EFFECT OF 2,450 MHz MICROWAVE RADIATION ON MICROORGANISMS

DISSERTATION

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The effect of microwave radiation on soil bacteria *in situ* has been studied in both lab and field conditions. Radiation and thermal profiles show that heterotrophic bacteria, spores, fungi, and actinomycetes were not affected by total microwave radiations over the range 0 to 80 seconds of exposure at a net input of 1 KW of intensity. Nitrogen-fixing bacteria and nitrifying bacteria were also resistant to these doses. The soil microorganisms were inactivated as a function of microwave radiation in the range of 80 to 480 seconds of exposure to 1 KW of continuous radiation.

The degree of moisture content exerted the primary effect on resistance to microwave radiation. The 2,450 MHz radiation employed appears to leave no residual effect in the soil and the effects on microorganisms seem to be non-selective except that fungi appeared to be more susceptible. A "heat shock" activation of microbial spores was observed.

These data imply that microwave treatment of agricultural fields for killing of weed seeds and insect population has no effect on the soil microorganisms.
By studying the relationship between temperature generated in dry and wet organisms and the pattern of destruction of inoculated bacteria by microwave radiation, it was found that inactivation was a function of cell hydration. It also revealed that bacterial cells do not absorb microwave energy and that the lethal effect of microwaves is due to direct energy transfer to cell water and the temperature increase of the suspending medium.
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INTRODUCTION

The influence of the various forms of energy on living organisms has always been an attractive field of investigation for biologists. Radiation biology began the same year that "x-rays" were discovered by the German physicist Konrad Roentgen (1895). Radiation has proven to be beneficial in many ways, but it can also produce deleterious effects in all living organisms.

Electromagnetic radiations may be divided into two categories: (1) Particulate radiation such as alpha particles, electrons, protons, neutrons, and certain fission fragments. (2) Electromagnetic radiation, which forms a continuous spectrum of varying wavelengths. The work of Maxwell and confirming observations of Hertz widened enormously the known spectrum ranging in wavelength from less than 0.001 Å to more than 10,000 meters, a range of some $10^{15}$ -fold in magnitude. The exact limits of the electromagnetic spectrum are not known. All electromagnetic waves travel at a velocity of $3 \times 10^8$ meters per second in free space regardless of differences in
wavelength, and hence in frequency. Characteristic names have been given to the various parts of the electromagnetic spectrum because of their special uses or means of generation or detection, but the demarcation lines are not sharp and a given radiation in a boundary region can often be considered from either side. The range of the electromagnetic spectrum from the very shortest to the very longest waves is known experimentally and shows no discontinuities or interruptions. The entire spectrum of electromagnetic radiation is present in our environment. The popular names according to commercial uses or popular science descriptions are cosmic rays, gamma rays, x-rays, ultraviolet light, visible light, infrared rays, microwaves and radio waves, roughly corresponding to the wavelengths shown in Fig. 1.

Cosmic rays consist of primary radiation entering the atmosphere from outer space and of secondary radiation produced by interaction of the primary radiation with matter in the atmosphere. Primary cosmic-ray particles seldom reach the surface of the earth because they interact violently with nuclei of the gases in the air and produce a variety of reactions. The primary component of cosmic
Fig. 1. The electromagnetic spectrum.
radiation consists largely of high-energy, positively-charged nuclei which may be lethal or may induce mutation and hence may be the primary cause of evolutionary processes. These radiations are associated with wavelengths up to approximately 0.01 Angstrom units (Å).

The electromagnetic radiations with shorter wavelengths in the range $10^{-3}$ to $10$ Å, produce both ionization and energy state excitation in the media through which they travel. Radiations which originate in atomic nuclei are called gamma rays while those which originate outside the atomic nucleus are termed x-rays. The wavelengths of the latter extend from about $10^{-2}$ Å up to about $10^2$ Å and are produced when high-energy electrons or other charge particles strike target atoms. The best-known characteristic of x-rays is, of course, their ability to penetrate matter. The high energy-bearing radiations are known to be lethal to all living things when they are exposed to sufficient quantities under the proper conditions. In low doses, they have great practical use in routine medical diagnostic procedures. Gamma rays are electromagnetic radiations of wavelengths shorter than about $10^{-2}$ Å. They have most
of the properties of x-rays but are more penetrating. Two prominent sources of gamma ray radiation are natural and induce radioactivity and as well as those coming from outer space. Studies of x-ray and gamma ray radiation, in relation to the effect on living matter, especially in microorganisms, can be found in many publications, including Bless 1937, Bridges et al. 1956, Davis et al. 1956, Dunn et al. 1948, Gowen 1936, Gunter and Kohn 1956, Kelner 1948, Koh et al. 1956, McLaren et al. 1953.

