EFFECTS OF STERILIZATION PROCEDURES ON THE BIOLOGICAL, CHEMICAL, AND PHYSICAL PROPERTIES OF SOILS: A REVIEW[1] /

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Resumen

Una revisión de literatura sobre esterilización de suelos demostró que existe muy poca información sobre los efectos de secado al calor sobre las propiedades químicas de suelo. La vaporización, fumigación e irradiación han sido estudiadas más detenidamente. Cada tratamiento tiene efecto sobre algunas propiedades físicas, químicas y biológicas de los suelos.

La esterilización por vapor, especialmente por autoclave, afecta la disponibilidad de los macronutrimentos, micronutrimentos y agregación del suelo. Los fumigantes tienen efectos variables en las propiedades químicas del suelo, bromuro de metilo es el que menos causa cambios en los micronutrimentos. La radiación gama destruye organismos dañinos del suelo. Nuevos datos en suelos de Puerto Rico demuestran que ocurren cambios en las propiedades químicas de suelo por esterilización secado-calor a 121°C por dos horas. Los resultados siguen a aquellos informados por vaporización, autoclave y fumigación con bromuro de metilo.

Introduction

Common soil sterilization procedures are aerated steam (5), autoclaving (28), or fumigating with various chemicals (3), especially methylbromide. Gamma radiation (15) and dry heating (53) have also been used, though not for commercial sterilization operations. Dry-heat sterilization at 121°C for two hours is the prescribed technique used by the USDA Animal, Plant, and Health Inspection Service (APHIS) for treating imported soils (Table 1).

Effects of sterilization methods, or simple air drying and storage, on plant growth and on soil properties are well documented (1, 3, 5, 8, 19, 22, 32, 53). Conflicting results are usually dependent on soil type and kind of plant grown afterwards on the sterilized materials. Few references explain any effects of dry heating on soil properties (49, 53).

Many universities in the United States now conduct cooperative agricultural, horticultural or forestry research with institutions in tropical countries. Such cooperative efforts are expected to increase in the future (40, 45, 50), and larger numbers of soil samples will be imported and subjected to laboratory tests. Because of strict phytosanitary requirements for imported soils, it seems opportune to review advantages and disadvantages of various soil sterilization techniques and to take a new look at dry-heat sterilization and its effects on soil chemical properties.

Historical overview of soil sterilization procedures

Soil roasting, soil burning, heat sterilization, soil steaming, soil disinfection, soil fumigation, and partial sterilization are terms that have been used to characterize soil sterilization. Sterilization treatments were originally used to destroy plant parasites or harmful soil microorganisms and to improve fertility of treated growing media (22). Two major approaches in sterilization research were (a) drastic soil treatment in which most organisms were killed, followed by inoculation or introduction of desired microorganisms or (b) minimal soil treatment to destroy only selected pathogens, which is still done with

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Table 1. Summary of USDA Animal, Plant, and Health Inspection Service (APHIS) treatment schedules for soils arriving at ports of entry in the United States without previous arrangements.

Treatment	Exposure Period
Dry Heat[1]	
110-120 5°C	16 hours
121-154°C	2 hours
154 5-192 5°C	30 minutes
193-220°C	4 minutes
221-232°C	2 minutes
Steam Heat[2]	
1.05 g per cm² pressure	30 minutes

1 Counting time not started until entire soil mass reaches the required temperature

2 Counting time not started until pressure reaches the required limit within an autoclave chamber

treating of mushroom casing soils (5). The first approach is appropriate to plant quarantine work, but the latter predominates in commercial agricultural or horticultural work. Drastic and minimal treatments affect growth of plants on sterilized soils (1, 3, 53). Observed plant responses on sterilized soil are best understood by studying several disciplines, including soil chemistry, soil microbiology, soil physics, and phytopathology.

Sterilization effects on soil properties can be separated into five classes (22): 1) Sterilization almost totally destroys undesirable and desirable soil flora and fauna such as bacteria, fungi, protozoans, and viruses or advanced life forms like propagative organs or seeds. Farmers in many parts of the world capitalized on this effect by regularly burning fields. This practice helped eliminate or at least reduce certain local pests; it also helped control root rot disease in certain crops (32).

