      |             | T1                  | 8.2                  | 6.7  | 3.3  | 2.7  | 1.0  | 0.2  | 4.5  | 3.8  | 0.02 | 0.01 | 0.16 | 0.30 | 0.04 | 0.01 | 0.04 | 0.01 | 3.1  | 2    |
|      |             | I                   | 4.9                  | 1.5  | 1.9  | 1.1  | 1.1  | 0.3  | 2.1  | 3.2  | 0.01 | 0.01 | 0.03 | 0.04 | 0.03 | 0.03 | 0.03 | 0.02 | 2.2  | 3    |
|      |             | T2                  | 8.9                  | 11.9 | 2.0  | 0.9  | 1.5  | 0.3  | 0.8  | 1.0  | 0.01 | 0.00 | 0.29 | 0.57 | 0.02 | 0.00 | 0.02 | 0.03 | 0.7  | 0    |
| 6    | Water-Flood | P                   | NA                   |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|      |             | T1                  | 24.3                 | 5.9  | 13.7 | 4.0  | 0.8  | 0.1  | 0.3  | 0.2  | 0.02 | 0.01 | 0.19 | 0.11 | 0.02 | 0.00 | 0.02 | 0.01 | 0.3  | 0    |
|      |             | I                   | 4.2                  | 5.1  | 1.7  | 2.0  | 0.4  | 0.2  | 3.7  | 4.9  | 0.03 | 0.03 | 0.07 | 0.07 | 0.04 | 0.01 | 0.04 | 0.02 | 1.7  | 2    |
|      |             | T2                  | 3.3                  | 2.1  | 0.6  | 0.5  | 0.4  | 0.2  | 0.7  | 0.3  | 0.02 | 0.01 | 0.03 | 0.03 | 0.02 | 0.01 | 0.02 | 0.01 | 0.6  | 0    |
| 7    | Water-Flood | P                   | 23.2                 | 0.0  | 3.3  | 0.0  | 0.8  | 0.0  | 3.3  | 0.0  | 0.01 | 0.00 | 0.59 | 0.00 | 0.03 | 0.00 | 0.03 | 0.00 | 2.4  | 0    |
|      |             | T1                  | 7.2                  | 1.1  | 4.7  | 1.4  | 0.5  | 0.1  | 7.5  | 12.9 | 0.02 | 0.03 | 0.05 | 0.07 | 0.03 | 0.04 | 0.03 | 0.02 | 3.2  | 5    |
|      |             | I                   | 3.8                  | 3.4  | 1.9  | 1.7  | 0.5  | 0.2  | 1.6  | 1.7  | 0.00 | 0.00 | 0.02 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | 0.7  | 0    |
|      |             | T2                  | 2.4                  | 1.8  | 1.5  | 1.4  | 0.4  | 0.2  | 1.7  | 2.7  | 0.01 | 0.01 | 0.05 | 0.05 | 0.03 | 0.01 | 0.03 | 0.04 | 0.8  | 0    |
| 8    | CMA-Flood   | P                   | 14.0                 | 0.0  | 9.2  | 0.0  | 0.5  | 0.0  | 0.2  | 0.0  | 0.01 | 0.00 | 0.01 | 0.00 | 0.02 | 0.00 | 0.03 | 0.00 | 0.1  | 0    |
|      |             | T1                  | 5.6                  | 3.0  | 4.0  | 2.5  | 0.6  | 0.1  | 5.0  | 6.4  | 0.01 | 0.01 | 0.03 | 0.02 | 0.03 | 0.02 | 0.03 | 0.01 | 2.4  | 2    |
|      |             | I                   | 6.1                  | 5.3  | 1.1  | 1.4  | 1.3  | 0.6  | 8.7  | 12.0 | 0.05 | 0.03 | 0.13 | 0.10 | 0.06 | 0.04 | 0.08 | 0.03 | 8.1  | 7    |
|      |             | T2                  | 38.0                 | 21.3 | 0.2  | 0.1  | 1.7  | 0.2  | 0.2  | 0.2  | 0.02 | 0.00 | 0.03 | 0.03 | 0.02 | 0.00 | 0.02 | 0.01 | 2.3  | 0    |

<sup>a</sup> P--Prior to first treatment (1/86-2/86)

I--Intermediate period between T1 and T2

T1--First treatment period and two months afterward (3/4/86-5/2 5/86)

(5/26/86-12/17/86)

NA--Not available

T2--Second treatment period and afterward as long as data are available (12/18/86-3/12/87)

Table C-8. Means and Standard Deviations of Five Constituents in Groundwater  
Over Four Periods During Field Plot Experiments

| Plot<br>No. | Treatment   | Period <sup>a</sup> | pH   |      | Spec. Cond.<br>( $\mu\text{mho/cm}$ ) |      | Alkalinity<br>( $\text{mg/l CaCO}_3$ ) |      | Acetate<br>( $\text{mg/l}$ ) |      | Total P<br>( $\text{mg/l}$ ) |      |
|-------------|-------------|---------------------|------|------|---------------------------------------|------|--|------|------------------------------|------|------------------------------|------|
|             |             |                     | Mean | S.D. | Mean                                  | S.D. | Mean                                   | S.D. | Mean                         | S.D. | Mean                         | S.D. |
| 1           | CMA-Spray   | P                   | 7.0  | 0.0  | NA                                    |      | NA                                     |      | 0                            | 0    | 1635                         | 0    |
|             |             | T1                  | NA   |      | 131                                   | 0    | NA                                     |      | 0                            | 0    | 179                          | 210  |
|             |             | I                   | 9.8  | 0.0  | 199                                   | 112  | NA                                     |      | 12                           | 5    | 755                          | 0    |
|             |             | T2                  | 7.8  | 0.0  | 183                                   | 4    | NA                                     |      | 1                            | 3    | NA                           |      |
| 2           | Water-Spray | P                   | 6.7  | 0.0  | 154                                   | 0    | 43                                     | 0    | 0                            | 0    | 51                           | 0    |
|             |             | T1                  | 7.5  | 0.0  | 128                                   | 12   | 64                                     | 6    | 0                            | 0    | 26                           | 20   |
|             |             | I                   | 7.4  | 0.1  | 110                                   | 10   | 82                                     | 0    | 6                            | 4    | 29                           | 0    |
|             |             | T2                  | 7.6  | 0.2  | 147                                   | 43   | NA                                     |      | 0                            | 0    | NA                           |      |
| 3           | CMA-Spray   | P                   | 7.2  | 0.0  | NA                                    |      | NA                                     |      | 0                            | 0    | 214                          | 0    |
|             |             | T1                  | 7.7  | 0.0  | 64                                    | 10   | NA                                     |      | 0                            | 0    | 285                          | 152  |
|             |             | I                   | 8.0  | 0.0  | 92                                    | 32   | NA                                     |      | 9                            | 7    | 40                           | 0    |
|             |             | T2                  | 7.7  | 0.3  | 105                                   | 65   | NA                                     |      | 0                            | 0    | NA                           |      |
| 4           | Water-Spray | P                   | NA   |      | NA                                    |      | NA                                     |      | 0                            | 0    | 278                          | 0    |
|             |             | T1                  | 6.8  | 0.0  | 115                                   | 6    | NA                                     |      | 0                            | 0    | 221                          | 274  |
|             |             | I                   | 7.5  | 0.1  | 85                                    | 21   | 58                                     | 0    | 7                            | 5    | 34                           | 0    |
|             |             | T2                  | 8.2  | 0.6  | 114                                   | 57   | 0                                      | 0    | 1                            | 2    | NA                           |      |
| 5           | CMA-Flood   | P                   | 7.5  | 0.0  | NA                                    |      | NA                                     |      | 0                            | 0    | 425                          | 0    |
|             |             | T1                  | 7.5  | 0.0  | 157                                   | 23   | NA                                     |      | 0                            | 0    | 235                          | 153  |
|             |             | I                   | 7.9  | 1.3  | 128                                   | 10   | 62                                     | 0    | 8                            | 5    | 50                           | 0    |
|             |             | T2                  | 8.6  | 0.7  | 140                                   | 48   | NA                                     |      | 3                            | 3    | NA                           |      |
| 6           | Water-Flood | P                   | 6.2  | 0.0  | 363                                   | 0    | 18                                     | 0    | 0                            | 0    | 41                           | 0    |
|             |             | T1                  | 6.4  | 0.0  | 232                                   | 45   | NA                                     |      | 0                            | 0    | 31                           | 25   |
|             |             | I                   | 7.7  | 0.6  | 147                                   | 43   | 56                                     | 0    | 18                           | 25   | 15                           | 0    |
|             |             | T2                  | 8.5  | 0.8  | 135                                   | 51   | NA                                     |      | 0                            | 0    | NA                           |      |
| 7           | Water-Flood | P                   | 6.9  | 0.0  | 147                                   | 0    | NA                                     |      | 0                            | 0    | 133                          | 0    |
|             |             | T1                  | 7.4  | 0.0  | 103                                   | 6    | NA                                     |      | 0                            | 0    | 132                          | 178  |
|             |             | I                   | 8.0  | 0.0  | 64                                    | 19   | NA                                     |      | 12                           | 9    | 43                           | 0    |
|             |             | T2                  | 7.7  | 0.4  | 86                                    | 54   | NA                                     |      | 1                            | 2    | NA                           |      |
| 8           | CMA-Flood   | P                   | 7.5  | 0.0  | NA                                    |      | NA                                     |      | 0                            | 0    | 89                           | 0    |
|             |             | T1                  | 7.2  | 0.0  | 181                                   | 9    | NA                                     |      | 0                            | 0    | 258                          | 219  |
|             |             | I                   | 11.6 | 0.0  | 1962                                  | 0    | NA                                     |      | 71                           | 91   | NA                           |      |
|             |             | T2                  | 11.8 | 0.1  | 1522                                  | 370  | NA                                     |      | 0                            | 0    | NA                           |      |

<sup>a</sup> P--Prior to first treatment (1/86-2/86)

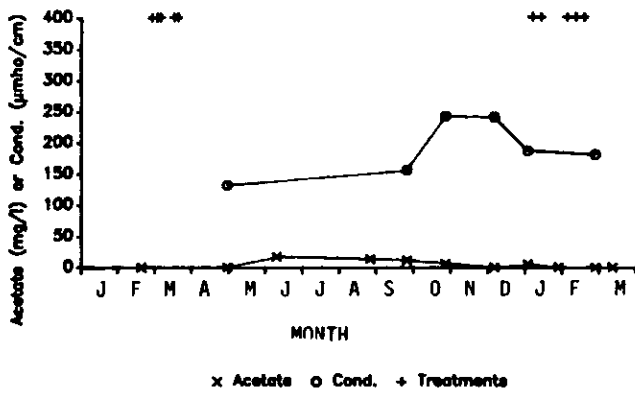
T1--First treatment period and two months afterward (3/4/86-5/2 5/86)

I--Intermediate period between T1 and T2 (5/26/86-12/17/86)

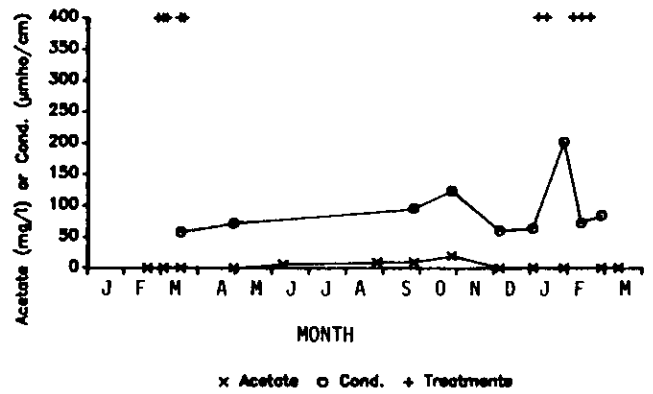
T2--Second treatment period and afterward as long as data are available  
(12/18/86-3/12/87)

NA--Not available (some analyses were not run when sample volume was insufficient)

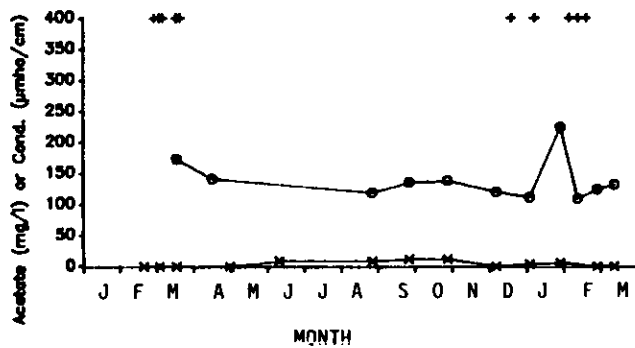
Plot 1 – CMA Spray



Plot 3 – CMA Spray



Plot 5 – CMA Flood

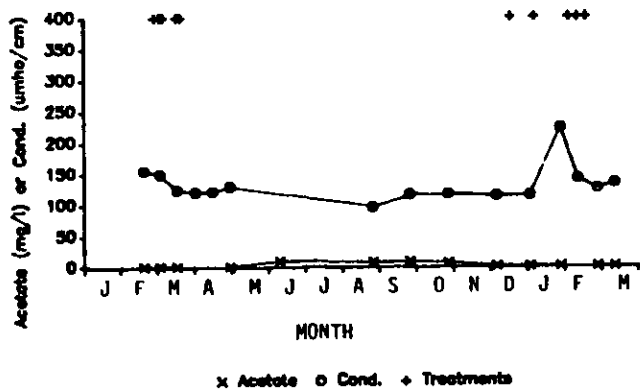


Plot 8 – CMA Flood

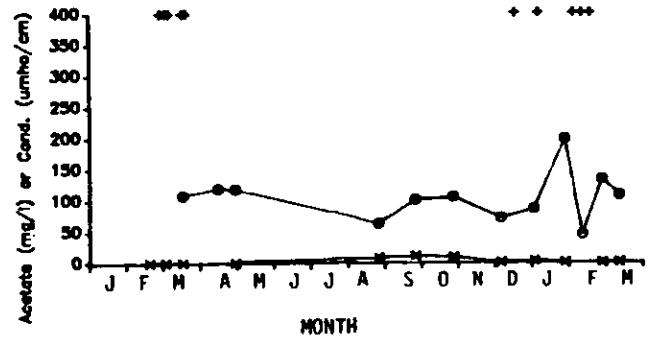


Figure C-5. Specific Conductivity and Acetate in CMA Plot Groundwater

Plot 2 – Water Spray



Plot 4 – Water Spray



Plot 6 – Water Flood



Plot 7 – Water Flood

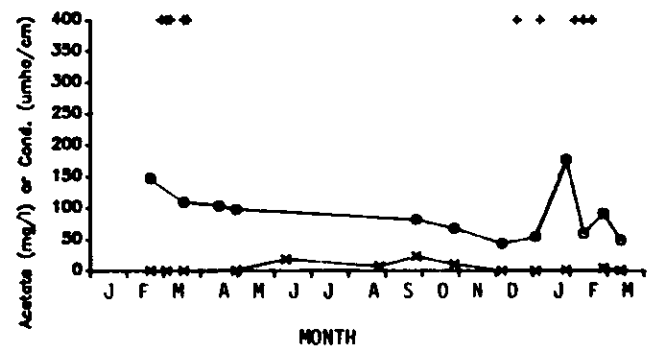


Figure C-6. Specific Conductivity and Acetate in Water Plot Groundwater

however, that these changes were due to CMA migration. CMA could not have raised the pH to > 11, and conductivities were often higher than even the CMA tank contents. The only explanation we have is that the plot may overlie an old lime dump from the former ranch, although no investigation of such a dump was undertaken. The extra water supply could have mobilized the lime. Therefore, no validity can be attached to cation measurements in Plot 8 groundwater.

Acetate sometimes appeared in groundwater drawn from wells on control plots long after treatments. Obviously, the speed of groundwater movement was sufficient to cover the approximately 10 m between wells in less than a year. It is notable that small amounts of acetate remained undegraded in the saturated zone after months.

## DISCUSSION

Observation of controlled plot and bin runoff demonstrated that acetate was marginally but consistently reduced during flow over the short test slopes. Since the hydraulic and CMA mass loading rates were in the test scale, similar removals (up to 25% in just a few meters) could also be expected in full-size systems. Greater reductions could be postulated on longer slopes, but there is no basis to forecast what acetate (and butyrate) decreases might occur. The potential exists to achieve substantial removals with a moderate length of overland flow. These removals would be important in reducing the oxygen depletion potential in receiving waters, which has been demonstrated (see Appendix I).

2  
With the limited data, the model fitting acetate removal in overland flow is unclear. These data fit several models, including the exponential relationship proposed in the literature for BOD (5, 6).

In contrast to the volatile fatty acid salts, metals were not consistently removed by the plot and bin vegetation and soils. This finding is in disagreement with the sewage and stormwater treatment literature (3, 4). However, the situation represented in these experiments may not be comparable to those other cases. Ca, Mg, and K are probably higher in CMA runoff and the trace metals are likely lower than in the other waste streams. The dynamic ion exchange and other physicochemical processes both capture and release metals. In particular, a potential for mobilization of some trace metals by Ca and Mg ion exchange has been noted (see Appendix E). No final conclusions can be drawn until CMA flow over longer vegetated slopes has been observed.

The analyses of subsurface water demonstrated that acetate in small concentrations can penetrate the soil to the saturated zone (here at 3 m). While it degraded and disappeared in a few weeks in the unsaturated zone, it existed measurably for months in the groundwater. The plot soils were moderate in texture, and coarser soils would pass more acetate. Studying the decomposition reactions of acetate in groundwater, their by-products, and effects on dissolved oxygen was beyond this project's scope. Objectionable by-products and oxygen reduction are possible (see Appendix I) and should be considered in a highway-scale study, in which deep groundwater is monitored.

Laboratory testing (see Appendix E) indicated that capture of the major cations in soils can release some preexisting metals (Fe, Al, Cu, and Zn). These releases occurred under the relatively severe flushing conditions applied in laboratory procedures. They were only partially borne out in the field data. Releases of these metals were not at all evident in the lysimeters. Fe and Al usually did occur in higher concentrations in groundwater below CMA plots after treatments. Zn was elevated in only one plot, and Cu never was. As discussed fully in Appendix E, this possibility of metal mobilization is of some concern and should also be investigated further in full-scale tests.

#### CONCLUSIONS

The range of concentrations and mass loadings of CMA in actual highway runoff were projected in a modeling exercise. CMA was applied to field plots in quantities relatively high in these ranges. Its transport and fate were observed, and observations were compared with control plots receiving only water. It was seen that components of CMA are largely retained in soils but can reach at least shallow groundwater in relatively small amounts. Also, there is evidence that native soil metals can be released through exchange reactions and increase in groundwater. On the surface, vegetation and soils can remove acetate from runoff, but not the cations with consistency.

Implications of the subsurface results for use of CMA and environmental impact assessment are considered in Appendix E. The observation of acetate removal in overland flow encourages providing vegetated drainage courses between highways that will receive the deicer

and receiving waters. The potential CMA impact of greatest concern is oxygen depletion in those waters (see Appendix I), and drainage over vegetation could offer significant protection against deoxygenation.

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## APPENDIX D

### EFFECTS OF CMA ON SOIL PHYSICAL PROPERTIES

#### INTRODUCTION

Laboratory tests on four soil types and field tests on one soil type were conducted to determine the effect of CMA on their physical properties. Classification tests were conducted on each soil type and on samples from eight plots at the field site. Subsequent tests were done to determine the effects of CMA on soil plasticity, moisture-density characteristics, hydraulic conductivity, and shearing resistance. Solutions containing calcium and magnesium compounds have been seen in the past to effect the properties of fine-grained soils (e.g., 1).

#### EXPERIMENTAL METHODS

##### Analysis of General Soil Properties

Soil samples were obtained from four sites to represent a variety of soil conditions representative of roadsides nationwide. These soils were used in the laboratory investigations of the effects of CMA on soil physics and chemistry, its decomposition in soils, and on vegetation. The four soils were:

1. A loam from the controlled field plot site at Pack Forest, Washington.
2. A silt loam from Lee Forest, Washington.
3. A sandy loam from the Washington State Agricultural Experiment Station at Puyallup, Washington.
4. A calcareous sandy loam from Vantage, Washington.

These soils were analyzed for organic content, cation exchange capacity (CEC), and exchangeable cations. Organic content was determined by the weight loss on ignition method. CEC and exchangeable cations were measured with an ammonium acetate extraction procedure. CEC was read colorimetrically, and Ca, Mg, and K were analyzed in extracts by ICP (see Appendix A). Calcium carbonate was measured in the Vantage soil by sodium hydroxide titration of hydrochloric acid extract.

Soil composites from 0-30 and 30-60 cm depths in each of the eight controlled field plots were analyzed for organic content as above. This analysis was performed to judge the uniformity of the plots.

##### Classification Tests

Laboratory testing was performed on the four soils to provide information for use in classifying the soils according to the Unified Soil Classification System. The samples were also classified according to the USDA SCS ternary plot scheme.

Gradation tests were conducted on each of the four soil types. The tests were run in accordance with the ASTM Method for Particle-Size Analysis of Soils [D 422-63(1972)].

Plasticity tests, including liquid limit and plastic limit tests, were conducted on each soil type and for selected samples from field test plots. These tests were conducted in accordance with the ASTM Test Method for Liquid Limit, Plastic Limit, and Plasticity Index of Soils [D 4318-84]. Tests were performed using deionized tap water (control) and a 5000 ppm solution of CMA in deionized tap water.



### Compaction Tests

The moisture-density characteristics of the four soil types were determined in accordance with the ASTM Suggested Method of Test for Moisture-Density Characteristics of Soils using Harvard Miniature Compaction Apparatus [STP 479(1970)]. The samples were air-dried and sieved on a #10 (2 mm) standard sieve, and then were mixed with the compacting fluid and allowed to season in air-tight containers for at least a 24 h period. Deionized tap water (control) and 5000 ppm solution of CMA in deionized tap water were used for the tests. All samples were compacted in five layers, 25 tamps per layer, with a 25 lb. (111 N) spring force. The Harvard compaction apparatus was chosen because it also provided a convenient means of preparing reproducible samples for permeability and strength tests.

### Permeability Tests

Permeability tests were conducted on each soil type. Samples were tested in specially constructed Plexiglas permeameters sized to the dimensions of the Harvard miniature apparatus. The body of the permeameter mold was lined with a thin coating of high vacuum grease to prevent piping along the side walls during the test. Soil specimens were compacted in accordance with ASTM STP 479(1970), as described in the previous section. The samples were compacted 3% to 5% dry of the optimum moisture content using the permeant fluid. Mitchell et al. (2) studied a range in compaction water content and found that storage time had more effect on the hydraulic conductivity of samples compacted wet of optimum. Since the samples in this testing program were to be run over a period of time, it was decided to compact them slightly dry of

optimum in order to reduce the number of variables. The top of each layer was scarified to prevent discontinuities and to form a homogeneous sample. After compaction, the top of the sample was trimmed and the mold was bolted between end plates, forming a pressure tight seal. The end plates were fitted with saturated porous disks and #4 Whatman filter paper.

Two series of tests for each soil were conducted in temperature-controlled rooms at 2 C and 20 C. Permeants used for testing all four soil types were: deionized Seattle tap water, and a 5000 ppm solution of dried CMA dissolved in deionized Seattle tap water. Additional tests were run on the calcareous (Vantage) soil using CMA solution concentrations of 50 and 500 ppm. In these tests, the flow of the permeant from the reservoir tank was from the bottom to the top of the permeameter. A falling head permeability test set-up was utilized. The reservoir was taken as a constant head source, and the outflow was measured in a vertically placed burette. Head difference was measured with a millimeter scale. The sample was initially saturated by soaking with the permeant from bottom-to-top for a period of 12-to-18 hours. The actual sample saturation was measured upon completion of the test. After saturation, a gradient was established in the sample, and head difference readings were taken at specific time intervals (usually every hour). After the test was finished, the valves were closed and the sample was allowed to age. For the samples cured at 20 C, tests were conducted at 1, 9, 17, 28, and 49 days after compaction for the Murphy's Ranch and Puyallup soils and at 1, 7, and 14 days for the Lee Forest and Vantage soils. For the samples cured at 2 C, tests were performed at 1,

7, and 14 days for all four soil types. The additional tests performed on the Vantage soil were also conducted at 2 C, with measurements taken at 1, 7, and 14 days.

#### Soil Strength Index

Unconfined compression tests were conducted on remolded samples of each soil type. Samples were compacted in the Harvard miniature apparatus according to ASTM STP 479(1970). The four soil types were compacted at approximately their optimum moisture content using deionized tap water or a 5000 ppm solution of CMA. Samples of each soil type were compacted, wrapped, sealed, and cured in a temperature-controlled room at 20 C for 1, 7, and 14 days. Additional samples of the Pack Forest soil were made and cured at 2 C for 1, 7, 14, and 28 days. The unconfined compression tests were conducted in accordance with ASTM Test Methods for Unconfined Compressive Strength of Cohesive Soil [D 2166-66(1979)]. The strain-controlled procedure was used.

#### Test Procedures for Field Test Plots

Composite grab samples were taken at the outset of the research program from the eight Pack Forest test plots at depth intervals of 0-30 cm and 30-60 cm. Gradation tests were conducted on each of these samples. A composite sample from test Plots 1 and 7 was used for the detailed laboratory analyses outlined previously.

Shelby tube samples were taken from test Plots 1, 4, 5, 6, and 8 in March of 1986, after the first season of CMA application. Samples from these tubes were visually classified. Moisture content and plasticity tests were conducted on selected samples.

Tests to determine the in-situ shear strength of the test plot soils were conducted using a hand-operated shear vane. The tests were run in accordance with ASTM Method for Field Vane Shear Test in Cohesive Soil [D 2573-72(1978)]. The test hole was advanced with a 3-inch diameter bucket auger. The vane used was tapered and had a diameter of 2 inches; the rod diameter was 1/2 inch. Tests were attempted on test Plots 1 to 5 at depths of 30 cm and 60 cm. Samples have not been taken as of this writing for the 1986-87 application season.

#### LABORATORY TESTING PROGRAM RESULTS

##### General Soil Properties

Table D-1 summarizes the general properties of the test soils. The four types represented an order-of-magnitude range in organic content and a three-fold difference in CEC.

Table D-2 provides data on organic content of field plot soils over two ranges of depth. Surface soils ranged from 4.2-8.1 percent organics. At depth the range was narrower (3.6-6.0%).

##### Classification Test Results

The particle-size analysis results for the four soils are shown in Table D-3. The soils are sandy loams, loam, and silt loam. Clay contents range from 3 to 19 percent. In general, the test plot samples exhibit good interplot particle-size uniformity.

Liquid limit, plastic limit, and plasticity index test results are summarized in Table D-4. The Pack Forest sample was found to have a liquid limit of 29% and a plasticity index of 6%. The limits of this soil were very similar to the control values when tested with a 5000 ppm

Table D-1. Summary of General Properties of Four Test Soils

| Soil                 | % Organics | Cation Exchange Capacity<br>(meq/100 g) | Exchangeable Cations(ppm) |     |     |
|----------------------|------------|---|---------------------------|-----|-----|
|                      |            |   | Ca                        | Mg  | K   |
| Pack Forest          | 6.0        | 12.6                                    | 754                       | 125 | 217 |
| Lee Forest           | 12.3       | 26.5                                    | 1030                      | 94  | 148 |
| Puyallup             | 5.2        | 8.8                                     | 899                       | 65  | 155 |
| Vantage <sup>a</sup> | 2.3        | 12.8                                    | 1320                      | 365 | 337 |

<sup>a</sup>Calcium carbonate percentage = 1.25%.

Table D-3. Particle-Size Analysis of Test Soils

| Sample                  | Gravel<br>(> 2 mm) | % Sand<br>(0.074-2 mm) | % Silt<br>(0.002-0.074) | % Clay<br>(<0.002 mm) | USDA SCS<br>Classification |
|-------------------------|--------------------|------------------------|-------------------------|-----------------------|----------------------------|
| Lee Forest              | 0                  | 43                     | 54                      | 3                     | Silt loam                  |
| Puyallup                | >Trace             | 53                     | 38                      | 9                     | Sandy loam                 |
| Vantage                 | 0                  | 51                     | 41                      | 5                     | Sandy loam                 |
| Pack Forest             |                    |                        |                         |                       |                            |
| Plot 5 (0-30 cm)        | >Trace             | 52                     | 30                      | 18                    | Sandy loam                 |
| Plot 5 (30-60 cm)       | Trace              | 57                     | 30                      | 13                    | Sandy loam                 |
| Plot 6 (0-30 cm)        | Trace              | 38                     | 44                      | 18                    | Loam                       |
| Plot 6 (30-60 cm)       | Trace              | 50                     | 34                      | 16                    | Loam                       |
| Plot 7 (0-30 cm)        | Trace              | 36                     | 49                      | 15                    | Loam                       |
| Plot 7 (30-60 cm)       | Trace              | 47                     | 39                      | 14                    | Loam                       |
| Plot 8 (0-30 cm)        | Trace              | 44                     | 44                      | 12                    | Loam                       |
| Plot 8 (30-60 cm)       | Trace              | 48                     | 35                      | 17                    | Loam                       |
| Combined Plots<br>1 & 7 | Trace              | 42                     | 39                      | 19                    | Loam                       |

Table D-2. Organic Content of Field Plot Soils Over  
Two Ranges of Depth

| Plot No. | % Organics |          |
|----------|------------|----------|
|          | 0-30 cm    | 30-60 cm |
| 1        | 8.0        | 3.9      |
| 2        | 4.2        | 3.6      |
| 3        | 5.3        | 4.5      |
| 4        | 6.4        | 5.9      |
| 5        | 6.7        | 5.7      |
| 6        | 4.5        | 4.8      |
| 7        | 8.1        | 5.9      |
| 8        | 6.8        | 6.0      |

Table D-4. Plasticity Test Results<sup>a</sup>

| Soil                                    | Liquid Limit (%) | Plastic Limit (%) | Plasticity Index (%) |
|---|------------------|-------------------|----------------------|
| <u>Tests with Deionized Tap Water</u>   |                  |                   |                      |
| Pack Forest                             | 29               | 22                | 7                    |
| Lee Forest                              | NP               | NP                | NP                   |
| Puyallup                                | NP               | NP                | NP                   |
| Vantage                                 | 20               | NP                | NP                   |
| <u>Tests with 5000 ppm CMA Solution</u> |                  |                   |                      |
| Pack Forest                             | 29               | 23                | 6                    |
| Lee Forest                              | NP               | NP                | NP                   |
| Puyallup                                | NP               | NP                | NP                   |
| Vantage                                 | 20               | NP                | NP                   |

<sup>a</sup>NP--Non-plasticTable D-5. Compaction Test Results<sup>a</sup>

| Soil        | Maximum Density (pcf) <sup>b</sup> | Optimum Moisture Content (%) |
|-------------|------------------------------------|------------------------------|
| Lee Forest  | 67.0 <sup>c</sup>                  | 45.0 <sup>c</sup>            |
| Pack Forest | 106.0                              | 19.9                         |
| Puyallup    | 100.2                              | 18.6                         |
| Vantage     | 118.6                              | 14.1                         |

<sup>a</sup>The Harvard miniature method was used; specifications: 5 layers, 25 tamps/layer, 25 lb. spring<sup>b</sup>Conversion factor: 1 pcf = 16.01 kg/m<sup>3</sup><sup>c</sup>Variable due to high (12.3% by weight) organic content.

CMA solution. The Vantage soil was found to have a very similar liquid limit for both water and a 5000 ppm CMA solution. The plastic limit of this soil could not be determined due to its non-plastic nature. The Lee Forest and Puyallup soils were found to be non-plastic with both water and a 5000 ppm CMA solution.

#### Compaction Test Results

Results of compaction tests are summarized in Table D-5. When compacted with 5000 ppm CMA solution, samples of all four soil types exhibited negligible variation of their moisture-density characteristics from values determined with water.

#### Permeability Test Results

The calculated permeabilities for the 20 C and 2 C tests conducted on the four soil types are summarized in Table D-6. The values given for the 2 C tests have been corrected for the viscosity and density of water and normalized to 20 C.

Samples of the Lee Forest soil tested at 20 C show a distinct decrease in permeability with storage time. Permeability in the sample tested with deionized tap water decreased by a factor of about six; permeability in the sample tested with CMA solution decreased by a factor of about four. The CMA-treated sample after one day showed a permeability two-three times higher than the control sample. Samples tested at 2 C exhibited little change in permeability with time. The CMA-treated sample again showed a permeability about 2-3 times higher than the control.

Samples of the Pack Forest soil tested at 20 C also demonstrated a distinct decrease in permeability with storage time. Permeabilities

Table D-6. Summary of Permeability Test Results

| Soil        | Cure Temp.<br>(C) | Permeant       | Void Ratio | Cure Time<br>(days) | Permeability<br>$K_{20}$ (cm/s) |
|-------------|-------------------|----------------|------------|---------------------|---------------------------------|
| Lee Forest  | 20                | Water          | 1.55       | 1                   | 2.0E-05                         |
|             |                   |                |            | 7                   | 8.1E-06                         |
|             |                   |                |            | 14                  | 5.4E-06                         |
|             | 20                | CMA (5000 ppm) | 1.55       | 1                   | 1.8E-05                         |
|             |                   |                |            | 7                   | 1.0E-05                         |
|             |                   |                |            | 14                  | 7.6E-05                         |
|             | 2                 | Water          | 1.67       | 1                   | 3.6E-05                         |
|             |                   |                |            | 7                   | 3.6E-05                         |
|             |                   |                |            | 14                  | 3.6E-05                         |
|             | 2                 | CMA (5000 ppm) | 1.65       | 1                   | 4.8E-05                         |
|             |                   |                |            | 7                   | 5.5E-05                         |
|             |                   |                |            | 14                  | 5.8E-05                         |
| Pack Forest | 20                | Water          | 0.78       | 1                   | 5.9E-06                         |
|             |                   |                |            | 9                   | 3.6E-06                         |
|             |                   |                |            | 17                  | 2.9E-06                         |
|             |                   |                |            | 28                  | 1.8E-06                         |
|             |                   |                |            | 49                  | 1.3E-06                         |
|             |                   |                |            | 1                   | 2.9E-05                         |
|             | 20                | CMA (5000 ppm) | 0.78       | 9                   | 2.7E-05                         |
|             |                   |                |            | 17                  | 1.7E-05                         |
|             |                   |                |            | 28                  | 1.3E-05                         |
|             |                   |                |            | 49                  | 4.5E-06                         |
|             |                   |                |            | 1                   | 8.8E-05                         |
|             |                   |                |            | 7                   | 9.1E-05                         |
|             | 2                 | Water          | 0.90       | 14                  | 9.2E-05                         |
|             |                   |                |            | 1                   | 2.1E-05                         |
|             |                   |                |            | 7                   | 1.8E-05                         |
|             |                   |                |            | 14                  | 1.8E-05                         |
|             | 2                 | CMA (5000 ppm) | 0.90       | 1                   | 2.1E-05                         |
|             |                   |                |            | 7                   | 1.8E-05                         |
|             |                   |                |            | 14                  | 1.8E-05                         |
| Puyallup    | 20                | Water          | 0.77       | 1                   | 1.1E-05                         |
|             |                   |                |            | 9                   | 1.1E-05                         |
|             |                   |                |            | 17                  | 1.2E-05                         |
|             |                   |                |            | 28                  | 8.3E-06                         |
|             |                   |                |            | 49                  | 7.2E-06                         |
|             |                   |                |            | 1                   | ---                             |
|             | 20                | CMA (5000 ppm) | 0.80       | 9                   | ---                             |
|             |                   |                |            | 17                  | 2.8E-05                         |
|             |                   |                |            | 28                  | 1.3E-05                         |
|             |                   |                |            | 49                  | 1.7E-05                         |
|             | 2                 | Water          | 0.82       | 1                   | 6.0E-05                         |
|             |                   |                |            | 7                   | 5.9E-05                         |
|             |                   |                |            | 14                  | 6.1E-05                         |
|             | 2                 | CMA (5000 ppm) | 0.83       | 1                   | 8.9E-05                         |
|             |                   |                |            | 7                   | 8.9E-05                         |
|             |                   |                |            | 14                  | 7.9E-05                         |

D-13

Continued

Table D-6 Continued

| Soil    | Cure Temp.<br>(C) | Permeant       | Void Ratio | Cure Time<br>(days) | Permeability<br>$K_{20}$ (cm/s) |
|---------|-------------------|----------------|------------|---------------------|---------------------------------|
| Vantage | 20                | Water          | 0.51       | 1                   | 2.8E-06                         |
|         |                   |                |            | 7                   | 5.0E-06                         |
|         |                   |                |            | 14                  | 5.2E-06                         |
|         | 20                | CMA (5000 ppm) | 0.51       | 1                   | 7.4E-05                         |
|         |                   |                |            | 7                   | 6.1E-05                         |
|         |                   |                |            | 14                  | 4.2E-05                         |
|         | 2                 | Water          | 0.53       | 1                   | 1.7E-06                         |
|         |                   |                |            | 7                   | 3.8E-06                         |
|         |                   |                |            | 14                  | 3.8E-06                         |
|         | 2                 | CMA (5000 ppm) | 0.54       | 1                   | 5.1E-05                         |
|         |                   |                |            | 7                   | 6.4E-05                         |
|         |                   |                |            | 14                  | 7.3E-05                         |
|         | 2                 | CMA (500 ppm)  | 0.49       | 1                   | 4.4E-05                         |
|         |                   |                |            | 7                   | 4.2E-05                         |
|         |                   |                |            | 14                  | 4.5E-05                         |
|         | 2                 | CMA (500 ppm)  | 0.48       | 1                   | 3.7E-05                         |
|         |                   |                |            | 7                   | 3.8E-05                         |
|         |                   |                |            | 14                  | 4.3E-05                         |
|         | 2                 | CMA (50 ppm)   | 0.49       | 1                   | 2.2E-05                         |
|         |                   |                |            | 7                   | 2.2E-05                         |
|         |                   |                |            | 14                  | 2.3E-05                         |
|         | 2                 | CMA (50 ppm)   | 0.49       | 1                   | 1.6E-05                         |
|         |                   |                |            | 7                   | 1.7E-05                         |
|         |                   |                |            | 14                  | 1.8E-05                         |

decreased by a factor of about five in the control and approximately seven in the CMA test. The CMA-treated sample exhibited a permeability about four times higher than the water-tested sample. Effect on permeability with time was again negligible at 2 C. The CMA sample showed a permeability about four times higher than the control.

Samples of the Puyallup soil tested at 20 C also showed a distinct decrease in permeability with storage time. Both the sample tested with deionized tap water and the sample tested with CMA solution had permeability decrease by a factor of about 2-3. The CMA-tested sample showed a permeability about 1-2 times higher than the water-tested sample. Samples tested at 2 C exhibited negligible changes in permeability with time. The CMA-tested sample showed a permeability about two times higher than the water-tested sample.

Samples of the Vantage soil tested at 20 and 2 C showed negligible changes in permeability with time for the water-tested samples and the three CMA solution concentrations. The CMA-treated sample at 20 C showed a permeability about 10 times higher than the control sample. The initial tests at 2 C using a 5000 ppm CMA solution produced a permeability about 20 times higher than the control. Additional tests showed that 500 ppm and 50 ppm solutions had permeabilities about ten and five times higher, respectively, than the water-tested sample.

#### Strength Test Results

Unconfined compression tests were performed on remolded samples of the four soil types cured at 20 C and the additional samples of the Pack Forest soil cured at 2 C. A representative result is shown by Figure D-1. No definite trend was seen in the samples cured at the two

temperatures. The differences in shear stresses measured are thought to be negligible and within the accuracy of the test method. It should be emphasized that these one-dimensional compression test results are not a measure of the shear strength of the non-plastic soils. However, these results can be interpreted to obtain a qualitative index of the effect of CMA on the stress-deformation characteristics of the soils.

#### FIELD TESTING PROGRAM RESULTS

Moisture content, liquid limit, plastic limit and plasticity index test results for the field plot samples are summarized in Table D-7. The samples for plasticity tests were taken from Plots 4, 6, and 8 at depths of 20 cm to 30 cm. The results of the tests show a distinct similarity to the combined grab sample tested earlier, having liquid limits in the 25% range and plasticity indices from 7 to 9%. The sample tested from the CMA plot showed a negligible plasticity difference from those taken from the water plots.

The shear vane test results are summarized in Table D-8. The strength characteristics appear to be similar for both the water and CMA plots. Data were limited due to gravelly conditions. The similarities in calculated shear strength seem to show, however, that the test plots have similar subsurface properties and that CMA had a negligible effect on the in-situ shear strength.

#### DISCUSSION

The 5000 ppm CMA solution and the CMA field applications appear to have had little effect on the plasticity characteristics and moisture-density relationships of the soils tested. In the classical explanation,

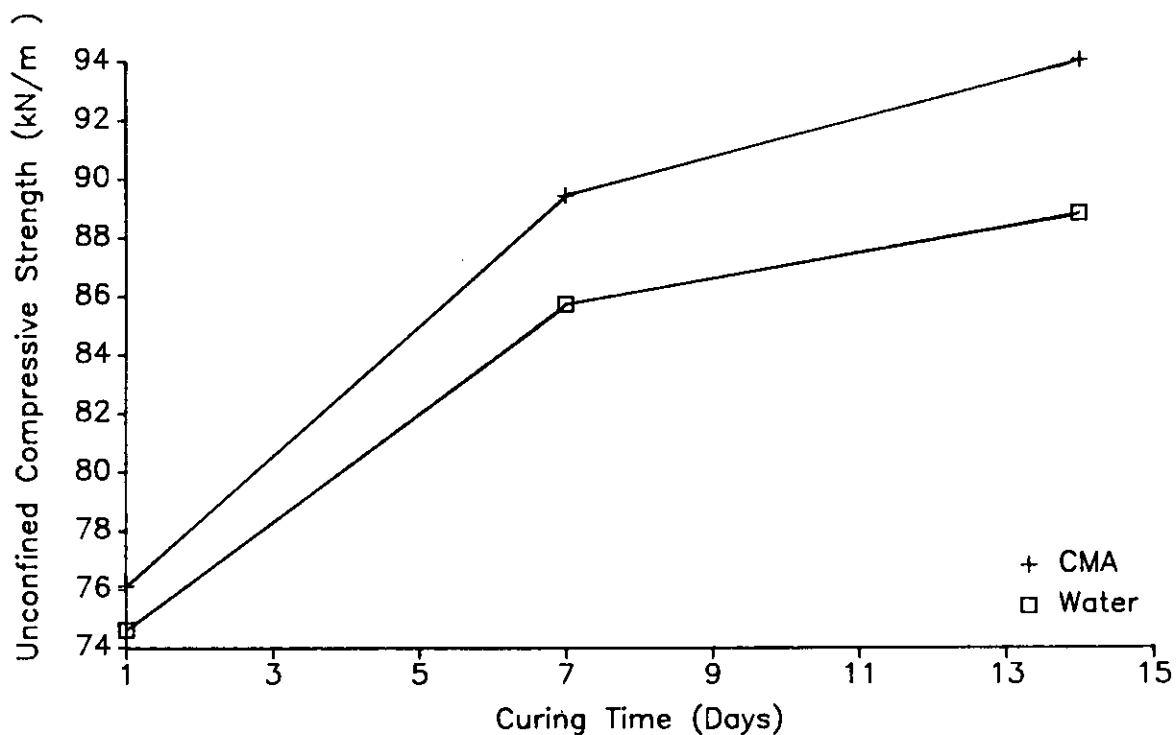


Figure D-1. Unconfined Compression Strength Versus Curing Time in Puyallup Soil

Table D-7. Summary of Physical Soil Data From Pack Forest Field Test Plots

| Plot No. | Treatment   | Depth (cm) | Natural Moisture <sup>a</sup> | Liquid Limit (%) | Plastic Limit (%) | Plasticity Index (%) |
|----------|-------------|------------|-------------------------------|------------------|-------------------|----------------------|
| 1        | CMA-Spray   | 5          | 22.1                          | N.T.             | N.T.              | N.T.                 |
|          |             | 30         | 23.8                          | N.T.             | N.T.              | N.T.                 |
| 4        | Water-Spray | 14         | 22.0                          | 25               | 18                | 7                    |
| 5        | CMA-Flood   | 20         | 26.6                          | N.T.             | N.T.              | N.T.                 |
|          |             | 40         | 25.1                          | N.T.             | N.T.              | N.T.                 |
| 6        | Water-Flood | 20         | 23.7                          | 25               | 17                | 8                    |
|          |             | 34-58      | 23.9                          | N.T.             | N.T.              | N.T.                 |
| 8        | CMA-Flood   | 24-36      | 22.2                          | 25               | 16                | 9                    |

<sup>a</sup>Samples were taken the last week of March, 1986.