Ultraviolet radiation (1 x 10^3 to 4 x 10^3 Å) affects biological processes in many ways; these include insect vision, erythema or sunburn of the human skin, pigmentation and tanning of the human skin, activation of unfertilized eggs of marine animals, increased mutation rates in all cells, and the conversion of ergosterol to vitamin D (Eisenstark 1971, Gates 1929, Koller 1965, Hollaender 1943, 1945, Stevens 1928).

In laboratory experiments conducted during the last 50 years it has been shown that the extent of the effect depends on dosage or, better, the total amount of energy absorbed by the irradiated material. Ultraviolet light does
not cause ionization of matter but rather causes excitation of electrons in their orbits. Ultraviolet light absorption by bacteria is due chiefly to the purines and pyrimidines of nucleic acids. The absorption maxima are found at an average of 2600 Å. Protein, in which the aromatic rings of tyrosine, tryptophan, and phenylalanine absorb UV light, have a maximum at approximately 2800 Å. The segment of the UV spectrum which is bactericidal coincides with the absorption spectrum of the bacteria, suggesting that absorption either by nucleic acid or protein can be the site of the primary lesion of lethal effects (Gates 1930). The outstanding property of this radiation is its ability to cause chemical changes; therefore, the major effect of ultraviolet absorption on deoxyribonucleic acid (DNA) molecules is the production of breaks in the polymer chains and chemical reactions resulting from these. Direct sunlight kills most of bacteria within a few hours because of its content of ultraviolet light. Ultraviolet radiation is used for sterilizing air in surgical rooms and recovery wards, in treating skin diseases, and in maintaining clean rooms in many industrial processes. Ultraviolet radiation is also used for the excitation of selected substances and the
subsequent fluorescence is characterized for analytical measurements.

Electromagnetic radiation of wavelengths in the visible region ($4 \times 10^3$ to $8 \times 10^3$ A) produces four well-known biological effects. They are:

(1) Photosynthesis. A few species of saprophytic, anaerobic bacteria which contain bacteriochlorophyll can utilize sunlight to build high energy compounds for biosynthesis (Frenkel 1970, Olson 1970, Vernon 1968, Van Niel 1962). The green sulfur bacteria (Chlorobiaceae) and purple sulfur bacteria (Chromatiaceae) use hydrogen sulfide as hydrogen donor for the reduction of carbon dioxide, according to equation:

$$2 \text{H}_2\text{S} + \text{CO}_2 \xrightarrow{\text{light}} (\text{CH}_2\text{O}) + \text{H}_2\text{O} + 2\text{S}$$

Some nonsulfur purple bacteria (Rhodospirillaceae) use an organic hydrogen donor, such as isopropanol, which is oxidized to acetone:

$$2 \text{CH}_3\text{CHOHCH}_3 + \text{CO}_2 \xrightarrow{\text{light}} (\text{CH}_2\text{O}) + 2 \text{CH}_3\text{COCH}_3 + \text{H}_2\text{O}$$

The photoreceptor pigments are collectively called bacteriochlorophylls. Auxiliary photosynthetic pigments are also
active; these fall into the chemical groups called carotenoids.

(2) Phototaxis. A gradient of light intensity has been shown to elicit differential flagellar activity in different parts of the cell of motile phytosynthetic bacteria resulting in motion toward the light. The moving cells enter the most physiologically favorable region of the light gradient by trial and error. Wavelengths in mediating phototaxis correspond exactly to the action spectrum of photosynthesis; therefore, the bacteria tend to accumulate where the wavelengths of light are those absorbed in the photosynthetic process (Clayton 1953, 1955).

(3) Photoreactivation. The reversal of ultraviolet radiation injury by the exposure of irradiated microorganisms to wavelengths in the visible spectrum \(3 \times 10^3\) Å is called photoreactivation. The treatment by secondary light activates enzymes that cleave thymine dimers formed following ultraviolet absorption and allows deoxyribonucleic acid replication (or photorepair), but only a fraction of the affected organisms recover. The degree of reactivation is proportional to time, intensity, and the temperature during exposure to visible light. (Dulbecco 1949, Kelner
1949, Rupert et al. 1958). Recovery from radiation damage may also occur in the dark. Such ultraviolet light-damaged DNA thymine dimer lesions can be repaired by specific enzymes including endonuclease, exonuclease, DNA polymerase and ligase present in all bacterial cells (Harm and Hillebrandt 1962, Setlow and Carrier 1964, Witkin 1969).