2) Sterilization causes various chemical reactions in the soil, including formation of toxic or other compounds. Decomposition of soil organic matter accelerates, causing liberation of ammonia, carbon dioxide, and organic products. Inorganic materials break down or change; nitrates and nitrite are reduced to ammonia and nutrient solubility or availability changes. 3) Biochemical changes occur in the soil; ammonification generally increases and nitrification or denitrification processes are modified. 4) Changes in soil physical properties can occur, modifying absorptive and capillarity capacities, soil structure, color, and odor. 5) If total destruction of soil microorganisms is not achieved, there are still subtle or even drastic changes in population levels, species, or numbers of surviving species because available food or substrates are altered. Surviving populations increase or decrease rapidly depending on whether other microorganisms are added after soil sterilization. Higher plants grown on sterilized soil can exhibit qualitative and quantitative changes in growth, form, color, or other factors as compared to similar plants grown on non-sterilized soil.

Soil fumigants also cause changes in soil properties. Chemical treatments are seldom uniformly administered throughout the soil, especially when injected at single or a few points instead of under pressure within enclosed containers (5). When using fumigants, one must also be aware of physico-chemical relationships between soils and individual chemicals. Each chemical has a distinct structure which determines its unique boiling temperature, vapor pressure and diffusion coefficients (12). Their sorption by growth media is also affected by existing soil moisture, porosity, tortuosity, and proportion of mineral and organic solids in the medium (20).

Steam heating has at least three advantages over most other sterilization methods (5, 6, 53): it is nonselective and kills all pests or microorganisms; it does not leave behind toxic residues as do some chemical treatments, although it can produce toxic levels of certain soil nutrients like Mn (19) that can hinder or even preclude plant growth after treatment; and steam produces uniform heat at low intensity within soils in a short time inside and around all soil pore spaces and solids.

Steam sterilization procedures have changed substantially since first used for research or commercial purposes (5). Initially, steam temperature was high and the treatment period long: 120 °C for six to eight hours in autoclaves. Later, both steam temperatures and treatment times were reduced: 100°C for 30 minutes if flowing steam was directed through soil, or 83°C for 30 minutes if moving soil was passed through steam. A treatment of 100°C for 30 minutes was high enough so that even if large amounts of wet soil with many clods were treated, a minimum temperature of 60°C was obtained for the same period. The common method used today is aerated steam at 60°C for 30 minutes (21).

Even at lower temperatures, aerated steam destroys most pathogenic plant fungi, bacteria, and nematodes, and nearly all viruses are inactivated.

Some fungal flora are drastically reduced in numbers and types; bacteria and actinomycetes are less affected. Fungistasis is largely destroyed, and phytotoxicity found at the higher temperature of 100°C for 30 minutes is practically eliminated. Most weed seeds are killed if soils are kept moist for three days prior to treatment. Weed molds are greatly suppressed at lower temperatures although they are quite prevalent after steaming at 100°C for 30 minutes (5).

Disadvantages of dry-heat sterilization are: heat intensity is usually quite high, the quantity of soil that can be effectively treated is small, and heat distribution throughout voids and solids in the soil matrix is poor and influenced by moisture content (6). Detrimental effects of high temperatures in dry heating are reduced by using ovens where the soil is continuously turned or moved and by using forced-draft ovens (6). Dry-heat sterilization with resistance cables or covered resistance heaters buried in the soil have also been used (32). Limiting factors were originally the size of service lines needed to achieve sterilization in a short time; now, soaring rates for electricity in most parts of the world preclude dry heating by resistance methods.

Dry-heat sterilization was chosen as the optimum plant quarantine procedure by USDA-APHIS in the 1940's. At the temperatures and durations precribed (Table 1), the safety margin for effectively killing all harmful soil organisms is quite high. This explains why APHIS dry- and steam-heat procedures are more vigorous than those used by commercial horticultural and agronomic operations; it also explains resistance to change in existing regulations. Although dry-heat sterilization of large soil volumes is difficult, particularly when they are wet and have large clods, it is convenient for small sample volumes. The method uses simple apparatus requiring less maintenance than that for equipment required in steaming.

Microwave ovens have recently been tested by USDA-APHIS because industry and commercial facilities want quick, simple, and effective procedures to dispose of unsterilized waste soils or their extracts. Microwave sterilization has not been very effective since it is difficult to maintain microwave temperature $\geqslant$ 121°C for long periods of time unless all soil moisture is removed first.

