

[Research]

Evaluation of chemical and biological consequences of soil sterilization methods

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ABSTRACT

Sterilized soils are commonly used for the study of xenobiotic sorption and as an abiotic control in biodegradation experiments. They are also used for the chemical study of nitrogen and carbon cycle processes and the elimination of the biological factors. In this research the effects of different soil sterilization methods such as autoclaving, fumigating and exposure to ultraviolet and microwave irradiation on soil chemical and biological properties (soil acidity, electrical conductivity, cation exchange capacity, optical density, extractable carbon and nitrogen and colony forming unit) were examined under laboratory conditions. All the sterilization methods significantly (at $P < 0.05$) decreased pH and increased electrical conductivity (EC), optical density (OD), extractable carbon and nitrogen as compared to non-sterilized soil samples. The results showed that autoclaving method was the most effective method in eliminating soil microorganisms and ultra violet irradiation was the less effective one. These induced changes were likely due to release of soluble organic acids from humic materials and dead microorganisms.

Keywords: Autoclaving, Colony forming unit, Fumigation, Microwave irradiation, Soil sterilization, Ultraviolet irradiation.

INTRODUCTION

Soil sterilization includes eradication or control of any major class of soil borne pests such as weeds, weed seeds, nematodes, fungi, bacteria and plant diseases (Nutter, 1957). There are different methods of soil sterilization in order to inhibit microbial activity in soils (Trevors, 1996; Shaw, 1998). Most commonly, these include autoclaving, gamma irradiation, chloroform fumigation, ethylene or propylene oxide, ultraviolet and microwave irradiation. Since these methods are greatly different in terms of properties and usefulness, the choice of the best sterilization method may significantly vary from one situation to another.

It has been reported that soil sterilization methods commonly alter soil physical and chemical properties. For example, ethylene and propylene oxide sterilants react with acidic hydrogen of organic matter to induce an increase in soil pH and organic carbon

content (Dao *et al.*, 1982; Wolf *et al.*, 1989; Negre *et al.*, 1995). These induced changes alter the sorptive behavior of compounds with functional groups whose reactivity to organic matter is sensitive to pH change (Dao *et al.*, 1982) or organic carbon content. Gamma irradiation causes depolymerization of carbohydrates such as cellulose and creates free hydrogen and hydroxyl radicals that are reactive as reducing and oxidizing agents to cleave C-C bonds (Puri and Barraclough, 1993). Jenkinson and Powlson (1985) reported that chloroform fumigation of soil caused an immediate increase in ammonium and organic carbon extractable with 1N K_2SO_4 . Some chemicals such as methyl bromide and chemical gases are toxic in their natural forms (Venkinson and Powlson, 1985). A number of different criteria such as toxicity, adaptability, cost, residual period and hazardness of the method are also important factors in

selecting a specifying soil sterilization method. Because of the cost, equipment involved and time and labor required, some sterilants are more adaptable than others. For example, bacterial spores are more resistant to dry heat sterilization than autoclaving, therefore, it may be necessary to incubate the wet soil samples for 1-2 days to allow spores to germinate prior to dry heat sterilization. Cost of chemicals, labor, equipment and the danger of human toxicity must all be considered in choosing a sterilization operation.

Although the soil sterilization is an important process in some scientific experiments, however, there is no report on chemical and biological consequences of soil sterilization in calcareous soils of Iran. Therefore, the aim of this research was to investigate the effects of four different soil sterilization methods (autoclaving, chloroform fumigating and exposure to ultraviolet and microwave irradiation) on some soil chemical properties.

MATERIALS AND METHODS

Soil sampling

Soil samples were collected from 0 to 25 cm depth of a *Haplcambid* soil, air dried, sieved (2 mm) and placed in plastic bags and stored at 4 °C until analysis were performed.

Experimental design and analytical procedures

Five subsamples were made from each soil sample (with 3 replicates for each subsample). One set of subsamples were left with no treatment as control samples (C). The second set of subsamples were autoclaved (AU) for 15 min (121 °C, 15 psi). The other set of subsamples were exposed to microwave irradiation (MW) at full power (1100 w) (Wang *et al.*, 2001). The fourth set of subsamples were placed in vacuum desiccator containing chloroform (CHCl₃) vapor for 24 hour (FU). The last series of subsamples were exposed to ultraviolet irradiation (UV) for 45 minutes in a laminar flow cabinet. Immediately, the moisture contents of all the treated samples were determined and they were covered with sterile aluminum foil.

A 20 gram subsample of each treatment was mixed with 50 ml distilled water and were shaken for about 15 minutes and sat

aside for 3 hours and the soil acidity (pH) and electrical conductivity (EC) of soil samples were determined in a 1:2.5 soil suspension by using EC and pH meter. Extractable carbon was determined by potassium dichromate oxidation method adapted from Vance *et al.*, (1987) and Dalal (1979). Potassium dichromate (1 ml, 1 N) and 7 ml concentrated H₂SO₄ were added to 5 ml extracts and heated, and residual potassium dichromate was determined by titration against ferrous ammonium sulphate (0.5 N) using o-phenanthroline ferrous complex as the indicator. Extractable nitrogen was determined by Micro-Kjeldhal digestion (Pace *et al.*, 1982; Davidson *et al.*, 1989). Cation exchange capacity (CEC) was measured by using Chopman (1956) method. Optical density of 0.5 M K₂SO₄ extracts of different treatments was determined at 420 nm. In order to investigate different treatment effects on the bacterial population, each treatment was serially diluted and cultured on Nutrient agar. The number of colony forming units (CFU) of all the treatments was calculated periodically.