N.T.--Not tested.

Table D-8. Results of Shear Vane Tests on Field Plots

| <u>Plot No.</u> | <u>Treatment</u> | <u>Depth<br/>(cm)</u> | <u>Torque<br/>(in-lb)</u> | <u>Shear Stress<br/>(kN/m<sup>2</sup>)</u> |
|-----------------|------------------|-----------------------|---------------------------|--|
| 1               | CMA-Spray        | 30                    | 345                       | 76.5                                       |
|                 |                  | 60                    | 375                       | 83.1                                       |
| 2               | Water-Spray      | 30                    | 360                       | 80.0                                       |
| 3               | CMA-Spray        | 30                    | 375                       | 83.1                                       |
| 4               | Water-Spray      | 30                    | 400                       | 88.6                                       |
|                 |                  | 60                    | 430                       | 95.3                                       |
| 5               | CMA-Flood        | 30                    | 350                       | 77.6                                       |
|                 |                  | 60                    | 375                       | 83.1                                       |

increases (decreases) in the liquid limit of a fine-grained soil with a specific fluid demonstrate that the repulsive forces between particles will increase (decrease), resulting in a dispersed (flocculated) structure (3). The minor differences in the shear strength index and vane shear tests also tend to indicate that CMA would have a minor effect on the mechanical behavior of these soils. This conclusion is borne out by the fact that the plasticity characteristics are negligibly changed with CMA application.

The decreases of permeability with time, seen during the 20 C tests on all soil types, are probably explained by the growth of microorganisms in the soils. Prolonged performance of permeability tests has been seen to result in a substantial reduction in hydraulic conductivity due to clogging of the flow channels by organic matter that grows in the soil during the test [4]. Workers have reported on tests in which a variety of disinfectants were added to the permeating water to stop such organic growth [e.g., 5]. It was found that in sterile samples there was an insignificant decrease in permeability with time. It was also found that the long-term conductivities of sterile soils were 8 to 50 times the values in unsterile soil. However, since organic growth could be expected in real-life field conditions, the soil samples used for this testing program were not sterilized in order to simulate those natural conditions.

There was insignificant change of permeability with time during the 2 C tests, probably due to low biological growth rates at this temperature. It is believed that the tests conducted at 2 C represent



conductivities that are affected minimally by organic growth in the soil.

The hydraulic gradients used for the permeability tests (about 20) are considered to be reasonable. Tests run at excessively high hydraulic gradients ( $> 100$ ) show that permeability can decrease, apparently as a result of particle migration causing clogging [6,7]. Such clogging does not appear to have happened during this testing program, due to the low gradients that were used.

The permeabilities calculated for tests conducted at 2 C were corrected for the effect of temperature on the density and viscosity of water and normalized to 20 C. A study measuring the effect of temperature on conductivity of three fine-grained soils found that simple viscosity and density adjustments are generally adequate for taking into account the effects of temperature [4]. It should be noted that the viscosities of the CMA solutions were not measured and were assumed to be similar to that of water.

Higher permeabilities occurred to some degree in all four soil samples when permeated with 5000 ppm CMA solution. It is believed that this increase is probably due to the reaction of  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  from the CMA solution with the exchangeable bases and organics of these soils. The Vantage soil, which showed a permeability increase at a concentration as low as 50 ppm, contained calcium in the form of  $\text{CaCO}_3$  (1.25% by weight). Since these soils contain relatively low percentages of clay, the observed increases in permeability were small except for the Vantage soil. Even in this case, the prevailing rates of about  $10^{-5}$

cm/s will still be restrictive to rapid passage of CMA solution through soils.

The cation exchange capacity of the soils is considered to be relatively low, which correlates with a low clay content. However these soils do contain relatively large amounts of major exchangeable bases, specifically,  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$ . These ions are attached to the clay and organic constituents of the soils and can be exchanged with each other and with other positively charged ions in the soil and permeant [8]. These exchanges can create changes in hydraulic conductivity caused by differences in the structure of the compacted soil. These structural changes are initiated by changes in the surface forces of interaction, due to variation in the attractive and repulsive forces between clay minerals [3]. The system variables that control these forces are defined by the development of the Gouy-Chapman theory [9] of the double diffuse layer around clay minerals. The model predicts that as the electrolyte concentration in the pore fluid increases, the thickness of the double layer tends to decrease. A tendency towards a more flocculated structure is caused by the decrease in the double-layer thickness. Therefore, an increasingly more flocculated and more permeable structure would be expected with increasing ion concentration [1]. Studies conducted with Ca and Mg sulfates and chlorides on bentonite [1] have shown this general relationship.

The low-clay soils tested with CMA in this study also exhibited an increased permeability, which can be related to a more flocculated and more permeable structure. However, the relative absence of clay reduces this effect, because the soil pores are filled with fines rather than

swollen clay particles. The free  $\text{CaCO}_3$  that exists in the Vantage soil probably has a tendency to cause an even higher ion concentration in the pore fluid, and therefore an even more flocculated and permeable structure.

## CONCLUSIONS

Soil plasticity was unaffected by CMA addition of 5000 ppm. Soil unconfined compressive strength and shear strength were not changed by CMA doses of 5000 ppm in the laboratory and 3000 ppm in the field. The permeability of a calcareous soil substantially increased after various CMA additions as low as 50 ppm, presumably because of particle flocculation by Ca and Mg, creating more pore space. In noncalcareous soils, permeability decreased over time at 20 C to lower levels in controls than in CMA-treated samples. The decrease was apparently due to clogging bacterial growths that were partially overcome by the opposing particle flocculation trend in CMA-treated soil. There was little difference in permeability between controls and CMA treatments at 2 C. None of these effects represent impacts on soil physical properties of particular concern.

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## APPENDIX E

### PHYSICOCHEMICAL REACTIONS INVOLVING CMA IN SOILS

#### INTRODUCTION

A review of literature pertaining to CMA and its component materials (see Chapter 1) raised four potential concerns relative to CMA-physicochemical activity in soils: (1) changes in soil chemical properties as a result of exposure to CMA; (2) transport through soils of major cations, volatile fatty acid salts, and trace elements present in CMA; (3) the fate of these substances, especially acetate, in soils; and (4) release of substances, especially trace metals, native to soils as a result of reactions involving CMA. These issues were studied both in the laboratory and through measurements at controlled field plots.

One laboratory experiment involved applying CMA-containing water solutions to soils in batch and incubating for two weeks, in order to determine changes in soil chemical properties over time. A second laboratory experiment was run to determine the extent of acetate and metals adsorption by soils and subsequent desorption by water flushing. Related to these experiments is the series of acetate decomposition studies that are reported in Appendix F.

Transport and fate issues were studied further through measurements taken on soil water and groundwater collected from the controlled field plots. Their methods and results are reported and discussed in Appendix C but will be also referenced in this appendix when relevant.

#### EXPERIMENTAL METHODS

##### Batch Laboratory Incubations

Batch incubations were performed using the Pack Forest, Lee Forest, Puyallup, and Vantage soils whose properties are summarized in Appendix D. These experiments consisted of duplicated additions of four CMA concentrations in water solution (12.5, 125, 1250, and 5000 mg/l) and water (controls) to the soils in extraction thimbles and incubations at 2 and 20 C for 14 days. Additions were to field moisture capacity. Soil solutions were extracted on days 0, 1, 7, and 14 for chemical analyses. Analyses were performed for pH; specific conductivity; total alkalinity; volatile fatty acid salts; metals; and, on some samples, chloride, nitrate-nitrogen, and sulfate. Analyses were according to the procedures specified in Appendix A.

##### Adsorption/Desorption Experiment

The adsorption/desorption experiment was performed by applying duplicate 30 ml volumes of CMA solutions of various concentrations (0-5000 mg/l for metals analysis; 0-500 mg/l for acetate analysis) to 10 g of soil in centrifuge tubes. A wider concentration range was applied for the metals study than for acetate because of the much greater cation than anion exchange capacity of soils. This experiment was conducted with the Lee Forest, Pack Forest, and Vantage soils, which were considered to provide an adequate range of texture and organic and calcium carbonate contents for the intended purpose. Each tube was stoppered with a freshly washed rubber cork and shaken every ten minutes for one hour. The tubes were then centrifuged at 5000 rpm for 10-to-15 minutes. The liquid in each tube was filtered using clean, 0.45 micron

Millipore filter paper, and the filtrate was then analyzed for acetate and metal concentrations.

To determine desorption, 30 ml of distilled water was added to the solids. Shaking, centrifuging, filtration, and analyses were repeated.

## RESULTS

### Batch Laboratory Incubations

Table E-1 summarizes the results of the batch laboratory incubations for metals. Among the metals, Ca, Mg, and K were considerably higher than in control samples on all days during the incubation period only with additions of > 1250 mg CMA/l to the Vantage (calcareous, low in organics) and Lee (high in organics) soils. In the other two soils the differences in these elements among treatments were pronounced only near the beginning of incubation and tended to decrease with time. Cu and Pb exhibited no patterns of differences among treatments or over time. The fact that Fe and Al were inversely related to CMA addition in the Vantage samples suggests that Ca and Mg displaced Fe and Al from that soil. This effect was weak or nonexistent in the other three soils, however. Zn and Se increased with time in both controls and CMA-treated samples; there was little difference among treatments.

In general, the trends described for the metals pertain to both 2 and 20 C incubations. However, there was a slightly greater tendency for Fe and Al displacement from the Pack Forest, Lee Forest, and Puyallup soils at 2 than at 20 C.

Table E-1. Metals Measured in Batch Soil Incubations

| Initial<br>CMA Conc.         |     |      |      |      |      |      |      |      |      |      | Initial<br>CMA Conc.        | Mean Concentration (mg/l) |      |      |      |      |      |      |      |      |     |  |
|------------------------------|-----|------|------|------|------|------|------|------|------|------|-----------------------------|---------------------------|------|------|------|------|------|------|------|------|-----|--|
| (mg/l)                       | Day | Ca   | Mg   | K    | Fe   | Cu   | Zn   | Se   | Pb   | Al   | (mg/l)                      | Day                       | Ca   | Mg   | K    | Fe   | Cu   | Zn   | Se   | Pb   | Al  |  |
| <u>Pack Forest Soil, 2 C</u> |     |      |      |      |      |      |      |      |      |      |                             |                           |      |      |      |      |      |      |      |      |     |  |
| 0                            | 0   | 4.2  | 1.7  | 5.1  | 1.52 | 0.06 | 0.11 | 0.00 | 0.00 | 2.7  | 1250                        | 0                         | 18.1 | 7.1  | 9.5  | 1.22 | 0.02 | 0.06 | 0.00 | 0.00 | 0.7 |  |
| 0                            | 1   | 8.7  | 1.3  | 4.6  | 2.61 | 0.83 | 0.44 | 0.03 | 0.23 | 4.2  | 1250                        | 1                         | 66.6 | 6.2  | 8.5  | 0.73 | 0.00 | 4.03 | 0.00 | 0.00 | 0.4 |  |
| 0                            | 7   | 3.1  | 1.1  | 3.3  | 4.71 | 0.04 | 0.12 | 0.05 | 0.06 | 8.8  | 1250                        | 7                         | 11.0 | 4.4  | 7.6  | 2.86 | 0.15 | 0.07 | 0.02 | 0.05 | 1.4 |  |
| 0                            | 14  | 3.4  | 1.2  | 4.5  | 3.21 | 0.02 | 0.29 | 0.03 | 0.01 | 5.4  | 1250                        | 14                        | 6.3  | 2.6  | 5.6  | 1.87 | 0.03 | 0.90 | 0.03 | 0.04 | 1.1 |  |
| 12.5                         | 0   | 3.8  | 1.2  | 5.3  | 2.52 | 0.01 | 0.07 | 0.00 | 0.00 | 4.5  | 5000                        | 0                         | 54.8 | 24.7 | 17.6 | 0.08 | 0.08 | 0.10 | 0.00 | 0.00 | 0.0 |  |
| 12.5                         | 1   | 2.8  | 0.9  | 3.8  | 2.85 | 0.08 | 0.02 | 0.04 | 0.03 | 5.6  | 5000                        | 1                         | 54.4 | 18.3 | 16.0 | 0.43 | 0.12 | 0.21 | 0.00 | 0.00 | 0.2 |  |
| 12.5                         | 7   | 3.5  | 1.2  | 4.0  | 4.84 | 0.04 | 0.31 | 0.08 | 0.18 | 8.6  | 5000                        | 7                         | 37.6 | 16.4 | 13.3 | 1.06 | 0.12 | 0.13 | 0.01 | 0.10 | 0.2 |  |
| 12.5                         | 14  | 3.2  | 0.9  | 3.8  | 1.94 | 0.06 | 0.41 | 0.03 | 0.04 | 2.7  | 5000                        | 14                        | 42.5 | 18.5 | 16.7 | 0.51 | 0.22 | 0.96 | 0.03 | 0.10 | 0.2 |  |
| 125                          | 0   | 3.8  | 1.4  | 4.4  | 6.84 | 0.01 | 0.05 | 0.00 | 0.00 | 14.0 | <u>Lee Forest Soil, 2 C</u> |                           |      |      |      |      |      |      |      |      |     |  |
| 125                          | 1   | 4.0  | 1.1  | 4.9  | 1.76 | 0.31 | 0.13 | 0.02 | 0.08 | 2.7  | 0                           | 0                         | 9.3  | 1.9  | 6.1  | 0.23 | 0.08 | 0.39 | 0.00 | 0.00 | 0.8 |  |
| 125                          | 7   | 3.4  | 1.1  | 3.7  | 4.30 | 0.03 | 0.21 | 0.04 | 0.04 | 7.5  | 0                           | 1                         | 8.1  | 1.8  | 3.7  | 0.21 | 0.01 | 0.21 | 0.01 | 0.02 | 0.7 |  |
| 125                          | 14  | 3.2  | 1.0  | 3.9  | 2.47 | 0.03 | 0.80 | 0.03 | 0.04 | 4.0  | 0                           | 7                         | 6.6  | 1.7  | 3.1  | 0.26 | 0.09 | 0.49 | 0.01 | 0.06 | 0.7 |  |
| 1250                         | 0   | 20.1 | 4.9  | 8.4  | 0.86 | 0.02 | 0.00 | 0.00 | 1.70 |      | 0                           | 14                        | 5.6  | 1.4  | 3.3  | 0.36 | 0.22 | 0.35 | 0.03 | 0.11 | 0.8 |  |
| 1250                         | 1   | 12.0 | 3.3  | 7.5  | 0.52 | 0.02 | 0.08 | 0.02 | 0.05 | 0.8  | 12.5                        | 0                         | 8.6  | 1.8  | 4.2  | 0.17 | 0.00 | 0.06 | 0.00 | 0.00 | 0.8 |  |
| 1250                         | 7   | 10.8 | 3.8  | 7.2  | 1.44 | 0.02 | 0.24 | 0.02 | 0.02 | 2.5  | 12.5                        | 1                         | 7.9  | 1.6  | 3.5  | 0.19 | 0.06 | 0.18 | 0.01 | 0.04 | 0.8 |  |
| 1250                         | 14  | 3.9  | 1.4  | 4.4  | 4.03 | 0.04 | 1.15 | 0.05 | 0.05 | 7.6  | 12.5                        | 7                         | 6.2  | 1.4  | 3.0  | 0.26 | 0.07 | 0.24 | 0.01 | 0.04 | 0.7 |  |
| 5000                         | 0   | 32.0 | 17.6 | 15.5 | 0.40 | 0.01 | 0.04 | 0.00 | 0.00 | 0.5  | 12.5                        | 14                        | 5.2  | 1.2  | 3.0  | 0.49 | 0.04 | 0.43 | 0.03 | 0.05 | 0.7 |  |
| 5000                         | 1   | 29.8 | 15.3 | 13.7 | 0.46 | 0.22 | 0.21 | 0.02 | 0.08 | 0.8  | 125                         | 0                         | 10.2 | 2.3  | 4.7  | 0.32 | 0.00 | 0.12 | 0.00 | 0.00 | 1.1 |  |
| 5000                         | 7   | 30.7 | 16.8 | 14.8 | 0.43 | 0.20 | 0.18 | 0.01 | 0.13 | 0.6  | 125                         | 1                         | 7.7  | 1.8  | 3.6  | 0.32 | 0.12 | 0.13 | 0.01 | 0.08 | 0.8 |  |
| 5000                         | 14  | 32.9 | 18.0 | 15.0 | 0.66 | 0.06 | 1.10 | 0.03 | 0.03 | 1.2  | 125                         | 7                         | 7.3  | 1.7  | 3.4  | 0.32 | 0.08 | 0.09 | 0.01 | 0.06 | 0.7 |  |
| <u>Vantage Soil, 2 C</u>     |     |      |      |      |      |      |      |      |      |      | 125                         | 14                        | 6.8  | 1.7  | 3.4  | 0.32 | 0.17 | 0.75 | 0.03 | 0.05 | 0.8 |  |
| 0                            | 0   | 3.3  | 1.3  | 4.1  | 2.75 | 0.02 | 0.10 | 0.00 | 0.00 | 1.2  | 1250                        | 0                         | 19.6 | 5.7  | 6.8  | 0.50 | 0.01 | 0.18 | 0.00 | 0.00 | 0.9 |  |
| 0                            | 1   | 3.7  | 1.9  | 4.6  | 5.77 | 0.03 | 0.17 | 0.04 | 0.05 | 3.8  | 1250                        | 1                         | 31.8 | 5.6  | 6.2  | 0.18 | 0.01 | 0.78 | 0.02 | 0.05 | 0.8 |  |
| 0                            | 7   | 3.6  | 1.9  | 4.3  | 4.85 | 0.10 | 0.15 | 0.02 | 0.05 | 3.0  | 1250                        | 7                         | 12.8 | 4.7  | 5.0  | 0.26 | 0.10 | 0.17 | 0.01 | 0.06 | 0.8 |  |
| 0                            | 14  | 3.5  | 1.6  | 4.0  | 2.50 | 0.02 | 0.38 | 0.03 | 0.04 | 1.4  | 1250                        | 14                        | 10.5 | 2.1  | 6.8  | 0.29 | 0.20 | 1.02 | 0.03 | 0.08 | 0.2 |  |
| 12.5                         | 0   | 4.1  | 1.8  | 5.7  | 4.49 | 0.02 | 0.12 | 0.00 | 0.00 | 2.6  | 5000                        | 0                         | 45.0 | 25.5 | 15.7 | 0.19 | 0.04 | 0.45 | 0.00 | 0.00 | 1.0 |  |
| 12.5                         | 1   | 3.3  | 1.7  | 4.2  | 5.29 | 0.51 | 0.18 | 0.04 | 0.19 | 3.4  | 5000                        | 1                         | 34.6 | 23.4 | 14.8 | 0.21 | 0.07 | 0.18 | 0.02 | 0.05 | 1.0 |  |
| 12.5                         | 7   | 3.7  | 2.0  | 4.4  | 6.45 | 0.09 | 0.20 | 0.04 | 0.05 | 4.2  | 5000                        | 7                         | 31.7 | 20.9 | 13.0 | 0.31 | 0.02 | 0.48 | 0.02 | 0.03 | 1.0 |  |
| 12.5                         | 14  | 3.4  | 1.6  | 4.3  | 3.93 | 0.02 | 0.40 | 0.03 | 0.05 | 2.4  | 5000                        | 14                        | 30.3 | 21.1 | 13.7 | 0.22 | 0.02 | 1.03 | 0.04 | 0.05 | 1.0 |  |
| 125                          | 0   | 3.9  | 1.6  | 4.6  | 1.88 | 0.10 | 0.06 | 0.00 | 0.00 | 1.1  | <u>Puyallup Soil, 2 C</u>   |                           |      |      |      |      |      |      |      |      |     |  |
| 125                          | 1   | 3.5  | 1.6  | 4.3  | 3.32 | 0.12 | 0.03 | 0.03 | 0.08 | 2.0  | 0                           | 0                         | 6.3  | 1.2  | 5.6  | 0.18 | 0.01 | 0.15 | 0.00 | 0.00 | 0.2 |  |
| 125                          | 7   | 4.3  | 2.3  | 4.9  | 6.27 | 0.04 | 0.20 | 0.03 | 0.03 | 4.0  | 0                           | 1                         | 5.8  | 0.9  | 4.8  | 0.18 | 0.04 | 0.05 | 0.01 | 0.04 | 0.2 |  |
| 125                          | 14  | 4.2  | 1.9  | 4.6  | 2.86 | 0.09 | 0.84 | 0.03 | 0.06 | 1.6  | 0                           | 7                         | 5.7  | 0.9  | 5.0  | 0.38 | 0.15 | 0.34 | 0.01 | 0.09 | 0.3 |  |
|                              |     |      |      |      |      |      |      |      |      |      | 0                           | 14                        | 7.2  | 0.9  | 4.1  | 0.29 | 0.02 | 0.40 | 0.04 | 0.04 | 0.2 |  |

Continued

Continued

Continued

Table E-1 Continued

| Initial<br>CMA Conc.<br>(mg/l) | Day | Ca   | Mg   | K    | Fe   | Cu   | Zn   | Se   | Pb   | Al   |
|--------------------------------|-----|------|------|------|------|------|------|------|------|------|
| 12.5                           | 0   | 19.3 | 2.6  | 8.0  | 0.15 | 0.01 | 0.14 | 0.00 | 0.00 | 0.1  |
| 12.5                           | 1   | 36.5 | 2.5  | 4.1  | 0.36 | 0.13 | 0.17 | 0.01 | 0.16 | 0.2  |
| 12.5                           | 7   | 5.3  | 0.8  | 4.5  | 0.36 | 0.03 | 0.10 | 0.01 | 0.02 | 0.2  |
| 12.5                           | 14  | 6.0  | 0.8  | 4.7  | 0.38 | 0.30 | 0.49 | 0.03 | 0.09 | 0.3  |
| 125                            | 0   | 10.3 | 1.5  | 6.6  | 0.21 | 0.01 | 0.06 | 0.00 | 0.00 | 0.1  |
| 125                            | 1   | 6.8  | 1.0  | 5.3  | 0.42 | 0.08 | 0.08 | 0.01 | 0.04 | 0.2  |
| 125                            | 7   | 6.2  | 0.9  | 4.5  | 0.31 | 0.04 | 0.27 | 0.02 | 0.04 | 0.3  |
| 125                            | 14  | 6.8  | 1.0  | 5.1  | 0.29 | 0.02 | 0.71 | 0.04 | 0.05 | 0.3  |
| 1250                           | 0   | 16.8 | 3.2  | 8.1  | 0.22 | 0.00 | 0.07 | 0.00 | 0.00 | 0.1  |
| 1250                           | 1   | 15.6 | 3.2  | 7.2  | 0.14 | 0.13 | 0.13 | 0.01 | 0.06 | 0.2  |
| 1250                           | 7   | 16.6 | 3.3  | 7.7  | 0.48 | 0.10 | 0.08 | 0.02 | 0.06 | 0.4  |
| 1250                           | 14  | NA   |      |      |      |      |      |      |      |      |
| 5000                           | 0   | 51.8 | 18.1 | 18.8 | 0.46 | 0.04 | 0.07 | 0.00 | 0.00 | 0.5  |
| 5000                           | 1   | 49.6 | 18.8 | 18.0 | 0.29 | 0.30 | 0.12 | 0.01 | 0.18 | 0.2  |
| 5000                           | 7   | 45.4 | 16.4 | 15.9 | 0.14 | 0.02 | 0.28 | 0.03 | 0.02 | 0.2  |
| 5000                           | 14  | 48.1 | 17.1 | 16.0 | 0.16 | 0.02 | 1.25 | 0.04 | 0.04 | 0.2  |
| <u>Pack Forest Soil, 20 C</u>  |     |      |      |      |      |      |      |      |      |      |
| 0                              | 0   | 4.2  | 1.7  | 5.1  | 1.52 | 0.06 | 0.11 | 0.00 | 0.00 | 2.7  |
| 0                              | 1   | 3.0  | 0.9  | 3.8  | 2.21 | 0.04 | 0.09 | 0.02 | 0.03 | 3.2  |
| 0                              | 7   | 2.4  | 0.9  | 2.8  | 3.56 | 0.09 | 0.22 | 0.03 | 0.05 | 6.2  |
| 0                              | 14  | 2.3  | 0.8  | 2.7  | 3.71 | 0.03 | 1.38 | 0.06 | 0.06 | 6.4  |
| 12.5                           | 0   | 3.8  | 1.2  | 5.3  | 2.52 | 0.01 | 0.07 | 0.00 | 0.00 | 4.5  |
| 12.5                           | 1   | 3.3  | 1.4  | 3.8  | 2.60 | 0.22 | 0.14 | 0.01 | 0.08 | 1.4  |
| 12.5                           | 7   | 3.0  | 0.9  | 3.9  | 4.14 | 0.25 | 0.24 | 0.04 | 0.08 | 7.3  |
| 12.5                           | 14  | 2.4  | 0.7  | 2.7  | 2.83 | 0.02 | 1.25 | 0.05 | 0.05 | 4.2  |
| 125                            | 0   | 3.8  | 1.4  | 4.4  | 6.84 | 0.01 | 0.05 | 0.00 | 0.00 | 14.0 |
| 125                            | 1   | 3.6  | 1.4  | 4.2  | 2.44 | 0.10 | 0.09 | 0.02 | 0.05 | 2.8  |
| 125                            | 7   | 2.8  | 0.8  | 3.7  | 1.68 | 0.29 | 0.10 | 0.01 | 0.09 | 2.1  |
| 125                            | 14  | 8.9  | 0.9  | 3.2  | 4.05 | 0.03 | 1.92 | 0.06 | 0.06 | 7.6  |
| 1250                           | 0   | 20.1 | 4.9  | 8.4  | 0.86 | 0.02 | 0.04 | 0.00 | 0.00 | 12.7 |
| 1250                           | 1   | 12.6 | 3.8  | 7.7  | 0.64 | 0.02 | 0.35 | 0.01 | 0.02 | 1.0  |
| 1250                           | 7   | 4.5  | 1.2  | 4.0  | 4.29 | 0.03 | 0.27 | 0.03 | 0.05 | 6.5  |
| 1250                           | 14  | 2.3  | 0.9  | 2.8  | 4.25 | 0.02 | 1.75 | 0.07 | 0.05 | 7.7  |

Continued

| Initial                      | Mean Concentration (mg/l) |      |      |      |      |      |      |      |      |     |  |
|------------------------------|---------------------------|------|------|------|------|------|------|------|------|-----|--|
| CMA Conc.                    | Day                       | Ca   | Mg   | K    | Fe   | Cu   | Zn   | Se   | Pb   | Al  |  |
| (mg/l)                       |                           |      |      |      |      |      |      |      |      |     |  |
| 5000                         | 5                         | 32.0 | 17.6 | 15.5 | 0.40 | 0.01 | 0.04 | 0.00 | 0.00 | 0.5 |  |
| 5000                         | 5                         | 33.0 | 18.0 | 15.9 | 0.54 | 0.02 | 0.19 | 0.02 | 0.02 | 0.9 |  |
| 5000                         | 7                         | 5.0  | 2.8  | 5.5  | 3.67 | 0.16 | 0.13 | 0.02 | 0.06 | 4.0 |  |
| 5000                         | 14                        | NA   |      |      |      |      |      |      |      |     |  |
| <u>Vantage Soil, 20 C</u>    |                           |      |      |      |      |      |      |      |      |     |  |
| 0                            | 0                         | 3.3  | 1.3  | 4.1  | 2.75 | 0.02 | 0.10 | 0.00 | 0.00 | 1.2 |  |
| 0                            | 1                         | 3.1  | 1.4  | 3.7  | 2.05 | 0.03 | 0.04 | 0.01 | 0.02 | 1.4 |  |
| 0                            | 7                         | 3.0  | 1.6  | 3.5  | 3.93 | 0.05 | 0.08 | 0.02 | 0.04 | 2.2 |  |
| 0                            | 14                        | 2.5  | 1.3  | 3.1  | 3.64 | 0.01 | 0.98 | 0.05 | 0.05 | 2.3 |  |
| 12.5                         | 0                         | 4.1  | 1.8  | 5.7  | 4.49 | 0.02 | 0.12 | 0.00 | 0.00 | 2.6 |  |
| 12.5                         | 1                         | NA   |      |      |      |      |      |      |      |     |  |
| 12.5                         | 7                         | 2.9  | 1.6  | 3.6  | 5.36 | 0.11 | 0.30 | 0.03 | 0.07 | 3.0 |  |
| 12.5                         | 14                        | 2.5  | 1.4  | 3.3  | 4.23 | 0.02 | 1.31 | 0.05 | 0.06 | 2.7 |  |
| 125                          | 0                         | 3.9  | 1.6  | 4.6  | 1.88 | 0.10 | 0.06 | 0.00 | 0.00 | 1.1 |  |
| 125                          | 1                         | 4.2  | 1.9  | 4.6  | 2.97 | 0.03 | 0.12 | 0.01 | 0.03 | 1.5 |  |
| 125                          | 7                         | 3.3  | 1.6  | 4.9  | 3.68 | 0.06 | 0.05 | 0.02 | 0.04 | 2.0 |  |
| 125                          | 14                        | 2.1  | 1.1  | 3.1  | 3.30 | 0.01 | 1.31 | 0.05 | 0.05 | 2.1 |  |
| 1250                         | 0                         | 18.1 | 7.1  | 9.5  | 1.22 | 0.02 | 0.06 | 0.00 | 0.00 | 0.7 |  |
| 1250                         | 1                         | 12.2 | 4.7  | 7.8  | 1.10 | 0.03 | 0.16 | 0.01 | 0.02 | 0.6 |  |
| 1250                         | 7                         | 5.2  | 2.2  | 4.7  | 3.03 | 0.02 | 0.24 | 0.02 | 0.03 | 1.7 |  |
| 1250                         | 14                        | 3.9  | 1.9  | 4.0  | 3.36 | 0.01 | 1.58 | 0.06 | 0.05 | 2.0 |  |
| 5000                         | 0                         | 54.8 | 24.7 | 17.6 | 0.08 | 0.08 | 0.10 | 0.00 | 0.00 | 0.0 |  |
| 5000                         | 1                         | 44.0 | 18.7 | 15.7 | 0.33 | 0.01 | 0.21 | 0.01 | 0.01 | 0.2 |  |
| 5000                         | 7                         | 37.5 | 16.2 | 14.6 | 0.63 | 0.19 | 0.13 | 0.02 | 0.07 | 0.3 |  |
| 5000                         | 14                        | NA   |      |      |      |      |      |      |      |     |  |
| <u>Lee Forest Soil, 20 C</u> |                           |      |      |      |      |      |      |      |      |     |  |
| 0                            | 0                         | 9.3  | 1.9  | 6.1  | 0.23 | 0.08 | 0.39 | 0.00 | 0.00 | 0.8 |  |
| 0                            | 1                         | 3.6  | 0.8  | 2.0  | 0.62 | 0.02 | 0.09 | 0.01 | 0.03 | 0.4 |  |
| 0                            | 7                         | 4.1  | 1.0  | 3.1  | 0.25 | 0.02 | 0.14 | 0.00 | 0.03 | 0.4 |  |
| 0                            | 14                        | 2.7  | 0.7  | 2.9  | 0.31 | 0.01 | 1.00 | 0.04 | 0.04 | 0.4 |  |
| 12.5                         | 0                         | 35.7 | 2.3  | 5.4  | 0.19 | 0.00 | 0.96 | 0.00 | 0.00 | 0.9 |  |
| 12.5                         | 1                         | 6.7  | 1.5  | 3.4  | 0.21 | 0.01 | 0.07 | 0.02 | 0.03 | 0.7 |  |
| 12.5                         | 7                         | 3.8  | 0.8  | 2.9  | 0.26 | 0.01 | 0.05 | 0.01 | 0.02 | 0.4 |  |
| 12.5                         | 14                        | 2.8  | 0.6  | 2.8  | 0.36 | 0.01 | 1.12 | 0.04 | 0.05 | 0.4 |  |

**Continued**

Table E-1 Continued

| Initial   |        | Mean Concentration (mg/l) |      |      |      |      |      |      |      |     |    |
|-----------|--------|---------------------------|------|------|------|------|------|------|------|-----|----|
| CMA Conc. | (mg/l) | Day                       | Ca   | Mg   | K    | Fe   | Cu   | Zn   | Se   | Pb  | Al |
| 125       | 0      | 10.2                      | 2.3  | 4.7  | 0.32 | 0.00 | 0.12 | 0.00 | 0.00 | 1.0 |    |
| 125       | 1      | 8.0                       | 1.9  | 3.8  | 0.39 | 0.15 | 0.07 | 0.02 | 0.11 | 0.8 |    |
| 125       | 7      | 3.2                       | 0.7  | 2.7  | 0.24 | 0.03 | 0.07 | 0.01 | 0.03 | 0.4 |    |
| 125       | 14     | 4.3                       | 0.7  | 2.8  | 0.33 | 0.01 | 1.40 | 0.04 | 0.05 | 0.4 |    |
| 1250      | 0      | 19.6                      | 5.7  | 6.8  | 0.50 | 0.01 | 0.18 | 0.00 | 0.00 | 0.9 |    |
| 1250      | 1      | 13.9                      | 4.7  | 5.3  | 0.27 | 0.01 | 0.22 | 0.02 | 0.03 | 0.8 |    |
| 1250      | 7      | 3.6                       | 1.3  | 3.5  | 0.44 | 0.22 | 0.11 | 0.01 | 0.10 | 0.5 |    |
| 1250      | 14     | 2.3                       | 0.8  | 3.1  | 0.50 | 0.01 | 1.48 | 0.05 | 0.05 | 0.6 |    |
| 5000      | 0      | 45.0                      | 25.5 | 15.7 | 0.19 | 0.04 | 0.95 | 0.00 | 0.00 | 1.0 |    |
| 5000      | 1      | 32.5                      | 21.0 | 13.9 | 0.20 | 0.01 | 0.17 | 0.03 | 0.03 | 0.9 |    |
| 5000      | 7      | 5.9                       | 5.1  | 5.7  | 0.20 | 0.02 | 0.19 | 0.02 | 0.03 | 0.4 |    |
| 5000      | 14     | NA                        |      |      |      |      |      |      |      |     |    |

Puyallup Soil, 20 C

|      |    |      |      |      |      |      |      |      |      |     |  |
|------|----|------|------|------|------|------|------|------|------|-----|--|
| 0    | 0  | 6.3  | 1.2  | 5.6  | 0.18 | 0.01 | 0.15 | 0.00 | 0.00 | 0.2 |  |
| 0    | 1  | 5.9  | 0.8  | 4.7  | 0.44 | 0.10 | 0.08 | 0.01 | 0.05 | 0.3 |  |
| 0    | 7  | 4.6  | 0.7  | 4.5  | 0.26 | 0.01 | 0.06 | 0.01 | 0.02 | 0.2 |  |
| 0    | 14 | 3.8  | 0.6  | 4.4  | 0.46 | 0.02 | 1.26 | 0.05 | 0.05 | 0.3 |  |
| 12.5 | 0  | 19.3 | 2.6  | 8.0  | 0.15 | 0.01 | 0.14 | 0.00 | 0.00 | 0.1 |  |
| 12.5 | 1  | 6.3  | 0.9  | 5.0  | 0.23 | 0.11 | 0.08 | 0.01 | 0.05 | 0.3 |  |
| 12.5 | 7  | 3.6  | 0.7  | 3.9  | 1.30 | 0.02 | 0.05 | 0.02 | 0.05 | 1.8 |  |
| 12.5 | 14 | 5.1  | 0.7  | 4.7  | 0.32 | 0.01 | 1.25 | 0.04 | 0.06 | 0.2 |  |
| 125  | 0  | 10.3 | 1.5  | 6.6  | 0.21 | 0.01 | 0.06 | 0.00 | 0.00 | 0.1 |  |
| 125  | 1  | 7.4  | 1.1  | 5.6  | 0.38 | 0.16 | 0.30 | 0.02 | 0.07 | 0.3 |  |
| 125  | 7  | 5.6  | 0.8  | 5.0  | 0.32 | 0.06 | 0.13 | 0.01 | 0.06 | 0.2 |  |
| 125  | 14 | 6.6  | 1.1  | 4.8  | 0.96 | 0.02 | 1.21 | 0.04 | 0.09 | 0.5 |  |
| 1250 | 0  | 16.8 | 3.2  | 8.1  | 0.22 | 0.00 | 0.07 | 0.00 | 0.00 | 0.1 |  |
| 1250 | 1  | 25.3 | 4.2  | 8.9  | 0.39 | 0.02 | 0.37 | 0.02 | 0.03 | 0.6 |  |
| 1250 | 7  | 4.9  | 1.1  | 4.9  | 0.26 | 0.03 | 0.06 | 0.01 | 0.04 | 0.2 |  |
| 1250 | 14 | 5.6  | 1.1  | 4.9  | 0.39 | 0.01 | 1.70 | 0.04 | 0.05 | 0.2 |  |
| 5000 | 0  | 51.8 | 18.1 | 18.8 | 0.46 | 0.04 | 0.07 | 0.00 | 0.00 | 0.5 |  |
| 5000 | 1  | 36.3 | 13.8 | 12.4 | 0.16 | 0.02 | 0.24 | 0.01 | 0.02 | 0.2 |  |
| 5000 | 7  | 5.0  | 1.8  | 6.3  | 0.38 | 0.31 | 0.08 | 0.01 | 0.07 | 0.2 |  |
| 5000 | 14 | NA   |      |      |      |      |      |      |      |     |  |

NA--Not available

Table E-2 summarizes the results of the batch laboratory incubations for constituents other than metals. CMA additions of 12.5 and 125 mg/l initially (day 0) raised pH, alkalinity, and conductivity slightly. Higher concentrations raised alkalinity and conductivity substantially but did not have a noticeable further effect on pH. By day 7 there were definite declines from day 0 levels in alkalinity and conductivity in all treatments at 20 C (e.g., alkalinity decreased by 86 percent in the 1250 mg CMA/l Pack Forest soil treatment). This trend was even visible at day 1 and continued through day 14. It was evident in all soils but was slightly more pronounced in the three noncalcareous soils. The alkalinity and conductivity decreases were much smaller with 2 C incubation (e.g., alkalinity declined by 23 percent in the 1250 mg CMA/l Pack Forest soil treatment by day 7 at 2 C). These changes were accompanied by a slow rise in pH at 20 C, whereas pH was quite stable over time at 2 C.

Among the volatile fatty acid salts, only acetate was detected in substantial concentrations in the extracts. Some acetate values were unreliable as a consequence of insufficient preservative acid addition to overcome the high buffering capacity of the solutions. As a result, biodegradation was possible during sample storage, and acetate was lost before measurement. These occurrences were evident and were removed from consideration before the acetate data were interpreted.