Sterilization and air-drying effects on soil properties

Specific examples of changes that sterilization treatments exert on soil pysical, chemical, and biological properties are now summarized by the four major sterilization procedures: steam, chemical, irradiation, and dry heating. Effects of air drying of soils are also examined.

Steam sterilization usually causes certain macronutrients and micronutrients to become more available in the soil. Usually affected are Ca, K, P, Zn, Cu, N, Mn, Fe, and B (26, 32, 53). High steam temperatures generally increase availability of affected elements more so than do low temperatures, particularly for soils with high organic matter contents (33, 43).

Manganese levels are often increased after steaming and Mn toxicity frequently occurs on both calcareous and acid soils (11, 19). This phenomenon could be related to the original Fe: Mn ratio in the soil (41). Less information on other micronutrients exists, but steaming sometimes increases soil Fe availability (26). Steaming causes little or no effect upon soil acidity (53) whereas autoclaving has increased soil acidity values over those for air-dried soil (28). Autoclaving also increases the amount of 1 N K_2SO_4-extractable organic carbon found in soils treated with fumigants or air-dry procedures (34). Slightly increased aggregation has been reported after steaming (53).

Plant growth responses on steam treated soils are quite variable and seem dependent on species, varieties, and kind of growth medium. Use of 100 percent autoclaved soil caused significant Ca accumulation in and distortion of sainfoin (*Onobrychis vicifolia*) roots, both in growth chamber and field studies (37). Shoot and root elongation decreased for three plant species grown on autoclaved compost, with neither storage of the treated material for up to 12 months nor addition of N-P-K and Fe fertilizer altering the poor growth (24). Measured N in tomato foliage increased after steaming, the increase becoming more pronounced each year over a three year period (54). Average lettuce headweights obtained on light soil types heated to 70°C were about 20 percent greater than those of lettuce grown on soils heated to 100°C.

In other trials, P uptake decreased in avocado (*Persea americana*) seedlings planted on steamed sandy loam soil (30). Growth of two desert plants on steamed calcareous soil decreased and was attributed to induced P deficiency; no adverse growth response occurred for the desert plants grown on steamed non-calcareous soil (52). And P uptake in barley (*Hordeum vulgare*) increased after eliminating microorganisms from basaltic soil by steaming; the basaltic soils normally required P fertilizer for adequate plant growth (9).

The most common **chemical sterilants**, methylbromide and propylene oxide, are equally effective in destroying soil bacteria, fungi, and actinomycetes (28). They and other chemicals temporarily impede nitrifying bacteria (53) and affect beneficial mycorrhizal fungi (47). Yet fumigation followed by planned reinoculation with desired mycorrhizal strains can be an effective management tool for commercial forest nurseries (31) or horticultural operations (46). Some chemicals, especially the epoxides, leave behind harmful residues after sterilization, and treated material must first be detoxified before being used (42). Other chemicals like biomonomethane, dazomet, and etridiazole have no adverse effect on plant growth, even after repeated soil treatment for several years (35).

Considering effects of chemical sterilants on soil physical properties, fumigation with four different chemicals had little or no effect upon soil aggregation (29). Both propylene oxide (28, 42) and ethylene oxide (2) increased soil acidity. Lopes and Wollum (28) recorded a significant decrease in soil acidity after using methylbromide, but Eno and Popenoe (18) found no significant effect, using a nursery soil that was low in organic matter content.

Propylene oxide and methylbromide produce only small changes in the amounts of extracted soil Ca, Mg, K, and Mn; three other chemicals produced substantial changes in the extractability of these elements (1). All five increased ammonium-N. Methylbromide produced no significant effect on soil cations or on total N and P (18, 28), but propylene oxide reduced exchangeable bases, effective CEC and Al saturation (28).

Of five chemicals tested, ethylene oxide had the least effect on ammonium acetate soluble Mn and Cu; formaldehyde increased both Mn and Cu to toxic levels (16). Treating with methylbromide caused no significant change in four micronutrients, including Mn and Cu (23, 28). Fumigants seem to have little effect upon anions except when chemicals contain S or Cl (1).