Statistical analysis

Statistical significance of difference between treatment means were assessed by one way ANOVA. Treatment means were compared using Duncan and the least significant difference test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of different treatments on soil chemical and biological properties

Optical density changed depending on the soil sterilization method. Optical density of extracted soil solution varied from 0.057 to 0.343 for control and autoclave treatments, respectively. However, all the treatments except ultraviolet irradiation caused significant increase ($p \leq 0.05$) in optical density in comparison to control (fig. 1). The yellowish brown substances were likely humified compounds (Puri and Barraclough, 1993), and the increase in optical density in all the treatments may be due to depolymerization of carbohydrates and cleave of C-C bonds. It seems that high temperature and steam pressure involved in autoclaving treatment resulted in highest values of optical density. An appreciable

portion of extractable carbon released by treatments may have originated from the non-biomass fractions of soil organic matter which is consistent with the findings of Puri and Barraclough (1993) and Islam and Weil (1998).

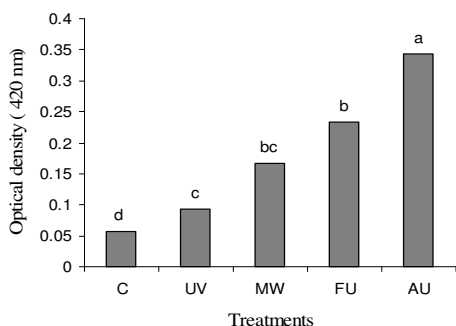


Fig 1. Influence of different soil sterilization methods on optical density. Means followed by the same letter are not significantly different at $P < 0.05$.

C=control, AU=autoclaving, MW=microwave irradiation, FU=chloroform fumigation and UV=ultra violet irradiation.

Release of extractable carbon followed a pattern similar to that of optical density. Extractable carbon of different treatments varied from 494 to 1155 mg Kg⁻¹ for control and autoclave treatments, respectively, and were statistically significant ($p \leq 0.05$) for all the treatments in comparison to control samples (fig. 2). As stated before, this increase may be due to the release of soluble organic acids presumably from humic matter and dead microorganisms. Salonius *et al.*, (1967) and Dao *et al.*, (1982) also found that autoclave and other treatments release soluble organic carbon from soil samples.

High temperature and steam pressure from autoclaving had a significant effect on C flushes, but release of high content of extractable carbon in fumigation treatment may be due to the long time exposure to chloroform vapor (24 h) that killed microorganisms and released their carbon. The difference between the values of extractable carbon of microwave and ultraviolet irradiation was not significant ($p > 0.05$). In this respect, it seems that low length of time for these treatments may be resulted in low extractable carbon values as compared with autoclave and fumigation treatments.

Similarly, in almost all the treatments there was increase in the extractable nitrogen

in comparison to control treatment (fig. 3). Autoclave and fumigation treatments were not significantly different ($P < 0.05$). Cleavage of nitrogen bonds in biomass and non-biomass components of soil need higher energy compared to C-C bonds. The high temperature and long time in autoclaving and fumigation treatments presumably resulted in release of high content of extractable nitrogen in comparison to control, ultraviolet and microwave irradiation treatments.

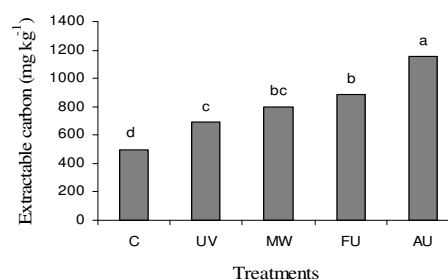


Fig 2. Influence of different soil sterilization methods on 0.5 M K₂SO₄ extractable carbon. Means followed by the same letter are not significantly different at $P < 0.05$.

C=control, AU=autoclaving, MW=microwave irradiation, FU=chloroform fumigation, and UV=ultra violet irradiation.

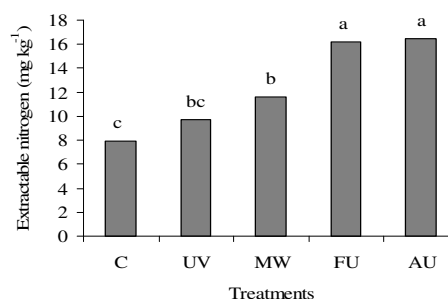


Fig 3. Influence of different soil sterilization methods K₂SO₄- extractable nitrogen. Means followed by the same letter are not significantly different at $P < 0.05$.

C=control, AU=autoclaving, MW=microwave irradiation, FU=chloroform fumigation, and UV=ultra violet irradiation.