(4) Photoinactivation. The sterilizing effect of sunlight is due mainly to its content of UV light, as stated previously, the killing of bacteria depends directly on the absorption of radiant energy. Violet and blue lights are highly destructive to bacteria, green light is much less so, and red and yellow lights have little bactericidal action. Intense visible light is capable of killing bacteria, presumably via physiologically occurring photosensitizing substances such as riboflavin and prophyrins (Burchard and Dworkin 1966, Elkind and Sutton 1957). Survival of bacteria exposed to the adverse effects of light sometimes is enhanced by the presence of carotenoid pigments. It was suggested that this causes the structural changes in the photosensitizer so that photooxidation cannot occur (Kunisawa and Stranier 1958, Mathews
and Sistron 1959). Sunlight can also cause damage to microorganisms; it is well established that this is due primary to UV light but there is also evidence that some effect resides in the spectrum of visible light.

Infrared radiation occupies the next segment of the electromagnetic spectrum. Matter at temperatures greater than a few degrees centigrade emits infrared radiation. It occupies the range from $7.6 \times 10^3$ to $5 \times 10^6$ Å. Its absorption results in increased rotation and vibrations of atoms and molecules and therefore increased temperature. It is largely associated with hot bodies and is sometimes inaccurately called "heat radiation", "heat rays" or "radiant heat". It is employed for warming foods, air, and other materials and has long been used as an important therapeutic agent for treatment of physical injury.

Recently, biologists have become interested in the effects of radio waves on living organisms. These are commonly called Hertzian waves. It is customary to classify radiowaves as "long" when the wavelength is more than 200 meters and as "short" when it is less than 200 meters. There are a few reports which indicate that radio waves may have an effect on biological systems. The only
well known effect is the use of these radiations for transmission of music and information by man.

1. Definition of Microwave Radiation

1.1. Development and Definition of Microwaves

The historical development of microwaves is a gradual evolution of the concept and application of electromagnetic waves in general. The long electromagnetic waves predicted by the Scottish physicist James Clerk Maxwell in 1864 were discovered by the German physicist Heinrich Hertz in 1887. In 1897 (still before the days of radio communications as known today), Hertz showed that electromagnetic waves may be propagated in hollow tubes or, in fact, in any medium having specific boundaries between two electrically different media. Finally, at the turn of this century, Guglielmo Marconi demonstrated the first practical method of communication by the use of radio waves.

The development of radar equipment for military application during World War II resulted in the introduction of a new range of electromagnetic radiation frequencies into man's environment, microwaves. In one generalization it is said that Hertzian waves include long, short and ultra-short wavelengths. The ultra-short radio range is called
"microwaves". They are situated between the extreme infrared and short radio waves in the electromagnetic spectrum and fall in the band of frequencies from roughly 30 megahertz to 300,000 megahertz (MHz). This corresponds to wavelengths from $10^7$ to $10^{10}$ Å. Therefore, it is sometimes labelled with such names as decimeter, centimeter, or millimeter radio waves.

In another generalization they are considered apart from radio waves and are called the radar range. Microwaves are also referred to as ultra-high frequency electromagnetic radiation (UHF).

1.2. Generation of Microwaves

Microwaves can be generated directly from electrical sparks across electrode gaps by applying a high electric potential. Microwaves can also be derived from the thermal radiation of warm bodies. But these sources are unsatisfactory because of the impurity of the wave and the low power of the radiation. In contrast to these, all modern microwave generators are electromagnetic devices which produce a continuous-wave (CW) of a single tunable frequency. Some important microwave generators are known as magnetrons, klystrons, and traveling-wave oscillators. The output of
power ranges from microwatts to thousands of kilowatts depending on the type and design of the generator and the frequency used. The schematic diagram of a basic microwave generator such as was used in this investigation is shown in Fig. 2.

1.3. Measurement of Microwaves

A microwave detector is a device to demonstrate the presence of microwaves by a specific effect that the waves produce. One of the most effective means of detection makes use of a device which converts the microwave field intensity into either a direct current or a low-frequency alternating current. A silicon crystal making a pinpoint contact with a tungsten wire is the most commonly used detector for microwaves. A typical crystal detector may give a current of the order of 1 milliampere (mA) for a microwave power input of 1 milliwatt (mW).

In the range of 0.01 to 10 mW, power measurements are customarily made with a bolometer, which operates in a bridge circuit and changes radio-frequency energy into heat energy. It is a device whose resistance changes with temperature, the latter being a function of the absorbed microwave power.
Fig. 2. Block diagram of the microwave generator.
In the range above 10 W, power measurements are generally made using calorimetric techniques. Either a dry calorimeter or a waterflow calorimeter is used. The waterflow calorimeter is a device which uses the increase in the temperature of a given volume of water as a means of measuring microwave dose.

The reflectometer used in this research, power range of 0 to 3 KW, gives direct, instantaneous readings of forward power from the generator. That is, the total power output, reflected power or power lost can be deduced when it is connected to a suitable bolometer mount.