As an illustration of acetate decline during the 14-day experiment, Table E-3 presents a comparison of acetate decomposition in the Pack Forest soil at the two temperatures. Decomposition was calculated as the difference between day 0 and day 1, 7, or 14 acetate



Table E-2. Seven Constituents  
Measured in Batch Soil Incubations

| Initial<br>CMA Conc.<br>(mg/l) | Day | Mean<br>Spec. Cond.<br>pH | Mean<br>Cond. Acetate<br>(μmho/cm) | Mean<br>Cl<br>(mg/l) | NO <sub>3</sub> -N<br>(mg/l) | SO <sub>4</sub><br>(mg/l) | Alkalinity<br>(mg/l) |    |
|--------------------------------|-----|---------------------------|------------------------------------|----------------------|------------------------------|---------------------------|----------------------|----|
| <u>Pack Forest Soil, 2 C</u>   |     |                           |                                    |                      |                              |                           |                      |    |
| 0                              | 0   | 4.6                       | 67                                 | 9.5                  | 2.77                         | 10.2                      | 5.68                 | 29 |
| 0                              | 1   | 5.6                       | 60                                 | 9.5                  |                              |                           |                      | 29 |
| 0                              | 7   | 4.9                       | 50                                 | 5.0                  | 1.64                         | 5.88                      | 0.00                 | 30 |
| 0                              | 14  | 6.2                       | 47                                 | 6.5                  | 2.00                         | 6.48                      | 6.29                 | 29 |
| 12.5                           | 0   | 5.4                       | 67                                 | 3.5                  | 2.50                         | 13.8                      | 5.44                 | 33 |
| 12.5                           | 1   | 5.6                       | 57                                 | 12.8                 |                              |                           |                      | 29 |
| 12.5                           | 7   | 5.7                       | 48                                 | 6.5                  |                              |                           |                      | 29 |
| 12.5                           | 14  | 6.2                       | 48                                 | 21.5                 | 2.99                         | 6.24                      | 0.00                 | 29 |
| 125                            | 0   | 5.5                       | 63                                 | 16.0                 |                              |                           |                      | 29 |
| 125                            | 1   | 5.6                       | 66                                 | 18.9                 |                              |                           |                      | 29 |
| 125                            | 7   | 5.7                       | 49                                 | 0.5                  | 1.11                         | 4.87                      | 3.25                 | 29 |
| 125                            | 14  | 6.1                       | 51                                 | 7.5                  | 3.32                         | 38.3                      | 9.38                 | 29 |
| 1250                           | 0   | 5.3                       | 134                                | 91.1                 | 1.62                         | 6.59                      | 0.00                 | 29 |
| 1250                           | 1   | 5.4                       | 104                                | 47.8                 |                              |                           |                      | 29 |
| 1250                           | 7   | 5.3                       | 96                                 | 83.0                 | 1.47                         | 8.20                      | 3.70                 | 29 |
| 1250                           | 14  | 6.0                       | 62                                 | 21.2                 | 2.50                         | 9.73                      | 7.82                 | 29 |
| 5000                           | 0   | 5.3                       | 307                                | 270.0                | 1.48                         | 9.57                      | 8.85                 | 29 |
| 5000                           | 1   | 5.3                       | 267                                | 119.3                |                              |                           |                      | 29 |
| 5000                           | 7   | 5.3                       | 255                                | 140.0                | 1.21                         | 7.95                      | 0.00                 | 29 |
| 5000                           | 14  | 5.8                       | 325                                | 273.5                | 1.51                         | 9.63                      | 9.35                 | 29 |
| <u>Vantage Soil, 2 C</u>       |     |                           |                                    |                      |                              |                           |                      |    |
| 0                              | 0   | 6.5                       | 47                                 | 2.0                  | 0.60                         | 4.05                      | 2.03                 | 22 |
| 0                              | 1   | 6.9                       | 40                                 | 9.0                  |                              |                           |                      | 22 |
| 0                              | 7   | 6.6                       | 39                                 | 9.0                  |                              |                           |                      | 22 |
| 0                              | 14  | 7.0                       | 39                                 | 0.0                  | 0.00                         | 0.00                      | 0.00                 | 22 |
| 12.5                           | 0   | 6.7                       | 46                                 | 1.5                  | 1.20                         | 1.57                      | 2.60                 | 22 |
| 12.5                           | 1   | 7.0                       | 40                                 | 9.6                  |                              |                           |                      | 22 |
| 12.5                           | 7   | 6.8                       | 36                                 | 4.5                  | 0.46                         | 2.57                      | 0.00                 | 22 |
| 12.5                           | 14  | 7.1                       | 39                                 | 7.5                  |                              |                           |                      | 22 |
| 125                            | 0   | 6.8                       | 47                                 | 15.5                 | 0.00                         | 0.00                      | 0.00                 | 22 |
| 125                            | 1   | 6.9                       | 43                                 | 11.9                 |                              |                           |                      | 22 |
| 125                            | 7   | 6.8                       | 46                                 | 39.5                 | 0.60                         | 1.50                      | 2.70                 | 22 |
| 125                            | 14  | 7.1                       | 46                                 | 7.0                  | 0.44                         | 3.60                      | 0.00                 | 22 |

| Initial<br>CMA Conc.<br>(mg/l) | Day | Mean Spec. Cond.<br>pH<br>(μmho/cm) | Mean<br>Acetate<br>(mg/l) | Cl<br>(mg/l) | NO <sub>3</sub> -N<br>(mg/l) | SO <sub>4</sub><br>(mg/l) | Alkalinity<br>(mg/l) |
|--------------------------------|-----|-------------------------------------|---------------------------|--------------|------------------------------|---------------------------|----------------------|
| <u>Lee Forest Soil, 2 C</u>    |     |                                     |                           |              |                              |                           |                      |
| 1250                           | 0   | 6.9                                 | 158                       | 73.6         |                              |                           | 22                   |
| 1250                           | 1   | 6.7                                 | 96                        | 217.2        |                              |                           | 22                   |
| 1250                           | 7   | 6.8                                 | 87                        | 44.8         | 0.46                         | 0.27                      | 4.22                 |
| 1250                           | 14  | 7.3                                 | 70                        | 9.6          | 0.37                         | 0.49                      | 1.83                 |
| 5000                           | 0   | 6.9                                 | 402                       | 337.3        |                              |                           | 22                   |
| 5000                           | 1   | 6.8                                 | 311                       | 233.6        |                              |                           | 22                   |
| 5000                           | 7   | 6.8                                 | 270                       | 163.9        | 0.25                         | 0.51                      | 6.12                 |
| 5000                           | 14  | 7.3                                 | 336                       | 257.4        | 0.33                         | 0.22                      | 7.28                 |
| <u>Lee Forest Soil, 2 C</u>    |     |                                     |                           |              |                              |                           |                      |
| 0                              | 0   | 4.9                                 | 134                       | 2.5          | 3.01                         | 9.91                      | 0.74                 |
| 0                              | 1   | 5.2                                 | 94                        | 13.0         |                              |                           |                      |
| 0                              | 7   | 5.2                                 | 71                        | 5.5          | 0.00                         | 0.00                      | 0.00                 |
| 0                              | 14  | 5.3                                 | 72                        | 0.0          | 1.80                         | 23.5                      | 6.27                 |
| 12.5                           | 0   | 5.1                                 | 90                        | 10.5         | 1.58                         | 11.5                      | 0.00                 |
| 12.5                           | 1   | 5.1                                 | 96                        | 15.7         |                              |                           |                      |
| 12.5                           | 7   | 4.9                                 | 80                        | 4.0          | 2.05                         | 7.56                      | 0.00                 |
| 12.5                           | 14  | 5.7                                 | 70                        | 6.0          | 2.05                         | 22.8                      | 6.82                 |
| 125                            | 0   | 5.0                                 | 116                       | 12.5         | 0.00                         | 0.00                      | 0.00                 |
| 125                            | 1   | 5.0                                 | 88                        | 16.4         |                              |                           |                      |
| 125                            | 7   | 5.3                                 | 81                        | 6.0          | 1.40                         | 6.39                      | 0.00                 |
| 125                            | 14  | 5.6                                 | 82                        | 5.0          | 1.74                         | 22.00                     | 5.65                 |
| 1250                           | 0   | 5.0                                 | 150                       | 59.6         | 2.44                         | 30.5                      | 9.69                 |
| 1250                           | 1   | 4.9                                 | 127                       | 54.2         | 1.35                         | 5.82                      | 0.00                 |
| 1250                           | 7   | 4.7                                 | 131                       | 46.5         | 2.33                         | 49.8                      | 8.12                 |
| 1250                           | 14  | NA                                  | 83                        | 18.8         | 1.70                         | 20.9                      | 0.35                 |
| 5000                           | 0   | 5.0                                 | 380                       | 213.5        |                              |                           |                      |
| 5000                           | 1   | 5.1                                 | 320                       | 279.1        |                              |                           |                      |
| 5000                           | 7   | 5.1                                 | 289                       | 268.0        | 1.35                         | 12.6                      | 0.00                 |
| 5000                           | 14  | NA                                  | NA                        | 286.1        | 1.63                         | 17.1                      | 10.3                 |
| <u>Puyallup Soil, 2 C</u>      |     |                                     |                           |              |                              |                           |                      |
| 0                              | 0   | 5.3                                 | 70                        | 1.5          | 2.81                         | 19.5                      | 4.45                 |
| 0                              | 1   | 5.6                                 | 64                        | 16.1         |                              |                           |                      |
| 0                              | 7   | 5.6                                 | 56                        | 6.0          | 0.65                         | 5.38                      | 0.00                 |
| 0                              | 14  | 6.0                                 | 58                        | 3.0          | 0.60                         | 16.6                      | 4.69                 |

Table E-2 Continued

| Initial<br>DMA Conc.<br>(mg/l) | Day | Mean<br>Spec. Cond.<br>pH | Mean<br>Cond.<br>( $\mu$ mho/cm) | Mean<br>Acetate<br>(mg/l) | Cl<br>(mg/l) | NO <sub>3</sub> -N<br>(mg/l) | SO <sub>4</sub><br>(mg/l) | Mean<br>Alkalinity<br>(mg/l) |
|--------------------------------|-----|---------------------------|----------------------------------|---------------------------|--------------|------------------------------|---------------------------|------------------------------|
| 12.5                           | 0   | 5.5                       | 84                               | 7.5                       | 1.22         | 20.7                         | 5.02                      | 30                           |
| 12.5                           | 1   | 5.5                       | 59                               | 5.0                       |              |                              |                           | 30                           |
| 12.5                           | 7   | 5.5                       | 54                               | 60.0                      | 0.88         | 21.4                         | 5.77                      | 27                           |
| 12.5                           | 14  | 5.9                       | 63                               | 0.0                       | 0.03         | 0.32                         | 0.57                      | 27                           |
| 125                            | 0   | 5.5                       | 100                              | 29.0                      | 1.02         | 175.6                        | 4.65                      | 30                           |
| 125                            | 1   | 5.4                       | 68                               | 7.0                       |              |                              |                           | 30                           |
| 125                            | 7   | 5.7                       | 58                               | 10.5                      | 0.80         | 16.2                         | 5.09                      | 32                           |
| 125                            | 14  | 6.0                       | 64                               | 0.0                       | 0.38         | 9.95                         | 3.25                      | 27                           |
| 1250                           | 0   | 5.3                       | 134                              | 127.5                     | 0.63         | 15.3                         | 5.24                      | 83                           |
| 1250                           | 1   | 5.3                       | 110                              | 17.0                      |              |                              |                           | 30                           |
| 1250                           | 7   | 5.5                       | 108                              | 130.1                     | 0.49         | 14.7                         | 5.66                      | 27                           |
| 1250                           | 14  | NA                        | NA                               | 24.4                      | 0.62         | 9.05                         | 0.00                      | 27                           |
| 5000                           | 0   | 5.3                       | 383                              | 167.1                     | 0.00         | 0.00                         | 0.00                      | 30                           |
| 5000                           | 1   | 5.4                       | 360                              | 298.3                     |              |                              |                           | 30                           |
| 5000                           | 7   | 5.4                       | 302                              | 244.1                     | 0.47         | 11.2                         | 0.00                      | 27                           |
| 5000                           | 14  | NA                        | NA                               | 240.0                     | 0.77         | 17.3                         | 11.5                      | 27                           |
| <u>Pack Forest Soil, 20 C</u>  |     |                           |                                  |                           |              |                              |                           |                              |
| 0                              | 0   | 4.6                       | 82                               | 9.5                       |              |                              |                           | 157                          |
| 0                              | 1   | 5.6                       | 47                               | 6.6                       |              |                              |                           | 29                           |
| 0                              | 7   | 4.6                       | 50                               | 0.0                       |              |                              |                           | 29                           |
| 0                              | 14  | 5.8                       | NA                               | 2.0                       | 1.74         | 7.32                         | 5.05                      | 29                           |
| 12.5                           | 0   | 5.4                       | 67                               | 3.5                       |              |                              |                           | 33                           |
| 12.5                           | 1   | 5.8                       | 50                               | 0.0                       |              |                              |                           | 29                           |
| 12.5                           | 7   | 4.8                       | 50                               | 0.0                       |              |                              |                           | 29                           |
| 12.5                           | 14  | 5.7                       | 50                               | 1.9                       | 0.00         | 0.00                         | 0.00                      | 29                           |
| 125                            | 0   | 5.5                       | 63                               | 16.0                      | 2.05         | 9.04                         | 5.09                      | 29                           |
| 125                            | 1   | 5.6                       | 54                               | 16.3                      |              |                              |                           | 29                           |
| 125                            | 7   | 5.9                       | 43                               | 0.0                       |              |                              |                           | 29                           |
| 125                            | 14  | 5.6                       | 38                               | 4.4                       | 2.83         | 11.3                         | 6.69                      | 83                           |
| 1250                           | 0   | 5.3                       | 134                              | 12.4                      |              |                              |                           | 29                           |
| 1250                           | 1   | 5.4                       | 117                              | 38.2                      |              |                              |                           | 29                           |
| 1250                           | 7   | 5.8                       | 39                               | 284.9                     |              |                              |                           | 29                           |
| 1250                           | 14  | 6.1                       |                                  | 23.4                      | 1.55         | 3.96                         | 5.46                      | 29                           |

| Initial<br>DMA Conc.<br>(mg/l) | Day | Mean<br>Spec. Cond.<br>pH | Mean<br>Cond.<br>( $\mu$ mho/cm) | Mean<br>Acetate<br>(mg/l) | Cl<br>(mg/l) | NO <sub>3</sub> -N<br>(mg/l) | SO <sub>4</sub><br>(mg/l) | Mean<br>Alkalinity<br>(mg/l) |
|--------------------------------|-----|---------------------------|----------------------------------|---------------------------|--------------|------------------------------|---------------------------|------------------------------|
| 5000                           | 5   | 5.3                       | 307                              | 270.0                     |              |                              |                           | 29                           |
| 5000                           | 5   | 5.3                       | 314                              | 168.4                     |              |                              |                           | 29                           |
| 5000                           | 7   | 6.4                       | 74                               | 4.5                       | 1.78         | 3.92                         | 11.9                      | 29                           |
| 5000                           | 14  | 6.5                       | 63                               |                           |              |                              |                           | 29                           |

Vantage Soil, 20 C

|      |    |     |     |       |      |      |      |    |
|------|----|-----|-----|-------|------|------|------|----|
| 0    | 0  | 6.5 | 47  | 2.0   | 1.89 | 1.20 | 2.73 | 22 |
| 0    | 1  | 6.8 | 36  | 0.0   |      |      |      | 22 |
| 0    | 7  | 6.6 | 29  | 0.0   |      |      |      | 22 |
| 0    | 14 | 6.6 | 29  | 0.0   | 0.38 | 5.08 | 2.81 | 22 |
| 12.5 | 0  | 6.7 | 46  | 1.5   |      |      |      | 22 |
| 12.5 | 1  | 6.9 | 36  | 13.4  |      |      |      | 22 |
| 12.5 | 7  | 6.7 | 29  | 0.0   |      |      |      | 22 |
| 12.5 | 14 | 6.8 | 29  | 0.0   | 0.30 | 2.65 | 0.00 | 62 |
| 125  | 0  | 6.8 | 47  | 15.5  |      |      |      | 22 |
| 125  | 1  | 7.0 | 43  | 15.6  |      |      |      | 22 |
| 125  | 7  | 6.9 | 35  | 3.0   |      |      |      | 22 |
| 125  | 14 | 6.6 | 31  | 0.0   | 0.25 | 2.62 | 2.09 | 61 |
| 1250 | 0  | 6.9 | 158 | 14.9  | 0.38 | 0.94 | 4.26 | 22 |
| 1250 | 1  | 6.9 | 111 | 67.5  |      |      |      | 22 |
| 1250 | 7  | 7.0 | 45  | 20.6  |      |      |      | 22 |
| 1250 | 14 | 7.2 | 47  | 0.0   | 1.21 | 31.5 | 9.80 | 22 |
| 5000 | 0  | 6.9 | 402 | 314.5 |      |      |      | 22 |
| 5000 | 1  | 6.9 | 321 | 305.5 |      |      |      | 22 |
| 5000 | 7  | 7.0 | 260 | 214.3 |      |      |      | 22 |
| 5000 | 14 | 7.4 | 208 |       |      |      |      | 22 |

Lee Forest Soil, 20 C

|   |    |     |     |      |      |      |      |    |
|---|----|-----|-----|------|------|------|------|----|
| 0 | 0  | 4.9 | 134 | 2.5  |      |      |      | 36 |
| 0 | 1  | 5.0 | 57  | 55.0 |      |      |      | 36 |
| 0 | 7  | 5.4 | 61  | 0.0  |      |      |      | 36 |
| 0 | 14 | 5.5 | 58  | 1.9  | 1.55 | 17.8 | 6.24 | 37 |

Table E-2 Continued

| Initial<br>DMA Conc.<br>(mg/l) | Day | Mean<br>Spec. Cond.<br>pH | Mean<br>Cond.<br>(µmho/cm) | Mean<br>Acetate<br>(mg/l) | Cl<br>(mg/l) | NO <sub>3</sub> -N<br>(mg/l) | SO <sub>4</sub><br>(mg/l) | Mean<br>Alkalinity<br>(mg/l) |
|--------------------------------|-----|---------------------------|----------------------------|---------------------------|--------------|------------------------------|---------------------------|------------------------------|
| 12.5                           | 0   | 5.1                       | 90                         | 10.5                      |              |                              |                           | 36                           |
| 12.5                           | 1   | 5.1                       | 101                        | 13.1                      |              |                              |                           | 36                           |
| 12.5                           | 7   | 5.3                       | 60                         | 6.1                       |              |                              |                           | 37                           |
| 12.5                           | 14  | 5.6                       | 53                         | 4.3                       | 1.47         | 16.1                         | 6.65                      | 36                           |
| 125                            | 0   | 5.0                       | 116                        | 12.5                      |              |                              |                           | 18                           |
| 125                            | 1   | 5.2                       | 83                         | 15.2                      |              |                              |                           | 36                           |
| 125                            | 7   | 5.3                       | 57                         | 0.0                       |              |                              |                           | 36                           |
| 125                            | 14  | 5.4                       | 50                         | 0.0                       | 1.68         | 19.5                         | 5.74                      | 36                           |
| 1250                           | 0   | 5.0                       | 150                        | 156.0                     |              |                              |                           | 36                           |
| 1250                           | 1   | 5.1                       | 125                        | 0.0                       |              |                              |                           | 36                           |
| 1250                           | 7   | 5.3                       | 61                         | 18.8                      |              |                              |                           | 36                           |
| 1250                           | 14  | 4.7                       | 78                         | 0.0                       | 1.45         | 19.4                         | 7.25                      | 36                           |
| 5000                           | 0   | 5.0                       | 380                        | 213.5                     | 0.00         | 0.00                         | 0.00                      | 36                           |
| 5000                           | 1   | 5.1                       | 328                        | 189.9                     |              |                              |                           | 36                           |
| 5000                           | 7   | 6.0                       | 96                         | 16.0                      |              |                              |                           | 36                           |
| 5000                           | 14  | 5.9                       | 85                         |                           |              |                              |                           | 36                           |

Puvallup Soil, 20 C

|      |    |     |     |       |      |      |      |    |
|------|----|-----|-----|-------|------|------|------|----|
| 0    | 0  | 5.3 | 70  | 1.5   |      |      |      | 30 |
| 0    | 1  | 5.5 | 57  | 14.4  |      |      |      | 33 |
| 0    | 7  | 4.9 | 65  | 3.3   |      |      |      | 27 |
| 0    | 14 | 6.0 | 47  | 2.0   | 0.00 | 0.00 | 0.00 | 27 |
| 12.5 | 0  | 5.5 | 84  | 7.5   |      |      |      | 30 |
| 12.5 | 1  | 5.5 | 55  | 13.7  |      |      |      | 30 |
| 12.5 | 7  | 5.5 | 49  | 0.0   |      |      |      | 27 |
| 12.5 | 14 | 5.9 | 56  | 0.0   | 2.17 | 43.6 | 7.54 | 27 |
| 125  | 0  | 5.5 | 100 | 29.0  |      |      |      | 30 |
| 125  | 1  | 5.6 | 62  | 8.0   |      |      |      | 30 |
| 125  | 7  | 5.1 | 59  | 3.1   | 0.00 | 0.00 | 0.00 | 27 |
| 125  | 14 | 5.5 | 67  | 0.0   | 0.75 | 26.7 | 6.70 | 29 |
| 1250 | 0  | 5.3 | 134 | 134.5 |      |      |      | 83 |
| 1250 | 1  | 5.4 | 126 | 81.5  |      |      |      | 30 |
| 1250 | 7  | 6.0 | 55  | 18.6  |      |      |      | 27 |
| 1250 | 14 | 6.1 | 65  | 12.4  | 0.75 | 10.8 | 0.00 | 29 |
| 5000 | 0  | 5.3 | 383 | 15.6  |      |      |      | 30 |
| 5000 | 1  | 5.4 | 259 | 267.3 |      |      |      | 30 |
| 5000 | 7  | 6.9 | 68  | 8.7   | 13.8 | 0.00 | 0.00 | 27 |
| 5000 | 14 | 6.6 | 73  |       |      |      |      | 27 |

Table E-3. Acetate Decomposition During Incubation of CMA Solutions with the Pack Forest Soil at 2 and 20 C

| CMA<br>(mg/l)          | Treatment         |                           | Acetate Extracted (mg/10 g Soil) |        |        |        | % Decomposition |       |        |
|------------------------|-------------------|---------------------------|----------------------------------|--------|--------|--------|-----------------|-------|--------|
|                        | Acetate<br>(mg/l) | Acetate<br>(mg/10 g soil) | Day 0                            | Day 1  | Day 7  | Day 14 | Day 1           | Day 7 | Day 14 |
| <u>2 C Incubation</u>  |                   |                           |                                  |        |        |        |                 |       |        |
| 125                    | 71.4              | 0.41                      | 0.307                            | 0.382  | 0.285  | 0.015  | --              | 7.2   | 95.0   |
| 1250                   | 714.0             | 4.07                      | 3.426                            | 2.377  | 2.625  | 0.015  | 30.6            | 23.4  | 99.5   |
| 5000                   | 2857.1            | 16.28                     | 10.926                           | 10.050 | 10.575 | 10.815 | 8.0             | 3.2   | 1.0    |
| <u>20 C Incubation</u> |                   |                           |                                  |        |        |        |                 |       |        |
| 125                    | 71.4              | 0.41                      | 0.307                            | 0.270  | 0.307  | 0      | 12.0            | 0     | 100    |
| 1250                   | 714.0             | 4.07                      | 3.426                            | 3.112  | 0.150  | 0      | 9.2             | 95.6  | 100    |
| 5000                   | 2857.1            | 16.28                     | 10.926                           | 11.797 | 1.425  | 0.517  | --              | 87.0  | 95.3   |

concentrations. In a few instances relatively small experimental errors in combination with small changes produced inconsistencies. Overall, though, several trends are clear. Nearly all of the applied acetate can be degraded in one week at 20 C, when a large initial application stimulates rapid microorganism growth. At a lower concentration, decomposition lagged at day 7 but was complete by day 14. The lag was more noticeable at 2 C, but acetate was nearly fully degraded within two weeks at all but the highest concentration. There, the high osmotic stress, in conjunction with the low temperature, likely retarded bacterial action severely.

Results for chloride, nitrate-nitrogen, and sulfate reported in Table E-2 exhibit no trends for elevation of these anions in relation to applied CMA concentration, temperature, or time.

#### Adsorption/Desorption Experiment

Tables E-4, 5, and 6 summarize the adsorption/desorption results for metals for the three soils. Each table reports the quantities of metals adsorbed per unit mass of soil, the quantities subsequently desorbed, and the fractions of originally applied metals that were desorbed. These data represent a highly complex and dynamic ion exchange process involving many cations. Therefore, they cannot be used to predict magnitudes of adsorption and desorption in simple systems involving paired ions. Still, they illustrate certain trends that deserve attention.

The total quantity of metals applied even in the most concentrated solution (5000 mg CMA/l) was less than 0.2 milliequivalents per gram of soil (meq/g), while the cation exchange capacities of the soils were

Table E-4. Summary of Adsorption and Desorption of Metals in the Pack Forest Soil

| <u>CMA</u><br><u>(mg/l)</u>                | <u>Ca</u> | <u>Mg</u> | <u>K</u> | <u>Fe</u> | <u>Cu</u> | <u>Zn</u> | <u>Se</u> | <u>Pb</u> | <u>Al</u> |
|--|-----------|-----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|
| <u>Adsorption in Soil (µg/g of soil)</u>   |           |           |          |           |           |           |           |           |           |
| 0  | -19       | -5        | -16      | -2        | -0.040    | -0.174    | -0.100    | -0.082    | -5.430    |
| 10   | -16       | 0         | -14      | -2        | -0.036    | -0.199    | -0.099    | -0.110    | -4.134    |
| 100  | 8         | 38        | -4       | -1        | -0.030    | -0.200    | -0.071    | -0.092    | -3.306    |
| 500  | 106       | 193       | 50       | 0         | -0.025    | -0.310    | 0.002     | -0.042    | -1.539    |
| 1000                                       | 250       | 378       | 129      | 1         | -0.024    | -0.250    | 0.104     | -0.010    | -1.467    |
| 5000                                       | 1267      | 1603      | 687      | 7         | -0.035    | -0.409    | 0.881     | 0.326     | -1.811    |
| <u>Desorption from Soil (µg/g of soil)</u> |           |           |          |           |           |           |           |           |           |
| 0  | 8         | 2         | 11       | 3         | 0.015     | 0.133     | 0.075     | 0.053     | 6.315     |
| 10   | 6         | 2         | 9        | 2         | 0.014     | 0.107     | 0.056     | 0.045     | 2.665     |
| 100  | 9         | 3         | 11       | 1         | 0.015     | 0.174     | 0.091     | 0.060     | 3.551     |
| 500  | 14        | 6         | 14       | 1         | 0.015     | 0.133     | 0.094     | 0.100     | 2.106     |
| 1000                                       | 24        | 14        | 22       | 2         | 0.015     | 0.126     | 0.086     | 0.069     | 4.198     |
| 5000                                       | 90        | 85        | 73       | 0         | 0.017     | 0.152     | 0.104     | 0.076     | 0.971     |
| <u>Fraction Desorbed</u>                   |           |           |          |           |           |           |           |           |           |
| 0  | -0.41     | -0.44     | -0.66    | -1.32     | -0.37     | -0.76     | -0.74     | -0.64     | -1.16     |
| 10   | -0.39     | -4.04     | -0.65    | -1.02     | -0.39     | -0.54     | -0.57     | -0.41     | -0.64     |
| 100  | 1.18      | 0.08      | -2.65    | -1.52     | -0.52     | -0.87     | -1.27     | -0.66     | -1.07     |
| 500  | 0.13      | 0.03      | 0.28     | 2.80      | -0.61     | -0.43     | 49.85     | -2.41     | -1.37     |
| 1000                                       | 0.10      | 0.04      | 0.17     | 2.00      | -0.64     | -0.50     | 0.83      | -7.30     | -2.86     |
| 5000                                       | 0.07      | 0.05      | 0.11     | 0.05      | -0.49     | -0.37     | 0.12      | 0.23      | -0.54     |

Table E-5. Summary of Adsorption and Desorption of Metals in the Lee Forest Soil

| <u>CMA</u><br><u>(mg/l)</u>                | <u>Ca</u> | <u>Mg</u> | <u>K</u> | <u>Fe</u> | <u>Cu</u> | <u>Zn</u> | <u>Se</u> | <u>Pb</u> | <u>Al</u> |
|--|-----------|-----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|
| <u>Adsorption in Soil (µg/g of soil)</u>   |           |           |          |           |           |           |           |           |           |
| 0  | -42       | -9        | -16      | -1        | -0.050    | -0.181    | -0.110    | -0.106    | -5.430    |
| 10   | -42       | -5        | -16      | -1        | -0.036    | -0.267    | -0.125    | -0.108    | -4.134    |
| 100  | -15       | 34        | 0        | -1        | -0.041    | -0.290    | -0.091    | -0.099    | -3.306    |
| 500  | 112       | 196       | 68       | 0         | -0.038    | -0.299    | -0.021    | -0.066    | -1.539    |
| 1000                                       | 262       | 382       | 141      | 1         | -0.039    | -0.445    | 0.105     | -0.010    | -1.467    |
| 5000                                       | 1429      | 1658      | 737      | 6         | -0.052    | -0.736    | 0.860     | 0.327     | -1.811    |
| <u>Desorption from Soil (µg/g of soil)</u> |           |           |          |           |           |           |           |           |           |
| 0  | 14        | 3         | 9        | 0         | 0.016     | 0.262     | 0.053     | 0.020     | 2.296     |
| 10   | 37        | 7         | 12       | 0         | 0.015     | 0.202     | 0.053     | 0.050     | 2.582     |
| 100  | 125       | 24        | 22       | 0         | 0.018     | 0.545     | 0.076     | 0.065     | 4.842     |
| 500  | 75        | 29        | 22       | 1         | 0.021     | 0.292     | 0.090     | 0.068     | 3.179     |
| 1000                                       | 45        | 26        | 24       | 1         | 0.048     | 0.184     | 0.091     | 0.082     | 2.679     |
| 5000                                       | 147       | 147       | 97       | 1         | 0.024     | 0.355     | 0.136     | 0.110     | 3.766     |
| <u>Fraction Desorbed</u>                   |           |           |          |           |           |           |           |           |           |
| 0  | -0.34     | -0.33     | -0.54    | -0.54     | -0.32     | -1.45     | -0.48     | -0.19     | -0.42     |
| 10   | -0.90     | -1.43     | -0.75    | -0.70     | -0.41     | -0.76     | -0.42     | -0.46     | -0.62     |
| 100  | -8.13     | 0.70      | -113.89  | -0.87     | -0.43     | -1.88     | -0.84     | -0.65     | -1.46     |
| 500  | 0.67      | 0.15      | 0.33     | 3.00      | -0.55     | -0.98     | -4.38     | -1.02     | -2.07     |
| 1000                                       | 0.17      | 0.07      | 0.17     | 1.14      | -1.23     | -0.41     | 0.87      | -8.36     | -1.83     |
| 5000                                       | 0.10      | 0.09      | 0.13     | 0.15      | -0.47     | -0.48     | 0.16      | 0.34      | -2.08     |

Table E-6. Summary of Adsorption and Desorption of Metals in the Vantage Soil

| <u>CMA</u><br><u>(mg/l)</u>                | <u>Ca</u> | <u>Mg</u> | <u>K</u> | <u>Fe</u> | <u>Cu</u> | <u>Zn</u> | <u>Se</u> | <u>Pb</u> | <u>Al</u> |
|--|-----------|-----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|
| <u>Adsorption in Soil (µg/g of soil)</u>   |           |           |          |           |           |           |           |           |           |
| 0  | -14       | -4        | -13      | -3        | -0.044    | -0.113    | -0.100    | -0.116    | -2.221    |
| 10   | -13       | -1        | -12      | -4        | -0.049    | -0.270    | -0.103    | -0.105    | -2.461    |
| 100  | 6         | 36        | -3       | -2        | -0.030    | -0.065    | -0.096    | -0.091    | -1.833    |
| 500  | 64        | 180       | 43       | 0         | -0.037    | -0.089    | 0.021     | -0.059    | -0.156    |
| 1000                                       | 148       | 350       | 121      | 1         | -0.040    | -0.121    | 0.103     | -0.027    | -0.152    |
| 5000                                       | 951       | 1550      | 699      | 7         | -0.072    | -0.054    | 0.872     | 0.316     | 0.154     |
| <u>Desorption from Soil (µg/g of soil)</u> |           |           |          |           |           |           |           |           |           |
| 0  | 13        | 4         | 11       | 12        | 0.041     | 0.564     | 0.100     | 0.061     | 6.315     |
| 10   | 40        | 18        | 26       | 40        | 0.091     | 0.284     | 0.228     | 0.153     | 2.665     |
| 100  | 18        | 5         | 12       | 12        | 0.033     | 0.244     | 0.111     | 0.183     | 3.551     |
| 500  | 20        | 10        | 21       | 25        | 0.045     | 0.155     | 0.177     | 0.132     | 2.106     |
| 1000                                       | 29        | 13        | 24       | 4         | 0.024     | 0.104     | 0.099     | 0.075     | 4.198     |
| 5000                                       | 100       | 75        | 83       | 1         | 0.030     | 0.131     | 0.106     | 0.102     | 0.971     |
| <u>Fraction Desorbed</u>                   |           |           |          |           |           |           |           |           |           |
| 0  | -0.93     | -0.98     | -0.87    | -3.90     | -0.93     | -4.98     | -1.00     | -0.53     | -2.84     |
| 10   | -2.97     | -18.76    | -2.22    | -10.12    | -1.86     | -1.05     | -2.21     | -1.46     | -1.08     |
| 100  | 3.17      | 0.14      | -4.07    | -4.77     | -1.12     | -3.73     | -1.16     | -2.01     | -1.94     |
| 500  | 0.32      | 0.06      | 0.48     | 66.92     | -1.24     | -1.74     | 8.62      | -2.23     | -13.50    |
| 1000                                       | 0.19      | 0.04      | 0.20     | 7.33      | -0.61     | -0.86     | 0.96      | -2.77     | -27.70    |
| 5000                                       | 0.11      | 0.05      | 0.12     | 0.07      | -0.42     | -2.40     | 0.12      | 0.32      | 6.30      |

approximately 1-2 meq/g (see Appendix D). This observation indicates that the amount of metal adsorption would increase approximately linearly with the concentration of the CMA solution, since the metal concentrations in the CMA solutions were much lower than the maximum possible levels that can be adsorbed. Examining the computed adsorption data in the tables confirms this behavior for all but three of the metals studied.

Exceptions to linear adsorption were observed for copper, zinc, and aluminum at all CMA concentrations in all soils. This result suggests that these ions were released from the soil rather than adsorbed. One explanation is that they were replaced by other cations (e.g., Ca and Mg). Another possible explanation is that the concentrations of all metals except calcium, magnesium, and potassium were too low to detect with sufficient accuracy.

Table E-7 summarizes the adsorption and desorption of acetate. The Vantage soil (lowest in organics) had the lowest anion exchange capacity, being able to adsorb less than 30 percent of the applied acetate. In other soils approximately 40-65 percent (mean 47.8) was adsorbed. The anion exchange capacity of soils is normally below 1 meq/100 g, which corresponds to 0.6 mg acetate/g soil. Acetate adsorption values in this experiment ranged up to 0.327 mg/g. An adsorption plot for the Pack Forest soil (Figure E-1) indicates that the maximum adsorption capacity was not reached with 500 mg CMA/l addition (the curve did not approach a horizontal asymptote). It may be presumed that larger quantities could be adsorbed, at least in the more highly organic soils.

Table E-7. Acetate Adsorption and Desorption for Three Soils

| <u>CMA<br/>(mg/l)</u>   | <u>Acetate<br/>(mg/l)</u> | <u>Equilibrium<br/>Acetate<br/>(mg/l)</u> | <u>Acetate<br/>Adsorbed<br/>(mg/g soil)</u> | <u>% Acetate<br/>Adsorbed</u> | <u>% Acetate<br/>Desorbed</u> |
|-------------------------|---------------------------|---|---|-------------------------------|-------------------------------|
| <u>Pack Forest Soil</u> |                           |   |   |                               |                               |
| 10                      | 5.8                       | 2.0                                       | 0.011                                       | 65.5                          | 54.5                          |
| 20                      | 11.6                      | 5.5                                       | 0.018                                       | 52.6                          | 44.4                          |
| 40                      | 23.2                      | 8.5                                       | 0.044                                       | 63.4                          | 34.1                          |
| 80                      | 46.4                      | 23.0                                      | 0.070                                       | 50.4                          | 31.4                          |
| 100                     | 58.0                      | 29.5                                      | 0.086                                       | 49.1                          | 12.8                          |
| 160                     | 92.8                      | 54.5                                      | 0.115                                       | 41.3                          | 41.7                          |
| 500                     | 290.0                     | 197.5                                     | 0.278                                       | 31.9                          | 34.5                          |
| <u>Lee Forest Soil</u>  |                           |   |   |                               |                               |
| 100                     | 58.0                      | 35.5                                      | 0.068                                       | 38.8                          | 30.9                          |
| 500                     | 290.0                     | 181.0                                     | 0.327                                       | 37.6                          | 40.4                          |
| <u>Vantage Soil</u>     |                           |   |   |                               |                               |
| 100                     | 58.0                      | 41.0                                      | 0.051                                       | 29.3                          | 5.9                           |
| 500                     | 290.0                     | 250.5                                     | 0.118                                       | 13.6                          | 38.1                          |

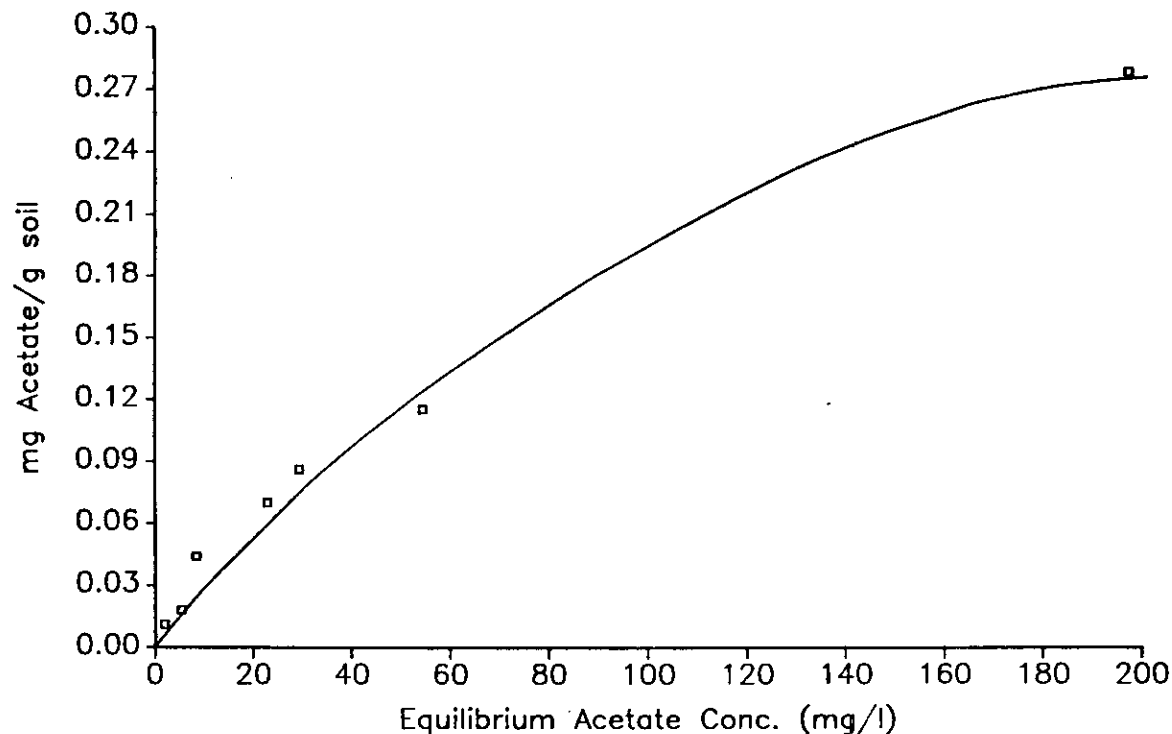


Figure E-1. Pack Forest Soil Acetate Adsorption Isotherm

With the exception of two values, 30-55 percent (mean 38.9) of the adsorbed acetate was subsequently desorbed and flushed from the soil. Working with mean values, on an overall basis, then, approximately 70 percent of applied acetate could eventually be mobile, even in relatively organic soils. In a soil like Vantage, perhaps 85 percent or more could be transported in soil water.

#### DISCUSSION

The batch incubation results suggest that initial additions of cations and acetate raised alkalinity and conductivity, but that decomposition of the acetate at 20 C decreased these quantities fairly rapidly. Often, values of these measures were not much above the untreated soil by day 14. Loss of the acid also made the soil solutions somewhat more basic.

The observations on acetate decomposition in these experiments were in general agreement with those of the respiration experiments in soils (see Appendix F). Both indicated that the organic can normally be essentially fully degraded at temperatures well above freezing within about two weeks. Of more interest in terms of CMA use on highways are the results near freezing, where oxidation was substantially slower.

Also of potential interest from the environmental impact standpoint is the indication that the majority of applied acetate could be mobile in water rather than bound to soil. This finding means that during periods of high runoff and soil percolation at low temperatures, when biological activity is at a minimum, there may be a high potential



to carry acetate to surface water or groundwater. There, decomposition would tend to reduce dissolved oxygen and diminish water quality.

The field measurements (see Appendix C) offer an opportunity to check the realism of laboratory findings. When acetate was measured in soil water at 30 and 60 cm depths, it never exceeded 8 percent of the concentration applied on the surface, despite the fact that at least 90 percent of the water normally percolated. When seen in groundwater at 3 m, acetate was never higher than 3 percent of the surface concentration, except in one measurement where it rose to 10 percent. Clearly, mechanisms in natural soil columns assist the removal of acetate to a greater degree than predicted by laboratory-scale work. These mechanisms probably include biodegradation and chemical reactions in addition to adsorption working in concert.

This finding, to a large extent, relieves the concern about high acetate mobility in soils. Nevertheless, its demonstration in the laboratory shows the potential for extensive transport with high water flushing (although dilution would also be high under these conditions). Coarse soils with low exchange capacities would be most subject to passing acetate to a surface receiving water or groundwater.

Another result of potential environmental significance apparent in the laboratory data is the tendency for displacement of some trace metals from soils when contacted by CMA. This tendency was also noted in the CalTrans soils experiments (1). In the present work, the batch experiment on the calcareous soil with low organic content exhibited a distinct tendency toward iron and aluminum release. In the

adsorption/desorption experiment, copper, zinc, and aluminum were released from all soils upon adsorption of other cations.

Displaced metals would be mobilized in the soil water and would have a tendency to be transported to surface water or groundwater. Iron and aluminum both have implications for drinking water quality, and Al, Cu, and Zn can be toxic to aquatic life.

Again, field results provide an opportunity to check the existence and extent of a laboratory-based finding. As observed in Appendix C, groundwater exhibited elevated Fe and Al, and occasionally Zn, after CMA treatments. Although lysimeters in the unsaturated zone did not exhibit this tendency, there is some consistency in the observation of metal mobility in independent laboratory and field observations.

Soils for laboratory experimentation were obtained from rural, forested or open, unused lands and lacked the substantial concentrations of metals that can be found in soils next to high-traffic highways (2). The field plots were located in one of these rural areas. Therefore, mobilization of metals in actual roadside soils cannot be directly evaluated from the results of this research. For example, lead release was never observed in any of these experiments, but its concentration was often barely above detection. It is unknown whether the relative insolubility of Pb would prevent its release in concentrations of any concern in a more contaminated soil. What can be said is that the mechanism for release has been demonstrated in both laboratory and field conditions, and that this mechanism applies, with varying strength, to all the cations. The potential for soil metal mobilization is one of

the leading reasons that CMA environmental effects should be tested in actual highway applications before it is put in widespread use.

## CONCLUSIONS

Laboratory soil chemistry experiments and confirmatory observations at the controlled field plots considered changes in soil chemical properties and mobility of various substances in soils as a result of CMA additions. Among the effects studied of greatest concern from an environmental impact standpoint are: (1) an indication that acetate could be quite mobile and remain undegraded in coarse soils with low exchange capacities at low temperature, especially when water flushing rates are high; and (2) evidence of displacement of some bound trace metals by ion exchange reactions involving calcium and magnesium.

The first observation is not considered particularly limiting to CMA usage. However, it might dictate some caution in the location and timing of CMA application. Consideration should be given to avoiding its use when runoff will pass over very coarse material that overlies a sensitive aquifer or is adjacent to a receiving water that should be protected from dissolved oxygen depletion. This caution should be emphasized when water infiltration could occur in large volumes over short time spans.

Relative to trace metal mobilization, more information is needed to develop definitive CMA use and environmental impact assessment guidelines. Obtaining this information could include laboratory desorption experiments on soils taken from heavily trafficked highway rights-of-way. The investigation should definitely include measurements

at sites of actual highway trial applications. Observations are needed on trace metal dynamics in soil water, groundwater, and surface runoff after CMA dosing. For now, the results of this research dictate caution in applying CMA above important aquifers and adjacent to surface waters with protected beneficial uses, especially where high traffic flows probably have contaminated roadside soils relatively heavily with metals.

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## APPENDIX F

### CMA DECOMPOSITION IN SOILS

#### INTRODUCTION

Decomposition of calcium magnesium acetate in soils has not been studied previously. However, since acetate is a common constituent of soils (1), it is expected to be oxidized at high rates. Soil acetate has been measured at levels ranging from 60-600 ppm (1, 2, 3). Levels of acetate usually increase temporarily when soils are amended with glucose, straw, or cellulose (1). Acetate oxidation proceeds rapidly without lag in temperate (25-30 C) agricultural and forest soils (4, 5, 6, 7), but little is known about acetate respiration rates in soils at temperatures near freezing.

This study was designed to quantify the rate and extent of acetate oxidation in four soils at low temperatures. The oxidation rates and the total amount of acetate oxidation per unit soil volume were determined for use in a model of the fate of acetate added to soils. Respiration rates were also determined at several horizons in soil core samples taken from the controlled field plots. These respiration rates indicate the reaction of soil organisms to acetate leaching through the soil column.

#### EXPERIMENTAL METHODS

##### Laboratory Batch Experiments

Laboratory batch experiments were performed using the Pack Forest, Lee Forest, Puyallup, and Vantage soils, whose properties are summarized

in Appendix D. Soil samples (1 liter) were air-dried, sieved through a 2 mm mesh screen, and mixed.

Twenty grams of air-dried soil were placed in 60 x 15 mm sterile, plastic, disposable Petri dishes. CMA was added in solution form, by pipet. The liquid volume that would moisten the soil to field capacity was used. A range of CMA concentrations (0, 125, 1250, 2500, and 5000 ppm) was applied to represent CMA in highway runoff under varying dilutions. The expected average concentration was approximately 125 ppm, while 5000 ppm represented the maximum predicted concentration.

The Petri dishes were placed in sealed containers. Acetate decomposition was determined by sampling CO<sub>2</sub> in the container head space. Respiration experiments were done at 2 C and 10 C, using cold rooms to simulate winter and spring soil temperatures, respectively. Container size and the amount of soil used were chosen to avoid a CO<sub>2</sub> concentration exceeding one percent in 24 hours. Wide-mouth pint mason jars were used at 2 C, and quart mason jars were selected at 10 C.

Gas samples were obtained through a septum port glued into the lids of the jars. Vacutainer tubes (4 ml, BD6490) were used to collect gas samples.

CO<sub>2</sub> was measured by isothermal (80 C) gas chromatography using a thermal conductivity detector. A Perkin-Elmer Sigma 300 gas chromatograph was used with a Chromosorb 104 (80/100 mesh) column and helium as the carrier gas. A 6-inch pre-column packed with HF-254 silica gel type 60 was used to remove water vapor. Gas samples were removed from the vacutainer and injected into the gas chromatograph using a 1 ml disposable plastic syringe with a side-port needle.

The calibration standards were a 1 percent  $\text{CO}_2$  gas mix (Scotty II, Supelco, Inc.) and  $\text{CO}_2$ -free cylinder air. Standard errors of replicate standards analyses were less than 0.3 percent.

After each set of measurements, the jars were flushed were four jar volumes of  $\text{CO}_2$ -free air, so that  $\text{CO}_2$  accumulation started from 0 ppm in each interval. Carbon dioxide never exceeded 1.3 percent.

Respiration volumes were calculated at the end of each interval on the basis of mass of  $\text{CO}_2$  per unit mass of soil. Rates were established by dividing this quantity by the hours of incubation.

#### Controlled Plot Soil Core Incubations

Thin-walled PVC pipe, 1-1/4 inch in diameter, was used to collect 30 cm deep soil cores from plots. The 1986 cores were collected 10 days after the last CMA application. The 1987 cores were collected 15 minutes after the final application of the winter. Soil temperatures at the time of core sampling in both years were 7-9 C. The 1986 loading was  $450 \text{ g/m}^2$ , while the 1987 loading was  $900 \text{ g/m}^2$ . The cores were chilled and transported from the field in an upright position, as in the soil. They were then cut into pieces corresponding to 0-3, 3-6, 8-16, 16-24, and 24-30 cm depths. The samples from each depth were placed in mason jars and incubated in a 2 C cold room as described previously for the batch experiments.

