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COMPARATIVE EFFECTS OF PROPYLENE OXIDE, SODIUM AZIDE, AND AUTOCLAVING ON SELECTED SOIL PROPERTIES

HORACE D. SKIPPER* and DALE T. WESTERMANN†

Department of Soils, Oregon State University, Corvallis, Oregon, U.S.A.

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Summary—Samples of soil (25 g) were treated with 1 or 2 ml of propylene oxide, 400 or 800 parts/10^6 of sodium azide, or autoclaved for 1·5 or 3·0 h. Soil sterilization was achieved by the propylene oxide and autoclaving treatments. Sodium azide inhibited the bacteria and actinomycetes and drastically reduced the fungal population. The autoclaving treatment decreased the soil pH 0·2 unit, while propylene oxide and sodium azide treatments increased it 0·5–1·1 units. Extractable manganous—Mn was increased 2- to 3-fold by all treatments except for a 90- to 120-fold increase in an autoclaved soil; extractable Ca was not affected; and the extractable K changes were slight. Total extractable N was increased 10–20 parts/10^6, and available P was generally increased by the treatments. Propylene oxide induced the least chemical alterations upon sterilization and is considered an appropriate sterilant to study chemical transformations in soils; but, germination and growth of wheat and alfalfa were retarded in propylene oxide treated soil.

INTRODUCTION

NUMEROUS papers deal with the beneficial and harmful effects of partial sterilization of soils by steam, chemical fumigants, or disinfectants (Warcup, 1957); and as with partial sterilization, agents that render the soil sterile have both helpful or deleterious effects. Steam sterilization increased extractable N, P and S (Eno and Popenoe, 1964), and water-soluble Mn, Cu, B, Ca, Mg and Na (Salonius, Robinson and Chase, 1967). As a result, plant and microbial growth may increase; however, the toxic nature of certain organic materials (Rovira and Bowen, 1966) and Mn (Fujimoto and Sherman, 1948) contribute to poor plant growth in autoclaved soils.

The epoxides, ethylene oxide and propylene oxide, sterilize soils by alkylation of functional groups in proteins of microorganisms (Fraenkel-Conrat, 1944). Soil sterilization by ethylene oxide increased soluble organic matter, available P (Clark, 1950), and Cu and Mn (Dalton and Hurwitz, 1948). Propylene oxide, an epoxide with better handling properties, increased soil pH (Bartlett and Zelazny, 1967) and was used to kill pathogenic organisms; but extended treatment time reduced the germination of club wheat (Ark, 1947). Unless adequate steps are taken for detoxification, residual epoxides may hinder subsequent plant growth (Bartlett and Zelazny, 1967; Bowen and Rovira, 1961).

Gamma irradiation increased water-soluble Mn, Ca, Mg, B, Cu, NH_4, conductivity, soluble carbohydrates, organic matter (Salonius *et al.*, 1967); extractable N, P and S (Eno and Popenoe, 1964). Ethylene is also produced during sterilization by gamma-irradiation (Rovira and Vendrell, 1972). Methyl bromide sterilization generally increased extractable N,

* Present address: Monsanto Company, Agricultural Division, 800 N. Lindbergh Boulevard, St. Louis, Missouri 63166.

† Present address: USDA, Agricultural Research Service, Snake River Conservation Research Center, Route 1, Box 186, Kimberly, Idaho 83341.

P and S in soils with a high organic matter content (Eno and Popenoe, 1964), whereas fumigation of a forest nursery soil low in organic matter had no significant effect on soil acidity, total N, or extractable K, Ca, Mn, Mg or P (Danielson, 1966).

Sodium azide has been used as a general enzyme inhibitor to study chemical transformations of Mn (Mann and Quastel, 1946), cellulose decomposition (Perlin, Michaelis and McFarland, 1947), and detoxification of herbicides (Audus, 1951; Harris, 1967). However, we have found no description of the effects of sodium azide on soil chemical properties.

The need and interest to study uptake of compounds by aseptic plant roots and chemical transformations of nutrients and pesticides in sterile soils prompted this research to define the effects of propylene oxide, sodium azide, and autoclaving on the soil pH, extractable Ca, K, Mn, N, available P, and the soil microbial population. Autoclaving was included as a standard comparison. Propylene oxide was used as a liquid-gaseous sterilant and sodium azide provided an option of enzyme inhibition without complete sterility.