2. The Application of Microwave Radiation.

In addition to the well known medical use of microwaves for diathermy treatment, or heat therapy, by delivering the energy which is transformed into heat directly into the deep tissues of the subcutaneous layer (Schwan and Piersol 1954), there are more recent applications in food and material industries. Freeze drying, a process in which microwaves are used as a source of heat, retains the fidelity and authenticity of the original
flavors and preserves more of the heat-sensitive vitamins and protein value than heating by any other method (Copson 1958, Hoover et al. 1966). Thawing of frozen food (Cathcart and Parker 1946, Bengston 1963), cooking, baking, blanching (Decareau 1956, Proctor and Goldblith 1948), and drying have all been accomplished with microwaves for some time. Sealing of plastics and processing of products, pasteurization of dairy products, and sterilization of many substances (Belbrough 1969, MacDonald 1947, Olsen 1965) have also been accomplished economically by microwaves.

Further application may include the use of synthetic (modulated) noise production, and plasma heating and confinement in nuclear reactions (Hatch 1968). Also radar application in such areas as weather forecasting, tracking and communications for satellites and missiles, and wave-guided high-speed vehicle transport system in the exploration of outer space (Arden et al. 1968, Stockman 1963) have been proposed and some of these applications are now under serious consideration or research investigation.
3. Microwave Problems.

With the growing use of microwave radiation in domestic, military and commercial operations, the potential hazard of microwave radiation for man and other organisms has received a significant degree of attention. A large amount of research into the biological effects of microwave radiation has been performed in the past. The general biological effects of microwaves have been reviewed by several authors (Barron and Baraff 1958, Cleary 1973, Follis 1946, Michaelson and Dodge 1971, Michaelson 1971, Milroy and Michaelson 1972) and will only be summarized here. Microwaves may affect a variety of organisms from microorganisms to man. Effects may occur at various frequencies and power densities characterized by responses that involve reactions of the entire organism to changes at the molecular level.

3.1. Effect on Animals

Microwave irradiation at high energy levels is lethal to both small animals and humans (Lappenbusch and Gellespie 1973, McAfee et al. 1973). Some important physiological health hazards include cataract formation (Cogan et al. 1958, Kurz and Einaugler 1969, Milroy and Michaelson 1972), lenticular opacities (Daily et al. 1950, 1951), testicular
degeneration (Imig et al. 1948), hematologic effects (Michaelson et al. 1963, 1964, McRee et al. 1975), and nervous system effects (Silverman 1973, Nelson 1973). Such effects have been induced in test animals by exposure to microwaves in the frequency range from 2,800 to 9,000 MHz for various time exposures. Many countries now have safety limits for microwave exposure (Swanson et al. 1970). These limits are based largely on the heating effects of microwaves observed in animal experiments. The most commonly accepted upper level of safety is 10 millijoules per cm² per sec.

3.2. Effects on Insects

Knowledge of the effect produced by high-frequency radiations on insects is obviously of great economic importance. Studies on this subject indicate that microwaves can be and actually are utilized to control pests such as cotton weevils and some moths. Early in 1930, McKinley and Charles subjected parasitic wasps, Habrobracon juglandis of different ages, sexes and conditions of feeding to radiation of 85.71 MHz (power levels not given). The wasps were killed in an average time of 11.41 seconds. In other experiments, Davis (1933) found that the radiation of 10
MHz (output power 20 kilowatts) was effective in exterminating adult weevils in wheat within a period of about 90 seconds, but eggs later hatched. However, with radiation of 50 MHz, an exposure of only 6 seconds was sufficient to exterminate eggs, larva, and adults.

Recently, microwave radiation was proposed as an alternative method of obtaining grain insect control (Hamid et al. 1968, Kirkpatrick et al. 1972). Since it is a non-chemical pesticide and causes no environmental pollution, it may play a new and increasingly important role in agriculture in the near future.

3.3. Effects on Microorganisms

The action of electromagnetic fields induced by high or ultra-high frequency radiation on microorganisms is of considerable interest.

When the temperature of the broth containing bacteria (colon bacilli) was maintained between 17 and 19 C, Fabian and Graham (1933) found that the 15 MHz radiation (power levels not given) was most effective in killing bacteria and 7.5 MHz radiation was less effective.

In studies of the effects of low-power-density
radiation of *Escherichia coli* B at 136,000 MHz (power levels not given), it was found that the rates of cell division were lower.

In the pasteurization of raw milk, Hamid *et al.* (1969) found that 14 seconds of exposure to the 2,450 MHz microwave radiation (1.2 KW output power) will completely destroy all of the bacteria in milk.