Gamma radiation effectively destroys soil microorganisms and removes fungistasis (25); its effects upon soil physical and chemical properties are not well documented. Ethylene production in the air above irradiated soils significantly increased within two hours of treatment; high amounts of ethylene that could affect root growth were still being released from the treated soil some 10 weeks later (39).

Gamma radiation did not change cation exchange capacity of two soils but increased release of N, P, and S over a non-treated soil (18). Radition decreased soil acidity in one soil slightly (4).

Extreme **dry-heat** temperatures or long duration heating can affect soil chemical properties or soil mineralogy. After dry-heat sterilization at 100°C for six months, the cation and anion exchange capacities of fine soil and clay minerals $< 2\mu$ were less than those for unheated soils; differential thermal analysis curves made before and after heating showed that dry-heat sterilization caused modifications to micellar structure and clay colloids (38). Dry heating at 600°C for six minutes or 700°C for three minutes changed clay mineral structure, particularly for montmorillonite and kaolinite; these changes caused slight decreases in exchange capacity and available nutrients and also produced strong, permeable, and frost-resistant aggregates (51). High dry-heat temperature can cause curling of minerals, exposing greater surface area for release or absorption of certain nutrients, particularly K (17).

Dry-heat treatments increased soluble salts by two to 10 times levels found in non-heated samples (32). Above-normal incubation temperatures of 80 to 127°C increased exchangeable K levels (13). Dry-heat sterilization of 19 Puerto Rican soils at 121° for two hours affected several soil chemical properties as compared to those found after simple air drying (27). For example, Mehlich-1 P was significantly lower in dry-heated than in air-dried soils. Dry-heat sterilization did not alter soil N or soil bases including Na (Table 2).

Soil micronutrients in the Puerto Rican soils were more influenced by dry-heated sterilization than were macronutrients (Table 2). Mehlich-1 Cu values were similar in air-dried and dry-heated soils, but Olsen Cu was significantly lower in dry-heated samples. Both Olsen and Mehlich-1 Fe values were significantly higher in heat-dried than in air-dried soils. Soil acidity values were significantly lower and Mn levels significantly higher in heat-dried than in air-dried soils. Boron was significantly higher in heat-dried than in air dried soils. Soil organic matter values were not affected by treatments.

In general, effects of dry-heat sterilization on soil chemical properties for Puerto Rican soils followed results published elsewhere for steaming and autoclaving procedures. Considering data on chemical sterilization, those from methylbromide followed dry-heat sterilization most closely. Observed micronutrient differences between air drying and dry heating were dependent upon the kind of extractant used (27).

Dry-heat sterilization is effective in killing bacteria spores (36) and causes certain decreases in plant growth. For example, sour orange (*Citrus aurantium*) seedlings became stunted after being planted on seedbed material treated by dry heat at 80°C for three hours (46). Dry heating caused a significant decrease in sour orange seedling height growth at eight months when compared to growth in soil having methylbromide sterilization. Dry heating did not significantly reduce infection by mycorrhizal fungi below levels measured after using methylbromide or other chemical treatments (47).

Even simple air drying of soils at low temperatures can affect soil physical and chemical properties. Alternate wetting and drying decreased amounts of soil aggregates, but this was compensated somewhat through microbial activity in nonsterilized soil (48). Air drying has increased levels of exchangeable soil K (10) and Mn (19) over non-dried soils. The sum of exchangeable and soluble ammonium in soils increased after air drying as long as there was no subsequent incubation period (44). Air drying decreased P absorption (7), and P deficiencies seemed more pronounced at lower soil temperatures (14). Air drying can alter soil surface chemical properties and organic matter content: chemical characteristics of dry soils continue to change with time and remoistening air-dried samples between one to three months after initial storage may or may not return soil chemical properties to the original field-state condition (8).

Summary and conclusions

Steam sterilization affects availability of certain macronutrients and micronutrients and soil aggregation as compared to results from air drying only. Steaming usually increases soil Mn levels several times; Mn toxicity is a common problem for plants grown on steam-treated soils. Other plant responses on steam-treated soils are quite variable and are dependent upon species or varieties used and soil type.

Chemical fumigants exert little or no influence on soil physical properties. Of five commonly used

Table 2. Effects of air drying (A) and heat drying at 121°C for two hours (H) on chemical properties of 19 Puerto Rican soils analyzed by several methods.