MATERIALS AND METHODS

The surface horizons of Dayton (Typic Albaqualfs, depositional planasol), Jory (Xeric Haplohumults, reddish-brown lateritic), and Woodburn (Aquultic Argixerolls, grey-brown podzolic) soils from the Williamette Valley were used in this study (Table 1). Samples of soil (25 g dry weight) were added to 125-ml Erlenmeyer flasks, moistened to field capacity, and allowed to equilibrate for 2 days. The samples were then treated with propylene oxide, $\overline{OCH_3CH}CH_3$, density of 0·859 g/ml (1 or 2 ml/25 g of soil), or sodium azide, NaN_3 (400 or 800 parts/10^6 on a soil basis, in 1 or 2 ml of water). The flasks were stoppered with cotton plugs and covered with 1 mil polyvinylidene chloride for 2 days at 27 ± 2°C. The film was then removed and the flasks were evacuated for 2 days in a laboratory exhaust hood.

TABLE 1. SOIL CHARACTERISTICS

Characteristic	Soil		
	Dayton silt loam	Jory silty clay	Woodburn silt loam
Organic matter* (%)	2·6	6·9	3·1
Field capacity (% H_2O)	24·8	24·7	20·2
CEC† (m-eq/100 g)	15·3	23·1	18·9
Fe_2O_3‡ (%)	1·2	7·9	2·1
Al§ (m-eq/100 g)	2·1	6·3	2·1
pH‖	5·0	5·8	5·6
Sand (%)	15	15	11
Silt (%)	71	42	72
Clay (%)	14	43	17
Clay minerals¶	Montmorillonite	Chloritic intergrades	Vermiculite, mica
Bacteria and actinomycetes** (× 10^6/g soil)	5·0	21·5	16·7
Fungi†† (× 10^4/g soil)	51·6	47·9	19·1

* Walkley and Black (1934).
† Schollenberger and Simon (1945).
‡ Sodium dithionite, citrate extractable Fe.
§ $NH_4C_2H_3O_2$, pH 4·8 extractable Al.
‖ Soil:H_2O ratio = 1:2.
¶ Dominant minerals, personal communication with Dr. M. E. Harward.
** Stevenson and Rouatt (1953).
†† Martin (1950).

Additional samples (*ca.* air-dried) were autoclaved at 121°C for 1·5 or 3·0 h. Preliminary experiments indicated these rates or times were generally needed for sterilization. Subsamples from treated and untreated soils were removed 2 days after each treatment for biological and chemical evaluations. Each sterilization method was studied in separate experiments.

Duplicate samples (*ca.* 0·25 g) of treated and untreated soils were transferred aseptically to: (1) nutrient broth (Difco), NB; (2) yeast extract agar, YEA (Stevenson and Rouatt, 1953); and (3) rose bengal agar + streptomycin, RBA (Martin, 1950). The soil was added to Petri plates before pouring the YEA and RBA. The sodium azide method was evaluated by addition of 400 or 800 parts/10^6 NaN_3 to the media, since microorganisms would resume growth if the NaN_3 inhibitory effects were removed. All media were examined for microbial growth after incubation for 11 days at 27 ± 2°C to ascertain sterility of treated soils. Viable populations of the untreated soils were counted by the dilution-plate method. Bacteria and actinomycetes were plated on YEA and fungi on RBA.

Soil pH was measured on the supernatant solution of a 1:2 soil–water slurry 30 min after mixing. Total extractable N (NH_4^+, NO_3^-, NO_2^-) was extracted from 5 g of soil with 50 ml of N-KCl and determined by MgO-Devarda's alloy method (Bremner and Keeney, 1965). Available P was determined by the method of Bray and Kurtz (1945). Watersoluble and exchangeable Mn, Ca and K were extracted from 10 g of soil with three 30-ml portions of N-sodium acetate, pH 7, filtered and diluted to a 100-ml volume. Manganese and Ca were analyzed by atomic absorption and K was determined by flame emission.

Each treatment and controls were performed in triplicate and analyses of variance were made within each sterilization method to test the effect of a sterilization method within a given soil.