In other experiments involving food sterilization, Watanabe (1969) indicated that 2,450 MHz microwave radiation (power levels not given) significantly reduced populations of streptococci, lactobacilli, coliforms and yeasts.

Aref (1973) pointed out that 60 seconds of 2,450 MHz microwave energy (1.8KW output) drastically reduced the number of viable organisms in flour.

Mayers and Habeshaw (1973) stressed that microwave radiation at a frequency of 2,450 MHz and an absorbed power level of 50 millijoules per cm² per sec resulted in the diminution of phagocytosis when the macrophages of mice were exposed to radiation for 30 minutes.

More recently, Vela *et al.* (1976) found that soil microorganisms, including heterotrophic bacteria, spore-forming bacteria, fungi, actinomycetes, nitrogen-fixing
bacteria, nitrifying bacteria, *Bdellovibrio bacteriovorus*, and a bacterial virus were inactivated as a function of 2,450 MHz microwave radiation exposure. The amounts of radiation required were in the range of $8.0 \times 10^4$ to $4.8 \times 10^5$ joules in a flux of 1 kW of continuous radiation.

Microwaves of 857.14 MHz (power levels not given) killed *Bacterium vesicatorium* when exposed for 24 hours, but neither 857.14 MHz nor 666.67 MHz had effect on *Saccharomyces* sp. and *Alternaria solani* (Gier 1937).

Using radiation of 81.08 to 150.78 MHz (power levels and temperature not given), Szymanowsk and Hicks (1932) found that three bacterial toxins; diphtheria, tetanus, and botulinus in raw broth filtrates were effectively attenuated.

Metlitzky and Soboleva (1936) showed that radiation at 136.36 MHz (power levels not given) on fungi (*Sclerotinia libertiana* and *Botrytis cinerea*) was ineffective in exposures from 1 second to 30 minutes. On the other hand, 53.57 MHz radiation did kill these fungi in 20 to 25 seconds.

Tverskoi (1937) discovered that microorganisms (*Fusarium solani*, *Sclerotinia libertiana*, *Botrytis cinerea* and *Phytophthora infestans*) exposed to 10 MHz or 15 MHz
radiations were not affected even with prolonged exposures. However, irradiations with 28.48 MHz and 53.57 MHz were effective in killing them.

4. Mechanisms of Killing by Microwave Radiation

There is extensive disagreement in the literature concerning the specific effects of microwaves on microorganisms. It has always been evident that inactivation of microorganisms by microwaves involves heat. That is, energy transfer from microwaves to water and other materials results in temperature increase of the absorbing material. Also, it has always been assumed that there must be other effects due to the radiation itself and that these effects are separate and distinct from the heat or thermal effects.

4.1. Thermal Effect

Imig et al. (1948) discovered that with a 10 minute exposure to 2,500 MHz microwave radiation (power levels not given), a temperature rise to 40 C in the testes of the adult albino rat was evident and resulted in tubular degeneration similar to that found from the heating of the testes by infrared radiation.

In 1954, Brown and Morrison stated that no significant destruction of bacteria in aqueous solution occurs from
application of radio-frequency fields in the frequency range up to 600 MHz, except for the destruction brought about by thermal effects. Kalant (1959) stressed that the physiological alterations produced by elevating the temperature for the various organisms by absorption of microwave radiation are macroscopically indistinguishable in character from those produced by the external application of heat.

Lystov et al. (1965), using 9,370 MHz radiation (power levels not given) and maintaining the temperature at -196 C throughout the time of irradiation, concluded that exposing the vegetative cells and spores of Bacillus subtilis (in glass tubes) produced no direct non-thermal effect on the genetic apparatus of the cell. In another experiment, Takashima (1966) studied the effects of 1 to 60 MHz radio-frequency energy (output Power was approximately 100 watts in continuous waves) on solutions of alcohol dehydrogenase and deoxyribonucleic acid (DNA) in a cooling system for one hour, and found no specific effects due to the radiation.

By using 2,450 MHz microwave radiation (power levels not given) without controlling temperature in the experiment, Goldblith and Wang (1967) claimed that the degree of
inactivation of *E. coli* and *B. subtilis* by radiation energy up to 100 second exposure was identical to that produced by conventional heat.

By keeping the temperature at 25 C (with coolant condenser) for various periods of time, Lechowich *et al.* (1969) found that continuous application of 2,450 MHz microwave radiation (energy levels not given) to suspensions of *Streptococcus faecalis* or *Saccharomyces cerevisiae* appeared to produce no lethal effects. Also, Aref *et al.* (1973) noticed that 60 seconds of exposure to 2,450 MHz (1.8 KW output energy) will inactivate the alpha-amylases in wheat and denature DNA by microwave heating. In experiments using 2,800 MHz microwave radiation (continuous wave, 1 KHz square wave modulation) at an incident power density of between 500 millijoules per cm$^2$ per sec and 1000 millijoules per cm$^2$ per sec carried out in a heat exchanger (inside the waveguide) at a fixed temperature for up to 18.5 minutes, Belkhole *et al.* (1974) reported the inactivation of human serum enzyme lactate dehydrogenase, acid phosphatase and alkaline phosphatase but no thermal effects were apparent.