## RESULTS

### Laboratory Batch Experiments

Figure F-1 shows the typical time course of  $\text{CO}_2$  production in a batch experiment with one of the soils. As expected, the peak

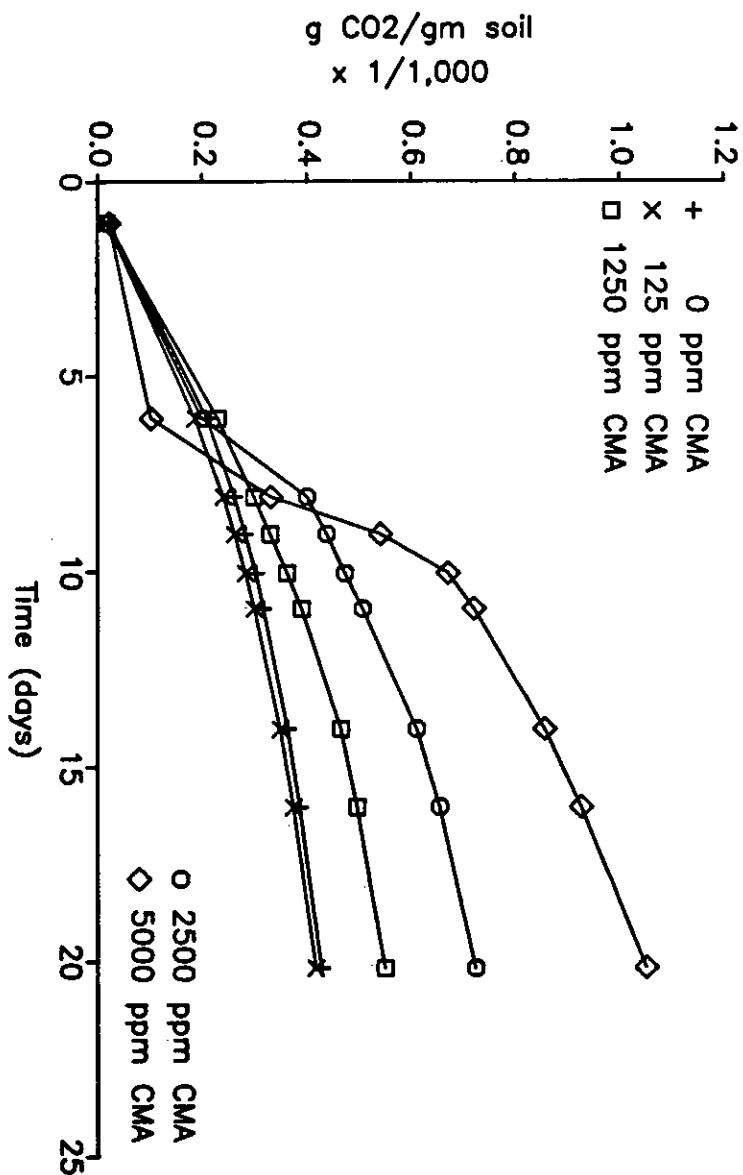


Figure F-1. Cumulative  $\text{CO}_2$  Production Over Time in One Batch Experiment

respiration rate increased with loading for each of the four soils, at both 2 and 10 C (Figure F-2). No saturation of rate with loading was observed up to 3000  $\mu\text{g}$  CMA/g soil. There was no significant difference in respiration rates at 0 and 38  $\mu\text{g}$  CMA/g soil.

The maximum respiration rates measured in the four soils were ordered as follows: Lee > Puyallup > Pack Forest > Vantage. At each loading the peak respiration rate was directly proportional to the percent organic matter found in the soil sample (Figure F-3).

Development of maximum respiration rates lagged by 13-29 days after the beginning of incubation at 2 C (Figure F-2a) and by 7-9 days at 10 C (Figure F-2b). Acetate was measured at the end of the experiment, when respiration had returned to the baseline level. Measured acetate concentration was below the detection limit.

Predicted  $\text{CO}_2$  yield, the mass of  $\text{CO}_2$  produced per unit mass of CMA, was calculated based on the stoichiometry of cell metabolic reactions, using McCarty's solution (8). In this model predicted  $\text{CO}_2$  yield varies with bacterial cell age, decreasing as the cell ages. Predicted  $\text{CO}_2$  yield was compared with the observed  $\text{CO}_2$  produced by the treated soil samples. The observed yield at 10 C for concentrations above 125 ppm (1 g  $\text{CO}_2$ /g CMA) was higher than the maximum predicted yield (0.7 g  $\text{CO}_2$ /g CMA), even in 30 days of growth. However, observed  $\text{CO}_2$  yield was equal to expectations at 2 C.

#### Controlled Plot Soil Core Incubations

Respiration rates on field core samples from CMA plots were higher than those of field core samples from water-treated plots. Respiration rates decreased with depth (Figure F-4). Most of the respiration was in

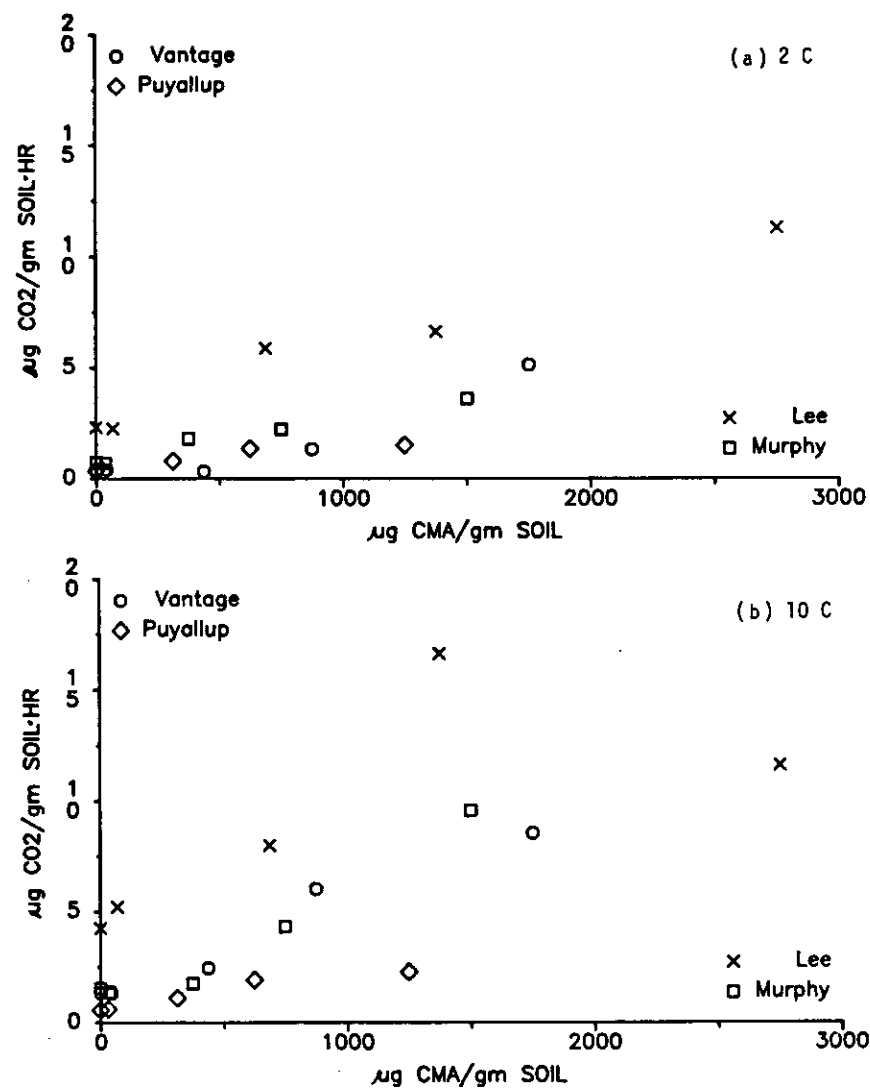


Figure F-2. Peak Respiration Rates Versus CMA Loadings

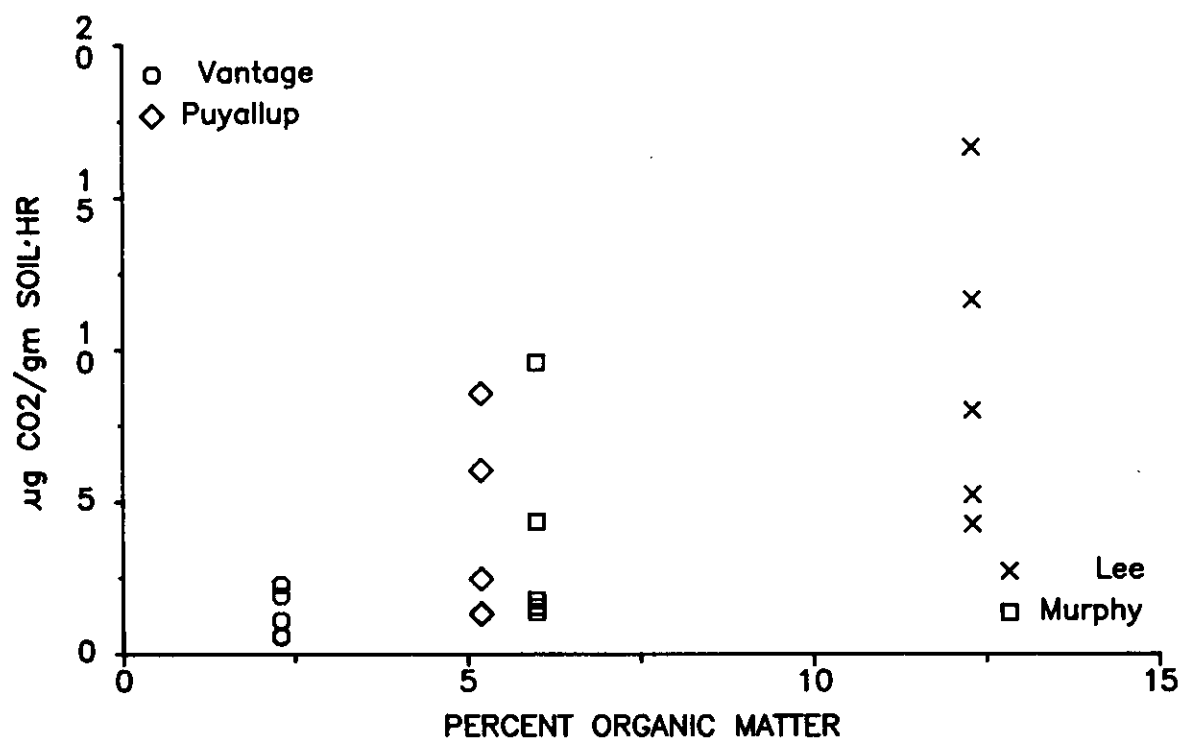


Figure F-3. Peak Respiration Rates at 10 C Versus Organic Content of Soils

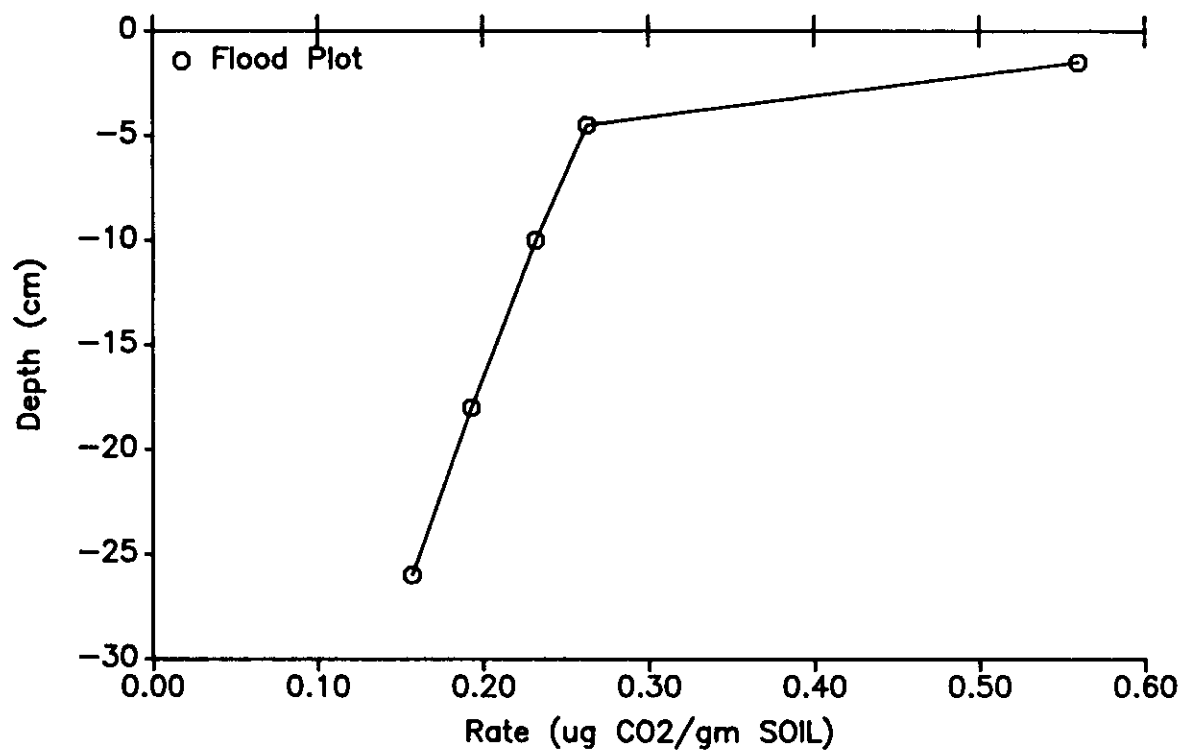


Figure F-4. Variation in Net Respiration Rate with Depth in a CMA Flood Plot

the 0-3 cm depth, with slightly less in the 3-6 cm depth. The net respiration rate in the top 3 cm was three-to-four times that at the 26-30 cm depth. Higher respiration rates occurred at lower depths in the CMA spray-plot cores than in the flood-plot cores.

Respiration rates in the CMA-treated soils from 1986 were approximately 10 times higher than those measured in the second season. This result occurred despite the higher CMA loading applied in 1987 and the much shorter time between application and incubation, which should have left more acetate to be oxidized in the laboratory.

#### DISCUSSION

Fungal hyphae were visible in soil samples at higher CMA loadings after incubation. This observation was consistent with the assumption that saprophytic bacteria and fungi were responsible for the decomposition of acetate.

There was no significant difference in batch experiment respiration rates between 38  $\mu\text{g}$  CMA/g soil and the 0  $\mu\text{g}$  soil controls at either temperature. This finding is consistent with the observations of high acetate levels extractable from untreated soils. The lag until respiration increased above the background corresponded to the peak size, which was also correlated with the organic matter content. Others have observed incomplete oxidation of acetate added to aerobic soils at higher temperature (30 C) (4). Stevenson and Ivarson found that only 25-30 percent of added carbon could be accounted for as carbon dioxide (5). In other experiments the remainder was recovered mainly in an acid-soluble fraction of the humic substances in the soil organic matter

(9) and in microbial carbon stores (6). However, Sorensen and Paul (7) found that 70 percent of acetate carbon added to a neutral clay soil evolved as carbon dioxide (at 25 C).

Maximum degradation rates were reached as loadings increased, except in the calcareous soil imported from Vantage in Eastern Washington. This result may be due to an osmotic effect on microbial activity at high calcium concentrations.

The effects of temperature on the rate of acetate decomposition (for concentrations > 125 ppm) were approximately as expected on the basis of a general Arrhenius relationship (10), i.e., an average 2.4-fold increase from 2 to 10 C. Carbon dioxide yield, however, was significantly lower than expected at 2 C. This result may have been due to more efficient metabolic processes at low temperature, creating more cell growth per unit acetate taken up. It could also have been due to low temperature storage of acetate carbon without oxidation (possibly as lipids).

The reason for the large drop in respiration rates in the field cores from the first to the second year of application is unknown. Unfortunately, there were insufficient direct acetate measurements in the soils before and after incubations to define the amount of substrate available to support respiration. One possibility is that buildup of saprophytic populations could have advanced decomposition rates in the second winter. However, insufficient time elapsed between the final treatment and measurement of respiration in that winter to allow any significant decay of the final dose. Another possibility is that an osmotic effect developed after ten treatments of high cation loadings

and interfered with microbial activity, although no direct measurements of soil osmolarity were made. This effect was suspected in the laboratory batch experiment conducted with the calcareous soil. It was also suspected in the pot tests on plants (see Appendix H). If true, accumulating cations over time could interfere with acetate decomposition and increase the possibility of its transport to groundwater or surface water. This possibility should be investigated in multi-year measurements at actual highway sites receiving CMA.

### CONCLUSIONS

The principal result of these experiments that has implications for the use and environmental impact assessment of CMA is that acetate decomposition in soils can be incomplete, even after a period of weeks. This observation is especially true at near-freezing temperatures. If undegraded acetate should be released to a receiving water by surface runoff or interflow, decomposition in water could diminish dissolved oxygen. Likewise, if acetate reaches groundwater and then decomposes, it could deteriorate its quality. If the groundwater is aerated, aerobic decay could reduce oxygen. If oxygen is already depleted, anaerobic decomposition could release undesirable by-products.

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## APPENDIX G

### EFFECTS OF CMA ON SOIL MICROBIAL ACTIVITY

#### INTRODUCTION

Being a readily biodegradable material, CMA has the potential to stimulate bacterial and fungal growths in soils to which it is applied, as well as animal consumers of those microorganisms. Containing high cation concentrations, it also could modify soil pH. To investigate the existence of these effects, soil cores were taken from each controlled field plot, analyzed for pH, examined for macro- and microfauna, and incubated to determine bacterial and fungal counts.

#### EXPERIMENTAL METHODS

##### Sampling

Soil cores were collected twice during the period of field experiments. The first collection occurred in the late summer following the first winter's treatments. This collection represented conditions during the period of highest microbial activity, but after acetate decomposition should have been completed. Therefore, it would indicate whether any long-lasting change occurred. The second collection occurred on the same day as the final treatment in the second winter (February, 1987). This collection represented conditions after heavy CMA loading, when acetate biodegradation had not been completed. While the relatively cool temperatures at that time would have retarded microbial activity, it was still expected to be in evidence.

Cores 10 cm in length (from the soil surface) were collected using a 2 cm diameter soil corer. On the first occasion several cores were

taken and composited from each of five locations on each plot (at the center and near each corner). After results were available, the data were analyzed statistically to determine whether a smaller sample size would have changed conclusions relative to the statistical significance of differences. It was shown that sampling from three locations per plot would increase errors of estimates marginally but not enough to change conclusions. Therefore, several cores were taken from each plot (near the top of the slope, at the center, and near the bottom of the slope) and composited on the second sampling occasion.

##### Laboratory Procedures

Analyses generally followed standard methods presented by Black (1), as adapted and modified by the University of Washington College of Forest Resources Laboratory. Essential features of these methods will be presented here.

Soil moisture content was determined gravimetrically so that results could be expressed on a dry-weight basis. Prior to pH measurement, 5 g soil subsamples were mixed in 10 ml distilled-deionized water for 10 seconds on a Vortex Genie Model 550-G mixer. After one hour of settling, pH readings were taken in water solution using a Fisher Accumet 210 pH meter and Orion 910500 electrode.

Petri dishes containing Yeast Extract Agar were inoculated with four serial dilutions of soil solution to make bacterial counts. Petri dishes containing Rose Bengal Streptomycin Agar were similarly inoculated to make fungal counts. Control plates were inoculated with autoclaved distilled-deionized water. Plates were incubated at room temperature for four days before counting.

Soil fauna were isolated using a Tullgren extraction system. Organisms were identified to order using a dissecting microscope.

#### Data Analysis

For statistical comparison of differences among treatments, results from Control-Spray, Control-Flood, CMA-Spray, and CMA-Flood plot pairs were pooled. Single-classification analyses of variance (anovas) were performed for pH, bacteria, and fungi to determine whether any significant differences existed among treatments. Where the anovas indicated the existence of significant differences among treatment means, Student-Newman-Keuls multiple comparisons tests were performed to determine which means differed significantly (2). Fauna results were patchy and were compared qualitatively.

#### RESULTS

Tables G-1 and G-2 present mean values for the first and second sampling occasions, respectively. No differences among treatments were significant on the late-summer occasion. Mites were best represented among the fauna, and were present in similar numbers in both CMA and control flood-plot soils, but in smaller numbers in spray-plot soils.

Results for the winter sampling occasion exhibited significant differences ( $P < 0.05$ ) between CMA and control plots, whether spray or flood, for pH. However, only the CMA flood plots had bacteria counts significantly higher than the controls. Differences among fungi in the four groups of plots were not statistically significant. Fauna were few and scattered, except for a large mite population in the CMA flood plot soils.

Table G-1. Mean Soil pH and Counts of Soil Bacteria, Fungi, and Fauna for Two CMA Treatments and Controls (September, 1986)<sup>a</sup>

| <u>Determination</u>           | <u>Control-Spray</u> | <u>Control-Flood</u> | <u>CMA-Spray</u>   | <u>CMA-Flood</u>   |
|--------------------------------|----------------------|----------------------|--------------------|--------------------|
| pH                             | 4.7                  | 4.8                  | 5.1                | 4.9                |
| Bacteria (No. col./g dry soil) | $8.86 \times 10^6$   | $6.66 \times 10^7$   | $2.57 \times 10^7$ | $3.25 \times 10^7$ |
| Fungi (No. col./g dry soil)    | $2.61 \times 10^5$   | $2.90 \times 10^5$   | $2.22 \times 10^5$ | $4.29 \times 10^5$ |
| Fauna (No./kg dry soil)        |                      |                      |                    |                    |
| Mite                           | 15.9                 | 30.5                 | 5.1                | 32.0               |
| Weevil                         | 2.5                  | 0                    | 0                  | 0                  |
| Lepidoptera                    | 0                    | 4.9                  | 0                  | 0                  |
| Isoptera                       | 0                    | 2.6                  | 2.5                | 2.5                |
| Larva                          | 0                    | 2.5                  | 0                  | 0                  |
| Centipede                      | 0                    | 0.3                  | 0                  | 2.5                |
| Spider                         | 0                    | 2.5                  | 2.5                | 0                  |
| Collembola                     | 0                    | 0                    | 0                  | 2.6                |
| Coleoptera                     | 0                    | 0                    | 0                  | 2.6                |

<sup>a</sup>Differences among treatments are not significant at  $P < 0.05$ .

Table G-2. Mean Soil pH and Counts of Soil Bacteria, Fungi, and Fauna for Two CMA Treatments and Controls (February, 1987)

| <u>Determination</u>                        | <u>Control-Spray</u> | <u>Control-Flood</u> | <u>CMA-Spray</u>   | <u>CMA-Flood</u>   |
|---|----------------------|----------------------|--------------------|--------------------|
| pH <sup>a</sup>                             | 5.1                  | 5.2                  | 6.1                | 5.8                |
| Bacteria (No. col./g dry soil) <sup>a</sup> | $5.35 \times 10^7$   | $2.88 \times 10^7$   | $3.40 \times 10^7$ | $1.41 \times 10^8$ |
| Fungi (No. col./g dry soil) <sup>a</sup>    | $5.85 \times 10^4$   | $5.94 \times 10^4$   | $1.04 \times 10^5$ | $2.14 \times 10^5$ |
| Fauna (No./kg dry soil)                     |                      |                      |                    |                    |
| Mite  | 4.2                  | 4.3                  | 4.5                | 63.3               |
| Protura                                     | 0                    | 0                    | 4.5                | 0                  |
| Diptera                                     | 4.5                  | 4.3                  | 0                  | 0                  |
| Collembola                                  | 0                    | 4.3                  | 8.7                | 4.3                |
| Millipede                                   | 0                    | 0                    | 0                  | 4.7                |

<sup>a</sup> \_\_\_\_\_ signifies means that are not significantly different at  $P < 0.05$ .

Figure G-1 illustrates the variation of soil pH over the CMA plots. On plots flooded with CMA at the top of the slope, pH declined nearly to the control plot mean by the bottom of the slope, whereas pH was more uniformly high on the homogeneously treated CMA spray plots. There was no strong tendency, however, for similar stratification of the biota on the CMA flood plots.

#### DISCUSSION

The analysis of the field plot soil microbial activity has demonstrated that flood application of CMA solution to soils can cause measurable increases in bacteria populations during the period of active acetate decomposition. However, the evidence indicates that these population increases do not persist, and differences between CMA-treated

and -untreated soils disappear well before a new winter application period. While fungi populations were elevated in CMA-treated plot soils relative to controls, differences were not statistically significant.

The rate at which bacteria populations return to control levels after rising in response to CMA addition is not known, but is not considered to be a significant factor in specifying how CMA should be used or its impacts assessed. Also unknown are the exact bacterial forms making up the populations. They are thought to be various saprophytes, and there is no reason to believe that pathogens are among them. Future work could test this hypothesis through identifications of laboratory- or field-grown cultures. This exercise was beyond the scope of the present work.

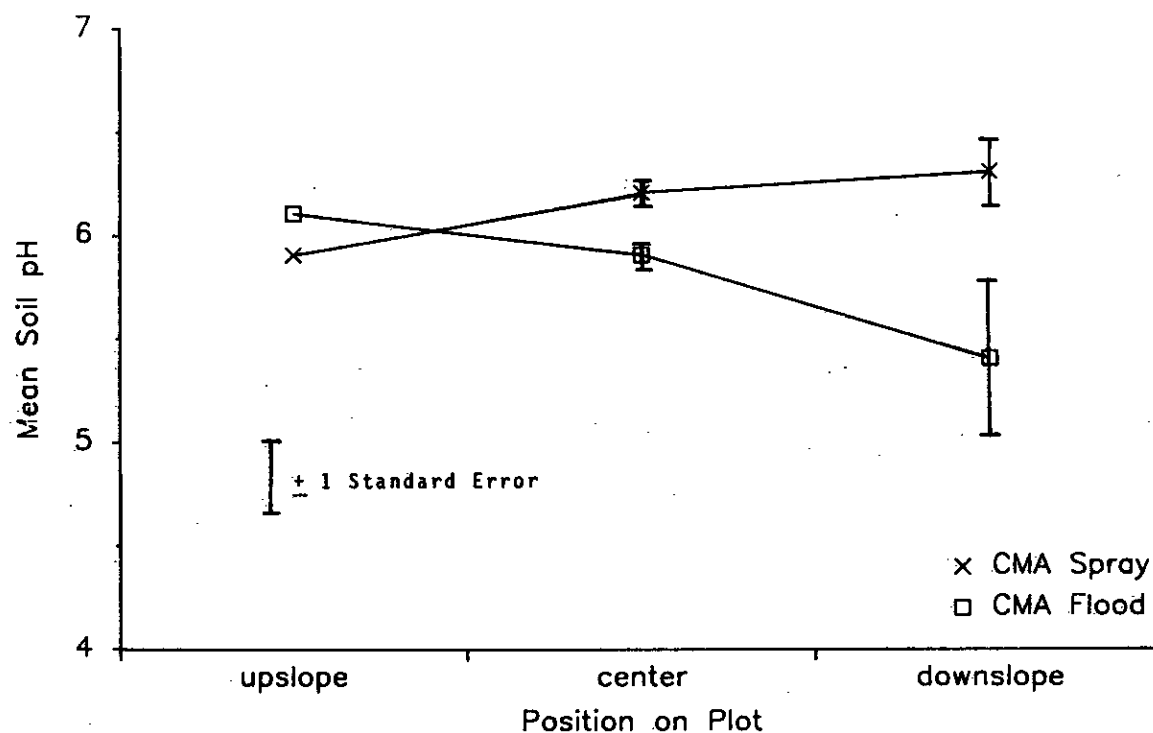


Figure G-1. Variation of Soil pH Over CMA Plots

The alkaline effect of calcium and magnesium on soil was evident in the data from the second sampling occasion. Again, this effect was not apparent some months after the last CMA treatment of the first winter. Apparently, plant uptake and chemical processes in the soil redistributed the cations over time. The fact that pH was notably higher near the point of application than downslope on the CMA flood plots demonstrated the rapid removal of the major cations from runoff by the soil. The observation that soil microbes were more evenly distributed over the CMA flood plots than was pH suggests that acetate was not as rapidly captured in soil as were calcium and magnesium. Analysis of runoff from bins yielded the same conclusion (see Appendix C).

#### CONCLUSIONS

The results reported and discussed do not suggest any specific guidelines to be applied to CMA use or provisions to note in environmental impact assessment. Soil bacteria populations increased with CMA addition by flood, but there is evidence that the increases were temporary and no reason to believe that pathogens were stimulated.

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## APPENDIX H

### EFFECTS OF CMA ON TERRESTRIAL VEGETATION

#### INTRODUCTION

Initial evaluations of the amounts of CMA needed for deicing effects, as well as a survey of the literature and pertinent reports (1, 2, 3, 4), convinced us that a number of potential influences of CMA should be studied further: viz., (1) potentially adverse influences of CMA as an osmoticum on foliage and on roots; (2) the effects of adding Ca and Mg to the soil; and (3) the effect of the readily decomposable acetate in the root zone on the roots themselves, as well as on soil microorganisms. These aspects were investigated using soil pot tests, roadside-simulating vegetation on field plots, and bins and boxes of soil seeded or planted with tree seedlings (with careful observations of vigor and harvests for dry matter yields). Also, specimen trees were sprayed for observation of any abnormalities. The results of the studies in each of these components are given in the sections below.

#### EXPERIMENTAL METHODS

##### Pot Tests

Pot tests were conducted in the University of Washington Botany Greenhouse with methods modified from Jenny et al. (5). These tests were conducted in two soils: (1) the sandy loam from Puyallup, WA, and (2) the Pack Forest (Murphy's Ranch) soil mixed 2:1 with Indianola sand, also from Pack Forest. The mixture was used to provide data from soils of substantially different texture. Appendix D lists the soil properties. A portion of soil (1000 to 1200 g, depending on the density

of the particular soil) was mixed with a suitable amount of NPK fertilizer to stimulate good growth. In our tests the soil was further mixed with the desired amount of dry CMA. Then, the soil was poured into a standard 5-inch plastic pot, moistened with distilled water, and planted with one or more seedlings. CMA quantities were 0 (control), 0.5, 1.0, 2.0, and 4.0 g/kg soil. These amounts corresponded to soil solution CMA concentrations of 0, 1250, 2500, 5000, and 10,000 ppm, respectively, when the soil was moistened to field capacity.

In most instances the planting was done shortly after the moistening, but in some treatments planting was delayed for 48 hours to permit cation exchange reactions between the CMA and the soil to take place. Species used for these trials were Romaine lettuce, buckhorn plantain (*Plantago lanceolata*), sunflower (*Helianthus annuus*), Douglas fir (*Pseudotsuga menziesii*), balsam fir (*Abies balsamina*), and red maple (*Acer rubra*). After a suitable growing period, the plants were harvested and dry weights of shoots and roots recorded.

#### Field Plots

In March 1985, eight replicate plots 3.17 X 3.17 m with 8 percent slope to the west were established at Pack Forest. Each was sown with the following: 84 g red fescue, 16.8 white Dutch clover, and 16.8 g common vetch. Prior to sowing 900 g of 5-10-10 NPK fertilizer was broadcast and raked in (=40 lb N, 80 lb P<sub>2</sub>O<sub>5</sub>, and 80 lb K<sub>2</sub>O per acre). Irrigation with stream water was performed as needed through the dry summer of 1985. Refertilization at one-half the above rates was made on each of the following dates: 8 October 1985; 13 May 1986; 11 August 1986. A satisfactory cover was present on each plot by December 1985,

and a vigorous grass-clover sward by June 1986. Indigenous Canada thistle had to be weeded out several times. A few indigenous but less aggressive species (especially, wild chrysanthemum and plantain) grew in the plots but were not removed because they contributed to the "typical roadside" nature of the vegetation. They were a minor component beside the fescue and clover.

In late July and early August 1985, we planted 16 each of Douglas-fir, red maple, plantain, and buttercup seedlings in a uniform pattern in each of the plots. These plantings were done to give a comparison between the greenhouse pot tests and the field growing plants.

On 9 July 1986, a biomass harvest was made by dividing the area into eight rectangles and then cutting all vegetation at 5 cm from the soil level. Prior to this total harvest, the individual buttercup and plantain plants were harvested (but central bud and developing young leaves were left intact). A repeat of the biomass harvest was made on 10 June 1987. In all cases the material was dried and then weighed.

In September, 1986, a partial harvest was made for determination of relative cover of the grass, clover, and other plants. Four locations on each plot were randomly identified for harvest by blindly tossing a 16 cm ring. All vegetation rooted within the ring was cut at 5 cm from the soil level, returned to the greenhouse, dried, and separated. Collections were then weighed to determine relative representation of grass, clover, and other species in the biomass.

#### Soil Bins

Bins served essentially as miniature parallels of the plots. They were intended to allow more flexible treatments and to test climatic

differences, since one set of six bins was installed at Pack Forest adjacent to the field plots (366 m elevation) with another set of six on the University of Washington campus at 20 m elevation. Each bin was 2 m X 1 m X 30 cm deep, constructed of 3/4-inch exterior grade plywood, supported on concrete blocks with 8 percent slope, and filled to 25 cm deep with soil collected near the field plots. Sowing of fescue, clover, and vetch, watering and fertilizing, refertilization, growing of additional specimens (buttercup, plantain, Douglas fir and red maple), and harvesting all followed the same procedures as the field plots, except that they were scaled down to the reduced area (1/5 that of the plots).

#### Boxes and Free-standing Saplings for Spray Treatment

In July 1986, four boxes each 0.5 m<sup>2</sup> were installed on concrete blocks and planted with Douglas fir, balsam fir, and red maple seedlings. These boxes were located at Pack Forest between Plots 4 and 5. Clumps of naturally growing red alder saplings ca 3 m tall were flagged for testing this species. Near the boxes, eight 4-year old Douglas-fir saplings ca 1 m tall were planted. Two of the boxes, four of the Douglas fir saplings, and one alder clump served as water controls. The others were sprayed with CMA during the winter of 1986-87.

#### Field Plot Treatments

Five treatments of CMA or water (controls) were made in each of two winters to each plot and bin, and in the final winter to each box and free-standing tree. Water volumes differed in the two winters, and were proportional to surface areas. CMA concentrations were 3000 mg/l,

except for one bin in each location, which received 1500 mg/l. Chapter 1 provides complete details on the various treatments.

## RESULTS

### Pot Tests

The objectives of the pot tests were: (1) to determine the amount of CMA that could be tolerated in the root zone, (2) to examine differences between plant species in their tolerance of such CMA, and (3) to determine the effects on dry-matter production of the CMA in the soil. Table H-1 summarizes the results of these tests. The principal points to be noted are:

1. The addition of 0.5 g CMA stimulated growth in most cases, although this stimulation was large enough for statistical significance only in a few instances, mostly with the coarser soil (Pack/sand).
2. Most plants tolerated 1.0 g CMA, and growth was mostly equal to or a little greater than in the controls. However, Douglas fir was sharply retarded by this addition on the Puyallup soil and died in the Pack/sand. Sunflower also died in the Pack/sand soil with this addition.
3. At 2.0 g addition, Douglas fir died in both soils, and sunflower died in the Pack/sand soil. Most of the species survived this addition, with yields depressed in plantain but increased in balsam fir and red maple.
4. All plants died in soil to which 4.0 g CMA was added, if planting occurred at once. However, all plants survived with

Table II-1. Summary of CMA Pot Tests

Note: All values are in percent of control, except last column, which gives control yields in g/pot.

| Plant Species                              | Soil <sup>1</sup> | Tissue | CMA Addition (g per kg soil) |                   |                    |                    |                    | Average Control Yield (g/pot) |      |
|--|-------------------|--------|------------------------------|-------------------|--------------------|--------------------|--------------------|-------------------------------|------|
|  |                   |        | 0 (Control)                  | 0.5               | 1.0                | 2.0                | 4.0 <sup>2</sup>   |                               |      |
| Romaine lettuce                            | Puyallup          | Shoot  | 100 <sup>a</sup>             | 106 <sup>a</sup>  | 103 <sup>a</sup>   | 85 <sup>b</sup>    | (68) <sup>3b</sup> | --                            | 5.84 |
|  |                   | Root   | 100                          | 147               | 222                | 180                | (190)              | --                            | 2.03 |
|  | Pack/Sand         | Shoot  | 100 <sup>ac</sup>            | 122 <sup>ac</sup> | (128) <sup>a</sup> | (160) <sup>b</sup> | (181) <sup>c</sup> | 95                            | 2.84 |
|  |                   | Root   | 100                          | 133               | (173)              | (118)              | (113)              | 55                            | 1.59 |
| <u>Plantain (Plantago lanceolata)</u>      | Puyallup          | Shoot  | 100 <sup>a</sup>             | 104 <sup>a</sup>  | 102 <sup>a</sup>   | 65 <sup>a</sup>    | Died               | 91 <sup>a</sup>               | 2.47 |
|  |                   | Root   | 100                          | 75                | 48                 | 24                 |                    | 53                            | 2.43 |
|  | Pack/Sand         | Shoot  | 100 <sup>a</sup>             | 162 <sup>a</sup>  | 137 <sup>a</sup>   | 55 <sup>a</sup>    | Died               | 183 <sup>a</sup>              | 1.22 |
|  |                   | Root   | 100                          | 147               | 130                | 25                 |                    | 102                           | 1.03 |
| <u>Sunflower (Helianthus annuus)</u>       | Puyallup          | Shoot  | 100 <sup>a</sup>             | 104 <sup>a</sup>  | 94 <sup>a</sup>    | 87 <sup>a</sup>    | Died               | 87 <sup>a</sup>               | 6.46 |
|  |                   | Root   | 100                          | 107               | 118                | 92                 |                    | 110 <sup>b</sup>              | 1.58 |
|  | Pack/Sand         | Shoot  | 100 <sup>a</sup>             | 181 <sup>b</sup>  | Died               | Died               | Died               | 227 <sup>b</sup>              | 2.01 |
|  |                   | Root   | 100                          | 115               |                    |                    |                    | 105                           | 0.73 |
| <u>Douglas Fir (Pseudotsuga menziesii)</u> | Puyallup          | Shoot  | 100 <sup>a</sup>             | 104 <sup>a</sup>  | 18 <sup>a</sup>    | Died               | Died               | 111 <sup>a</sup>              | 2.18 |
|  |                   | Root   | 100                          | 103               | 15                 |                    |                    | 97                            | 2.29 |
|  | Pack/Sand         | Shoot  | 100 <sup>a</sup>             | 177 <sup>a</sup>  | Died               | Died               | Died               | 65 <sup>a</sup>               | 1.67 |
|  |                   | Root   | 100                          | 175               |                    |                    |                    | 106                           | 1.65 |
| <u>Balsam Fir (Abies balsamina)</u>        | Pack/Sand         | Shoot  | 100 <sup>a</sup>             | 122 <sup>a</sup>  | 104 <sup>a</sup>   | 191 <sup>a</sup>   | Died               | 135 <sup>a</sup>              | 0.23 |
|  |                   | Root   | 100                          | 105               | 84                 | 115                |                    | 100                           | 0.39 |
| <u>Red Maple (Acer rubra)</u>              | Pack/Sand         | Shoot  | 100 <sup>a</sup>             | 141 <sup>a</sup>  | 149 <sup>a</sup>   | 234 <sup>a</sup>   | Died               | 63 <sup>a</sup>               | 2.01 |
|  |                   | Root   | 100                          | 106               | 98                 | 170                |                    | 88                            | 1.35 |

<sup>1</sup> The soils are characterized in Appendix D. "Pack/Sand" refers to a mixture of two volumes of soil from the Murphy Ranch site at Pack Forest to one volume of Indianola Sand (a fine sand from a natural deposit at Pack Forest).

<sup>2</sup> Plants were placed in the pots two days after the addition of CMA and moistening.

<sup>3</sup> Values in parentheses (for lettuce) are for plants that replaced the original ones. The replacements were planted one week after the CMA was added.

<sup>4</sup> Each pot received a fertilizer treatment of: 274 mg  $\text{NH}_4\text{NO}_3$ ; 324 mg  $\text{NH}_4\text{H}_2\text{PO}_4$ ; 107 mg  $\text{KNO}_3$ .

<sup>5</sup> Fertilizer was applied in solution and thoroughly mixed into the soil prior to CMA addition.

<sup>6</sup> For shoot yields, treatment means that have the same superscript letter are not significantly different statistically, but are significantly different from those with a different letter. Significance was determined at  $P < 0.05$  by anova followed where appropriate by the Student-Newman-Keuls multiple range comparison. Use of an underline with the superscript indicates that only a few plants survived.



this much CMA if planting was delayed 48 hours after the CMA addition. Probable reasons for this striking response are taken up in the discussion below.

#### Field Plots

At the harvest of July 1986, all plots showed a vigorous growth of fescue and clover, with a small admixture of volunteer weedy species. The growth of the grass and clover had become so tall that the plantain, buttercup, Douglas fir, and maple seedlings were so severely shaded that their harvest in sufficient numbers for comparisons of treatments was impractical. The values for the biomass of major species in the plots are given in Table H-2, part A. There were no statistically significant differences between these yields for the eight plots, although there was a trend toward higher yields with flood applications of either CMA or water. The results of the June 1987 harvest, after a second winter of treatments, exhibited no statistically significant differences.

All plots were vegetated with grass and clover to the near exclusion of other species, although there was always a very small percentage of weedy invaders. Grass "cover" exceeded clover "cover" somewhat on five of the eight plots, four of which received spray applications. Table H-3 summarizes the cover data. For grass, cover was significantly greater on water- than on CMA-treated plots, but mode of application made no difference. However, for clover, material (CMA or water) made no difference, while flooding with either material significantly increased cover over spraying. There was some spatial heterogeneity in cover of other plants, with the first three plots having more of these species than other plots.

Table H-2. Summary of Plot and Bin Harvest Biomass Yields<sup>1</sup>

Note: All values are in g dry weight/m<sup>2</sup>.