Chemical Property	Mehlich-1 Extractant			Olsen Extractant	
	A	H	Units	A	H
N[1]	0.213	0.221	%	–	–
Ca	625	592	ppm	660	651
K	101	94	ppm	130	115
Mg	273	244	ppm	356	328
Na	57	61	ppm	–	–
P	24	16**[2]	ppm	2	4**
B[3]	0.9	1.9**	ppm	-	-
Cu	2.6	2.4	ppm	6.4	5.2**
Fe	25	41**	ppm	105	147**
Mn	191	363**	ppm	175	707**
Zn	2.9	3.4	ppm	5	5
Al[4]	53	48	ppm	69	139[7]**
Organic matter	4.02	3.93[5]	%	–	–
pH	4.7	4.5[6]**			

1 Determined by micro-Kjeldahl procedure

2 Analysis of variance between air –and heat–dried treatments significant at 0.05 probability

3 Hot-water extractable, using modified procedure of U.C. Gupta. 1967. Soil Sci. 103:424-428

4 Atomic absorption analysis of 1 N KCl extract

5 Determined by Walkely-Black procedure

6 1:1 soil-water paste and a glass electrode

7 Titration of 1 N KCl extract with 0.01 N NaOH

8 Adapted from L.H. Liegel. 1983. Common. Soil Sci. Plant Anal. 14:277-286

fumigants, methylbromide has the least effect upon changes in soil chemical properties. Several authors have reported no significant changes in soil bases, total N and P, and even Mn over air-drying treatments. Methylbromide has increased and decreased soil acidity. Fumigants destroy both beneficial and harmful soil microorganisms. Yet fumigation with planned reinoculation with desired mycorrhizal strains is effective in managing commercial forest or horticultural nurseries.

Gamma radiation also effectively destroys soil microorganisms. Its effects upon soil physical or chemical properties have not been studied in detail. Gamma radiation did not affect cation exchange capacity over two non-treated soils. Yet gamma radiation has influenced N, P, S, and K availability and soil acidity, according to other studies.

Dry-heat sterilization at high temperatures and for long duration kills almost all soil pathogens. Dry-heating is therefore still the prescribed USDA-APHIS treatment for unsolicited soil entering the United States. Dry-heat temperatures of 600°C and above for only three minutes can change clay mineral structure, decrease exchange capacity, and affect soil aggregation. Lower dry-heat temperature effects on soil chemical properties are not well documented. Data from dry-heat sterilization of Puerto Rican soils at 121°C for two hours indicate that changes over air-dried treatment generally followed results reported for steaming, autoclaving, and fumigating with methylbromide. Some exceptions occurred, particularly for micronutrients like Mn and B, and depended on the soil extractant used after imposing dry-heat sterilization.

Potential importers or exporters of soils should be aware that any sterilization procedure will influence some soil physical, chemical, or biological properties. Different sterilization methods do not always produce similar changes. Therefore, a sterilization procedure should be chosen which has least influence on those properties of interest that are studied. When possible, a permit should be obtained to import soils in a nonsterilized condition through USDA-APHIS approved laboratory facilities. If a permit cannot be obtained, results reviewed here suggest that dry-heat sterilization at low temperatures would be preferable to autoclaving. Even if soils are received in an air-dried, unsterilized condition, chemical and organic matter properties might have changed for stored soils over time; and remoistening air-dried soils within a few months after initial storage may not return soil chemical properties to the original field-state condition.

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Notas y comentarios

Los hombres primitivos obtenían sus alimentos del suelo

Nuestros antepasados más remotos introdujeron de dos maneras: la variedad en sus comidas contolando y usando fuego para cocinar, e inventando herramientas para desenterrar bulbos, tubérculos y otros órganos subterráneos de almacenamiento que poseen muchas plantas. La importancia de estos alimentos vegetales enterrados, suponen los arqueólogos, creció cuando los habitats favorecidos por los homínidos en la región del Rift Valley de Africa Oriental llegaron a hacerse más abiertos y secos hace dos y medio millones de años.