As research has progressed in this area, it has become increasingly evident that, in contrast to many earlier
interpretations, at least the overwhelming majority of reported effects of microwaves on biological materials are of thermal origin. Proper temperature control is difficult in microwave experiments and local, temporary temperature rises may greatly enhance a lethal effect. Carefully controlled experiments done in the last few years have failed to give any support to arguments about non-thermal effects of microwaves as a cause of killing of organisms.

A direct approach to temperature-controlled microwave experiments was taken by Vela and Wu (1976), who studied the effect of microwaves on microorganisms including *Azotobacter chroococcum*, *Azotobacter vinelandii*, vegetative cells and spores of *Bacillus cereus*, *Bdellovibrio bacteriovorus*, *Escherichia coli*, *Pseudomonas aeruginose*, *Salmonella typhimurium*, *Serratia marcescens*, *Staphylococcus aureus*, commercial yeast, and bacteriophages. They discovered that the survival rate of the microorganisms exposed to 2,450 MHz microwave radiation (1kW of continuous radiation) was inversely proportional to the moisture content of the suspending medium.

The survival rates of the microorganisms in "dry" condition (lyophilized state) are nearly one hundred
per cent even after extensive exposure to microwave fields. The temperature of the "dry" microorganisms was found to be constant (23 C) in spite of prolonged irradiation; to the contrary, the temperature of "wet" microorganisms increased proportionally to the amount of radiation energy. They concluded that bacterial cells do not absorb microwave energy and that the lethal effect of microwaves is due to direct energy transfer to cell water and the temperature increase of the suspending water.

In a word, biological effects resulting from microwave exposure are primarily due to heat produced by the transfer of microwave energy to water molecules and subsequent temperature increase.

4.2. Nonthermal Effect

Although there is ample evidence which indicates that microwaves affect biological systems by energy transfer and the resultant temperature increases, it must be noted that there are many reports in the literature which show that not all the effects are associated with temperature increase. Nonthermal effects are caused by a biological response to a direct interaction between an electromagnetic field and the cell or its macromolecules.
By studying the effects of high frequency fields (8.95 MHz, power levels not given) on parasitic wasps, McKinley and Charles (1930) concluded that there exists a lethal effect other than heat. In 1944, Fleming studied the lethal effects of radio-frequency energy on bread mold by exposing cultures to frequencies from 11 to 350 MHz (power levels not given). The maximal temperature reported during the 1 minute exposure was 30°C; he observed that all frequencies tested had a lethal effect on the bread mold, with the greatest destruction (98 per cent) occurring at approximately 70 MHz.

Nyrop (1946) studied the effect of a 20 MHz field (modulated frequencies from 10 to 100 KHz; power levels not given) on stomatitis virus. The virus was completely inactivated when treated with the radiation for 10 seconds and the temperature was not allowed to rise above 36°C. When inactivating the same virus by heat, 60 hours were required at 37°C. This suggested that the electromagnetic treatment acts on the virus in ways quite different from those by which heat acts.

Heller and Teixeira-Pinto (1959) mentioned certain morphological changes in the nuclei and chromosomes of
cells of growing garlic root-tips when exposed for 5 minutes to 27 MHz radiation (power level not given). The effects were again described as non-thermal since no temperature increase in the water was noticed.

In vivo cellular transformations independent of heat were also detected by Valtonen (1966). He reported that after 5 minutes of treatment with 2,450 MHz microwave radiation (output power was 80 watts), some of the mast cells located in the peritoneal fluid of the rat were transformed into giant cells. Since the mere heating of the tissue did not produce giant mast cells, their formation must be a non-thermal effect of the microwave radiation.

By using a plexiglass thermostat system to maintain the experimental temperature at 37 C, Stodolnik-Baranska (1967) found human lymphocytes irradiated 4 hours daily at a power density of 7 millijoules per cm² per sec or 15 minutes at a power density of 20 millijoules per cm² per sec for a period of 3-5 days with 3,000 MHz microwave radiation, to undergo lymphoblastiod transformation in vitro. Since there was no rise in temperature under these conditions, the observed effects were attributed to the effect of microwave irradiation and not to the effects of heating.
Baranski (1971) reported that following chronic irradiation 3 hrs daily at a power density of 3.5 millijoules per cm$^2$ per sec with 3,000 MHz microwave radiation, guinea pigs and rabbits (in a constant temperature room) were observed to have nuclear structure and mitotic abnormalities in the erythroblastic and lymphocytic cell series. Since no significant temperature increase was observed in the irradiated animals, these changes were thought to be due to a cumulative effect of the repeated radiation. In addition, these abnormalities seem difficult to explain in terms of thermal effects while nonthermal complex interactions seem to be more probable.