#### A. Field Plots

| Plot                           | <u>1</u>                      | <u>2</u>                      | <u>3</u>                      | <u>4</u>                      | <u>5</u>                      | <u>6</u>                       | <u>7</u>                      | <u>8</u>                       |
|--------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|--------------------------------|
| Material Application           | CMA Spray                     | Water Spray                   | CMA Spray                     | Water Spray                   | CMA Flood                     | Water Flood                    | Water Flood                   | CMA Flood                      |
| <u>Harvest of 9 July 1986</u>  |                               |                               |                               |                               |                               |                                |                               |                                |
| Mean Biomass                   | 748 <sub>W</sub> <sup>a</sup> | 754 <sub>W</sub> <sup>a</sup> | 828 <sub>W</sub> <sup>a</sup> | 868 <sub>W</sub> <sup>a</sup> | 881 <sub>X</sub> <sup>a</sup> | 1026 <sub>X</sub> <sup>a</sup> | 944 <sub>X</sub> <sup>a</sup> | 1107 <sub>X</sub> <sup>a</sup> |
| Standard Error                 | 71                            | 74                            | 67                            | 52                            | 82                            | 120                            | 84                            | 83                             |
| <u>Harvest of 10 June 1987</u> |                               |                               |                               |                               |                               |                                |                               |                                |
| Mean Biomass                   | 245 <sub>W</sub> <sup>a</sup> | 342 <sub>W</sub> <sup>a</sup> | 350 <sub>W</sub> <sup>a</sup> | 361 <sub>W</sub> <sup>a</sup> | 318 <sub>W</sub> <sup>a</sup> | 273 <sub>W</sub> <sup>a</sup>  | 358 <sub>W</sub> <sup>a</sup> | 328 <sub>W</sub> <sup>a</sup>  |
| Standard Error                 | 19                            | 17                            | 13                            | 15                            | 26                            | 14                             | 23                            | 19                             |

#### B. Campus Bins

| Bin                            | <u>1</u>                      | <u>2</u>                      | <u>3</u>                      | <u>4</u>                       | <u>5</u>                       | <u>6</u>                       |
|--------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Material Application           | Water Combined                | Water Flood                   | Water Flood                   | CMA Combined                   | CMA Flood                      | CMA Flood                      |
| <u>Harvest of 2 June 1986</u>  |                               |                               |                               |                                |                                |                                |
| Grass + Clover Mean            | 415 <sub>W</sub> <sup>a</sup> | 346 <sub>W</sub> <sup>a</sup> | 492 <sub>W</sub> <sup>a</sup> | 281 <sub>W</sub> <sup>bc</sup> | 408 <sub>W</sub> <sup>bd</sup> | 389 <sub>W</sub> <sup>bd</sup> |
| Standard Error                 | 27 <sub>W</sub>               | 38 <sub>W</sub>               | 25 <sub>W</sub>               | 19 <sub>W</sub>                | 32 <sub>W</sub>                | 26 <sub>W</sub>                |
| Buttercup Mean                 | 1.2 <sub>W</sub> <sup>a</sup> | 1.6 <sub>W</sub> <sup>a</sup> | 3.0 <sub>W</sub> <sup>a</sup> | 1.7 <sub>W</sub> <sup>a</sup>  | 1.6 <sub>W</sub> <sup>a</sup>  | 1.3 <sub>W</sub> <sup>a</sup>  |
| Standard Error                 | 0.2                           | 0.4                           | 0.8                           | 0.2                            | 0.3                            | 0.8                            |
| Plantain Mean                  | 4.2 <sub>W</sub> <sup>a</sup> | 3.0 <sub>W</sub> <sup>a</sup> | 3.8 <sub>W</sub> <sup>a</sup> | 2.4 <sub>W</sub> <sup>a</sup>  | 4.0 <sub>W</sub> <sup>a</sup>  | 3.3 <sub>W</sub> <sup>a</sup>  |
| Standard Error                 | 0.5                           | 0.4                           | 0.8                           | 0.4                            | 0.8                            | 0.6                            |
| Vetch Mean                     | 225                           | 8.8                           | 444                           | 0.0                            | 10                             | 0.0                            |
| <u>Harvest of 4 Sept. 1986</u> |                               |                               |                               |                                |                                |                                |
| Grass + Clover Mean            | 310                           | 322                           | 391                           | 517                            | 366                            | 430                            |
| <u>Harvest of 29 June 1987</u> |                               |                               |                               |                                |                                |                                |
| Grass + Clover mean            | 206 <sub>W</sub> <sup>a</sup> | 226 <sub>W</sub> <sup>a</sup> | 277 <sub>W</sub> <sup>a</sup> | 440 <sub>W</sub> <sup>b</sup>  | 299 <sub>W</sub> <sup>b</sup>  | 370 <sub>W</sub> <sup>b</sup>  |
| Standard Error                 | 22 <sub>W</sub>               | 21 <sub>W</sub>               | 22 <sub>W</sub>               | 42 <sub>W</sub>                | 27 <sub>W</sub>                | 35 <sub>W</sub>                |

Continued

Table H-2. Continued

C. Pack Forest Bins

| Bin                            | <u>7</u>                       | <u>8</u>                      | <u>9</u>                      | <u>10</u>                      | <u>11</u>                     | <u>12</u>                     |
|--------------------------------|--------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|
| Material Application           | Water Combined                 | Water Flood                   | Water Flood                   | CMA Combined                   | CMA Flood                     | CMA Flood                     |
| <u>Harvest of 9 July 1986</u>  |                                |                               |                               |                                |                               |                               |
| Grass + Clover Mean            | 369 <sup>a</sup> <sub>w</sub>  | 370 <sup>a</sup> <sub>w</sub> | 434 <sup>a</sup> <sub>w</sub> | 348 <sup>b</sup> <sub>w</sub>  | 353 <sup>b</sup> <sub>w</sub> | 350 <sup>b</sup> <sub>w</sub> |
| Standard Error                 | 20                             | 9                             | 21                            | 26                             | 22                            | 36                            |
| Buttercup Mean                 | 0.7 <sup>a</sup>               | 1.0 <sup>a</sup>              | 1.7 <sup>a</sup>              | 0.8 <sup>a</sup>               | 0.8 <sup>a</sup>              | 1.6 <sup>a</sup>              |
| Standard Error                 | 0.1                            | 0.3                           | 0.5                           | 0.2                            | 0.2                           | 0.5                           |
| Plantain Mean                  | 2.8 <sup>a</sup>               | 2.1 <sup>a</sup>              | 2.3 <sup>a</sup>              | 1.8 <sup>a</sup>               | 1.2 <sup>a</sup>              | 1.8 <sup>a</sup>              |
| Standard Error                 | 1.0                            | 0.4                           | 0.4                           | 0.3                            | 0.4                           | 0.2                           |
| <u>Harvest of 10 June 1987</u> |                                |                               |                               |                                |                               |                               |
| Grass + Clover Mean            | 390 <sup>a</sup> <sub>wx</sub> | 417 <sup>a</sup> <sub>x</sub> | 410 <sup>a</sup> <sub>w</sub> | 441 <sup>a</sup> <sub>wx</sub> | 448 <sup>a</sup> <sub>x</sub> | 332 <sup>a</sup> <sub>w</sub> |
| Standard Error                 | 18 <sup>wx</sup>               | 18 <sup>x</sup>               | 22 <sup>w</sup>               | 21 <sup>wx</sup>               | 16 <sup>x</sup>               | 27 <sup>w</sup>               |

<sup>1</sup>Superscript series abcd signifies statistical significance of differences based on material (CMA or water), and subscript series wxyz signifies statistical significance of differences based on Application in a two-way anova. Where only the superscript series is used, a one-way anova based on Material was conducted. Where no indication is given, there was no replication. Treatment means that have the same letter are not significantly different, but are significantly different at  $P < 0.05$  from those with a different letter. Statistical procedures were as designated in footnote 5 of Table H-1.

Table H-3. Summary of Plant Cover on Field Plots<sup>1</sup>  
 Note: All values are in g dry weight.

| Plot           | <u>1</u>                       | <u>2</u>                       | <u>3</u>                       | <u>4</u>                       | <u>5</u>                       | <u>6</u>                       | <u>7</u>                       | <u>8</u>                       |
|----------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Material       | CMA                            | Water                          | CMA                            | Water                          | CMA                            | Water                          | Water                          | CMA                            |
| Application    | Spray                          | Spray                          | Spray                          | Spray                          | Flood                          | Flood                          | Flood                          | Flood                          |
| Grass Mean     | 2.99 <sup>a</sup> <sub>w</sub> | 5.30 <sup>b</sup> <sub>w</sub> | 2.30 <sup>a</sup> <sub>w</sub> | 3.17 <sup>b</sup> <sub>w</sub> | 1.99 <sup>a</sup> <sub>w</sub> | 2.69 <sup>b</sup> <sub>w</sub> | 4.38 <sup>b</sup> <sub>w</sub> | 2.22 <sup>a</sup> <sub>w</sub> |
| Standard Error | 0.66                           | 0.78                           | 0.31                           | 0.61                           | 0.50                           | 0.42                           | 0.87                           | 0.27                           |
| Clover Mean    | 2.57 <sup>a</sup> <sub>w</sub> | 1.56 <sup>a</sup> <sub>w</sub> | 1.80 <sup>a</sup> <sub>w</sub> | 2.57 <sup>a</sup> <sub>w</sub> | 3.05 <sup>a</sup> <sub>x</sub> | 3.22 <sup>a</sup> <sub>x</sub> | 2.06 <sup>a</sup> <sub>x</sub> | 3.94 <sup>a</sup> <sub>x</sub> |
| Standard Error | 1.00                           | 0.31                           | 0.28                           | 0.41                           | 0.55                           | 1.14                           | 0.48                           | 0.83                           |
| Other Mean     | 0.50                           | 0.32                           | 0.48                           | 0.16                           | 0.10                           | 0.11                           | 0.07                           | 0.07                           |
| Standard Error | 0.28                           | 0.11                           | 0.24                           | 0.05                           | 0.05                           | 0.04                           | 0.03                           | 0.01                           |

<sup>1</sup>See Note 1 of Table H-2 for an explanation of the superscript and subscript system for designation of statistical significance.

On all plots there was intense rooting to a depth of 5 to 7 cm., and some roots penetrated to 20 to 25 cm. No differences could be distinguished between the plots.

#### Campus Bins

It was possible to harvest the individually planted buttercup, plantain, and vetch from bins separate from the harvest of the grass/clover material. After the initial winter's treatments, the latter was harvested from eight segments of the area of each bin for replicate weighings. Table H-2, part B summarizes results. There was a small advantage in yield for water treatment as compared with CMA for the grass/clover, but this advantage did not show up with the buttercup or plantain. Vetch was harvested but was too scattered to evaluate.

Because the plants at lower elevation grew more during the season than those at the higher elevation at Pack Forest; a second harvest was made of the grass/clover from these bins in September. Since this harvest could not in any case be comparable to that at Pack Forest, the material was harvested from each bin as a single sample, so statistical treatment was not possible. However, there was a trend toward greater yield at this time with the CMA additions.

These bins were reharvested in June 1987, when grass/clover exhibited significantly greater growth with CMA compared to water treatment. Differences among CMA loading patterns were not significant.

#### Pack Forest Bins

The bins at Pack Forest were harvested as described for the campus bins.

As can be seen in Table H-2, part C, the yields of grass/clover after the first winter were somewhat higher on the controls than on the bins treated with CMA, and this difference was statistically significant. However, no such difference was apparent for the buttercup and plantain. Also, no differences showed up between the methods of application.

The harvest following the second winter demonstrated no significant differences between CMA and water. In this case there were some significant differences between loadings and as a result of the material-loading interaction. However, in conjunction with the campus bin results, there are no outstanding phenomena that indicate large differences in plant yield depending on material, means of application, or loading.

#### Boxes and Free-Standing Saplings

Only qualitative assessments can be made of the results of spraying these plants. They were carefully examined on 24 April and 10 June 1987, with no differences among treatments or adverse symptoms being apparent on controls or CMA sprayed specimens. Symptoms of injury would have included chlorosis, necrosis, abscission, unusual pigmentation (alder), and morphological changes.

## DISCUSSION

### Pot Tests

All of the plants used in the experiments, both in the greenhouse and in the field, are glycophytic species (i.e., are not salt tolerant or resistant). Such species were chosen because they are characteristic

of U.S. roadsides. Earlier experimentation with CMA (4) used artificial greenhouse "soil" mixes, so we worked with field-collected soils of two textures in the pot tests. One was a silt loam (Puyallup), representative of valley soils in most regions. The second was a sandy loam (Pack/Sand), representative of coarser soils in many areas. The latter was in fact a mixture of two parts by volume of the soil from the field plot area and one part of a natural fine sand. This mixture gave improved aeration for the pot tests, but at the same time brought the texture and chemical properties to a range often encountered in coarse soil materials.

A trend seen in most of the pot tests was for some improvement in growth and yield with the smaller additions of CMA (0.5 to 1.0 g/kg soil). This effect is not too surprising, because even on a fertile but somewhat acid soil such as the Puyallup, additions of calcium and/or magnesium are often recommended for maximizing yields. Although such additions are usually made in the form of calcic or dolomitic limestone, CMA can give a similar addition of Ca and Mg. The acetate is decomposed by microorganisms, and the Ca and Mg are left to go on to the exchange complex of the soil.

Adverse effects of larger CMA additions (2.0 and 4.0 g/kg soil) were evident in reduced growth or, often, death of the plants. Both of these additions are higher than ever expected to result from highway runoff or spray of CMA. Although direct osmolarity measurements were not made, these effects were almost certainly caused by osmotic water stress from concentrations of salts being too high in the soil solution in contact with the roots. The fertilizer used contributed somewhat to

this effect, but only with the larger CMA additions did the total osmoticum apparently become strong enough to prevent adequate water uptake by the seedlings.

Delay of planting for two days, after mixing of CMA into the soil and moistening, dramatically improved plant survival (Table H-1). This result can be explained by the exchange reactions between the added CMA (also the fertilizer) and the clay and organic matter of the soil, as well as the consumption of acetate by microorganisms. Both of these processes reduce the concentrations of ions in the soil solution and alleviate the osmotic stress.

#### Field Plots

There were no statistically significant effects of CMA applications on the yields of the plots. Rooting depth and intensity also were very similar in all of the plots. Further, there was no evidence of transitory injury such as leaf spotting, curling, or chlorosis from the treatments.

The means of treating was associated with a significant effect on plant cover. CMA versus water and spray versus flood appeared to shift the species composition somewhat more, according to the statistical analysis, than would be expected on chance. However, this shift is not believed to have any implications for the use of CMA or assessment of its environmental impacts. Both grass and clover provide good, stabilizing roadside covers.

The beneficial effect of small CMA additions in the pot tests was not seen in the plot studies. However, if applications were continued for another year or two, such a favorable influence might be detectable.

Not determinable in this two-year study was the potential effect of long-term applications at the rates used in these first two years. If such additions were made over a five-to-ten year period, Ca and Mg would be expected to saturate the exchange complex. This saturation could lead to a greater leaching of CMA when applied. It also could result in rather high adsorbed Mg in the soil, which could depress yields somewhat, although probably not to a pronounced degree.

#### Soil Bins

Although the rates of application were the same on an area basis to both bins and plots, the depth of soil is, of course, shallower in the bins (although as deep as appreciable rooting occurred in the plots). Also, soil temperatures in the field bins went lower in the winter but warmed up somewhat faster in the spring than soil in the pots. The soil in the bins at the lower elevation (on the campus) was a little warmer in the autumn, winter, and spring than the field bins.

In the bins in the field, the "controls" seemed to yield a little higher, but on the campus the CMA treatments yielded slightly better. This could be related to the temperature differences and the potentially more rapid degradation of acetate in the campus bins.

Even though there were these small differences in yields, the plants grew well in all bins. All treatments looked healthy and vigorous in April 1987, although the campus bins were considerably advanced in growth over the bins at Pack Forest (where frosts were still occurring at night).

In the harvest to be carried out in June 1987, the yields will certainly be higher on the campus bins, but at neither location do we anticipate large influences of the treatments.

#### Boxes and Free Standing Saplings

None of these plants (free-standing Douglas fir and alder saplings, and seedlings of Douglas fir, balsam fir, and maple in boxes) showed any adverse symptoms of spraying with CMA during the winter of 1986-87. Controls and CMA-treated plants all look healthy (observed 24 April 87). Further observations will be made in June 1987 when new growth is developed (buds were just swelling or beginning to burst in April).

#### CONCLUSIONS

Results of laboratory, bin, and controlled plot experiments warrant the following conclusions:

1. In pot tests, CMA in small amounts (0.5 to 1.0 g/kg soil) either had no effect on the plants, or often exerted a favorable influence on growth.
2. In pot tests, CMA in larger amounts (2.0 g/kg soil) depressed growth. CMA at 4.0 g/kg soil (~ 10,000 ppm CMA in the soil solution) killed all seedlings planted immediately after CMA was added and the soil moistened.
3. Most plants survived and grew reasonably well in pots to which 4.0 g CMA/kg soil was added, if planting was delayed for two or more days after CMA was added and the soil moistened. This effect is

attributable to reduction in osmotic stress by adsorption of salts by the soil and degradation of acetate by soil organisms.

4. Survival and growth were better at the higher CMA additions on a silt loam soil (Puyallup) than on a sandy loam soil (Pack/sand). This result is attributable to the greater exchange capacity of the Puyallup soil.
5. In the field plots the vegetation (a "typical roadside complement" of grass, clover, and a few weeds) was equally vigorous with water control and CMA applications, although some species composition shifts were noted.
6. The CMA applied to bins of soil again had no adverse effects on the vegetation (similar to that on the plots); there was a trend toward improved growth with CMA, but this was not significant statistically.
7. A variety of woody saplings and seedlings (Douglas fir, red alder, balsam fir, and red maple) were sprayed repeatedly during the winter with CMA. No adverse effects were noted; treated and control plants appear equally healthy.

Since prospective CMA concentrations in highway runoff and spray are lower than those found to affect plants negatively in these experiments, the results suggest no particular CMA application guidelines to protect roadside vegetation.

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# APPENDIX I

## DEPLETION OF AQUATIC DISSOLVED OXYGEN RESOURCES

### INTRODUCTION

Because acetate is such an excellent substrate for bacterial growth, depletion of oxygen in receiving waters by bacterial respiration presents a major concern in determining the environmental impacts of CMA. To assess this impact, two laboratory biochemical oxygen demand (BOD) experiments were conducted, and field ponds were monitored for oxygen concentrations before, during, and after treatments with CMA. The first BOD experiment was conducted to determine oxygen depletion with various concentrations of CMA, while the second was done to assess the effects of different temperatures on the rate of decomposition of CMA and the resulting oxygen depletion. Field monitoring of the ponds also included a diurnal study to determine if there were daily fluctuations in dissolved oxygen (DO).

### EXPERIMENTAL METHODS

#### Standard BOD Experiment

This experiment was conducted following the basic procedure described by the American Public Health Association (1), with some modifications. The experiment was run for twenty days rather than the standard five. Concentrations of CMA solutions used in the experiment were 10, 100, 1000, and 5000 mg CMA/l, made up by dissolving dry CMA in the prescribed nutrient-enriched dilution water. In addition to these concentrations, two sets of blanks were also prepared, one with the nutrient water and bacterial seed that was added to the CMA solutions,

and the other with nutrient water without the seed. The bacterial seed added to the bottles in a concentration of 1 ml/l was obtained from the primary clarifier at a local sewage treatment plant.

To determine a rate of decomposition, duplicate samples from each of the four CMA concentrations and both blanks were analyzed for dissolved oxygen concentration on each day of the twenty-day experiment. Sample bottles were placed in the dark in a constant temperature room set at 20 C. Dissolved oxygen analysis was done using the azide-modified Winkler method (1). Rate constants were determined by the method of Thomas (2).

#### Multiple-Temperature BOD Experiment

This experiment was set up in a similar manner to the first experiment, with the following exceptions. It was run using only one CMA concentration (10 mg CMA/l, the appropriate concentration determined from the first experiment), but with bottles incubated at three different temperatures, 2, 10 and again 20 C. Dissolved oxygen readings were made with a YSI dissolved oxygen meter, calibrated by the modified Winkler method. The meter was equipped with a probe that allowed readings to be made directly in the bottle without sacrificing its contents. This made it possible to do daily readings on the same bottle, and greatly reduced the number of bottles needed for the experiment. This savings permitted using three replicates in this experiment, rather than only two.

#### Pond Monitoring for Dissolved Oxygen

Dissolved oxygen was measured in the ponds approximately biweekly, with some additional determinations made during, and just after, the

period when CMA applications were made. Samples were collected near midday in standard 300 ml BOD bottles and analyzed by the modified Winkler method. Samples for the diurnal study were collected and analyzed in the same way.

## RESULTS

### Standard BOD Experiment

Figure I-1 illustrates the results of the first BOD experiment using the four different CMA concentrations. Oxygen was completely depleted in the 100, 1000, and 5000 mg CMA/l concentrations after only two days. According to Standard Methods (1), to have a valid BOD test one must have a residual dissolved oxygen concentration of at least 1 mg/l and a depletion of at least 2 mg/l after five days. The 10 mg CMA/l concentration fit this criterion.

Using the points from this curve, the calculated BOD rate constant of the decomposition reaction was  $k = 0.107/\text{day}$ , and the calculated ultimate BOD was 72% of the initial applied CMA concentration. Ultimate BOD is the theoretical maximum oxygen demand that would be exerted in an unlimited time. According to these results, approximately 69% of the ultimate BOD would be exerted in the first five days after entering a water body at 20 C, 83% in the first 10 days, 88% in 15 days, and 92% in 20 days.

### Multiple-Temperature BOD Experiment

The temperature at which the 10 mg CMA /l samples were incubated resulted in large differences in the degradation rate of CMA. Figure I-2 shows the amount of oxygen consumed in bottles incubated at each

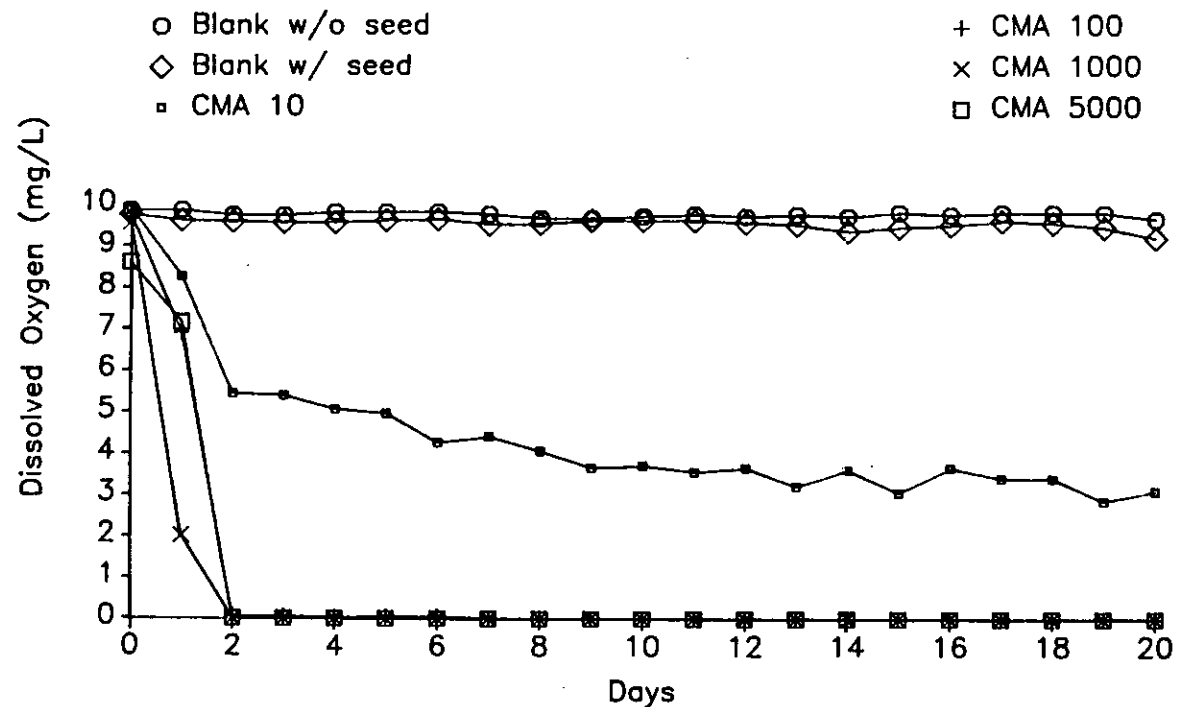


Figure I-1. Oxygen Remaining over Time in 20 C BOD Experiment with Several CMA Concentrations



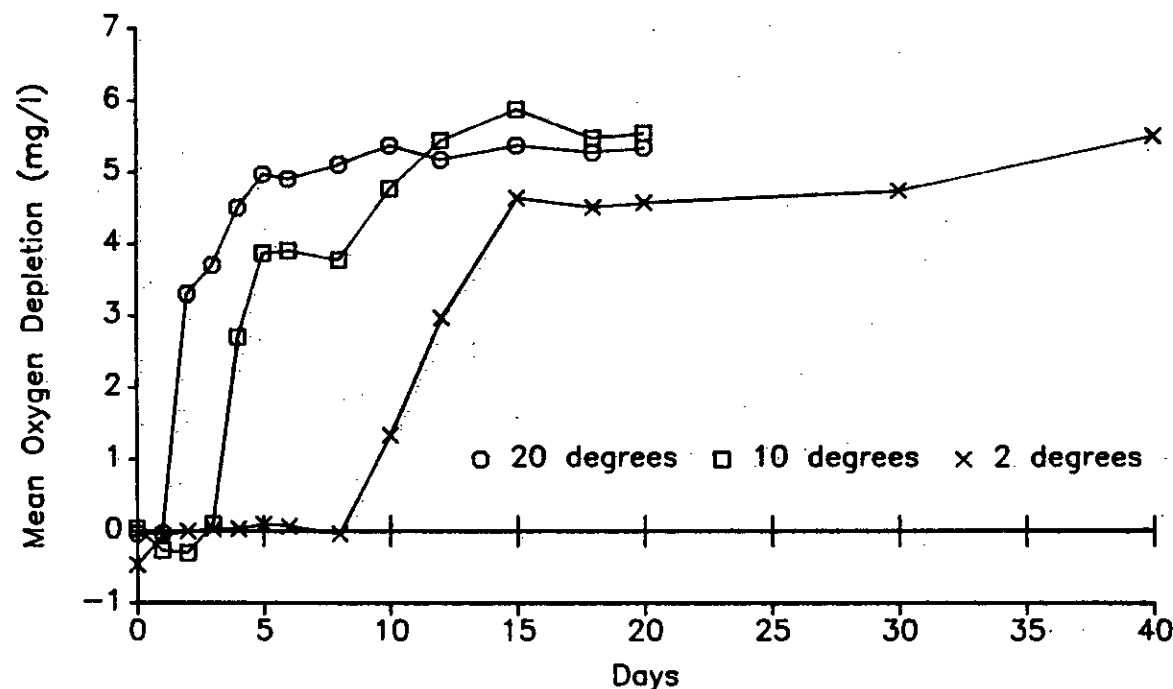


Figure 1-2. Oxygen Depletion over Time in Multiple-Temperature BOD Experiment (CMA Concentration = 10 mg/l)

temperature, calculated by subtracting the mean of the three CMA replicates from the mean of the three seeded blanks. A significant drop in DO was seen after a one-day lag in the 20 C samples, while the 10 C and 2 C samples had lag times before depletion of 3 and 9 days, respectively. Once oxygen depletion commenced at 10 C, it proceeded at approximately the same rate as at 20 C, reached a temporary plateau from days 5 to 8, and then rose to the level in the 20 C bottles by day 12. In addition to the much longer lag time at 2 C, the oxygen was depleted at a slower rate than at the other temperatures once depletion did start. Because of this slower rate, bottles were monitored after 30 and 40 days to determine if depletion would eventually reach the level of the other two temperatures, and indeed it did. Rate constants calculated for the 20, 10, and 2 C experiments were 0.130, 0.064, and 0.020/day, respectively.

#### Pond Monitoring for Dissolved Oxygen

Depletion of dissolved oxygen was very evident in the ponds as a result of CMA input. Figures I-3 and I-4 show dissolved oxygen from September, 1986, to March, 1987, in control and CMA ponds, respectively. Marks and values at the top of the CMA graphs indicate the times and volumes (liters) of direct CMA runoff that each pond received from treatments of the plots that drained into them.

None of the control ponds had DO's below 10 mg/l during or after the treatment period while all CMA ponds had measurements below this level, except Pond 5. This exception is significant, because Pond 5 received only a small amount of direct runoff during the applications. Totalling the measured amounts, Ponds 1, 3, 5, and 8 received 198, 71, 7,

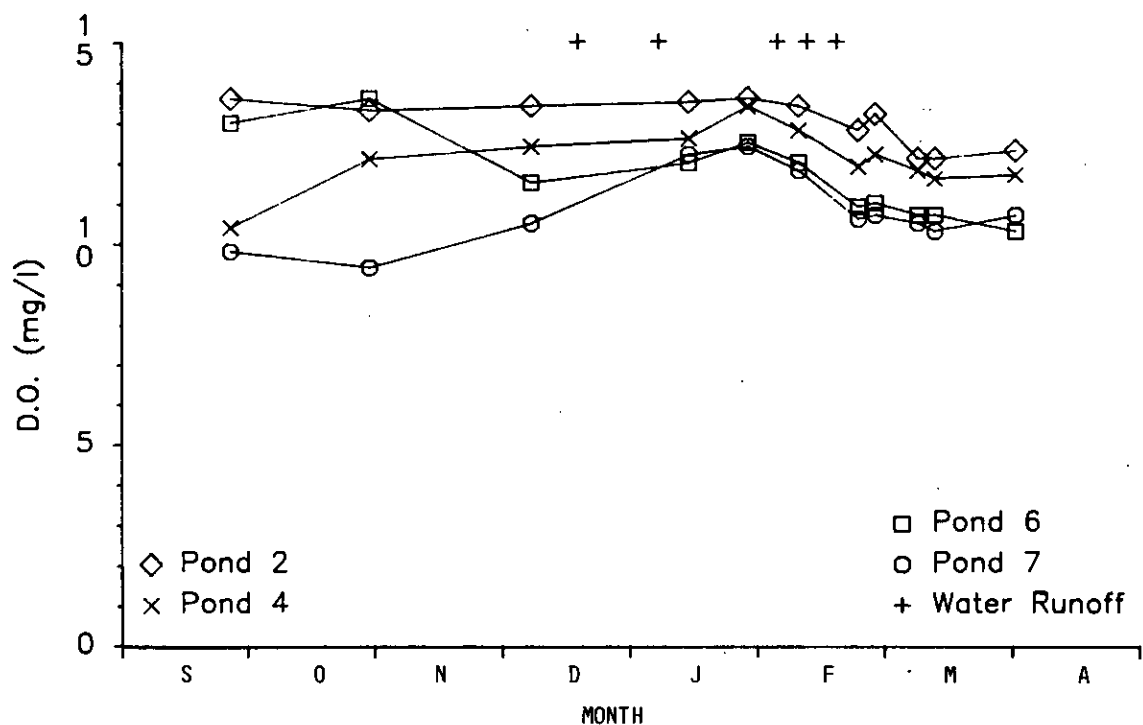


Figure I-3. Dissolved Oxygen in Control Ponds (September, 1986, to April, 1987)

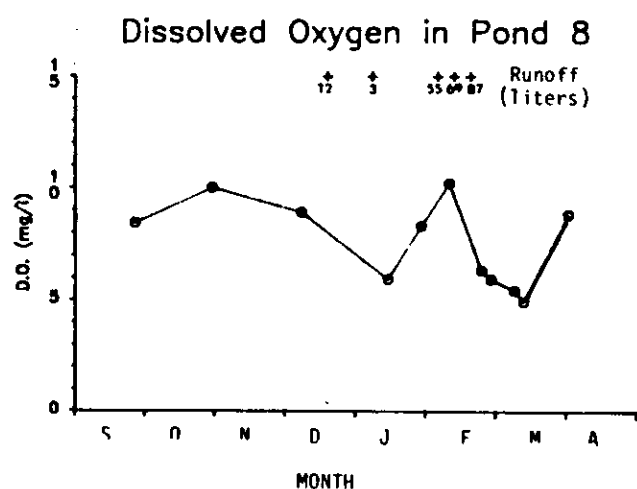
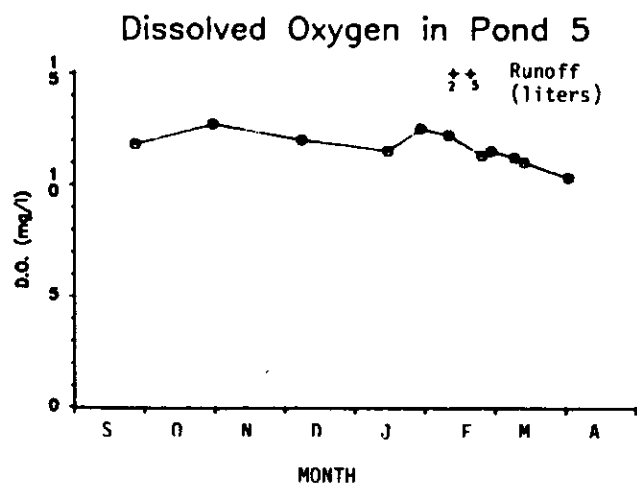
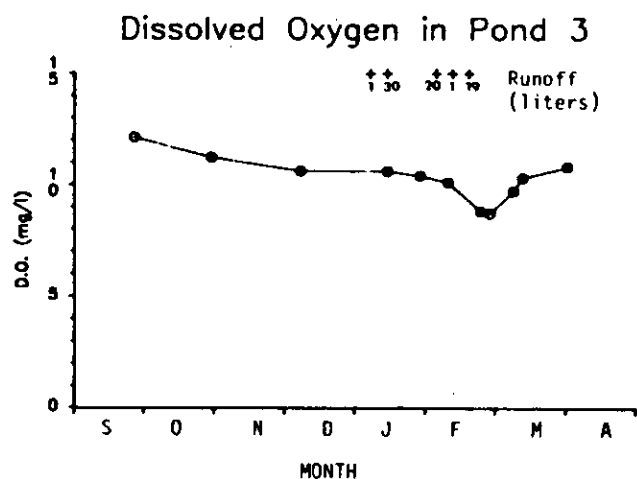
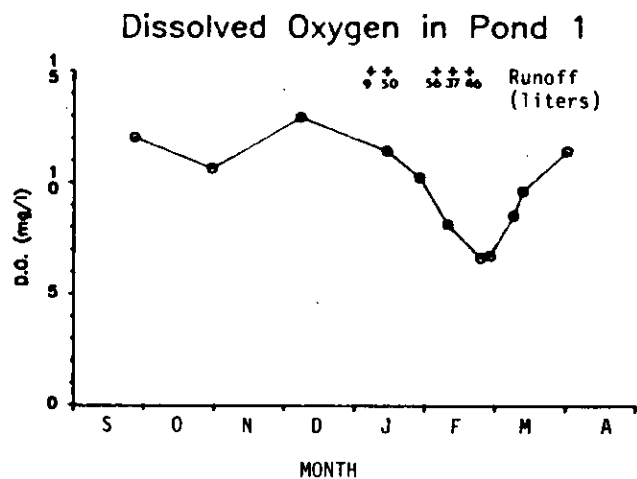


Figure I-4. Dissolved Oxygen in CMA Ponds (September, 1986, to April 1987)

and 227 liters of direct CMA runoff, respectively, or an average of 1.4-45 liters per treatment. These numbers correlate very well with the observed oxygen depletions in each pond. Assuming complete mixing, 33 liters of runoff would give a pond CMA concentration of about 10 mg/l after a treatment.

The ponds showed reasonably predictable responses to the inputs, with depletions starting just after the first loading and minimums recorded just after the final input. Pond 8 continued to show some slightly different responses, however. Dissolved oxygen concentrations in this pond were consistently lower than all the others after the first year's inputs, even extending into the summer that followed (note the low value in September). Its DO dropped after the first two inputs but then rapidly recovered and had a higher concentration on 2/8/87 (10.4 mg/l) than any measurement for that pond in the previous eight months. This increase was in spite of the fact that a large CMA input had been made on 2/3/87. This value is not likely a measurement error, because it was confirmed by several others in that pond during a preliminary diurnal study that day. This pond was also slower to recover after the last input, with concentrations continuing to decrease for several measurements after the last treatment, while DO in Ponds 1 and 3 was increasing at those times.

The full diurnal study was done on 2/21/87 to 2/22/87, with measurements made every six hours over a 24 hour period. Results are shown in the Table I-1. While there are no dramatic findings, slight diurnal fluctuations can be seen in all ponds. In general, ponds had peaks just before dark and minimums just before dawn. This pattern is

Table I-1. Diurnal Study on 2/21/87 - 2/22/87.

|                  |          | Dissolved Oxygen Concentrations (mg/l) at: |      |          |      |
|------------------|----------|--|------|----------|------|
|                  | Pond No. | Noon                                       | 6 PM | Midnight | 6 AM |
| CMA<br>Ponds     | 1        | 6.4  | 6.8  | 6.6      | 6.3  |
|                  | 3        | 8.4  | 8.9  | 8.6      | 8.1  |
|                  | 5        | 11.2                                       | 11.5 | 11.5     | 11.2 |
|                  | 8        | 5.9  | 6.1  | 5.9      | 5.7  |
| Control<br>Ponds | 2        | 12.6                                       | 13.0 | 13.0     | 12.8 |
|                  | 4        | 11.7                                       | 12.0 | 11.9     | 11.7 |
|                  | 6        | 10.9                                       | 11.2 | 10.9     | 10.9 |
|                  | 7        | 10.5                                       | 10.5 | 10.7     | 10.3 |

what might be predicted, due to photosynthesis throughout the day increasing oxygen and respiration through the night decreasing it. The two ponds with the greatest fluctuations were both CMA ponds, but the maximum fluctuation was still only 0.8 mg/l.

## DISCUSSION

There were some small differences in the results of the first BOD experiment for the 10 mg CMA/l concentration at 20 C compared to the later experiment at that temperature. The rate constant,  $k$ , for the first experiment was 0.107, while a value of 0.130 was calculated for the second experiment at 20 C. Although the estimate of this constant was higher for the second experiment, the first experiment actually exhibited a slightly higher peak value of oxygen depletion. There was also no depletion in oxygen in the 20 C run of the multiple-temperature experiment until day two, while the first experiment showed depletion after 1 day of incubation. This difference could be due to a difference in the bacterial seed used: Although both were obtained from the same sewage treatment plant, the seed for the first experiment was very fresh, while that for the multiple-temperature experiment was stored in a cold room for two days before the experiment was set up. Another factor that could account for the small differences was that two different lots of CMA were used for the experiments.

Using stoichiometry, it is estimated that the ultimate BOD should be approximately 75 percent of the initial applied CMA concentration. This concentration agrees very well with the experimentally determined value of 72 percent from the first experiment. Using the stoichiometric

value, the ultimate BOD would be exerted some time in the interval 30 to 40 days for both 10 C and 20 C temperatures, but not until approximately day 100 at 2 C, according to logarithmic or power regression equations derived from the data. The consequence of these results is that CMA runoff into cold surface waters would have an extended oxygen depletion effect compared to the situation at higher temperatures. On the other hand, the effect could occur quickly enough at higher temperatures to prevent sufficiently rapid reaeration from the atmosphere and, therefore, cause a greater effect on aquatic biota.

Although, ideally, runoff from all the plots into the CMA ponds would be identical for them to be true replicates, the fact that differences occurred actually yielded some interesting information. The lack of much oxygen depletion in the pond that received almost no direct CMA runoff indicates that runoff that may occur from rains sometime after CMA addition apparently causes no problems. The inequity in runoff to the ponds also showed the clear association between the amount of runoff and the oxygen depletion.

Large fluctuations were probably not seen in the diurnal study because of the time of year in which it was conducted. The ponds had little algal productivity in February to cause large increases in DO from photosynthesis in the day and DO drops from respiration at night.

It is important to consider the results of the multiple-temperature experiment and the possibilities of diurnal effects in management decisions concerning CMA application. Runoff from a spring snowstorm could reach a receiving water at a time when it is beginning to warm up. Increased water temperatures would cause more rapid

decomposition. Also, increased algal production corresponding to this period of higher light and temperature could cause increased respiration at night. These factors together could result in critical oxygen levels being reached, unless CMA runoff is highly diluted.

## CONCLUSIONS

CMA is quickly degraded at 20 C and rapidly removes oxygen from water. All concentrations higher than 10 mg CMA/l completely depleted oxygen in a 20-day BOD test. Approximately 72% of applied CMA mass is expected to be exerted as BOD ultimately, if the CMA enters water. At 20 C most (~70%) would be exerted within five days, and more than 90% in 20 days.

In a multiple-temperature BOD experiment, decomposition at 10 C was not as complete at day 5 as that at 20 C but caught up by day 20. The overall reaction rate at 10 C was half that at 20 C.

At 2 C nine days passed before any significant oxygen depletion occurred. The overall reaction rate was about one-sixth that at 20 C, and it was estimated that complete decomposition would take approximately 100 days.

Ponds receiving CMA runoff showed oxygen depletion to levels as low as 5 mg/l after input, while control ponds all remained above 10 mg/l during the period. Assuming complete mixing of runoff in the ponds, initial CMA concentrations would be of the order 10 mg CMA/l after a treatment. There was a clear relationship between the amount of runoff a pond received and the depletion of oxygen in the pond.

Diurnal variations in DO concentration were small during the study in February. However, it is likely that they would be larger in late spring and could result in increased depletion of DO from nighttime respiration.

It was estimated in modeling that CMA of order of magnitude 100 mg/l would often be reached in highway runoff. These experiments have shown that ten-fold dilution of such a concentration depleted dissolved oxygen substantially in both laboratory vessels and field ponds. This result, coupled with the demonstrated effects of low DO on aquatic life (see Appendix L) make this issue the most important potential environmental impact issue associated with CMA use. To prevent possible impacts to aquatic life, two guidelines are recommended:

1. CMA should not be applied in catchments when less than 100-fold dilution will be available in waters receiving direct runoff. This guideline holds particularly in waters inhabited by protected aquatic species, of which salmonid fishes are the most sensitive.
2. Extra precaution should be taken in applying CMA after the receiving water has already started to warm.

## REFERENCES

1. American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 16th Ed., American Public Health Association, Washington, D.C. (1985).
2. Metcalf and Eddy, Inc., Wastewater Engineering, McGraw-Hill, Inc., New York, NY, pp. 248-249 (1972).

## APPENDIX J

### OTHER SURFACE WATER CHEMISTRY EFFECTS OF CMA

#### INTRODUCTION

In addition to monitoring of the ponds to assess the impacts of CMA on dissolved oxygen (Appendix I) and aquatic biota (Appendices K and L), both field pond water and plot runoff were analyzed for other standard water quality parameters. These included temperature, pH, specific conductivity, alkalinity, total phosphorus (TP), and metals. Nontypical water quality analyses done, for obvious reasons, were acetate and other volatile fatty acid ions. A few measurements were also made for nitrate and soluble reactive phosphorus. This appendix presents pond water quality data, while Appendix C provides runoff characteristics.

#### EXPERIMENTAL METHODS

Water was collected from the ponds for determinations of conductivity, alkalinity, acetate, total phosphorus, and metals, by grab sampling. A one-liter polyethylene bottle was placed in a specially designed holder attached to a six-foot wooden pole in such a way that the bottle could be extended to the middle of the pond and opened about one-half meter below the surface for filling. Samples were transported to the laboratory in an ice chest. Measurements of pH were done in the field using a Cole-Parmer portable pH meter. Preservation of samples and chemical analyses were done as described in Appendix A.

Runoff samples were collected in clean, 20-liter plastic buckets suspended beneath the plot drainage channel on a long metal pipe. New

buckets were inserted as necessary as they filled. After measurement of runoff volume, a 500-ml polyethylene bottle was filled for analyses. If more than one bucket was needed to collect the runoff, a 500 ml composite sample was taken from all the buckets. Samples were analyzed as described in Appendix A.

#### RESULTS

Tables J-1 and J-2 give means and standard deviations of metals and other constituents, respectively, measured in the field ponds. Major cations and trace metals fluctuated but did not increase in association with treatments. Metals were almost always highest in the intermediate period between treatments, when ponds were being flushed from the supply pond to keep temperatures down and renew oxygen. Figures J-1 and J-2 illustrate time series of specific conductivity, alkalinity, and total phosphorus in all the ponds before, during, and after the winter 1986-87 applications. An obvious effect can be seen for all three parameters in the two ponds that received the most CMA runoff, Ponds 1 and 8. The other two CMA ponds were very similar to the control ponds, although a small increase in all three parameters can be seen in Pond 5 after its two small inputs. Conductivity increased substantially in Pond 3 after CMA input. Where CMA runoff raised TP in ponds, the increase was from approximately 20-25  $\mu\text{g/l}$  to about 40  $\mu\text{g/l}$ . No control ponds showed an effect of the runoff on conductivity and alkalinity, but all exhibited increases in total P after treatments, especially Pond 7.