RESULTS

Biological tests

Fungi did not grow in RBA that had been inoculated with soil treated with propylene oxide, sodium azide, or by autoclaving. Moreover, no visible growth, fungal or bacterial, was observed in NB containing any of the treated soils. A very small number of bacteria and actinomycetes (YEA) were present only in the soils treated with the 1-ml rate of propylene oxide. Addition of NaN_3 to the agar media completely inhibited the microbial growth; but when NaN_3 was omitted from the media, the bacteria and actinomycetes grew without any reduction in the number of colonies as a result of the NaN_3 soil treatment, whereas a 60–70% reduction in colony numbers was observed for the fungal population.

Chemical alterations

Both propylene oxide and sodium azide significantly increased the soil pH approx. 0·5 unit in each soil with a higher increase at the higher rates, whereas the autoclave treatment lowered the soil pH approx. 0·2 unit (Fig. 1a). Extractable Mn was increased approx. 1·5 times with the low rates of propylene oxide and NaN_3 in the Dayton and Jory soils. However, propylene oxide produced no significant changes in extractable Mn in the Woodburn soil, but NaN_3 increased Mn approx. 2·5 times (Fig. 1b). The release of Mn at the high rates of propylene oxide and NaN_3 was not significantly higher than the lower rates. Autoclaving resulted in a 3-fold increase in Mn for the Dayton and Jory soils, and approx. a 90- to 120-fold increase in the Woodburn soil, depending on the duration of treatment. Even with the large release of Mn in the autoclaved Woodburn soil, the extractable Mn level was still lower than that from either the autoclaved Dayton or Jory soil.

Propylene oxide had no significant effect on the extractable K status of the three soils. Sodium azide also did not produce any significant changes in the Dayton and Jory soils, but a significant increase was recorded for the extractable K content on the Woodburn soil at the 400 parts/10^6 rate of NaN_3. Autoclaving for 1·5 or 3·0 h significantly increased the extractable K in the Dayton and Jory soils; however, a significant decrease was noted for

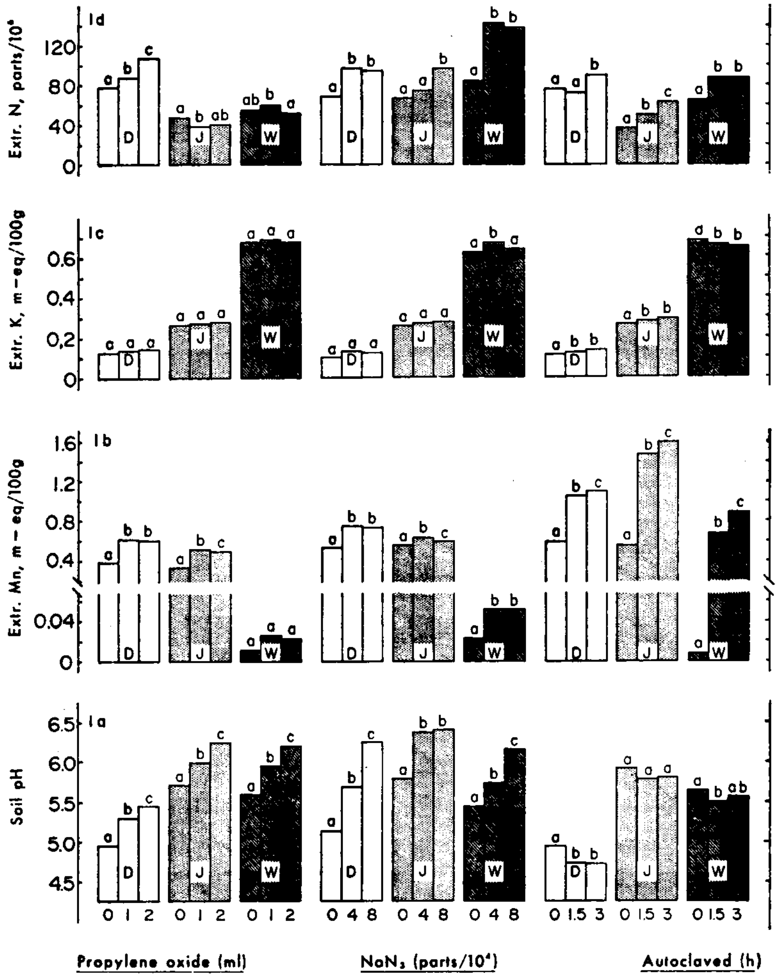

FIG. 1. Soil chemical changes caused by propylene oxide, sodium azide and autoclaving treatments. Significant differences (5 % level) for a given treatment within a given soil are indicated by different letters. D = Dayton, J = Jory, W = Woodburn.

the Woodburn soil (Fig. 1c). The extractable Ca levels of the soils were not altered by the agents used in this investigation. Total extractable N was significantly increased by all treatments except for very slight, inconsistent changes in propylene oxide treated Jory and Woodburn soils (Fig. 1d). Sodium azide produced the largest increase.