Another interesting report of non-thermal effects of microwaves was made by Mayers and Habeshow (1973). Using temperature control in a 37°C water bath, they noted that 30 minutes of microwave radiation at a frequency of 2,450 MHz (power density at 50 millijoules per cm$^2$ per sec) depressed the phagocytic activity of mouse macrophages which were perfused with suspensions of human red blood cells. The macrophage phagocytic activity was restored to normal if the radiation was discontinued.

More recently, Culkin and Fung (1975) studied the effects
of 915 MHz microwave radiation on *E. coli* and *S. typhemurium* at the various positions of a modified graduated cylinder (50 mm x 250 mm) with five glass side arms. For any given exposure time, the temperature of the middle region was warmest; that of the bottom, intermediate; and the temperature of the top, coolest. They found that the greater decrease in survival of organisms was in the top regions of the liquid as compared to the decreases in survival in the middle and bottom regions. If the lethal action of microwave energy on microorganisms was solely due to the heat generated by the waves, it would be expected that organisms sampled from the warmest regions would have resulted in the lowest survival values at any given time and those sampled from the coolest region should show the greatest survival rates. The data suggest that the heat generated during the microwave exposure alone was inadequate to fully account for the exact nature of the effects of microwaves on bacteria.

It seems evident from the above reports that the heating effect alone is insufficient in explaining the detrimental effects of microwaves on organisms. Non-thermal effects do exist and appear to be more evident in higher organisms than in microorganisms.
5. Purpose of the Experiment

The use of ultra-high frequency (UHF) electromagnetic radiation for the control of vegetation and its potential to kill pest populations in agricultural fields is considered to be a timely solution to a major problem. The prolonged use of insecticides and herbicides, which can eventually accumulate in water and soil, has led to some problems such as food chain accumulation, indirect effects, and unknown synergisms. These effects may well be detrimental to man.

Since microwave radiation is not harmful under controlled conditions and since it has no residual effects that cause further damage to living things either directly or indirectly, it can thereby serve as a fantastic tool in the capacity of non-chemical pesticide and herbicide.

The present study was undertaken to investigate the influence of 2,450 MHz microwave radiation over a wide dosage range on the activity of the soil microflora including heterotrophic bacteria, actinomycetes, spore-forming bacteria, fungi, nitrogen-fixing bacteria, nitrification bacteria, yeasts and viruses.

There were no generally accepted conclusions concerned
with the mechanism of killing by microwave radiation when this work was started. Improper experiments and local temporary temperature rises may greatly enhance a lethal effect. Fallacious experimental designs and methods have also caused a questioning of conclusions proposed in the past.

In the present research, a direct approach to temperature-controlled microwave experiments was undertaken by using the "dry" microorganisms (lyophilized state), in which the temperature was found to be constant throughout the experiment in spite of prolonged irradiation. It is assumed that the findings of this research will definitely settle the question of the mechanism of microwave radiation.
MATERIALS AND METHODS

1. Microwave Source

1.1. Laboratory

A specially constructed apparatus was used for all laboratory studies. It consisted of a 66 x 41 x 48 cm cavity made of stainless steel to which was coupled a magnetron tube capable of delivering 1 kW of absorbed energy (approximately 1.5 kW output and 0.5 kW reflected) at 2,450± 20 MHz. The field produced was uniform over a platform made of teflon positioned under the waveguide and rotated at approximately 60 rpm to insure uniformity of exposure. All samples irradiated weighed approximately 250 g for uniformity of energy absorption except for preparations of dry bacterial cells, which weighed approximately 0.5 g. The radiation field was maintained at a flux of 1 kW of absorbed energy and samples were placed in predetermined positions. Uniformity of radiation field and dose of radiation absorbed were confirmed by measuring increase in the temperature of 250 ml of distilled water at varying periods of time. Linear correlation between
absorbed energy and irradiation time for this type of microwave chamber was established in a separate study (Wayland et al., unpublished results).

1.2. Field

Field soils were irradiated with a commercial mobile microwave generator named "Zapper #2". The microwaves were produced by two 30-kilowatt klystron tubes powered by a diesel generator. The power package, supplied by the Bechtel Corporation (San Francisco, Cal.) was mounted on a 16-ton vehicle with speed-synchronized wheels which allowed it to move along the furrows at any desired speed.