Table J-1. Means and Standard Deviations of Metals in Ponds Over Four Periods During Field Plot Experiments

| Pond | No.         | Treatment | Period <sup>a</sup> | Concentration (mg/l) |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|------|-------------|-----------|---------------------|----------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
|      |             |           |                     | Ca                   |      | Mg   |      | K    |      | Fe   |      | Cu   |      | Zn   |      | Se   |      | Pb   |      | Al   |      |
|      |             |           |                     | Mean                 | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| 1    | CMA-Spray   | P         |                     | 2.7                  | 0.0  | 1.3  | 0.0  | 0.3  | 0.0  | 0.29 | 0.00 | 0.01 | 0.00 | 0.01 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.40 | 0.00 |
|      |             | T1        |                     | 6.4                  | 7.0  | 1.5  | 0.2  | 0.6  | 0.2  | 0.20 | 0.10 | 0.00 | 0.00 | 0.06 | 0.09 | 0.01 | 0.01 | 0.00 | 0.00 | 0.32 | 0.10 |
|      |             | I         |                     | 13.5                 | 18.3 | 1.8  | 0.8  | 1.1  | 0.9  | 0.43 | 0.42 | 0.01 | 0.01 | 0.31 | 0.60 | 0.02 | 0.01 | 0.02 | 0.01 | 0.17 | 0.10 |
|      |             | T2        |                     | 4.7                  | 1.4  | 3.1  | 1.6  | 1.2  | 0.5  | 0.19 | 0.07 | 0.00 | 0.00 | 0.02 | 0.01 | 0.02 | 0.01 | 0.02 | 0.01 | 0.11 | 0.07 |
| 2    | Water-Spray | P         |                     | 5.4                  | 0.0  | 1.5  | 0.0  | 0.9  | 0.0  | 0.45 | 0.00 | 0.01 | 0.00 | 0.42 | 0.00 | 0.01 | 0.00 | 0.02 | 0.00 | 0.58 | 0.00 |
|      |             | T1        |                     | 7.0                  | 6.7  | 1.5  | 1.3  | 1.4  | 1.9  | 0.18 | 0.02 | 0.00 | 0.00 | 0.25 | 0.41 | 0.01 | 0.00 | 0.01 | 0.00 | 0.29 | 0.05 |
|      |             | I         |                     | 5.0                  | 3.6  | 1.3  | 0.6  | 0.5  | 0.3  | 0.56 | 0.88 | 0.01 | 0.01 | 0.08 | 0.21 | 0.02 | 0.01 | 0.02 | 0.01 | 0.26 | 0.31 |
|      |             | T2        |                     | 2.5                  | 0.3  | 0.7  | 0.1  | 0.3  | 0.0  | 0.11 | 0.01 | 0.00 | 0.00 | 0.01 | 0.00 | 0.02 | 0.01 | 0.06 | 0.07 | 0.07 | 0.02 |
| 3    | CMA-Spray   | P         |                     | 4.6                  | 0.0  | 1.3  | 0.0  | 0.5  | 0.0  | 0.46 | 0.00 | 0.01 | 0.00 | 0.12 | 0.00 | 0.01 | 0.00 | 0.02 | 0.00 | 0.49 | 0.00 |
|      |             | T1        |                     | 2.5                  | 0.2  | 1.0  | 0.1  | 0.3  | 0.0  | 0.16 | 0.03 | 0.00 | 0.00 | 0.02 | 0.02 | 0.01 | 0.00 | 0.01 | 0.00 | 0.23 | 0.05 |
|      |             | I         |                     | 10.2                 | 15.0 | 2.3  | 3.1  | 2.1  | 4.3  | 0.28 | 0.20 | 0.01 | 0.01 | 0.36 | 1.01 | 0.03 | 0.02 | 0.02 | 0.01 | 0.15 | 0.08 |
|      |             | T2        |                     | 3.2                  | 0.4  | 1.5  | 0.5  | 0.9  | 0.1  | 0.24 | 0.07 | 0.00 | 0.00 | 0.02 | 0.01 | 0.02 | 0.01 | 0.03 | 0.03 | 0.15 | 0.10 |
| 4    | Water-Spray | P         |                     | 1.8                  | 0.0  | 0.7  | 0.0  | 0.4  | 0.0  | 0.41 | 0.00 | 0.01 | 0.00 | 0.05 | 0.00 | 0.01 | 0.00 | 0.01 | 0.00 | 0.50 | 0.00 |
|      |             | T1        |                     | 14.6                 | 26.4 | 1.7  | 2.4  | 0.5  | 0.4  | 0.33 | 0.31 | 0.00 | 0.00 | 0.42 | 0.81 | 0.01 | 0.00 | 0.38 | 0.76 | 0.39 | 0.21 |
|      |             | I         |                     | 5.3                  | 5.8  | 1.2  | 0.8  | 1.0  | 1.5  | 0.33 | 0.24 | 0.01 | 0.01 | 0.05 | 0.08 | 0.02 | 0.01 | 0.03 | 0.01 | 0.20 | 0.14 |
|      |             | T2        |                     | 1.9                  | 0.4  | 0.6  | 0.2  | 0.4  | 0.1  | 0.56 | 0.95 | 0.01 | 0.00 | 0.01 | 0.01 | 0.02 | 0.01 | 0.03 | 0.02 | 0.44 | 0.76 |
| 5    | CMA-Flood   | P         |                     | 3.1                  | 0.0  | 1.4  | 0.0  | 0.4  | 0.0  | 0.38 | 0.00 | 0.01 | 0.00 | 0.02 | 0.00 | 0.02 | 0.00 | 0.03 | 0.00 | 0.45 | 0.00 |
|      |             | T1        |                     | 1.6                  | 0.3  | 0.6  | 0.1  | 0.3  | 0.0  | 0.11 | 0.05 | 0.00 | 0.00 | 0.02 | 0.02 | 0.01 | 0.00 | 0.01 | 0.00 | 0.19 | 0.06 |
|      |             | I         |                     | 6.5                  | 5.7  | 1.2  | 0.6  | 0.7  | 0.4  | 0.27 | 0.14 | 0.01 | 0.01 | 0.07 | 0.10 | 0.03 | 0.01 | 0.02 | 0.01 | 0.20 | 0.14 |
|      |             | T2        |                     | 2.1                  | 0.3  | 0.8  | 0.1  | 0.7  | 0.1  | 0.11 | 0.04 | 0.00 | 0.00 | 0.02 | 0.01 | 0.02 | 0.01 | 0.05 | 0.07 | 0.14 | 0.05 |
| 6    | Water-Flood | P         |                     | 5.5                  | 0.0  | 1.0  | 0.0  | 0.4  | 0.0  | 0.36 | 0.00 | 0.01 | 0.00 | 0.20 | 0.00 | 0.01 | 0.00 | 0.02 | 0.00 | 0.44 | 0.00 |
|      |             | T1        |                     | 2.5                  | 0.4  | 1.0  | 0.2  | 0.4  | 0.2  | 0.34 | 0.27 | 0.01 | 0.00 | 0.03 | 0.02 | 0.02 | 0.01 | 0.02 | 0.01 | 0.47 | 0.33 |
|      |             | I         |                     | 5.3                  | 3.1  | 1.4  | 0.6  | 0.7  | 0.4  | 0.29 | 0.19 | 0.01 | 0.01 | 0.03 | 0.05 | 0.02 | 0.01 | 0.02 | 0.01 | 0.19 | 0.14 |
|      |             | T2        |                     | 2.9                  | 0.3  | 0.8  | 0.1  | 0.8  | 0.1  | 0.21 | 0.18 | 0.00 | 0.00 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | 0.03 | 0.23 | 0.31 |
| 7    | Water-Flood | P         |                     | 2.5                  | 0.0  | 1.0  | 0.0  | 0.4  | 0.0  | 0.42 | 0.00 | 0.01 | 0.00 | 0.04 | 0.00 | 0.02 | 0.00 | 0.02 | 0.00 | 0.45 | 0.00 |
|      |             | T1        |                     | 1.8                  | 0.2  | 0.7  | 0.1  | 0.4  | 0.0  | 0.10 | 0.06 | 0.00 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.00 | 0.13 | 0.08 |
|      |             | I         |                     | 4.7                  | 2.1  | 1.2  | 0.6  | 0.8  | 0.6  | 0.40 | 0.29 | 0.01 | 0.00 | 0.04 | 0.05 | 0.03 | 0.01 | 0.02 | 0.01 | 0.17 | 0.16 |
|      |             | T2        |                     | 1.9                  | 0.4  | 0.5  | 0.1  | 0.6  | 0.2  | 0.21 | 0.10 | 0.00 | 0.00 | 0.02 | 0.01 | 0.02 | 0.01 | 0.03 | 0.03 | 0.09 | 0.07 |
| 8    | CMA-Flood   | P         |                     | 2.3                  | 0.0  | 1.0  | 0.0  | 0.3  | 0.0  | 0.32 | 0.00 | 0.00 | 0.00 | 0.03 | 0.00 | 0.02 | 0.00 | 0.02 | 0.00 | 0.39 | 0.00 |
|      |             | T1        |                     | 3.7                  | 0.7  | 2.1  | 0.6  | 0.8  | 0.2  | 0.15 | 0.08 | 0.00 | 0.00 | 0.03 | 0.02 | 0.01 | 0.00 | 0.01 | 0.00 | 0.28 | 0.13 |
|      |             | I         |                     | 6.0                  | 4.3  | 1.6  | 0.3  | 0.9  | 0.7  | 0.37 | 0.22 | 0.01 | 0.00 | 0.06 | 0.15 | 0.03 | 0.01 | 0.03 | 0.02 | 0.14 | 0.13 |
|      |             | T2        |                     | 5.2                  | 2.3  | 3.4  | 2.2  | 1.6  | 0.7  | 0.41 | 0.14 | 0.00 | 0.00 | 0.01 | 0.00 | 0.02 | 0.01 | 0.03 | 0.02 | 0.08 | 0.01 |

<sup>a</sup> P--Prior to first treatment (1/86-2/86)

T1--First treatment period and one month after (3/4/86-4/25/86)

I--Intermediate period between T1 and T2

(4/26/86-12/17/86)

T2--Second treatment period and afterward as long as data are available (12/18/86-3/31/87)

Table J-2. Means and Standard Deviations of Eight Constituents in Ponds  
Over Four Periods During Field Plot Experiments

| Pond<br>No. | Treatment   | Period <sup>a</sup> | Temperature |      |        |      | Diss. Oxygen |      |                 |      | Spec. Cond.               |      |        |      | Alkalinity |      |        |      | Acetate |      |        |      | Total P |      |  |  | Chl a |  |  |  |
|-------------|-------------|---------------------|-------------|------|--------|------|--------------|------|-----------------|------|---------------------------|------|--------|------|------------|------|--------|------|---------|------|--------|------|---------|------|--|--|-------|--|--|--|
|             |             |                     | (C)         |      | (mg/l) |      | pH           |      | ( $\mu$ mho/cm) |      | (mg/l CaCO <sub>3</sub> ) |      | (mg/l) |      | (mg/l)     |      | (mg/l) |      | (mg/l)  |      | (mg/l) |      | (mg/l)  |      |  |  |       |  |  |  |
|             |             |                     | Mean        | S.D. | Mean   | S.D. | Mean         | S.D. | Mean            | S.D. | Mean                      | S.D. | Mean   | S.D. | Mean       | S.D. | Mean   | S.D. | Mean    | S.D. | Mean   | S.D. | Mean    | S.D. |  |  |       |  |  |  |
| 1           | CMA-Spray   | P                   | NA          |      | NA     |      |              | 6.5  | 0.0             | 41   | 0                         | 15   | 0      | 0    | 0          | 0    | 0      | 39   | 0       | 0.3  | 0.0    |      |         |      |  |  |       |  |  |  |
|             |             | T1                  | NA          |      | 10.5   | 0.8  | 7.2          | 0.6  | 45              | 5    | 14                        | 3    | 0      | 0    | 0          | 0    | 28     | 7    | 5.9     | 4.0  |        |      |         |      |  |  |       |  |  |  |
|             |             | I                   | 14.4        | 7.7  | 10.0   | 1.7  | 7.0          | 0.5  | 41              | 12   | 21                        | 6    | 0      | 0    | 0          | 0    | 46     | 24   | 4.9     | 2.6  |        |      |         |      |  |  |       |  |  |  |
|             |             | T2                  | 6.6         | 2.9  | 9.1    | 1.9  | 7.2          | 0.3  | 51              | 18   | 26                        | 9    | 1      | 2    | 33         | 6    | 6.9    | 2.5  |         |      |        |      |         |      |  |  |       |  |  |  |
| 2           | Water-Spray | P                   | NA          |      | NA     |      |              | 6.5  | 0.0             | 34   | 0                         | 20   | 0      | 0    | 0          | 0    | 0      | 40   | 0       | 0.1  | 0.0    |      |         |      |  |  |       |  |  |  |
|             |             | T1                  | NA          |      | 10.4   | 0.1  | 6.8          | 0.5  | 32              | 4    | 11                        | 2    | 0      | 0    | 0          | 0    | 26     | 6    | 1.1     | 1.0  |        |      |         |      |  |  |       |  |  |  |
|             |             | I                   | 14.4        | 7.5  | 10.4   | 2.3  | 7.3          | 1.0  | 35              | 15   | 20                        | 8    | 0      | 0    | 0          | 0    | 44     | 20   | 5.9     | 6.3  |        |      |         |      |  |  |       |  |  |  |
|             |             | T2                  | 6.6         | 3.0  | 12.9   | 0.6  | 8.8          | 0.3  | 23              | 5    | 10                        | 2    | 0      | 0    | 0          | 15   | 4      | 2.7  | 1.6     |      |        |      |         |      |  |  |       |  |  |  |
| 3           | CMA-Spray   | P                   | NA          |      | NA     |      |              | 6.5  | 0.0             | 35   | 0                         | 9    | 0      | 0    | 0          | 0    | 0      | 46   | 0       | NA   |        |      |         |      |  |  |       |  |  |  |
|             |             | T1                  | NA          |      | 10.4   | 1.1  | 7.0          | 0.4  | 36              | 5    | 12                        | 3    | 0      | 0    | 0          | 0    | 27     | 14   | 1.1     | 1.0  |        |      |         |      |  |  |       |  |  |  |
|             |             | I                   | 14.2        | 6.8  | 9.9    | 1.4  | 7.1          | 0.4  | 38              | 12   | 21                        | 8    | 0      | 0    | 0          | 0    | 48     | 27   | 5.0     | 3.7  |        |      |         |      |  |  |       |  |  |  |
|             |             | T2                  | 6.5         | 2.9  | 9.9    | 0.8  | 7.0          | 0.1  | 32              | 8    | 15                        | 2    | 0      | 0    | 0          | 25   | 4      | 8.1  | 1.7     |      |        |      |         |      |  |  |       |  |  |  |
| 4           | Water-Spray | P                   | NA          |      | NA     |      |              | 6.6  | 0.0             | 26   | 0                         | 6    | 0      | 0    | 0          | 0    | 0      | 64   | 0       | 1.2  | 0.0    |      |         |      |  |  |       |  |  |  |
|             |             | T1                  | NA          |      | 10.3   | 1.2  | 7.1          | 0.3  | 20              | 4    | 9                         | 2    | 0      | 0    | 0          | 0    | 40     | 11   | 4.7     | 3.1  |        |      |         |      |  |  |       |  |  |  |
|             |             | I                   | 14.4        | 6.9  | 10.0   | 1.9  | 7.1          | 0.5  | 33              | 17   | 18                        | 9    | 0      | 0    | 0          | 0    | 55     | 27   | 7.9     | 7.4  |        |      |         |      |  |  |       |  |  |  |
|             |             | T2                  | 6.6         | 2.9  | 12.3   | 0.6  | 7.5          | 0.1  | 18              | 5    | 7                         | 2    | 0      | 0    | 0          | 23   | 4      | 3.1  | 1.3     |      |        |      |         |      |  |  |       |  |  |  |
| 5           | CMA-Flood   | P                   | NA          |      | NA     |      |              | 6.5  | 0.0             | 43   | 0                         | 16   | 0      | 0    | 0          | 0    | 0      | 38   | 0       | 1.3  | 0.0    |      |         |      |  |  |       |  |  |  |
|             |             | T1                  | NA          |      | 11.4   | 0.5  | 7.1          | 0.5  | 25              | 3    | 7                         | 2    | 0      | 0    | 0          | 0    | 33     | 10   | 2.8     | 1.1  |        |      |         |      |  |  |       |  |  |  |
|             |             | I                   | 14.2        | 7.0  | 10.1   | 1.6  | 7.1          | 0.7  | 35              | 16   | 19                        | 8    | 0      | 0    | 0          | 0    | 51     | 32   | 6.4     | 4.6  |        |      |         |      |  |  |       |  |  |  |
|             |             | T2                  | 6.6         | 2.9  | 11.4   | 0.7  | 6.9          | 0.2  | 24              | 6    | 10                        | 1    | 0      | 0    | 0          | 29   | 5      | 6.8  | 2.0     |      |        |      |         |      |  |  |       |  |  |  |
| 6           | Water-Flood | P                   | NA          |      | NA     |      |              | 6.5  | 0.0             | 29   | 0                         | 6    | 0      | 0    | 0          | 0    | 0      | 53   | 0       | 2.6  | 0.0    |      |         |      |  |  |       |  |  |  |
|             |             | T1                  | NA          |      | 10.5   | 0.5  | 7.1          | 0.2  | 35              | 6    | 13                        | 5    | 0      | 0    | 0          | 0    | 48     | 13   | 2.5     | 2.6  |        |      |         |      |  |  |       |  |  |  |
|             |             | I                   | 14.4        | 6.9  | 10.8   | 2.0  | 7.1          | 1.1  | 38              | 14   | 21                        | 8    | 0      | 0    | 0          | 0    | 47     | 22   | 8.2     | 8.1  |        |      |         |      |  |  |       |  |  |  |
|             |             | T2                  | 6.6         | 2.9  | 11.3   | 0.8  | 7.0          | 0.2  | 25              | 5    | 11                        | 2    | 0      | 0    | 0          | 22   | 4      | 5.6  | 1.7     |      |        |      |         |      |  |  |       |  |  |  |
| 7           | Water-Flood | P                   | NA          |      | NA     |      |              | 6.5  | 0.0             | 33   | 0                         | 4    | 0      | 0    | 0          | 0    | 0      | 47   | 0       | 0.7  | 0.0    |      |         |      |  |  |       |  |  |  |
|             |             | T1                  | NA          |      | 10.5   | 0.3  | 6.9          | 0.4  | 28              | 4    | 15                        | 12   | 0      | 0    | 0          | 0    | 18     | 10   | 2.0     | 1.6  |        |      |         |      |  |  |       |  |  |  |
|             |             | I                   | 14.1        | 6.9  | 9.4    | 1.2  | 6.8          | 0.4  | 37              | 15   | 20                        | 8    | 0      | 0    | 0          | 0    | 51     | 18   | 7.5     | 4.8  |        |      |         |      |  |  |       |  |  |  |
|             |             | T2                  | 6.1         | 2.3  | 11.2   | 0.8  | 6.8          | 0.2  | 18              | 5    | 8                         | 2    | 0      | 0    | 0          | 34   | 9      | 8.3  | 2.4     |      |        |      |         |      |  |  |       |  |  |  |
| 8           | CMA-Flood   | P                   | NA          |      | NA     |      |              | 6.5  | 0.0             | 31   | 0                         | 3    | 0      | 0    | 0          | 0    | 0      | 39   | 0       | 0.4  | 0.0    |      |         |      |  |  |       |  |  |  |
|             |             | T1                  | NA          |      | 9.0    | 1.6  | 7.2          | 0.3  | 49              | 8    | 22                        | 11   | 0      | 0    | 0          | 0    | 25     | 4    | 0.5     | 0.3  |        |      |         |      |  |  |       |  |  |  |
|             |             | I                   | 13.8        | 6.8  | 8.9    | 1.3  | 6.8          | 0.5  | 42              | 9    | 23                        | 5    | 0      | 0    | 0          | 0    | 50     | 26   | 9.2     | 6.1  |        |      |         |      |  |  |       |  |  |  |
|             |             | T2                  | 5.7         | 2.7  | 7.0    | 1.9  | 7.0          | 0.1  | 55              | 24   | 29                        | 11   | 6      | 9    | 30         | 6    | 6.2    | 2.3  |         |      |        |      |         |      |  |  |       |  |  |  |

<sup>a</sup> P--Prior to first treatment (1/86-2/86)

T1--First treatment period and one month afterward (3/4/86-4/25/86)

I--Intermediate period between T1 and T2 (4/26/86-12/17/86)

T2--Second treatment period and afterward as long as data are available (12/18/86-3/31/87)

NA--Not available



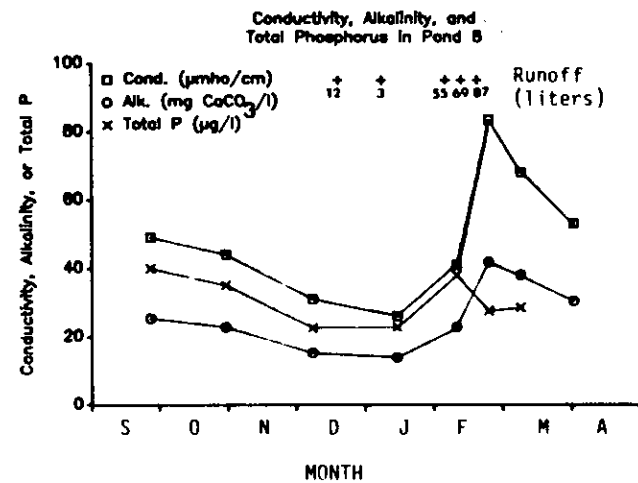
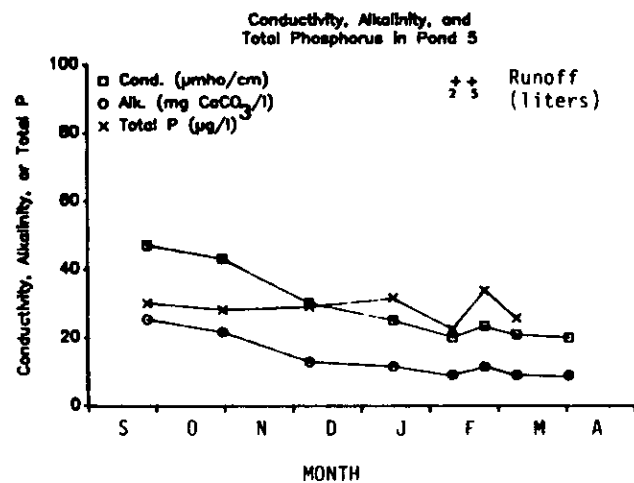
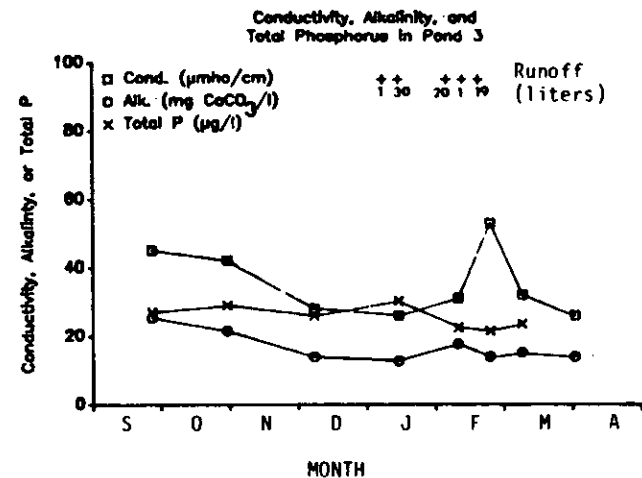
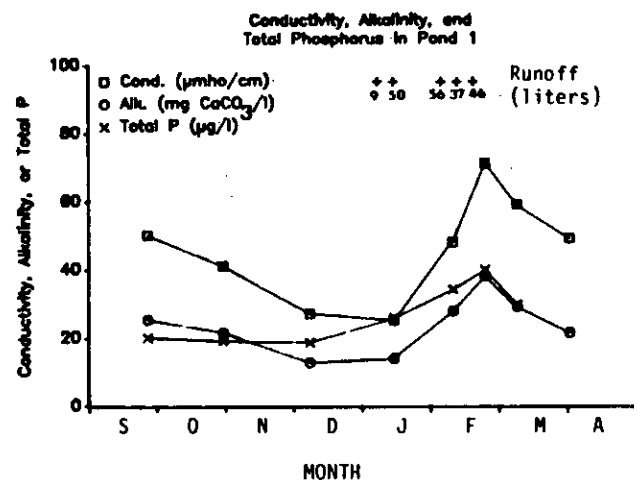


Figure J-1. CMA Pond Conductivity, Alkalinity, and Total Phosphorus

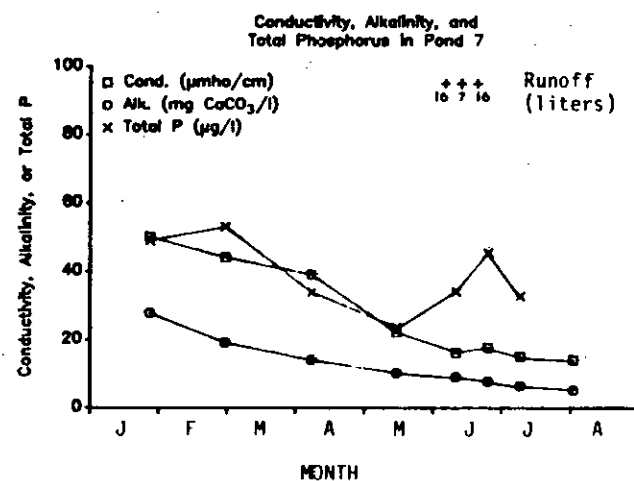
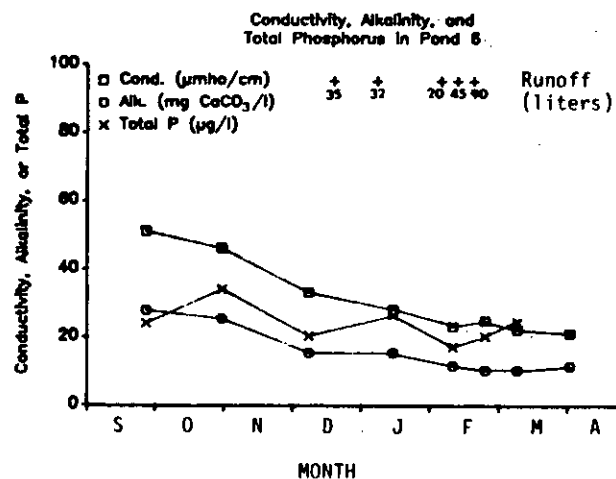
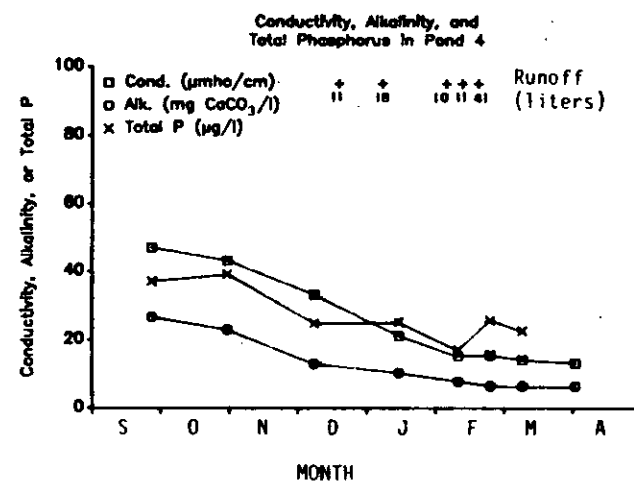
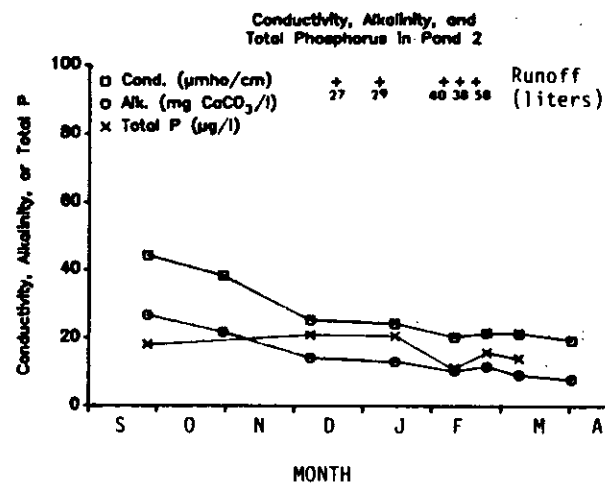


Figure J-2. Water Pond Conductivity, Alkalinity, and Total Phosphorus

Table J-3 shows combined phosphorus loadings to the ponds from all 1986-87 treatments, as well as volume-weighted total P concentrations in the original water and CMA solutions used to treat the plots. While total P in the CMA runoff was about equal to or less than the applied solution, the concentrations were much higher in the runoff from the control plots than in the original water from the tank. Apparently, CMA solutions either remained constant in P or declined due to removal processes on the plots, while the control plots contributed P to the runoff.

Although ponds received acetate in runoff, it apparently decomposed to below detectable concentration before pond sampling occurred on most occasions. In the first winter's treatments, the ponds received very little direct runoff, with the exception of Pond 8; and acetate was never measured. In the second winter, because of higher application rates, ponds received more direct runoff. Pond 1 had 4 mg acetate/l on 2/8/87, while Pond 8 had 4, 19, and 2 mg acetate/l on 2/8, 2/22, and 3/8/87, respectively. CMA treatment ponds 3 and 5 and all control ponds failed to show any detectable concentrations of acetate.

With the exception of a drop in pH that appeared to correspond with CMA runoff in Pond 1, there were no obvious effects of CMA on pond pH.

#### DISCUSSION

In general, it was observed that direct CMA runoff increased alkalinity, conductivity, and total P in the ponds in proportion to the amount of runoff they received. However, some results are not

Table J-3. Phosphorus Loadings to Field Ponds During 1986-87 Treatments

| <u>Pond or Tank</u>  | <u>Total Runoff (l)</u> | <u>Volume-Weighted Mean TP Concentration (µg/l)</u> | <u>TP Loading (mg)</u> |
|----------------------|-------------------------|---|------------------------|
| <u>CMA Ponds</u>     |                         |   |                        |
| 1                    | 198                     | 572   | 113                    |
| 3                    | 71                      | 533   | 38                     |
| 5                    | 7                       | 453   | 3                      |
| 8                    | 227                     | 531   | 121                    |
| <u>Control Ponds</u> |                         |   |                        |
| 2                    | 192                     | 255   | 49                     |
| 4                    | 91                      | 211   | 19                     |
| 6                    | 223                     | 137   | 30                     |
| 7                    | 39                      | 122   | 5                      |
| <u>Supply Tanks</u>  |                         |   |                        |
| CMA                  | --                      | 598   | --                     |
| Water                | --                      | 56  | --                     |

completely understood. It is difficult to explain why alkalinity and total P did not exhibit any increase in Pond 3 corresponding to the conductivity increase, especially considering that all ponds, including controls, showed some total P increase after treatments.

The increases observed in TP in control ponds after water treatments could be due to runoff transport of fertilizer and P-rich decaying plant matter from the plots. However, the very large increase seen in total P in Pond 7 is difficult to explain, considering it had the lowest volume-weighted mean P concentration, as well as the lowest P loading. It is not likely that this result is due to measurement error, because algal biomass as measured by chlorophyll *a* also increased during this time (see Appendix K). It could be the result of high precipitation and subsequent runoff simultaneous with the treatment period.

Results for Ponds 1 and 8, particularly, demonstrated that CMA of the characteristics used in this research has a potential to advance surface water eutrophication. Runoff quickly approximately doubled pond total phosphorus concentrations. This increase occurred at the onset of the growing season, when algae could make the most effective use of nutrient supplies. Development of the algal community will be followed this spring to determine the responses to the higher phosphorus additions accompanying the 1986-87 treatments.

Acetate was probably not observed in the first winter's treatments due to the small amounts of runoff and the sampling schedule. With the exception of a sampling occasion four days after the second treatment, ponds were sampled more than 10 days after any treatments were made.

Assuming complete mixing of all CMA runoff in the ponds, 20 liters of the 3000 mg CMA/l solution would yield a pond concentration of 6 mg CMA/l. Because acetate makes up about 2/3 of CMA by weight, a maximum of about 4 mg acetate/l would be expected immediately after application. With the time for decomposition, it is not surprising that it was not detected. In the second winter's applications, acetate was detected due to higher runoff volumes, and sampling dates less than one week after applications. Still, it was only observed in the two ponds with the highest runoff volumes.

A drop in pH might be anticipated as a result of decomposition of acetate producing CO<sub>2</sub>. This effect was seen in only one pond, however.

#### CONCLUSIONS

Three of the CMA ponds exhibited increases in total phosphorus, alkalinity, and conductivity coincident with CMA runoff, while one showed an increase only in conductivity. No obvious changes in pH were observed as a result of CMA input to ponds. Acetate was detectable only in the ponds with very high CMA loadings, and it was quickly decomposed.

Control ponds increased only in total phosphorus after water treatments, but this increase was not as great as in the CMA treatment ponds. This increase was probably due to transport of phosphorus generated from fertilizer and decaying vegetation on the plots. High phosphorus content in CMA manufactured from agricultural wastes could cause algal blooms, especially if applied in late spring just prior to optimal conditions for growth.

This possibility of enriching surface waters should be taken into account in using CMA and assessing its potential environmental impact. The strongest tendency toward eutrophication is likely to occur in small ponds and lakes that receive extensive CMA runoff, especially if they are poorly flushed. In these circumstances consideration should be given to avoiding CMA use, or at least avoiding its use when it could be delivered to the water body in significant quantities in the late winter or early spring. As discussed in Appendices I and L, this same precaution would protect against dissolved oxygen depletion in waters with sensitive animal life.

## APPENDIX K

### AQUATIC MICROORGANISM GROWTHS

#### INTRODUCTION

The effects of CMA on aquatic microorganisms was assessed in the laboratory with algal bioassays and in the field by monitoring growth in the ponds. The original project proposal designated only testing for the effects of CMA on planktonic algae in both the laboratory and field. However, because of some interesting observations and the relatively small amount of additional work involved, these tests were extended to include measurements of bacteria and periphyton.

Tests run in the laboratory included a single species phytoplankton bioassay using Selenastrum capricornutum, and a periphyton experiment run in artificial streams concurrently with the flow-through invertebrate bioassay discussed in Appendix L. Bacterial counts were also made in the test containers used in the Selenastrum bioassay.

In the ponds, phytoplankton and periphyton were quantified by using chlorophyll a as an indicator of biomass. Some indirect estimates of free-floating and attached bacteria were also made by determining ash-free dry weights (total volatile organics) of samples.

#### EXPERIMENTAL METHODS

##### Phytoplankton Bioassay

General test procedures for the laboratory phytoplankton bioassay were those for the standard EPA algal assay (1) and the American Public Health Association (2) using the green alga Selenastrum capricornutum. Culture medium containing macro- and micronutrients was prepared as in

these protocols. Test vessels utilized in the experiment were clean, 125 ml Erlenmeyer flasks loosely covered with aluminum foil.

Triplicate flasks were set up for a control and seven CMA concentrations. CMA concentrations used were 1, 10, 50, 100, 500, 1000, and 4000 mg CMA/l, made up by adding the appropriate amounts of dried CMA to the nutrient media. Although not within the defined scope of this study, an identical set of flasks was set up using sodium chloride in the same concentrations. These samples were intended to indicate whether or not osmotic effects were the likely explanation for algal growth observations. Solutions were filtered through a glass fiber filter to eliminate particulates, which would interfere with algal counts made on an electronic particle counter. Each flask received 40 ml of solution and was inoculated with an appropriate volume of a stock Selenastrum culture to yield an initial cell concentration of about 1000 cells/ml.

Test flasks were placed on a shaker table in a constant temperature room set at 24 C under continuous cool white fluorescent lighting at 3800 lux. Experiments were originally scheduled for 14 days but were terminated at 10 days for reasons explained in the Results section.

Algal biomass measurements were made daily for the first seven days of the experiment and every other day thereafter. Cell concentrations were determined indirectly using a Coulter Counter Model ZBI electronic particle counter, and cell volumes were calculated using a Coulter Channelyzer mean cell volume computer. Algal biomass (mg DW/l) was established according to Miller et al. (1) as follows:

$$\text{Biomass} = \text{Cell concentration (cells/ml)} \times \\ \text{Mean cell volume } (\mu\text{m}^3) \times 3.6 \times 10^{-7}$$

Analysis of variance was used to test for differences between means, and IC<sub>50</sub> values (concentration creating 50 percent inhibition relative to controls) were calculated according to the method of Joubert (3).

Bacteria populations were also enumerated using the Coulter Counter, but with a smaller size setting than used for the Selenastrum. Mean cell volumes were not computed for bacteria, however.

#### Laboratory Periphyton Bioassay

The periphyton bioassay in artificial streams was an added experiment run concurrently with the invertebrate bioassay described in Appendix L. A complete description of the system is found in that section. The channels were originally designed for periphyton experiments; thus, modifications to the streams were not necessary to add this test to the originally planned experiment.

Three plexiglass slides, 8 cm by 5 cm, were placed in specially designed holders in each channel. Slides were allowed to accumulate periphyton for the entire 14 days of the experiment. At that time, slides were removed and all the algae was washed and scraped off and filtered onto a glass fiber filter. The samples were then analyzed for chlorophyll a as described below. Scrapings were also taken from channel walls and preserved with Lugol's solution for microscopic identification of species composition.

#### Pond Sampling for Microorganisms

Pond water was grab sampled biweekly and analyzed for chlorophyll a. From the start of field sampling in January 1986 through September

of 1986, measurements were made using a Turner model 110 fluorometer calibrated with a spectrophotometer (4). At that point the fluorometer malfunctioned, and all subsequent measurements analyses were done directly on a spectrophotometer (5).

In the summer of 1986, periphyton samplers were installed in the ponds to provide an additional measurement of the aquatic community biomass. The same 8 cm by 5 cm plexiglass slides used in the laboratory periphyton bioassay were used in the ponds. Nine slides were suspended in each pond using specially constructed units. Each slide was held in a spring-jawed plastic clothespin that was hung via holes in its handles from a 1/8-inch diameter plexiglass rod. The rod was suspended by nylon cord 1/2 meter below a bottle used as a float. The entire unit was anchored to the pond bottom to prevent drift.

The nine slides per pond allowed three replicates to be sampled each month with a 3-month accumulation period before resampling. Samples were taken and analyzed in the same fashion as the laboratory periphyton.

Following the 1987 CMA applications, some measurements were also made of ash-free dry weights of particulates in pond water and on periphyton slides. Analyses were done following the total residual solids method outlined by the American Public Health Association (2). The purpose of these measurements was to get an indication of nonalgal suspended and attached organisms by relating the ash-free dry weight (AFDW) to chlorophyll *a*. Because AFDW includes both algae and other organisms such as bacteria and fungi, this ratio gives the proportion of biomass made up by algae. Ponds with the highest chlorophyll *a*-to-AFDW

ratio would have a higher percentage of their biomass composed of algae, while ponds with lower ratios would have higher abundances of other microorganisms.

## RESULTS

### Phytoplankton Bioassay

The algal bioassay showed very clear evidence of an inhibitory effect of CMA on the growth of *Selenastrum*. The IC<sub>50</sub> was 11.5 mg CMA/l. Figure K-1 displays the growth curves of the means of the three replicate flasks for the control and the four lowest concentrations of CMA. The 500, 1000, and 5000 mg CMA/l curves are not shown, because they were virtually indistinguishable from the 100 mg CMA/l treatment, showing essentially no growth of algae. Algal biomass reached its peak and then either leveled off or declined after day 7 of the experiment. Therefore, biomass levels from this day were used in statistical analyses of maximum standing crops. Analysis of variance showed a statistical difference between means ( $p < 0.001$ ). Using the Student-Newman-Keuls multiple comparison test with  $\alpha = 0.05$ , biomass on day 7 was significantly greater in the control than in the 1 mg CMA/l, greater in the 1 mg/l than in the 10 mg CMA/l, and greater in the 10 mg CMA/l than in the 50 mg CMA/l treatment. Significant differences could not be detected between the 50 mg CMA/l concentration and all higher concentrations. In summary:

Control > 1 > 10 > 50 = 100 = 500 = 1000 = 4000

The parallel experiment using sodium chloride in the same concentrations as CMA exhibited considerable differences from the

results of the CMA additions (Figure K-2). There was no significant difference ( $\alpha = 0.05$ ) in biomass on day 7 between the control and all concentrations up through 1000 mg NaCl/l. Inhibition from NaCl was only evident in the 4000 mg NaCl treatment, which had significantly lower biomass than all other treatments. Summarizing:

Control = 1 = 10 = 50 = 100 = 500 = 1000 > 4000

Measurements of bacteria populations in the control and CMA treatments on five days during the bioassay showed highest cell numbers in the three highest CMA concentrations (Figure K-3). Again using analysis of variance and the Student-Newman-Keuls test, cell counts on day 7 were significantly different between each of the three highest concentrations but not significantly different between the control, 1, 10, 50, and 100 mg CMA/l. In summary of bacteria counts:

4000 > 1000 > 500 > 100 = 50 = 10 = 1 = control

#### Laboratory Periphyton Bioassay

While the Selenastrum bioassay showed a strong inhibitory effect of CMA on that alga, the periphyton bioassay in the artificial streams showed quite a different result. As shown in Figure K-4, CMA greatly stimulated periphyton growth in the channels receiving CMA concentrations of 100 and 500 mg/l. Based on the measured percentage of phosphorus in the CMA (0.020 percent), these channels would have had approximately 20 and 100  $\mu\text{g P/l}$ , respectively. The control, 1000 and 2500 mg CMA/l channels all had significantly lower periphyton accumulation than either of these two channels.

The 100 and 500 mg CMA/l channels turned a dark blue-green by the end of the experiment from this periphyton accumulation. Microscopic

examination of samples scraped from channel walls revealed that the periphyton in both of these channels was indeed a blue-green alga from the genus Oscillatoria. This type of periphyton was not found in any of the other channels.

#### Pond Sampling for Microorganisms

Measurements to detect responses of pond microorganisms to CMA input produced far less obvious results than the laboratory experiments. Figure K-5 displays chlorophyll a concentrations in all ponds through the entire duration of field measurements. All ponds had an algal bloom during the months of June and July. Although two control ponds had the two highest peak concentrations, variability was too great to conclude that algal populations were suppressed in CMA ponds. One CMA Pond (Pond 8) had an autumn algal bloom, but this was not seen in any of the other CMA ponds.

Table K-1 summarizes responses of microorganisms in ponds on the three sampling dates immediately following completion of all five treatments in winter of 1986-87. The table shows means of the four control and four treatment ponds plus or minus one standard deviation for chlorophyll a, ash-free dry weight, and the ratio of the two.