The available P of the Jory soil was not affected by propylene oxide or sodium azide, whereas the P content of the Dayton soil was significantly increased 1·5-fold by both agents and also by steam sterilization for both soils (Fig. 2). A rate response was not observed with propylene oxide or sodium azide, whereas a significant difference was noted between the autoclaved treatments. Significant data were not obtained on the available P for the Woodburn soil because of its high initial P content (150 parts/10^6).

The Woodburn soil sterilized with propylene oxide was evaluated for subsequent use as a medium for plant growth. When seeds of alfalfa (*Medicago sativa*, var. DuPuits) and wheat (*Triticum aestivum*, var. Druchamp) were added directly to the treated soil, plant growth was

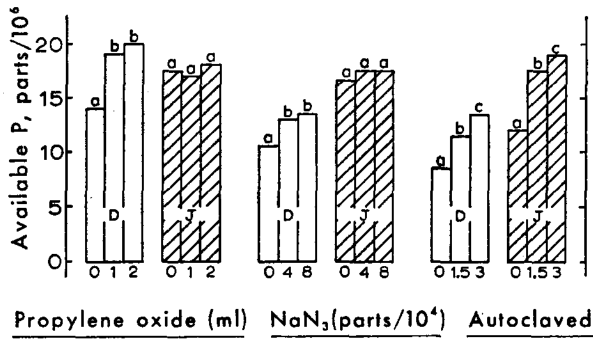

FIG. 2. Effect of sterilization treatments on available P. Significant differences (5% level) for a given treatment within a given soil are indicated by different letters. *D* = Dayton, *J* = Jory.

very restricted and abnormal. Soils were then sterilized by exposure to propylene oxide vapors (Bartlett and Zelazny, 1967); but even after severe detoxification at 45°C and 8·5 N/cm^2 (63·8 cm Hg) vacuum for 3 days, germination was reduced by 50–60% and the stems of the plants were twisted and distorted. Alfalfa appeared to be more resistant than wheat to the toxic residues of propylene oxide. In contrast, plant growth in autoclaved Woodburn soil was approximately equivalent to that in untreated soil.

DISCUSSION

Air contamination may be partially responsible for the lack of complete sterility in the soils treated with the low rate of propylene oxide since only 1–2 colonies/plate were observed in approximately one-third of the YEA agar plates. The absence of growth in NB inoculated with the propylene oxide treated soils also suggests the presence of some highly resistant spores that germinated in the YEA but not in NB. In view of these possibilities, one should consider the lower rate of propylene oxide as a questionable case of sterility.

Potassium azide has been shown to protect cowpeas from fungal pathogens (Gay, 1970). A reduction in fungal population was observed in our study that supports the fungicidal activity of KN_3. This decrease might be explained on the basis of differences in metabolic pathways or presence of resistant spores, because azide uncouples oxidative phosphorylation (Wilson and Chance, 1966).

The increase in soil pH from propylene oxide treatment results from its reaction with labile H-atoms in the inorganic and organic fractions of the soil, while sodium azide as a weak base would neutralize soil acidity. The lower pH after autoclaving apparently arises from the release of organic acids from the organic matter.

The significant increases in extractable N in the soils treated with sodium azide may be attributed to the reduction of the azide to the ammonium ion. Propylene oxide and autoclaving apparently solubilized some nitrogeneous components of microbial cells or organic matter. The increases in available P and extractable Mn could arise from solubilization of inorganic forms or also be released from organic matter and microbial cells. The reducing conditions of saturated steam under pressure may also contribute to the results of autoclaving (Conner, 1932), particularly for extractable Mn.

For studies of chemical transformations of nutrients or pesticides in sterilized soils, propylene oxide is considered an appropriate sterilant to use; however, residual toxicity may restrict its use for aseptic plant studies. The propylene oxide method has been used to study the effects of potassium salts on the availability of soil Mn (Westermann, Jackson and Moore, 1971). Sodium azide should be considered as a partial sterilization agent because of its effect on the fungal population.

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