Almost all the treatments caused a significant increase in electrical conductivity ($P < 0.05$) in comparison to control (fig. 4). The electrical conductivity of soil samples in autoclave, fumigation and microwave treatments were not significantly different ($P < 0.05$).

Cleave of different bonds in humic material and residual killed biomass induced by these treatments released different ions in

extracted soil solution and resulted in increase in electric conductivity. Salenius *et al.*, (1967) also found that sterile soils have higher electrical conductivity in comparison to non-sterilized soil samples.

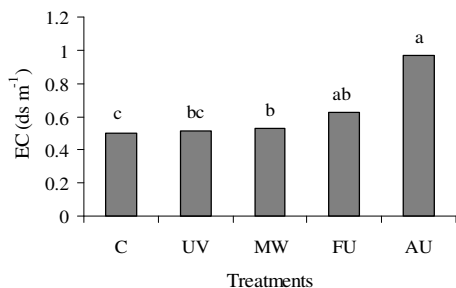


Fig 4. Influence of different soil sterilization methods on electric conductivity (EC). Means followed by the same letter are not significantly different at $P < 0.05$. C=control, AU=autoclaving, MW=microwave irradiation, FU=chloroform fumigation, and UV=ultra violet irradiation

Soil pH significantly decreased by autoclaving, fumigating and microwave irradiation treatments ($p \leq 0.05$) compared to the control. Autoclaving had the highest influence on pH; however, the differences between fumigation and microwave irradiation treatments were not significant ($P < 0.05$) (fig. 5). This decrease in pH in different treatments may have resulted from solubilization of organic acids, however, the magnitude of the decrease depended on acidic buffering capacity of the soils. In addition, the effect of change in pH on the surface charge and hydrophobicity of soil organic matter should be considered. Salenius *et al.*, (1967) found that autoclaving reduced the pH value of a clayey soil, whereas Wolf *et al.*, (1989) reported no change in pH for autoclaved soils.

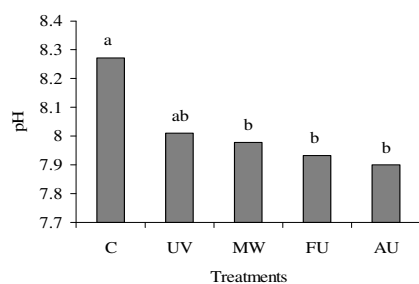


Fig 5. Influence of different soil sterilization methods on pH. Means followed by the same letter are not significantly different at $P < 0.05$. C=control, AU=autoclaving, MW=microwave irradiation, FU=chloroform fumigation, and UV=ultra violet irradiation.

Influence of different sterilization methods on cation exchange capacity (CEC) did not follow an obvious pattern (fig. 6). All the treatments caused significant increase ($P < 0.05$) in cation exchange capacity in comparison to control, but the differences between fumigation and ultraviolet irradiation were not significant ($p \geq 0.05$), while these treatments had the highest cation exchange capacity.

It is possible that through other effects induced by fumigation and ultraviolet treatments pH dependent charge of the soils increased and this may have resulted in increase of cation exchange capacity of these treatments.

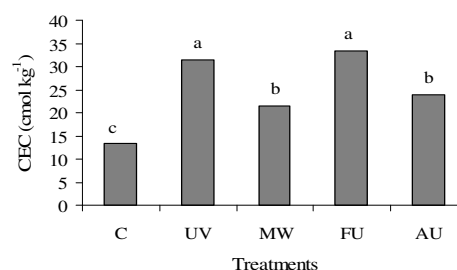


Fig 6. Influence of different soil sterilization methods on cation exchange capacity. Means followed by the same letter are not significantly different at $P < 0.05$. C=control, AU=autoclaving, MW=microwave irradiation, FU=chloroform fumigation, and UV=ultra violet irradiation.

The results of colony forming unit (CFU) of different soil sterilization treatments showed that the differences among the treatments and non sterilized soil samples were significant ($p < 0.05$). The colony forming units varied from 1×10^8 to 207×10^8 in one g of soil sample for autoclave and control treatments respectively, however, microwave and fumigation had the same effect (fig. 7).

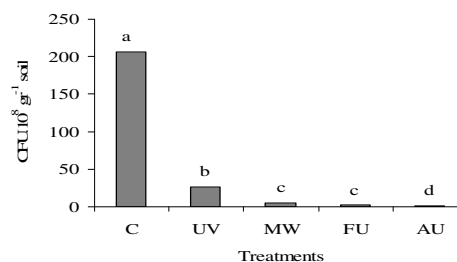


Fig 7. Influence of different soil sterilization methods on colony forming unit. Means followed by the same letter are not significantly different at $P < 0.05$. C=control, AU=autoclaving, MW=microwave irradiation, FU=chloroform fumigation, and UV=ultra violet irradiation.

It could be considered that all the treatments were effective in reducing microbial biomass and in this respect, autoclave was the strongest method.

Despite the limitation of these methods, they can still form a good basis for a better understanding of chemical and biological changes induced in sterilization methods especially with lack of reliable research information. It seems that further research is needed to evaluate physical consequences of soil sterilization methods.

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