Although it is not so evident in Figure K-5, this table shows higher algal populations in CMA ponds on all three dates following treatments. However, none of the differences on any of the dates is significant (two-sample t-test,  $\alpha = 0.05$ ). Similarly, none of the AFDW nor ratio measurements are significantly different. Overall, variability was large enough within both control and CMA ponds that



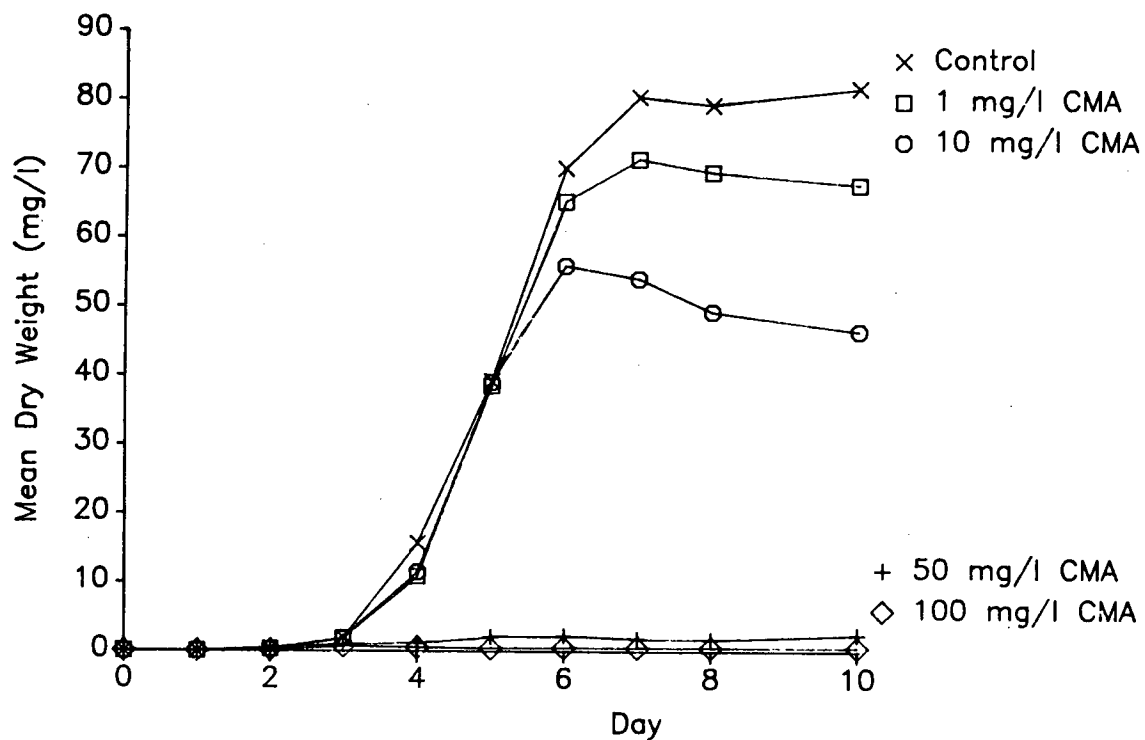


Figure K-1. Biomass Versus Time in CMA Algal Bioassay

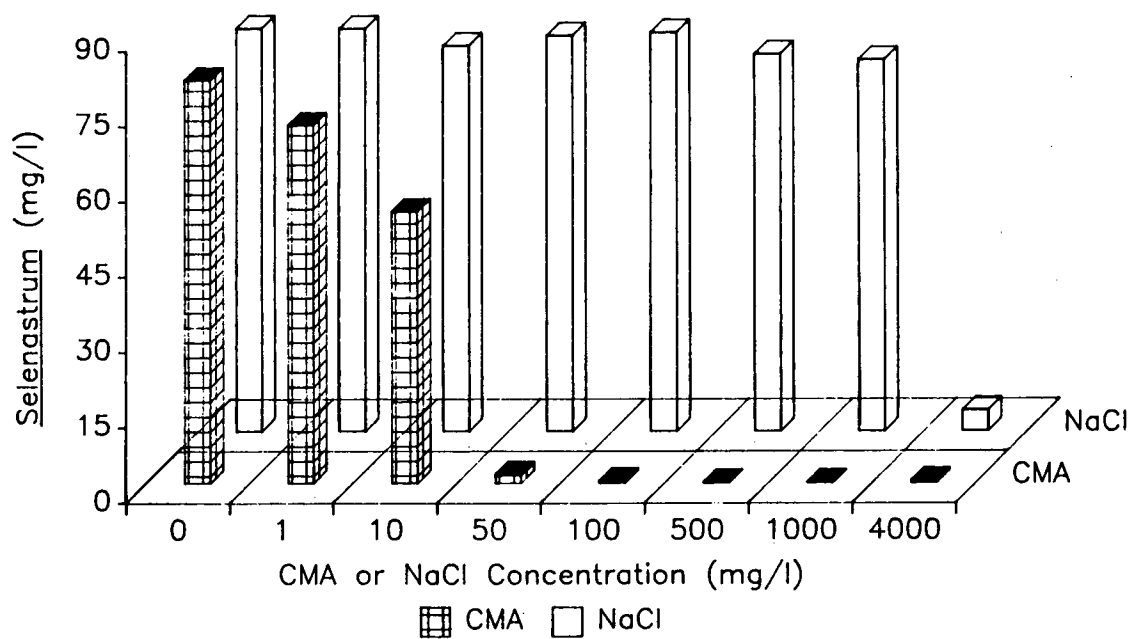


Figure K-2. Algal Biomass on Day 7 in CMA and NaCl Bioassays

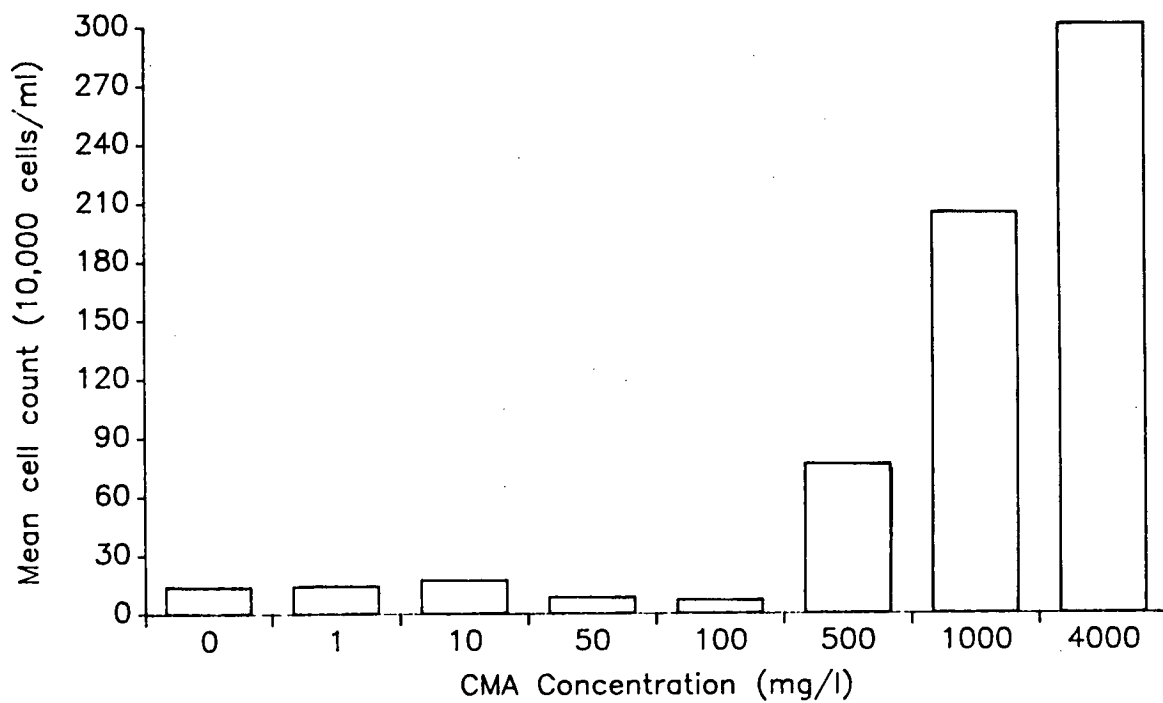


Figure K-3. Bacterial Biomass on Day 7 in CMA Algal Bioassay

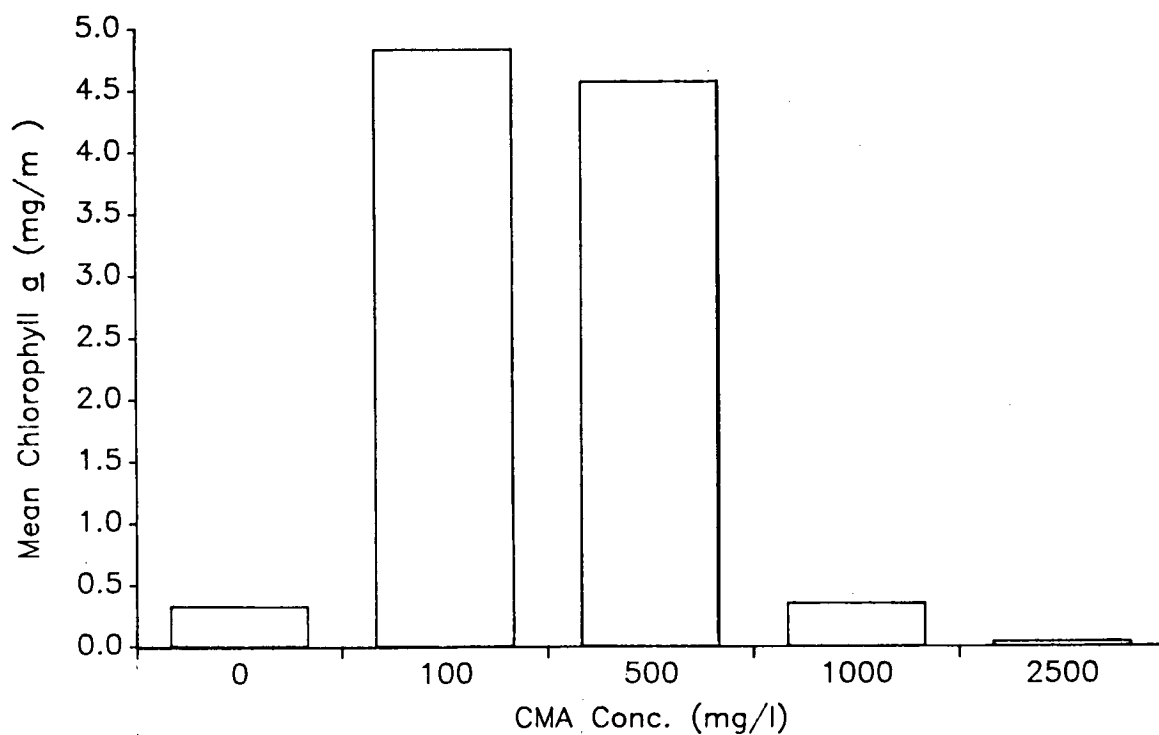


Figure K-4. Periphyton Biomass in Channels Used for Invertebrate Bioassay

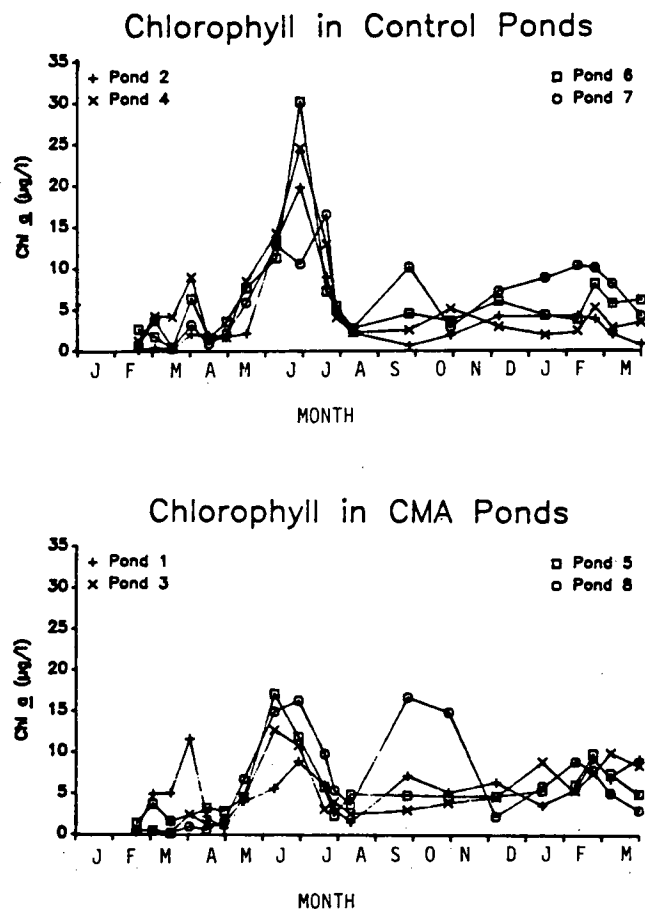


Figure K-5. Field Pond Chlorophyll Over Period of Field Experiments

Table K-1. Pond Microorganism Responses Following 1986-87 Treatments

| Ponds   | Date     | Mean<br>Chl a <sup>a</sup><br>(µg/l) | Mean<br>AFDW <sup>a</sup><br>(mg/l) | Mean<br>Chl a/<br>AFDW <sup>a</sup> |
|---------|----------|--------------------------------------|-------------------------------------|-------------------------------------|
| CMA     | 02-22-87 | 8.7 ± 1.1                            | 2.8 ± 1.4                           | 4.1 ± 2.6                           |
| Control | 02-22-87 | 6.8 ± 2.8                            | 1.5 ± 1.1                           | 6.1 ± 4.3                           |
| CMA     | 03-08-87 | 7.4 ± 2.0                            | 2.8 ± 0.1                           | 2.6 ± 0.7                           |
| Control | 03-08-87 | 4.7 ± 2.8                            | 2.2 ± 0.4                           | 2.0 ± 1.0                           |
| CMA     | 03-31-87 | 6.5 ± 3.0                            | 2.3 ± 0.6                           | 2.7 ± 0.7                           |
| Control | 03-31-87 | 3.6 ± 2.2                            | 1.5 ± 0.3                           | 2.4 ± 1.2                           |

<sup>a</sup> ± 1 standard deviation

there were no obvious differences in the response of planktonic algal communities to CMA input.

Periphyton measurements in the ponds were first made in autumn 1986. Figure K-6 shows periphyton chlorophyll *a* values 11 days before the first treatment of the winter 1986-87 and 19 days after the last of the five treatments. Again, it is difficult to see any obvious effects that can be attributed to CMA. There was more variability in the CMA ponds than in the control ponds on the before-treatment date, but variability was high in the control ponds after treatments. The only effect at all evident is that three of the four control ponds showed a substantial increase from the first date to the second, while only one of the CMA ponds (Pond 5) had a large increase. This observation may be even more significant, considering that Pond 5 received only a very small quantity of direct CMA runoff during applications (most percolated into the soil).

## DISCUSSION

Based on the results of these experiments, it is difficult to predict the effects of CMA on microorganisms in natural systems. While the *Selenastrum* bioassay showed strong inhibition of this planktonic species by CMA, the laboratory periphyton experiment showed a stimulatory effect on attached blue-green algae. The field tests demonstrated no obvious effect at all. These results are not necessarily conflicting, however, because the systems involved were very different. The *Selenastrum* bioassay was a static system with high control nutrients and a monoculture of green algae, while the periphyton

experiment was flow-through with low control nutrients and open to colonization by any indigenous organisms in the feedwater. The field experiments differed from either of these bioassays, in that CMA concentrations in the ponds even at their peak were much lower than in the bioassays (with the exception of the 1 mg/l *Selenastrum* treatment). The ponds also contained a much more diverse assemblage of organisms at several trophic levels.

The CalTrans bioassays exposing two algal species to CMA and NaCl demonstrated agreement with the bioassay reported here (6). CMA was inhibitory to both species at much lower concentrations than was NaCl.

It is not completely clear why CMA so strongly inhibited *Selenastrum* growth. Although it must be noted that equal concentrations of NaCl and CMA do not impose equivalent osmotic potentials, due to their differences in molecular weight and valence, it is very clear in comparing NaCl to CMA treatments that CMA inhibition was something other than osmotic stress. What is more likely is inhibition by the trace metals in CMA, or competition from bacteria.

Bacterial growth would be expected with CMA addition because of the excellent substrate that acetate provides. Therefore, just because *Selenastrum* decreased with increasing CMA concentrations while bacteria increased, it is not safe to assume that one caused the other. For instance, it may be speculated that these effects are independent of one another; bacteria may increase because of increased substrate while the algae decline due to some other chemical property of CMA, such as a toxic contaminant. However, competition stress for nutrients imposed by the bacteria is a very likely explanation for the results. An argument

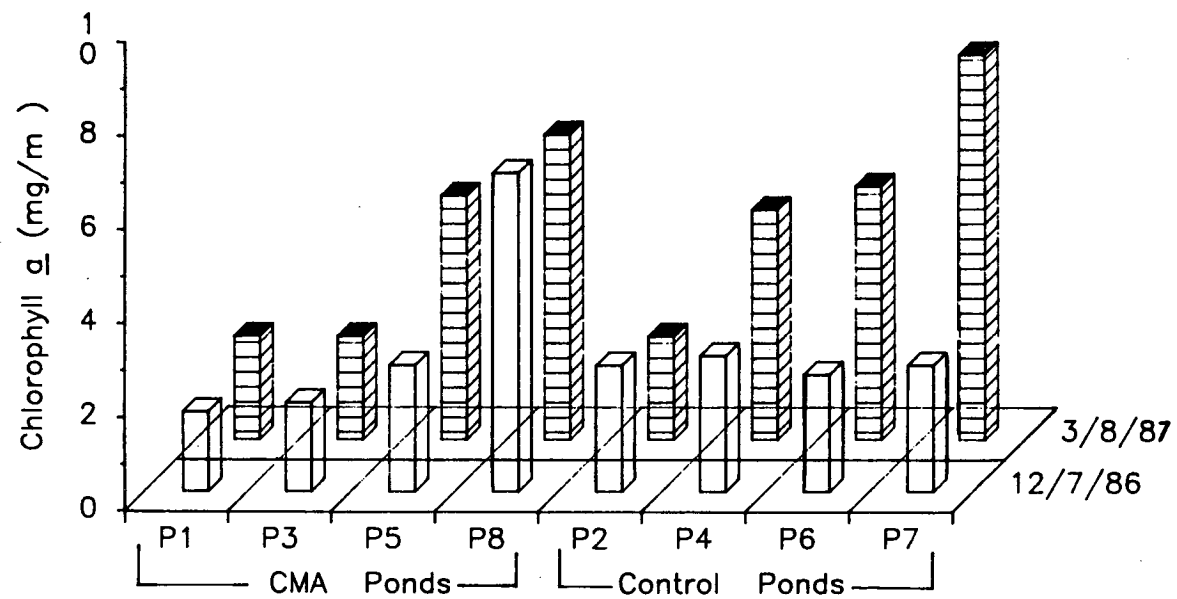


Figure K-6. Field Pond Periphyton Biomass Before and After 1986-87 Treatments

supporting this point is that Selenastrum concentrations were virtually identical in the Control, 1, and 10 mg CMA/l treatments on day 5, and differences became evident only after that time (Figure K-1). It is very likely that a surplus of nutrients was available for both algae and bacteria to coexist up to that time, but as populations increased and nutrients diminished, bacteria outcompeted the algae for the available nutrients.

A point that should be restated concerning this bioassay, particularly when comparing it to the other experiments discussed in this section, is that the solution used for the control and to make up all the CMA concentrations was a high-nutrient solution used specifically for culturing Selenastrum. Because this CMA was impure and made from agricultural wastes, it was relatively high in phosphorus (0.02 percent). Had the control water been low in nutrients, it is possible that CMA may have stimulated growth at low concentrations. It is difficult to project whether the inhibitory effect of other constituents of the CMA would counteract any stimulatory effect of its nutrient content.

This hypothesis of nutrient stimulation by CMA in low-nutrient systems is supported by the laboratory periphyton experiment. The pond water used in this experiment was relatively low in nutrients (7). It is possible that these algae were simply resistant to whatever factor inhibited the Selenastrum up to 500 mg CMA/l and could then take advantage of the increased nutrients provided by the CMA. Another explanation for the stimulatory response of CMA on periphyton growth in the 100 and 500 mg CMA/l channels is that the algal communities in these

two channels were made up of blue-green algae. These organisms are actually classified now by most taxonomists as cyanobacteria (blue-green bacteria), rather than as cyanophytes (blue-green algae), and there is evidence that some can grow heterotrophically (8, 9).

The failure to make any conclusive demonstrations about the effects of CMA on microorganism growth in the ponds, like those seen in the laboratory, has several explanations. As already stated, CMA concentrations in the ponds were much lower than those in the laboratory experiments. In addition to being lower, CMA additions to the ponds were not equal, due to the differences in runoff characteristics of the plots above them. This inequality could increase variability, making it difficult to detect statistically significant differences. The only comparable levels of CMA additions in laboratory experiments to those in the field were the 1 and 10 mg CMA/l treatments in the Selenastrum bioassay. While the differences from the control were detectable in these treatments, they were not substantial. They were significant primarily because the tightly controlled laboratory conditions reduced variability, allowing small differences to be detected.

## CONCLUSIONS

The planktonic green alga Selenastrum was inhibited by CMA concentrations as low as 1 mg/l in a static laboratory bioassay. This inhibition was not due to osmotic stress. Bacteria concentrations increased with increasing CMA concentrations, and it is hypothesized that they outcompeted the algae for nutrients, accounting for the inhibition observed. Parasitism by bacteria and toxic contaminants in

the CMA are also possible explanations for reduced growth of Selenastrum in CMA.

CMA at concentrations of 100 and 500 mg/l stimulated the growth of periphyton compared to a control in a flow-through bioassay in artificial streams. The periphyton in these channels was primarily composed of a blue-green alga. This stimulatory effect could be due to the resistance of these algae to any toxic effects of CMA, while taking advantage of the increased nutrients that are present in CMA. The blue-green algae may also have used the acetate as a substrate for heterotrophic growth.

No significant effects of CMA on microorganisms could be detected in the ponds. Maximum CMA concentrations in the ponds were much lower than almost all treatments in laboratory bioassays. It is possible that there are truly no effects from CMA at these concentrations in more realistic systems like the ponds, or that lack of true treatment replication makes variability too great to detect anything but large effects.

These experiments have revealed a tendency for CMA to stimulate fungal and bacterial growths unless highly diluted, which could suppress algae through competition. On the other hand, CMA was also found to stimulate attached blue-green algal growth under the right conditions. These possibilities should be noted in environmental impact assessments of CMA use, but it is proper to conclude that a number of factors make their actual development in natural systems somewhat unlikely.

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## APPENDIX L

### EFFECTS OF CMA ON AQUATIC ANIMALS

#### INTRODUCTION

Six single-species bioassays were conducted in the laboratory to assess the effects of CMA on aquatic fauna. Both acute and chronic static bioassays were done using the zooplankter Daphnia magna, and rainbow trout, Salmo gairdneri. A static acute toxicity bioassay was done with the fathead minnow, Pimephales promelas, and a two-week flow-through bioassay was run using the amphipod invertebrate Hyalolella azteca.

To determine if responses of aquatic organisms in more complete ecosystems were comparable to the results seen in laboratory single-species bioassays, the field site ponds were monitored for abundances and types of zooplankton, invertebrates, and bluegill sunfish (Lepomis macrochirus). The ponds also contained fathead minnows, although their population sizes were not assessed quantitatively.

Dissolved oxygen levels are instrumental to the responses of aquatic animals to a biodegradable organic like CMA. Extensive separate studies were performed on CMA biodegradation and associated oxygen levels and are reported in Appendix I.

#### EXPERIMENTAL METHODS

##### Acute Daphnia Bioassay

Acute bioassays using Daphnia magna were conducted as described by Standard Methods for the Examination of Water and Wastewater (1), with some modifications. Water from Lake Washington, which was obtained



through the University of Washington School of Fisheries Hatchery, was used as the control water and to make the various solutions from dried CMA. Rather than clear glass jars, cleaned, white porcelain evaporating dishes were used as containers. These dishes provided a much better background for viewing the Daphnia during counting. The dishes were covered with clear plastic Petri dishes to help prevent evaporative loss, although they were not tightly sealed to insure some air exchange.

A preliminary range-finding bioassay was run to determine the approximate range of CMA concentrations to use in the actual experiment. Concentrations of CMA used in the final bioassay were 0, 500, 1000, 2000, 3000, 4000, and 5000 mg CMA/l. Solutions were prepared from dry CMA and were glass-fiber filtered after mixing. Filtering was done to be consistent with the algal and fish bioassays, which required filtering for reasons discussed in those sections. Three replicate containers for each concentration were set up, each receiving 100 ml of the various solutions.

Ten neonates (less than 2 days old) were added to each container. Containers were placed in a constant temperature room set at 20 C with fluorescent lighting set on a diurnal cycle of twelve hours on and twelve hours off. Two drops of a very concentrated Selenastrum capricornutum algal culture were added for food each day. Daphnia were counted each day at the same time for the four days of the bioassay, and any dead Daphnia were removed immediately. As a measure of mortality, concentrations lethal to 50 percent of the organisms (LC<sub>50</sub>) were calculated as in Standard Methods (1).

#### Chronic Daphnia Bioassay

Containers, control water, lighting, temperature, and counting for the chronic Daphnia bioassay were identical to those for the acute Daphnia test. Because the objective of a chronic bioassay is to detect long-term effects on reproduction rather than direct toxicity, lower concentrations of CMA were used in this experiment: 0, 100, 500, 1000, and 1500 mg CMA/l. Also, six replicate containers, rather than three, were run for each concentration, and only one neonate was added to each container. One drop of a concentrated Selenastrum capricornutum algal culture was added daily to provide food.

Daily counts were made of the number of young each original organism produced for a duration of 45 days. The much smaller newly hatched neonates were immediately removed from each container, so that only the original adult remained. The day the original adult died, the total number of broods produced by each adult, and the total number of offspring produced were recorded.

#### Acute Fish Bioassays

The acute bioassays for both rainbow trout and fathead minnows were done identically, with the exception of being run at different temperatures. Rainbow experiments were done in a constant temperature room at 10 C, while the minnow bioassay was conducted at 20 C.

Experiments were done in a manner similar to the acute Daphnia bioassays, with the exceptions of different sizes of containers and volumes and concentrations of solutions. Clean, four-liter glass jars each holding two liters of solution were used. Following a preliminary range-finding bioassay, CMA concentrations used were 0, 10,000, 15,000,

20,000 and 25,000 mg CMA/l. Solutions were made up from dry CMA with Lake Washington water and filtered through a glass fiber filter. Filtering was done to prevent any undissolved particulates from clogging gills. Clogging is known to cause death in young trout (2). Fatalities could result from these undissolved particulate impurities and not from the actual CMA, which is completely soluble at these concentrations.

Three replicate jars for each concentration, each holding 10 fish, were used. Rainbow trout in the sac-fry stage were obtained from the University of Washington fish hatchery. The fathead minnows were adults about two inches in length obtained from the Chico Fish Farm in Chico, California.

Due to the relatively high density of fish in the chambers, jars were aerated using compressed air bubbled through aquarium airstones to insure survival of controls. Therefore, these experiments represent impacts of CMA attributed strictly to factors other than oxygen depletion. This consideration will be addressed further in the Discussion section.

Fish were counted every 12 hours for the four-day duration of each test, and dead fish were removed immediately. Temperature, pH, and dissolved oxygen were measured daily. Values of  $LC_{50}$  were calculated as in the acute Daphnia bioassay.

#### Chronic Rainbow Trout Bioassay

Chronic fish bioassays were done by assessing the effects of CMA on hatching and subsequent survival of rainbow trout eggs. The experimental set up was very similar to the acute trout bioassay, except that eyed eggs were used rather than sac fry and the CMA concentrations

used were 0, 10, 100, 500, 1000, and 5000 mg CMA/l. Also, each of the sets of 10 eggs in each jar were suspended in baskets. The baskets were constructed of 2-inch long sections of 2-inch diameter PVC pipe with one end covered with fine mesh plastic screen secured to the pipe with silicone seal. These baskets were suspended in the solutions from above by fine fishing line. The baskets were designed to be miniature replicates of the screen bottom buckets used to incubate eggs in fish hatcheries. Together with aeration as in the acute fish bioassays, the set up provided a constant water flow around the eggs. However, the water was not renewed during the experiment and degraded CMA was not replaced.

The experiment was monitored daily to determine hatching of the eggs and survival of the fry. Dead fish were removed immediately. Daily measurements were also made of pH, temperature, and dissolved oxygen. The experiment was run for 35 days with aeration and an additional 10 days with the air supply turned off.

#### Invertebrate Bioassay

The invertebrate bioassay using Hyalella azteca was conducted in artificial streams set up as continuous flow-through systems. Streams were rectangular Plexiglas channels, 1 meter long, 20 centimeters wide, and 8 centimeters deep.

CMA feed to the macroinvertebrate bioassays was controlled by a modification of the Mount and Brungs (3) proportional diluter developed by the Battelle Pacific Northwest Laboratory (4, 5). The apparatus consists of a Marriotte bottle, which supplies concentrated stock chemical solution; a Cole-Parmer Masterflex Model 7568 peristaltic pump

equipped with multiple discharge ports, which meters out stock solution at set flow rates; an inlet pond water manifold; and dilution cells, in which mixing of stock solution with pond water to desired concentrations occurs. Testing in the Battelle Laboratories demonstrated that use of this flow-through delivery system yielded variances in chemical concentration no larger among tanks receiving the same nominal concentration than among multiple samples from a single tank (4).

Following a static range-finding bioassay, the following scenario of pond water and CMA solution flows was used to arrive at the desired channel CMA concentrations of 0, 100, 500, 1000, 2500, and 5000 mg/l.

| Channel | CMA stock<br>bottle<br>Conc.<br>(mg/l) | CMA flow<br>(ml/min) | Pond water<br>flow<br>(ml/min) | Total<br>Flow<br>(ml/min) | Channel<br>CMA conc.<br>(mg/l) |
|---------|--|----------------------|--------------------------------|---------------------------|--------------------------------|
| 1       | -                                      | 0.00                 | 110                            | 110                       | 0                              |
| 2       | 2,100                                  | 5.25                 | 105                            | 110                       | 100                            |
| 3       | 10,500                                 | 5.25                 | 105                            | 110                       | 500                            |
| 4       | 21,000                                 | 5.25                 | 105                            | 110                       | 1,000                          |
| 5       | 52,500                                 | 5.25                 | 105                            | 110                       | 2,500                          |
| 6       | 52,500                                 | 10.50                | 100                            | 110                       | 5,000                          |

For this bioassay, CMA was used directly in its original liquid form, instead of being reconstituted from dry material. Another exception from all the other bioassays was that the CMA solution was not filtered, because filtering was impractical for the large quantities needed.

With the above inflows, the channels had an exchange rate of 10 times per day. The original intent of the experiment was to run the channels as lotic systems. However, in a trial run the air injection system used to create the current caused such excessive foaming of the CMA that this method was abandoned. Instead, the channels were operated

without a current but still as continuous flow-through systems. This was not a problem, because *Hyallolella* are naturally found in both lotic and lentic environments.

Two replicate cages each holding 10 invertebrates were placed in each channel. Cages were constructed from 8-inch long sections of 4-inch diameter PVC pipe. The pipe was cut longitudinally to remove an arc section of about 90 degrees from the cross section. This shape created an opening along the full length of the top for viewing the invertebrates during the experiment. Each end of the cage was sealed with fine mesh nylon screening attached with silicone seal. The white PVC provided an ideal background for counting the invertebrates.

The temperature for the experiment was  $18 \pm 2$  C, set by a thermostatically controlled heater in the pond water holding tank. Fluorescent lighting was on a diurnal cycle of 15 hours on and 12 hours off at a low level of approximately  $24 \mu\text{E m}^{-2} \text{s}^{-1}$  (100 ft-ca). No aeration was provided. Three, one-inch square sections of dried maple leaves were added to each cage to provide food and cover. Leaves were presoaked in pond water for three days to provide microorganism accumulation as a food source.

The experiment was run for 14 days. Invertebrate counts were made daily for the first 10 days and also on days 12 and 14. Dead organisms were immediately removed. Dissolved oxygen, pH, temperature, and volatile fatty acid salts were measured on each of the first 5 days and irregularly thereafter. Periphyton measurements made at the conclusion of the experiment are discussed in Appendix K.

### Pond Sampling for Aquatic Animals

Ponds at the Pack Forest site were monitored regularly for abundances and species composition of zooplankton and benthic invertebrates, and survival of bluegill sunfish.

Zooplankton were collected with a 30-cm diameter plankton net. Samples were preserved with 10 ml of 37 percent formaldehyde to each 100 ml of concentrated sample. Samples were counted using a channelled Plexiglas counting chamber under a dissecting microscope.

Estimates of benthic invertebrate populations were made using two artificial substrate multiplate samplers (Dendy type) placed in each pond (1). Each sampler was tethered to stakes on opposite sides of the pond. Units were sampled alternately on a monthly basis so that each one was given a two-month undisturbed period in which to accumulate fauna. Samplers were retrieved with a fine-mesh D-ring net to prevent motile organisms from escaping when the sampler was lifted by its string. The multiplate samplers were disassembled and all attached material from the plates and the net was washed into a 500 ml wide-mouth jar for transport back to the laboratory. There, the samples were immediately sorted while organisms were still alive. Invertebrates were placed in small vials and preserved with formaldehyde. Enumeration and identification to the taxa of order was done using a dissecting microscope.

Fish were added to the ponds on three separate occasions. The original plan was to use bluegill sunfish (Lepomis macrochirus) as the test species. However, due to their unavailability and legal restrictions enforced by the Washington State Department of Game, this

was not immediately possible. Therefore, extra fathead minnows (Pimephales promelas) not used in the laboratory bioassay were used as an alternative. Ten minnows were added to each pond. Later, a legal source of bluegills was found. However, the fish were shipped from California and many did not survive in transport. As a result, only three bluegills were added to each pond as test specimens for the first winter of treatments. Finally, another shipment was received and additional bluegills were added, so that each pond contained seven bluegills during the second winter of operation.

The fathead minnows and the first shipment of bluegills were left free in the ponds. However, netting the fish to do counts proved extremely tedious. Therefore, cages were built to contain the fish in each pond. These cages were constructed of wood frames covered with plastic screening and had dimensions of 1.5 m tall and 1 m square. Cages were placed directly on the pond bottoms and had open tops. It was hoped that this arrangement would let the fish maintain contact with both the benthic and terrestrial communities, thus providing food supplies. Each was then secured with staked guidelines to prevent them from blowing over in the wind. All bluegills were placed in these cages. Counts were then very easily done each time the ponds were sampled by lifting up the cages. Fathead minnows were not caged, because they had reproduced several times and the very small fry could easily escape.

## RESULTS

### Acute Daphnia Bioassay

The preliminary range-finding test produced poor survival in all three control replicates. In contrast, half or more of the test animals survived four days in CMA concentrations of up to 100 mg/l. Three more bioassays continued to show the same trend of poor survival in the controls but good survival in CMA concentrations of up to 1000 mg/l. Concentrations of greater than 1000 mg CMA/l also had poor survival. It is hypothesized that this result was due to the stimulation of microorganism growth in the CMA containers, which provided an ideal food source for the young neonates. At higher concentrations, however, the CMA was toxic, dissolved oxygen was too low due to CMA biodegradation, and/or osmotic pressure was excessive. Nevertheless, the bioassay protocol (1) dictates greater than 90 percent survival must be observed in controls for a valid test. Therefore, the experiment was repeated until the proper food allocation was determined to insure control survival.

The results of this experiment are shown in Figure L-1. All but two of the 30 Daphnia from the three control replicates survived the four days, thus meeting the control survival requirement. Only one of the 30 organisms died in the 500 mg/l replicates, and survival was greater than 80% even in 1000 mg CMA/l. None of the replicates at concentrations of 2000 mg/l or higher had greater than 50% survival after four days. The 96-hour  $LC_{50}$  calculated from probit analysis was 2000 mg/l.

### Chronic Daphnia Bioassay

The chronic Daphnia bioassay further supported the results seen in the acute test; i.e., that at CMA concentrations up to 500 mg/l, survival is as good or better than in controls. Figure L-2 summarizes these results. The longest survival, greatest number of broods, and largest total number of offspring occurred in the 500 mg CMA/l treatment. Survival was comparable at 100 mg/l, but reproduction was considerably less than at 500 mg/l. The 1000 mg/l treatment had reproduction similar to the 100 mg/l test vessels but only about half the length of survival. Animals in the 1500 mg/l test lived an average of only 5.5 days and did not reproduce. Control survival was not as long as in the intermediate CMA treatments, and none of the replicates reproduced.

### Acute Fish Bioassays

Preliminary range finding tests using rainbow trout indicated that a very high concentration series would have to be run to observe any mortality. Although the concentrations used were well above any realistic CMA concentration expected in regular highway runoff, the high concentrations were necessary to determine a 96-hour  $LC_{50}$  value.

Figures L-3 and L-4 show the results of the acute rainbow trout and fathead minnow bioassays, respectively. Both fish had greater than 90 percent survival in both the 5000 and 10,000 mg/l treatments but greatly reduced survival at 15,000 mg/l. None of either type of fish survived the 20,000 or 25,000 mg/l treatments through the 4-day experiment. The poor survival of the controls in the fathead bioassay appears unusual but has a very likely explanation. Soon after the fish

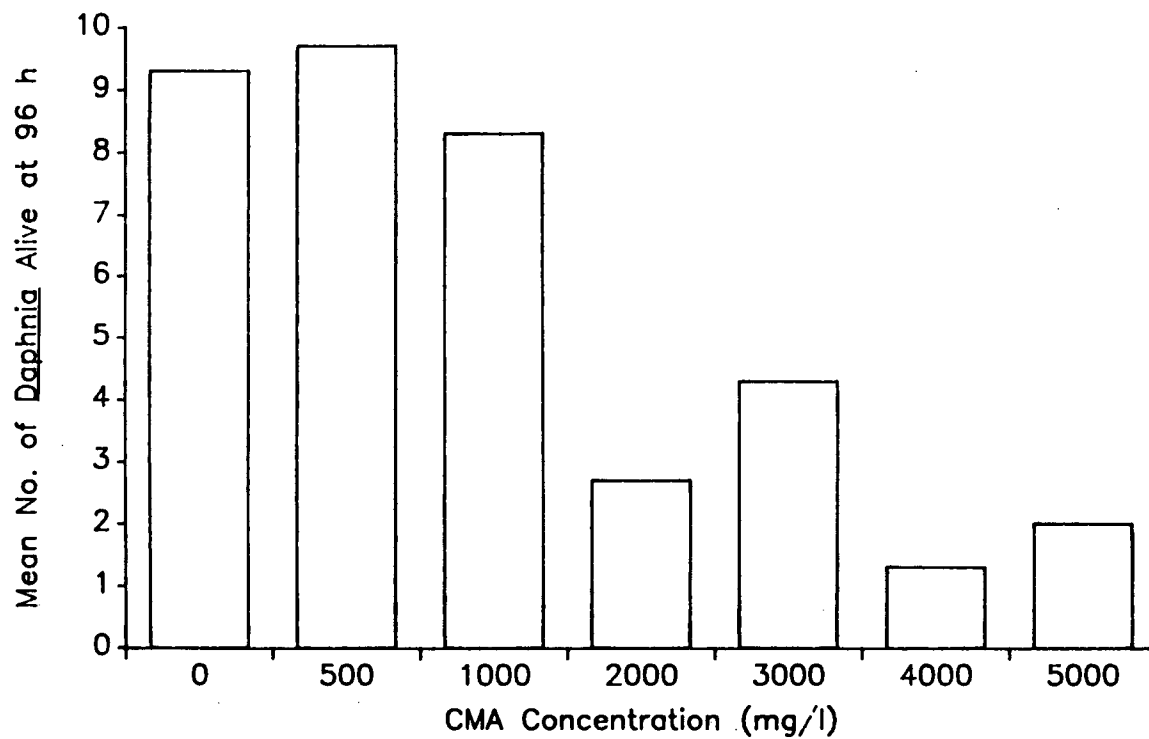


Figure L-1. Acute Daphnia Bioassay Results

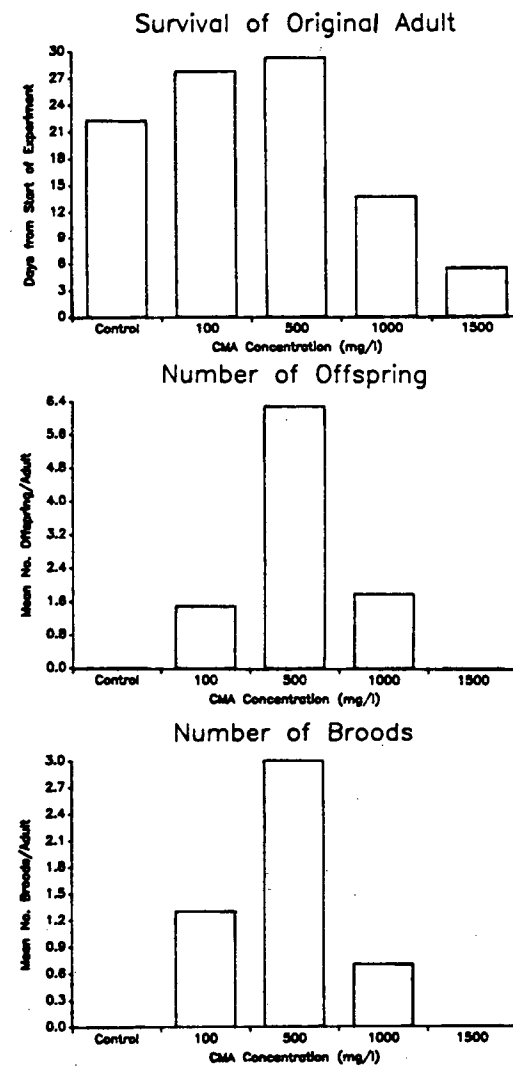


Figure L-2. Chronic Daphnia Bioassay Results

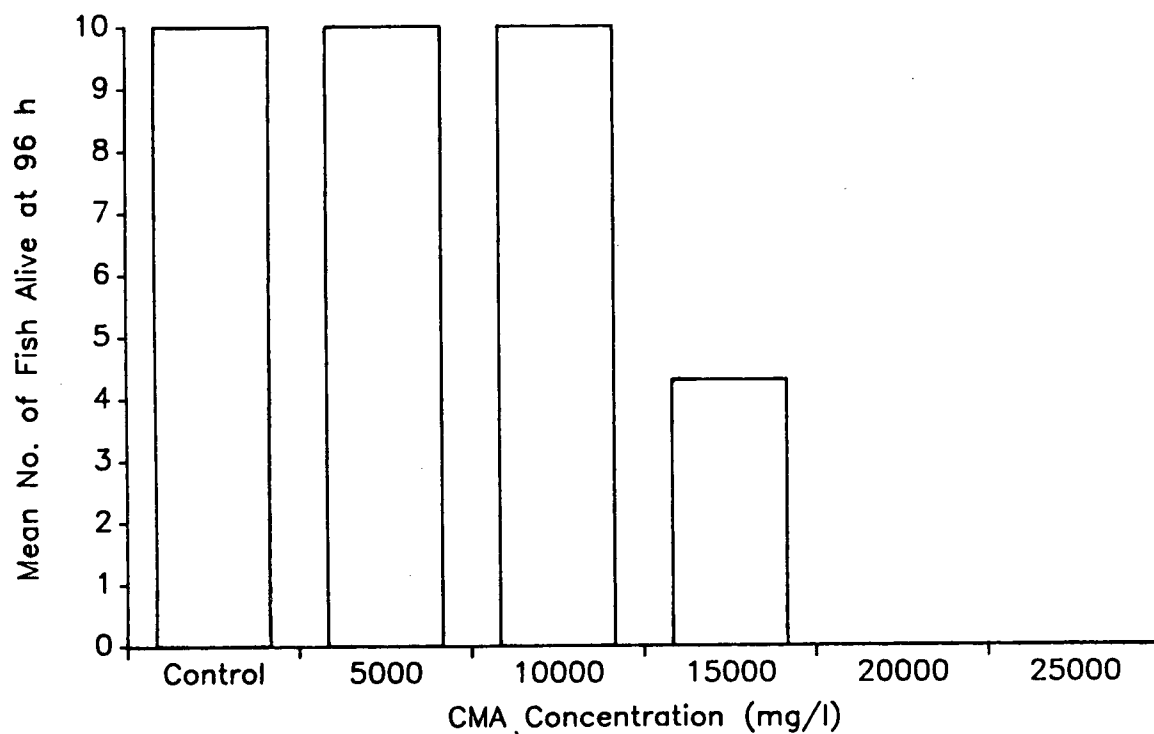


Figure L-3. Acute Rainbow Trout Bioassay Results

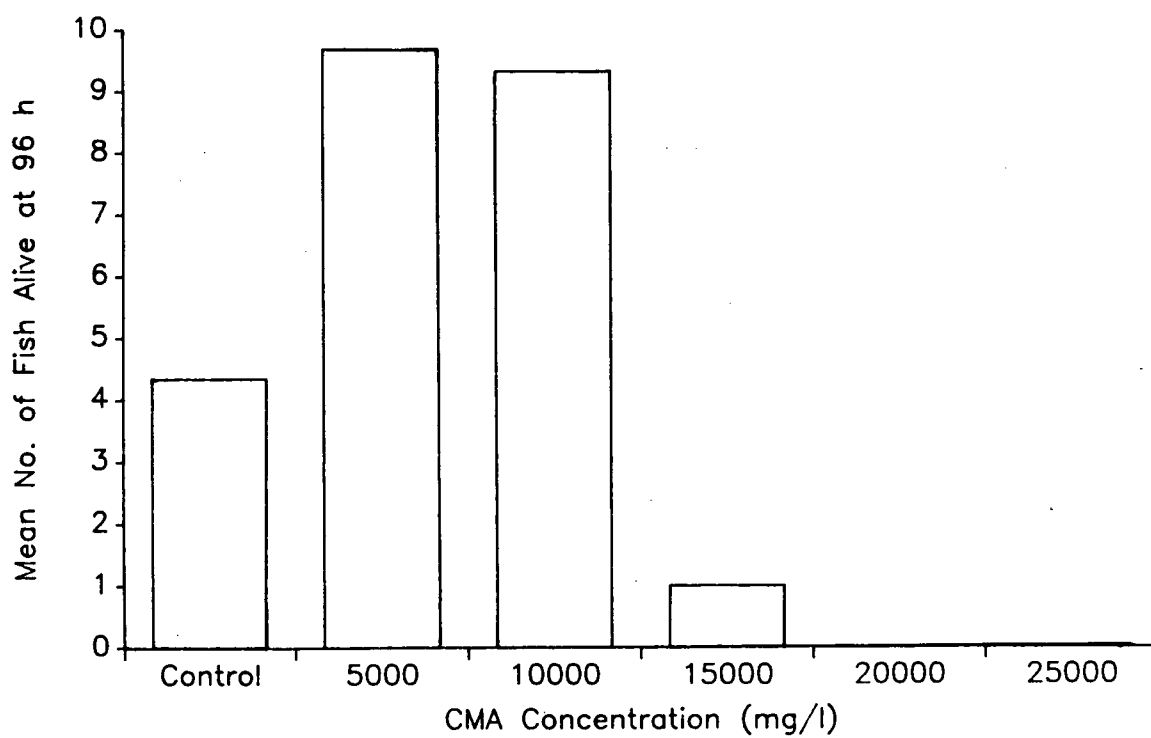


Figure L-4. Acute Fathead Minnow Bioassay Results

arrived in the laboratory, it was discovered that they were afflicted with a fatal parasitic fin-rot disease. The prescribed treatment for this condition is immersion in a high ionic strength salt solution. CMA treatments of the proper ionic strength apparently cured the fish, while control organisms died during the test period. High mortalities also occurred in the stock aquarium until those fish were given a sodium chloride treatment. The 96-hour  $LC_{50}$ 's calculated were 17,500 mg/l for the rainbow trout and 12,500 mg/l for the fathead minnows.