Extrapolar hacia atrás, desde los tiempos modernos hasta un pasado remoto de más de dos millones de años, puede ser engañoso, pero en ausencia de evidencia arqueológica, puede contribuir a elaborar modelos útiles del comportamiento de aquellos seres. Así, del estudio de tribus actuales, cazadoras y recogedoras, de esta región, que todavía extraen tubérculos silvestres del suelo, se logra una idea del comportamiento de los precusores del hombre.

Uno de estos grupos, los Hadzas, del Norte de Tanzania, son estudiados ahora por Anne Vincent como parte de su tesis doctoral en la Universidad de California, en Berkeley (**World Archaeology**, vol. 17, p. 131). La población total, de unos 600 Hadzas, vive en lo que se califica como ambiente sabana mosaico, que se considera similar al que habitaban los homínidos de Africa hace unos dos millones de años, aunque, ciertamente, no había entonces pastoreo de ganado de parte de tribus pastoriles.

Aunque los Hadzas comen carne de caza, también recolectan frutillas, miel y varios tipos de tubérculos. Vincent no duda que, con la ayuda de una tecnología simple (palos para excavar) y con un poco de trabajo, esta gente consigue un buen valor por sus esfuerzos de excavación. Los tubérculos pueden ser comidos crudos, aunque los Hadzas prefieren tostarlos ligeramente, y constituyen una buena y confiable fuente de alimentos durante todo el año, si es necesario.

En el área de estudio de Vincent, alrededor de Mangola, cerca del rio Barai y de los llanos circundantes regularmente inundados, se recoge (principal mente por mujeres) tubérculos de hasta cinco especies de leguminosas leñosas perennes. Todos son ricos en hidratos de carbono, son jugosos y dulces, y se requiere sólo pelarlos antes de comerlos crudos o tostados. Los de *Vigna macrorhyncha* y de *Vatovaea pseudolablab* son redondeados como las papas y se forman a profundidades no mayores de 50 centímetros. *Vigna frutescens* tiene tubérculos alargados que se encuentran un poco más profundo que los otros.

Las mujeres Hadzas necesitan cavar, a veces hasta metro y medio de profundidad, para encontrar estos tubérculos, pero pueden obtener hasta cinco kilogramos o más por cada hora de trabajo. Los palos cavadores tardan unos cuatro minutos en ser hechos con la ayuda de un machete de acero, y duran para unos ocho viajes de recolección.

A estas mujeres no les importa caminar hasta ocho kilómetros desde el campamento, para desenterrar tubérculos, recoger frutillas y colectar agua, aunque generalmente sólo caminan menos de cinco kilómetros.

Estos recursos que el hombre primitivo descubrió cuando el ambiente se volvió hostil y difícil nos recuerdan los tubérculos que el hombre andino aprendió a utilizar en el altiplano de los Andes, donde el maíz, las cucurbitáceas y los granos de leguminosas no podían resistir el frío, la sequedad y la fuerte irradiación de estos páramos a más de cuatro mil metros sobre el nivel del mar. La misma papa decrece en sus rendimientos en esas condiciones. Pero los habitantes de las punas encontraron tubérculos de plantas de diversas familias, adaptadas a ese ecosistema, a las que domesticaron estableciendo cultivados apropiados a ese peculiar ambiente. Así tenemos el olluco (*Ullucus tuberosus*) de la familia de las baseliáces, la oca (*Oxalis tuberosa*), quiba y huisisay en Venezuela, de las oxalidáceas, y la mashua *Tropaeolum tuberosum*), cubio en Colombia, de las tropoláceas, entre las más conocidas, cuya producción llega a venderse en los mercados urbanos, incluso en capitales como Lima, La Paz y Quito. Hay otros tubérculos de menor difusión, a veces conocidos sólo localmente.

Es claro que aquí se habla de comunidades pastoriles que cultivan esas plantas, mientras que en el caso de los Hadzas se trata de tribus que están todavía en la etapa de recolección y de caza. Pero nos gustaría también extrapolar hacia atrás, como hace Anne Vincent en Africa Oriental, y pensar que estos habitantes del Altiplano, quizás refugiados de conquista de los Incas, aprendieron a servirse, en sus refugios de las alturas, de los tubérculos que estas plantas habían desarrollado para asegurar su supervivencia en las épocas de sequía o de heladas. Adalberto Gorbitz.