#### Chronic Rainbow Trout Bioassay

The chronic rainbow trout bioassay was used to determine if CMA had an effect on hatching and survival of fry. Table L-1 and Figure L-5 summarize the results of the experiment. With the exception of one egg in a 1000 mg/l replicate, every egg in all concentrations hatched by day 8. The control and all treatments up to 1000 mg CMA/l had at least 90 percent survival of the sac fry through the end of the regular portion of the experiment. In the 5000 mg CMA/l treatment, all died by day 21.

After the 35 days of the regular experiment, the aeration was shut off and the systems continued to be monitored for another ten days, in order to determine the extent of oxygen depletion and impacts on the fry. All those alive in the control, 10, and 100 mg/l treatments survived the remaining 10 days, and oxygen never dropped below 8.5 mg/l in any of these vessels. Dissolved oxygen fluctuated unpredictably between replicates of both the 500 and 1000 mg/l treatments. When it remained above 2 mg/l, all or most of the remaining fish survived; when dissolved oxygen dropped below that level, however, most fish died quickly.

Table L-1. Chronic Rainbow Trout Eyed-Egg Bioassay Summary

| Treatment      | % Hatched by Day: <sup>a</sup> |          |          | % Alive by Day <sup>a</sup> |           |           |           |           |           |
|----------------|--------------------------------|----------|----------|-----------------------------|-----------|-----------|-----------|-----------|-----------|
|                | <u>6</u>                       | <u>7</u> | <u>8</u> | <u>12</u>                   | <u>14</u> | <u>18</u> | <u>21</u> | <u>24</u> | <u>28</u> |
| Control        | 43                             | 97       | 100      | 97                          | 97        | 97        | 97        | 97        | 97        |
| 10 mg CMA/l    | 40                             | 93       | 100      | 100                         | 100       | 100       | 97        | 97        | 90        |
| 100 mg CMA/l   | 33                             | 97       | 100      | 100                         | 100       | 100       | 97        | 97        | 97        |
| 500 mg CMA/l   | 43                             | 97       | 100      | 97                          | 97        | 97        | 90        | 90        | 90        |
| 1,000 mg CMA/l | 70                             | 97       | 97       | 97                          | 97        | 97        | 97        | 97        | 97        |
| 5,000 mg CMA/l | 83                             | 97       | 100      | 87                          | 67        | 40        | 0         | 0         | 0         |

<sup>a</sup>Mean of three replicates.



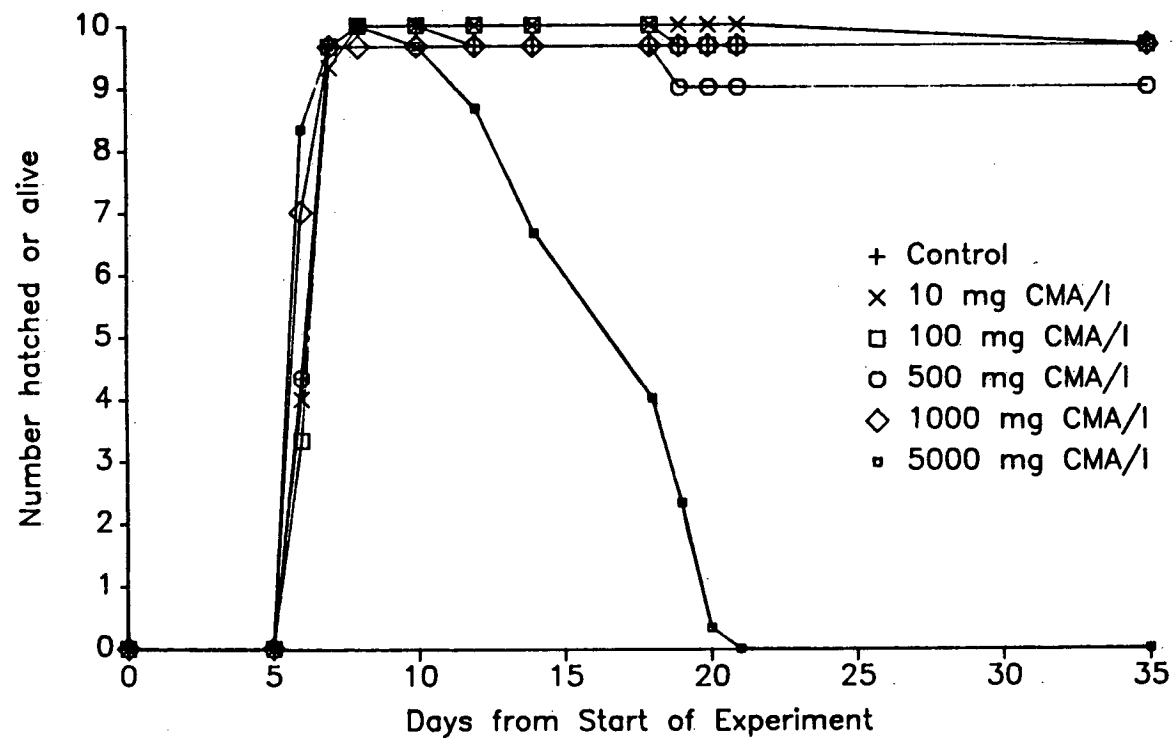


Figure L-5. Chronic Rainbow Trout Bioassay Results

Table L-3 summarizes the pond animal population measurements taken throughout the program. There are no notable differences associated with treatments, except zooplankton were suppressed in Pond 8 (CMA-Flood) in the summer between treatments relative to other ponds. Zooplankton generally were more abundant in CMA than control ponds.

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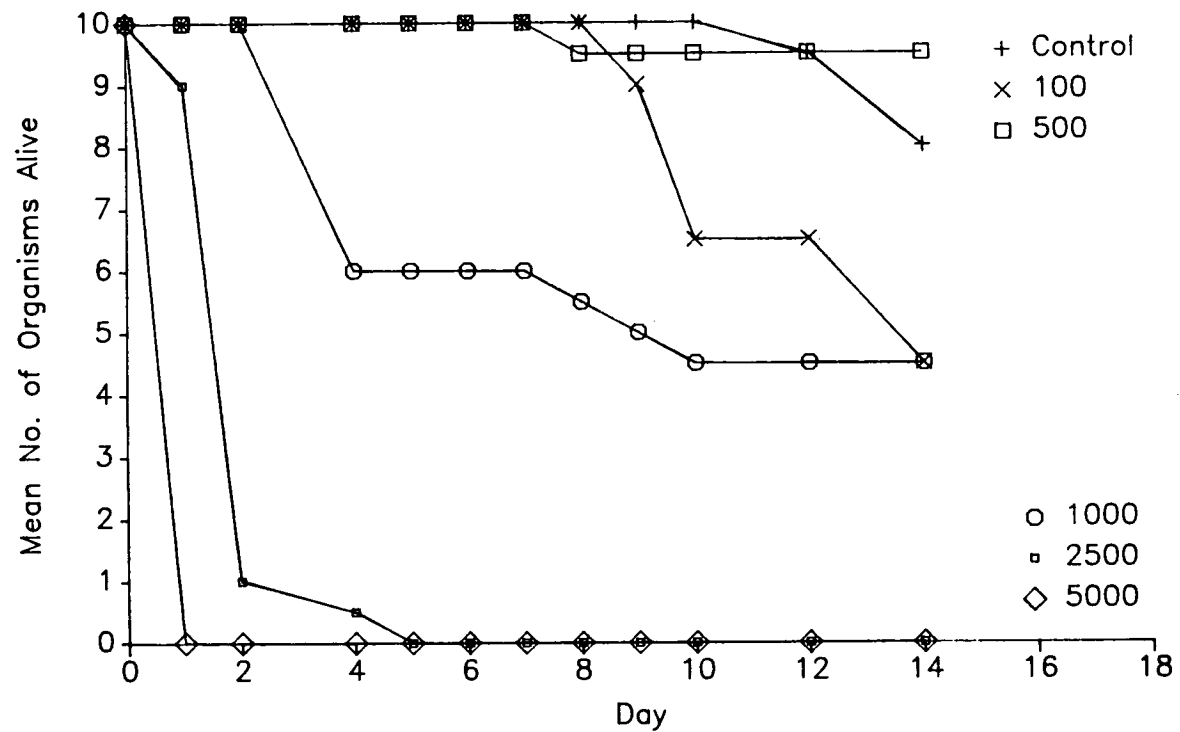


Figure L-6. Invertebrate Bioassay Results

Table L-3. Means and Standard Deviations of Zooplankton and Invertebrates in Ponds  
Over Four Periods During Field Plot Experiments

| Pond<br>No. | Treatment   | Period <sup>a</sup> | Rotifers<br>(org./l) |      | Copepods<br>(org./l) |       | Cladocera<br>(org./l) |       | Chironomids<br>(org./ft <sup>2</sup> ) |      | Ephemeroptera<br>(org./ft <sup>2</sup> ) |      |
|-------------|-------------|---------------------|----------------------|------|----------------------|-------|-----------------------|-------|--|------|--|------|
|             |             |                     | Mean                 | S.D. | Mean                 | S.D.  | Mean                  | S.D.  | Mean                                   | S.D. | Mean                                     | S.D. |
|             |             |                     | Mean                 | S.D. | Mean                 | S.D.  | Mean                  | S.D.  | Mean                                   | S.D. | Mean                                     | S.D. |
| 1           | CMA-Spray   | P                   | 0                    | 0    | 0.1                  | 0.0   | 0.00                  | 0.00  | NA                                     |      | NA                                       |      |
|             |             | T1                  | 9                    | 15   | 20.8                 | 35.2  | 0.03                  | 0.06  | NA                                     |      | NA                                       |      |
|             |             | I                   | 398                  | 845  | 106.0                | 110.0 | 54.30                 | 85.20 | 24                                     | 15   | 4  | 3    |
|             |             | T2                  | 42                   | 0    | 10.6                 | 0.0   | 0.10                  | 0.00  | 93                                     | 71   | 18                                       | 25   |
|             |             |                     |                      |      |                      |       |                       |       |  |      |  |      |
| 2           | Water-Spray | P                   | 0                    | 0    | 0.1                  | 0.0   | 0.00                  | 0.00  | NA                                     |      | NA                                       |      |
|             |             | T1                  | 3                    | 5    | 2.7                  | 4.5   | 0.00                  | 0.00  | NA                                     |      | NA                                       |      |
|             |             | I                   | 48                   | 78   | 60.5                 | 64.4  | 2.10                  | 2.40  | 25                                     | 17   | 22                                       | 21   |
|             |             | T2                  | 26                   | 0    | 0.4                  | 0.0   | 0.00                  | 0.00  | 13                                     | 14   | 17                                       | 6    |
|             |             |                     |                      |      |                      |       |                       |       |  |      |  |      |
| 3           | CMA-Spray   | P                   | 0                    | 0    | 0.1                  | 0.0   | 0.00                  | 0.00  | NA                                     |      | NA                                       |      |
|             |             | T1                  | 11                   | 19   | 7.0                  | 12.0  | 0.10                  | 0.10  | NA                                     |      | NA                                       |      |
|             |             | I                   | 137                  | 204  | 59.0                 | 67.0  | 1.30                  | 1.20  | 52                                     | 24   | 31                                       | 43   |
|             |             | T2                  | 19                   | 0    | 2.0                  | 0.0   | 0.30                  | 0.00  | 7                                      | 3    | 28                                       | 27   |
|             |             |                     |                      |      |                      |       |                       |       |  |      |  |      |
| 4           | Water-Spray | P                   | 23                   | 0    | 0.2                  | 0.0   | 0.00                  | 0.00  | NA                                     |      | NA                                       |      |
|             |             | T1                  | 116                  | 92   | 51.0                 | 86.0  | 0.03                  | 0.06  | NA                                     |      | NA                                       |      |
|             |             | I                   | 98                   | 139  | 65.0                 | 46.0  | 6.30                  | 11.50 | 40                                     | 21   | 7  | 4    |
|             |             | T2                  | 5                    | 0    | 7.1                  | 0.0   | 0.50                  | 0.00  | 21                                     | 10   | 12                                       | 14   |
|             |             |                     |                      |      |                      |       |                       |       |  |      |  |      |
| 5           | CMA-Flood   | P                   | 73                   | 0    | 0.0                  | 0.0   | 0.00                  | 0.00  | NA                                     |      | NA                                       |      |
|             |             | T1                  | 461                  | 531  | 71.0                 | 122.0 | 0.07                  | 0.12  | NA                                     |      | NA                                       |      |
|             |             | I                   | 18                   | 23   | 118.0                | 119.0 | 9.00                  | 10.90 | 24                                     | 7    | 5  | 4    |
|             |             | T2                  | 443                  | 0    | 21.0                 | 0.0   | 0.10                  | 0.00  | 17                                     | 11   | 3  | 4    |
|             |             |                     |                      |      |                      |       |                       |       |  |      |  |      |
| 6           | Water-Flood | P                   | 9                    | 0    | 0.2                  | 0.0   | 0.00                  | 0.00  | NA                                     |      | NA                                       |      |
|             |             | T1                  | 125                  | 141  | 2.1                  | 2.8   | 0.00                  | 0.00  | NA                                     |      | NA                                       |      |
|             |             | I                   | 20                   | 31   | 69.0                 | 63.0  | 0.40                  | 0.50  | 58                                     | 30   | 19                                       | 13   |
|             |             | T2                  | 240                  | 0    | 1.0                  | 0.0   | 0.00                  | 0.00  | 34                                     | 27   | 13                                       | 10   |
|             |             |                     |                      |      |                      |       |                       |       |  |      |  |      |

Continued

| Pond<br>No. | Treatment   | Period <sup>a</sup> | Rotifers<br>(org./l) |      | Copepods<br>(org./l) |      | Cladocera<br>(org./l) |      | Chironomids<br>(org./ft <sup>2</sup> ) |      | Ephemeroptera<br>(org./ft <sup>2</sup> ) |      |
|-------------|-------------|---------------------|----------------------|------|----------------------|------|-----------------------|------|--|------|--|------|
|             |             |                     | Mean                 | S.D. | Mean                 | S.D. | Mean                  | S.D. | Mean                                   | S.D. | Mean                                     | S.D. |
|             |             |                     | Mean                 | S.D. | Mean                 | S.D. | Mean                  | S.D. | Mean                                   | S.D. | Mean                                     | S.D. |
| 7           | Water-Flood | P                   | 5                    | 0    | 0.1                  | 0.0  | 0.00                  | 0.00 | NA                                     |      | NA                                       |      |
|             |             | T1                  | 70                   | 77   | 3.3                  | 5.5  | 0.40                  | 0.60 | NA                                     |      | NA                                       |      |
|             |             | I                   | 23                   | 21   | 89.0                 | 60.0 | 4.20                  | 5.00 | 36                                     | 16   | 5  | 3    |
|             |             | T2                  | 25                   | 0    | 3.1                  | 0.0  | 0.20                  | 0.00 | 12                                     | 10   | 16                                       | 12   |
|             |             |                     |                      |      |                      |      |                       |      |  |      |  |      |
| 8           | CMA-Flood   | P                   | 3                    | 0    | 0.0                  | 0.0  | 0.00                  | 0.00 | NA                                     |      | NA                                       |      |
|             |             | T1                  | 23                   | 22   | 0.1                  | 0.1  | 0.00                  | 0.00 | NA                                     |      | NA                                       |      |
|             |             | I                   | 378                  | 769  | 21.0                 | 21.0 | 2.30                  | 2.30 | 56                                     | 52   | 39                                       | 37   |
|             |             | T2                  | 32                   | 0    | 1.3                  | 0.0  | 0.00                  | 0.00 | 9                                      | 6    | 71                                       | 36   |
|             |             |                     |                      |      |                      |      |                       |      |  |      |  |      |

<sup>a</sup> P--Prior to first treatment (1/86-2/86).

T1--First treatment period and one month afterward (3/4/86-4/25/86)

I--Intermediate period between T1 and T2 (4/26/86-12/17/86)

T2--Second treatment period and afterward as long as data are available  
(12/18/86-3/31/87)

NA--Not available

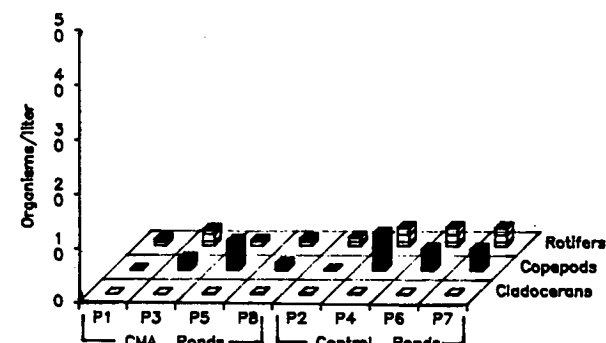
However, Pond 8 received more direct plot runoff than other ponds. Its dissolved oxygen declined more than others just after treatments, and fungal or bacterial growths were more in evidence during the summer. However, bluegills and minnows survived and reproduced (in the case of minnows) in Pond 8 as well as or better than other ponds.

Figures L-7 and L-8 display the abundances of zooplankton and benthic invertebrates, respectively, on a date just before CMA applications began (12/7/86) and just after all applications were completed (2/22/87). Neither zooplankton nor benthic invertebrate numbers or taxa show any obvious differences between control and CMA ponds. It should be noted that there is a major scale change between the zooplankton graphs on the two different dates. Zooplankton, particularly rotifers, increased greatly over the period but not with any correlation to pond treatment. Variability was so large within each treatment group that it is impossible to make any definitive statements concerning CMA effects on either invertebrates or zooplankton.

As might be expected from the laboratory fish bioassays, which demonstrated great tolerance to very high CMA concentrations, none of the bluegills in any of the ponds died. There was some concern that, because the laboratory bioassays were artificially aerated, low dissolved oxygen concentrations created by CMA input could result in mortalities in the field not seen in the laboratory. Dissolved oxygen concentrations never dropped below 4 mg/l in any of the ponds, however, a level sufficient for bluegills to survive.

After a short period following stocking, numbers of fathead minnows in each pond were not counted. With their rapid reproduction

## Zooplankton on 12/7/86



## Zooplankton on 2/22/87

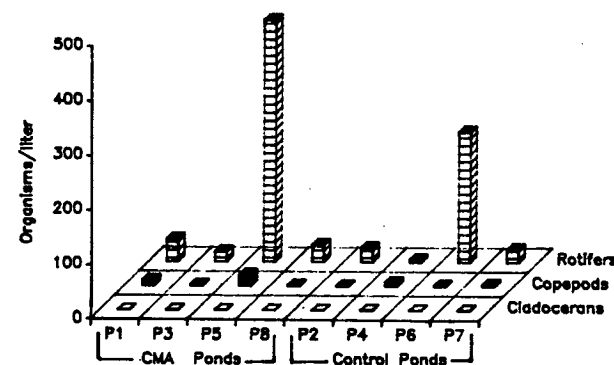
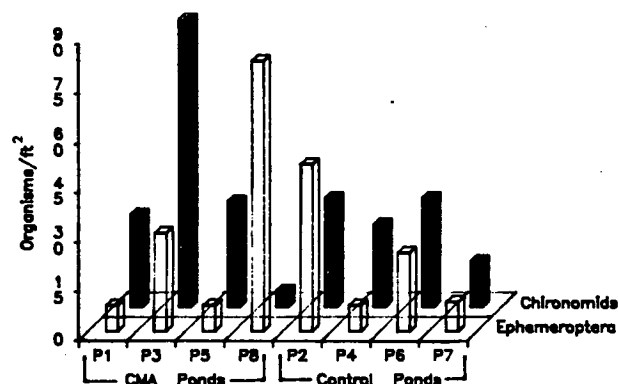


Figure L-7. Field Pond Zooplankton Before and After 1986-87 Treatments

### Benthic Invertebrates on 12/7/86



### Benthic Invertebrates on 2/22/87

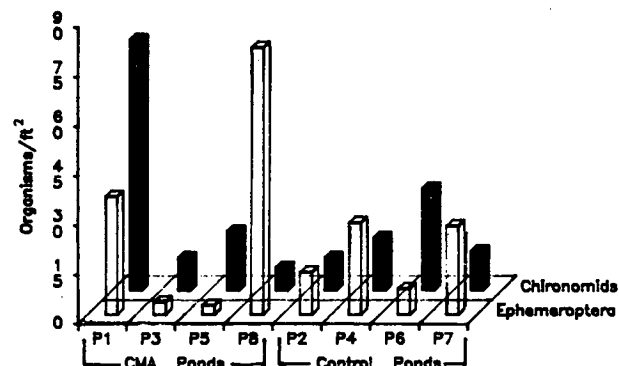


Figure L-8. Field Pond Benthic Invertebrates Before and After 1986-87 Treatments

and small size, netting the fish was too difficult. However, observations during pond sampling indicated that all ponds still have healthy populations of fathead minnows.

### DISCUSSION

A zooplankter in laboratory culture exhibited the best survival and reproduction at 500 ppm CMA, compared to controls and lower and higher CMA concentrations. This result was apparently due to the food supplement provided by fungi growing on the CMA. In chronic tests, the reason for the more successful survival and reproduction at moderate CMA concentrations also appears to be the supplementary food source provided by fungi, and possibly bacteria, living on acetate. Animals in all treatments were fed the green alga *Selenastrum capricornutum* in the proportions found to promote control survival in the previous acute experiments. However, the advantage of the supplementary food source became evident in the long-term tests, as more individuals competed for the available food.

These results differed somewhat from the CalTrans bioassays performed with reagent-grade CMA. The 96-hour  $LC_{50}$  in these earlier experiments was 445 mg/l, compared to 2000 mg/l in the present tests. In the chronic experiment CalTrans observed the onset of reproductive effects between 125 and 250 mg/l, a range below the optimum seen in these tests. Reasons for these differences are unknown, but there is no indication that potential production-run CMA would be more harmful to zooplankton than the reagent material.

These experiments demonstrated that, under laboratory test conditions, CMA at or somewhat above the concentrations expected in ordinary highway runoff benefited the zooplankter Daphnia over several generations. CMA concentrations between 500 and 1,000 mg/l began to reduce long-term survival and reproductive success. These concentrations, and higher, are easily imaginable in actual highway runoff but are not expected to be routine.

A similar result was noted with a macroinvertebrate exposed to CMA in a flow-through bioassay. High mortality occurred at > 1,000 ppm CMA, but good survival at lower concentrations occurred even when oxygen was low. It is hypothesized that both low DO and high osmotic potential are responsible for rapid complete mortality at the higher CMA concentrations.

Trace contaminants in the CMA also could be implicated. Because Hyalalella survived well at low DO in lower CMA concentrations, the osmotic effect may be of greater importance. To explain the better survival at 500 than at 100 mg CMA/l, it is hypothesized that the higher CMA concentration stimulated fungal growth, which served as a food source. At the same time, food quantity may have been declining in the 100 mg CMA/l chambers. It was observed that the amphipods did not seem to consume the shredded maple leaves provided as food but must have lived on microorganisms growing on the leaf surface. The hypothesis is given some support by the death occurring after about two weeks in the controls, which also may have begun to be depleted of food. Because control organisms were dying, the experiment was terminated at this point.

CMA concentrations up to the maximum level expected in actual highway runoff had no deleterious effect on rainbow trout survival or hatching success. Rainbow trout fry and fathead minnows exhibited nearly complete survival in acute bioassays at 5000 ppm CMA and even higher. The 96-hour LC<sub>50</sub> computed from these data for rainbow trout (17,500 mg/l) was very similar to that found by CalTrans (18,700 mg/l). In a chronic bioassay hatching was similar at 5000 ppm and lower concentrations, but the hatchlings exhibited lower survival at the higher concentration. A concentration (1,000 mg/l) well above the level routinely expected did not impede survival of the young. These results duplicated those of CalTrans with reagent-grade CMA. It must be noted that aerating the test chambers removed the effect of oxygen depletion that appears to pose the greatest threat to fish exposed to CMA. CMA concentrations expected routinely (up to 100 mg/l) did not reduce dissolved oxygen or increase long-term mortality after aeration ceased. Higher concentrations, that could occur from time-to-time in practice, did result in greatly reduced oxygen and rapid, high mortality. However, this hazard should be much smaller in natural running waters.

In field ponds no conclusive differences were noted in zooplankton, macroinvertebrates, bluegills, or fathead minnows between control and treatment ponds. Fish survival has been very high in all ponds, and minnows have reproduced a number of generations. These observations lend support to the conclusions reached after laboratory bioassays; viz., if oxygen depletion is avoided, CMA concentrations expected to result in natural waters after mixing of even relatively

concentrated highway runoff should not create short- or long-term impacts on aquatic animals.

Excessive oxygen depletion, however, is a possibility. It could occur prior to mixing and dilution of runoff, or as a consequence of rapid washoff of a large CMA volume. The latter effect would be most likely if the receiving water had already been warmed well above freezing, when acetate decomposition and oxygen removal would be most rapid. In the field ponds, dissolved oxygen did drop as low as 4 mg/l. This concentration was sufficient for the requirements of the resident organisms, but could affect salmonid fish negatively if prevalent for a period of days or longer.

#### CONCLUSIONS

CMA concentrations expected in most highway runoff have been shown not to cause harmful toxic or osmotic effects to a zooplankter, an amphipod invertebrate, and three fish species, if their dissolved oxygen requirements are met. The principal implications of these results for use and environmental impact assessment of CMA concern its effect on aquatic oxygen resources. Appendix I also discusses this issue. To ensure sufficient dissolved oxygen in receiving waters, several steps could be taken:

1. CMA should not be used on highway segments where mixing and dilution capacity in the receiving water is limited, i.e., it should not be drained into small ponds and lakes or streams that offer less than 100:1 dilution of the expected highway runoff rate in the late winter and spring.

2. CMA should not be applied in a late storm condition, when the receiving water has already heated somewhat above freezing, especially when dilution may be limited. In this situation the acetate decomposition rate could exceed the reaeration rate sufficiently to cause acute, severe oxygen decline and kill vertebrate and invertebrate aquatic animals.
3. Care should be taken to avoid CMA spills and runoff from stock piles in the vicinity of receiving waters at all times. These circumstances would create especially concentrated runoff streams.
4. Special precautions relative to points 1-3 above should be considered when the receiving water houses salmonids, because of the high degree of sensitivity of this family of fishes to low oxygen.

#### REFERENCES

1. American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 16th Ed., American Public Health Association, Washington, D.C. (1985).
2. Portele, G.J., B.W. Mar, R.R. Horner, and E.B. Welch, "Effects of Seattle Area Highway Stormwater Runoff on Aquatic Biota," Washington State Department of Transportation, Olympia, WA, FHWA WA-RD-39.11 (1982).
3. Mount, D.I. and W.A. Brungs, "A Simplified Dosing Apparatus for Fish Toxicology Studies," Water Research Vol. 1:21-29 (1967).



4. Anderson, D.R. and E.B. Lusty, "Acute Toxicity and Bioaccumulation of Chloroform to Four Species of Freshwater Fish," Battelle Pacific Northwest Laboratory, Richland, WA (1980)
5. Bean, R.M., C.I. Gibson, and D.R. Anderson, "Biocide By-products in Aquatic Environments," Battelle Pacific Northwest Laboratory, Richland, WA (1981).

## APPENDIX M

### DETAILED INSTRUCTIONS FOR FULL-SCALE MONITORING

#### APPENDIX M-1

#### RUNOFF COMPOSITE SAMPLING SYSTEM DESIGN AND CALIBRATION

##### Principles of Flow Splitter Design

Note: See illustrations on following pages.

1. The expected water volume yield from a design runoff event controls the fraction split, the number of divisions, and the width of divider openings.
2. A sufficient length of approach to the divider is required to dissipate disturbances.
3. A supercritical slope is required to prevent propagation of disturbances upstream and the settlement of solids in the flow splitter.

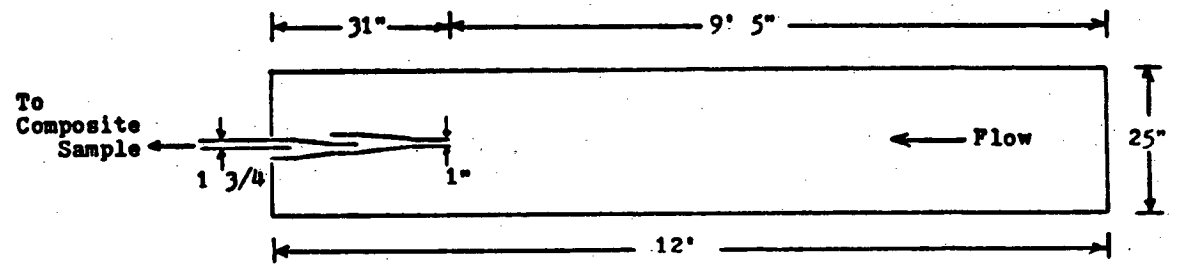
##### Determination of Divider and Tank Dimensions

1. Select a design runoff event and estimate the water volume yield (V) using a hydrologic model or approximations of the snow pack water release volume and rate.
2. Select the fraction split (F), collection tank volume (V'), divider opening (DO), and channel width (W) to approximate the dimensions of flow splitters shown to be functional (see the table below). Use a trial-and-error solution if necessary. The following equations relate the quantities:

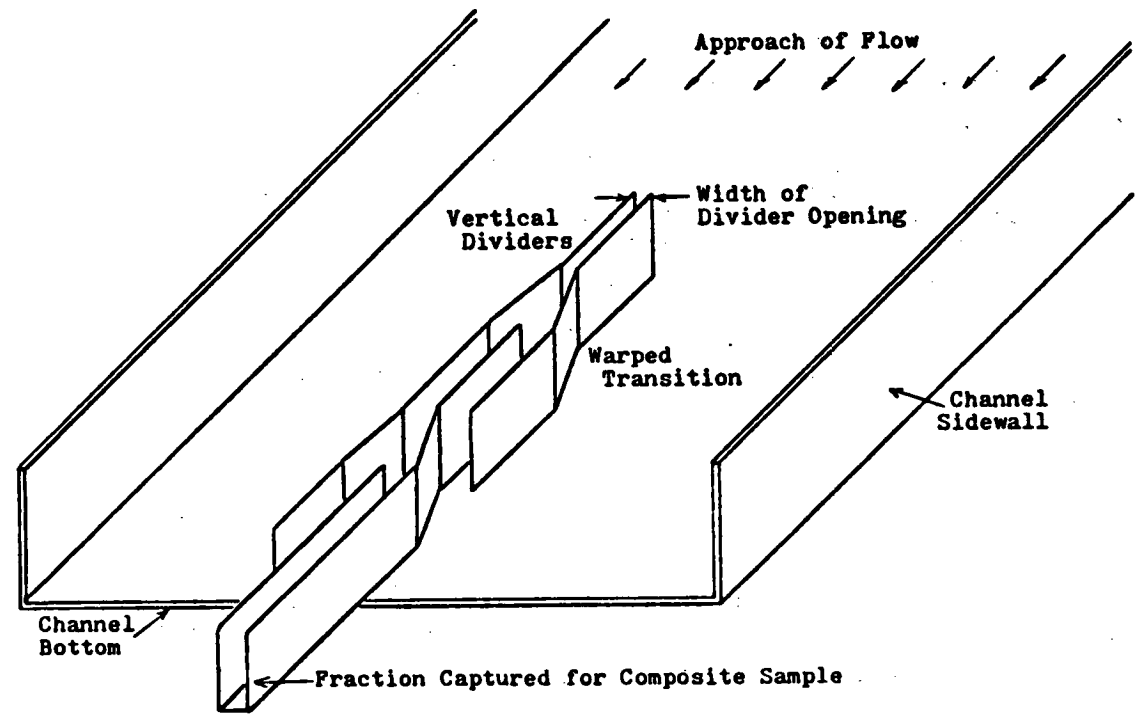
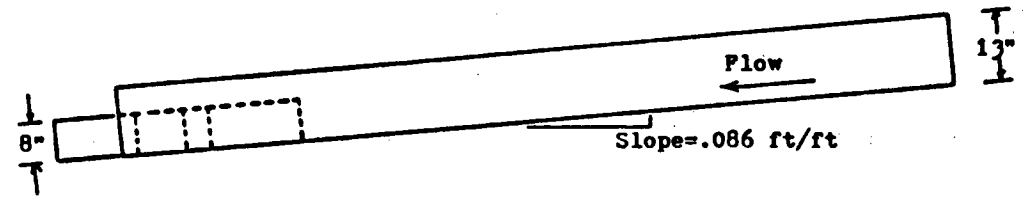
$$F = V'/V$$

$$F = DO/W$$

Note: DO should usually be 1 inch (smaller openings risk clogging).



Plan  
Elevation



### Design of Channel

1. Use depths of 13 inches for the channel and 8 inches for dividers.
2. Use divider lengths ( $L_d$ ) of 6 inches for one split, 17 inches for two splits, and 31 inches for three splits. Do not use more than three splits.
3. Select length of approach to dividers ( $L_a$ ) such that  $L_a/W > 4$  (4.5 - 5.5 has been found to work well).
4. Find total length ( $L_t$ ) =  $L_d + L_a$ .

Notes: (a) Flow splitters should be installed on a slope of 1 inch vertical:1 ft. horizontal to ensure supercritical flow.

(b) Following are the dimensions of some typical flow splitters.

| <u>F</u><br><u>(%)</u> | <u>W</u><br><u>(inches)</u> | <u><math>L_a</math></u><br><u>(ft)</u> | <u><math>L_a/W</math></u> | <u><math>L_t</math></u><br><u>(ft)</u> |
|------------------------|-----------------------------|--|---------------------------|--|
| 1.0                    | 25                          | 9.42                                   | 4.52                      | 12                                     |
| 20.0                   | 10                          | 4.50                                   | 5.40                      | 6                                      |
| 3.75                   | 20                          | 6.58                                   | 3.95                      | 8                                      |
| 5.0                    | 15                          | 6.58                                   | 5.26                      | 8                                      |
| 2.0                    | 25                          | 6.58                                   | 3.16                      | 8                                      |

### Materials of Construction

1. Flow splitter -- 3/4 inch marine plywood with exterior plywood lid
2. Dividers -- masonite
3. Collection tank -- 3/4 inch exterior plywood
4. Coating -- marine resin

### Flow Splitter Calibration

1. Select 8-12 flow rates in the range from zero to maximum design flow rate. These rates may be concentrated somewhat in the area of the range in which most actual flows are expected to occur.
2. Take five readings of the split flow rate for each total flow rate setting.
3. Average each set of five readings and plot measured fraction split versus total flow rate. Use this graph to determine a calibration factor to use in the field to estimate total runoff volume.

## APPENDIX M-2

RUNOFF COMPOSITE SAMPLING  
DATA SHEET

Station: \_\_\_\_\_

Date: \_\_\_\_\_ Observer: \_\_\_\_\_

Time: \_\_\_\_\_

Runoff is: \_\_\_\_\_ Finished  
                  \_\_\_\_\_ Continuing

Tank water depth (4 readings):

|       |       |            |       |
|-------|-------|------------|-------|
| _____ | _____ | Average -- | _____ |
| _____ | _____ |            |       |

Calculation of runoff volume collected:

Tank cross-sectional area = \_\_\_\_\_

Volume = area x average depth = \_\_\_\_\_

Estimation of total runoff volume:

Flow splitter calibration factor = \_\_\_\_\_

Total volume = tank volume/calibration factor

= \_\_\_\_\_

## APPENDIX M-3

TEST FOR SOIL RESPIRATION RATE (CO<sub>2</sub> EVOLUTION)

1. Place a 3 lb (7.6 cm diameter) coffee can in an inverted position on the soil surface at five locations at the center and around the circumference of a 1 m diameter circle.
2. For each 24 hours that a coffee can will remain in place, place 5 g of dry soda lime (predried at 75°C) in a 50 ml screw-cap plastic vial. Let the cap off and place the vial under the can. Note: It is best that vials be retrieved after 24 hours. If that is impossible, they may be left 48 hours with 10 g of soda lime.
3. Drive the edges of the cans into the litter layer to seal the soda lime CO<sub>2</sub> traps.
4. Place a second set of five cans on top of plexiglass sheets. Repeat step 2 for these cans. Seal the edges with vaseline.
5. After 24 or 48 hours, retrieve and cap the vials. Note: Vials must be retrieved after an even multiple of 24 hours because of diurnal fluctuation in CO<sub>2</sub> production. Record the number of hours the vials were in place.
6. Return vials to the laboratory for drying at 75°C. Reweigh to determine gain due to CO<sub>2</sub> absorption. Report results in g CO<sub>2</sub>/cm<sup>2</sup>/day, where the cm<sup>2</sup> divisor is the coffee can area.

APPENDIX M-4  
FISH POPULATION SURVEY PROCEDURE

Electrofishing

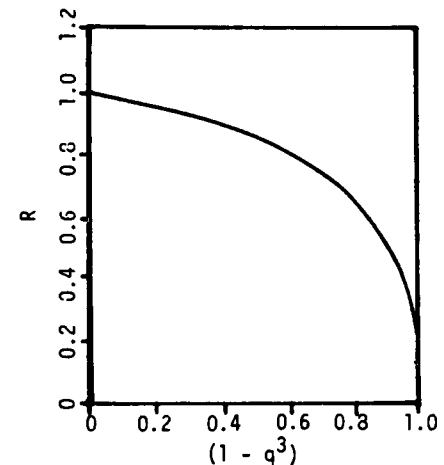
1. Block the stream with nets upstream and downstream of a reach of relatively uniform width. Make the length great enough to incorporate the various fish habitat types. Measure and record length and average width.
2. Use electrofishing gear and hand nets to capture all fish.
3. Place captured fish in a cage until finished.
4. Identify and count fish.
5. Measure the length of and weigh enough fish of each species to obtain length-frequency and weight-frequency distributions.
6. Return fish to the stream outside the blocked reach.
7. Repeat steps 2-4, 6, and, if necessary, 5 two more times.

Population Estimation

1. Find the total catch by summing the catch for all three passes. This is "T."
2. Add the catch in the second pass plus two times the catch in the third pass. This is "A."
3. Find the ratio A/T. This is "R."

4. Use the chart<sup>a</sup> to find  $(1 - q^3)$  for the calculated R.
5. Find the estimated population size =  $T/(1 - q^3)$ .

<sup>a</sup>Zippin, C., "The Removal Method of Population Estimation." Journal of Wildlife Management Vol. 22 (1958) pp. 82-91.



Length-Frequency and Weight-Frequency Distributions

1. Group data from all three passes and tabulate the number of each species falling in various length and weight ranges.
2. Arrange in frequency distributions and find mean and median lengths and weights.

Condition Factor

1. Calculate condition factor (K) for each specimen that was measured and weighed:

$$K = \frac{W}{L^3} \times 10^5$$

where: W = weight in grams

L = length in millimeters

## APPENDIX M-5

### ANALYSIS OF VOLATILE FATTY ACIDS

Volatile fatty acids may be determined by direct aqueous injection of acidified samples into a gas chromatograph with flame ionization detector (FID). Acid blanks should be run with each set of samples to check for contamination. Specifications for the equipment and settings follow:

Gas chromatograph column--6 ft x 2 mm ID glass column packed with

0.3 percent Carbowax 20M/0.1%  $H_3PO_4$  on 60/80 Carbopack C

(Supelco, Inc., Bellefonte, PA, or equivalent)

Oven temperature--120°C

FID temperature--250°C

Injection port temperature--200°C

Sample volume--1  $\mu$ l

Carrier gas--Nitrogen at 50 ml/min

## APPENDIX M-6

### ANALYSIS OF SOIL CATION EXCHANGE CAPACITY

1. Weigh out 10 g of soil (in duplicate) and transfer to a 300 ml Erlenmeyer flask.
2. Add 50 ml of 1 N ammonium acetate extracting solution.
3. Shake the suspension mechanically for 30 minutes.
4. Filter the suspension using a 5.5 cm Buchner funnel and Whatman No. 2 filter paper (adjust suction to get a filtration rate of 1-2 drops per second).
5. Rinse the Erlenmeyer flask four times with no more than 10 ml of extractant per rinse. Add rinsings to the funnel by thoroughly washing the soil on the filter after any previous addition has passed through.
6. Test the last drippings for calcium using saturated ammonium oxalate (one drop in test tube to see if precipitate forms). If precipitate forms, add additional extractant in 10 ml portions and recheck. Record the total volume of extractant used.
7. Before soil collected on funnel dries, remove excess ammonium acetate by washing the soil with 25 ml neutral 1 N ammonium chloride, vacuuming dry, and discarding ammonium chloride runnings. Then, leach with neutral methanol until leachate is free of chloride.
8. Wash the soil further with 450 ml of 10 percent NaCl, adding in successive small portions. Make the leachate up to 100 ml.

9. Pipet 0.5 ml of the NaCl extract into a 125 ml flask and add 45 ml of distilled water, 1 ml of 10 percent sodium tartrate solution, and 2.5 ml of Nessler's reagent, swirling well after each addition.
10. After 25 minutes, compare to ammonium standards on a colorimeter.
11. Calculate ammonium adsorbed per 100 g of soil.
12. Weigh out two 50 mg portions of air-dried soil into tared porcelain dishes. Dry in a 110°C oven for at least two days, cool in a dessicator, then quickly reweigh. Calculate percent moisture in the soil and convert the value found in step 11 from an air-dry to an oven-dry basis.

#### APPENDIX M-7

#### ANALYSIS OF SOIL MICROBIAL ACTIVITY

##### Determination of Soil Moisture Content

1. Place 20 g of wet soil in 80 ml vial for drying at 75°C for 24 hours.
2. Cool for one hour in a dessicator and then quickly reweigh.
3. Express moisture content as the difference in the wet and dry weights divided by the dry weight.
4. Use the moisture content to express all other results on a dry weight basis.

##### Soil pH

1. Place 5 g of wet soil in a 35 ml test tube.
2. Add 10 ml distilled-deionized water.
3. Mix on a vortex mixer for 10 seconds.
4. Settle for one hour.
5. Take pH reading in water solution.

##### Microfauna

1. Place 50 g of wet soil in double-layered cheesecloth and knot loosely.
2. Place cheesecloth bag on wire-mesh supports in 15 cm plastic funnel within a Tullgren extraction system.

3. Using plastic tubing connect funnel to a 30 ml vial half filled with 70% ethanol.
4. After 24 hours, remove and stopper the vial.
5. For counting, pour the contents of each vial into a 9 cm Petri plate.
6. Wash the residue in the vial onto the plate with 95% ethanol.
7. Identify organisms to the order level and count using a dissecting microscope under 10-20 times magnification.
8. Express population density on a per gram dry soil basis.
8. Autoclave media at 121°C for 15 minutes and cool in a water bath at 45°C.
9. Pour 20 ml of media into each dilution plate, plus control plates to which 1 ml autoclaved distilled-deionized water had been added.
10. After four days of incubation at room temperature, select a plate from each series that contains 30-300 colonies for counting.
11. Express results on a per gram dry soil basis.

#### Bacterial and Fungal Counts

1. Place 2.5 g wet soil in a 35 ml test tube containing 25 ml of autoclaved distilled-deionized water.
2. Mix on a vortex mixer for 10 seconds.
3. Transfer 2.5 ml of suspension to another 35 ml test tube containing 22.5 ml of autoclaved distilled-deionized water.
4. Vortex mix for 5 seconds.
5. Use an autoclaved pipet to make serial transfers of solution into 9 ml autoclaved distilled-deionized water to make dilutions of  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ , and  $10^{-6}$ .
6. Beginning with the most dilute solution in each series, place 1 ml of solution into each of two 9 cm Petri dishes (one for bacteria and one for fungi).
7. Prepare Rose Bengal Streptomycin Agar for fungi and Yeast Extract Agar for bacteria.



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