1.3. Laboratory Irradiation

Soil samples: Soils were collected from 10 different spots at a given location, each sample being a plug 2.5 cm in diameter and 2.5 cm in depth. They were placed in clean plastic bags and brought to the laboratory immediately. Rocks, leaves, and other debris were removed, the soils were ground to fine powder in clean mortars, and then sifted through a 50 mesh screen. Wet soil samples were homogenized by mixing and kneading in clean mortars. Approximately 250 g of soil were placed in 500 ml beakers and irradiated for
predetermined periods of time. The beakers were then covered with sterilized aluminum foil and allowed to cool to room temperature.

Activated sludge: sludge samples were collected from the Denton City Sewage Pond. Exactly 250 ml of serage water was placed in 500 ml beakers for irradiation.

Pure cultures: approximately 0.5 g of dry bacterial cells (pulverized sample) were weighed in polystyrene disposable dishes (characterized by their inability to absorb 2,450 MHz radiation), and then immediately exposed to the microwaves. Irradiation for wet bacterial cells was made by adding 0.5 g of the samples and 250 ml sterile distilled water to 500 ml beakers.

1.4. Field Irradiation

This was accomplished by driving the "Zapper #2" over selected agricultural fields at given velocities and power outputs. The radiation dose delivered was determined from the following empirical equation derived by Davis et al. (1971).

\[
\text{Joules/cm}^2 = \frac{\text{Power in watts} \times 0.85}{10 \text{ in.} \times 17.6 \times 2.54^2 \times \text{MPH}}
\]

where:

Power in watts = generator output
85% = power transfer efficiency due to guide position

MPH = velocity of "Zapper 2 over the ground

10 in. x 17.6 x 2.54^2 = microwave guide size and proportionality factor

The soil samples were taken from three layers: 0-2.5 cm, 2.5-5.0 cm and 5.0-10.0 cm, immediately following "zapping".
The applied energies were 200, 400, and 800 joules per cm^2 per sec respectively.

1.5. Temperature Measurement

Soil and water temperatures were measured by plunging a rapid-indicating mercury thermometer (20-100 C range) into the sample immediately after irradiation.

1.6. Determination of Radiation Energy

The classical equation for efficient power is:

\[ Q_T = Q_F - Q_R \]

where:

\[ Q_T = \text{efficient power (KW)} \]
\[ Q_F = \text{forward power (KW)} \]
\[ Q_R = \text{reflected power (KW)} \]

Therefore, the total energy absorbed by water (Q) can be expressed as:

\[ Q = (m_1 - m_2) \times c \times (100 - T_1) + 539 \times (m_1 - m_2) + m_2 \times c \times (T_2 - T_1) \]
Where $Q =$ total energy absorbed by water (cal)

$m_1 =$ initial mass of water (g)

$m_2 =$ final mass of water (g)

$c =$ specific heat of $H_2O$ (liq.)

$T_1 =$ initial temperature of water ($^\circ$C)

$T_2 =$ final temperature of water ($^\circ$C)

539 = evaporation heat of water (cal/g)

1.7. **Moisture Determination**

Soil moisture was determined by finding the weight difference before and after drying in a 105$^\circ$C oven for 24 hours; and using the following equation:

$$\text{Moisture (\%) = } \frac{\text{Weight of wet soil} - \text{Weight of dry soil}}{\text{Weight of wet soil}} \times 100$$
2. Measurement of Effect on Soil Microorganisms

2.1. Soil Dilution

The effect of graded doses of microwave radiation on the soil microflora was measured by comparing the number of viable microorganisms in a given soil sample before and after irradiation. Approximately 10 g of treated soil were transferred to a 90 ml sterile water dilution bottle and the mixture placed on a Kahn Reciprocal Shaker (Eberbach Corp., Ann Arbor, Michigan) for 10 minutes. Ten-fold serial dilutions were prepared and kept in an ice bath until use, generally within 10 to 30 minutes. The exact amount of soil suspended in the first dilution bottle was measured by transferring three 10 ml aliquotes to tared weighing aluminum dishes, drying at 105 C overnight, and averaging the three weights.

2.2. Total Bacterial Counts

Aliquants of 0.1 ml from each dilution prepared were plated on Tryptic Soy Agar (Difco Laboratories, Inc., Detroit, Michigan) in triplicate and incubated at 20-26 C for 96 hours. All counts reported were the average obtained from the appropriate triplicate set.
2.3. **Bacterial Spores**

Ten ml of each soil suspension were transferred to sterile test tubes and placed in a boiling water bath for 10 minutes. The tubes were cooled to room temperature and plated as for total bacterial counts.

2.4. **Fungi Population**

Aliquants of 0.1 ml of the soil suspensions used for total counts were plated in triplicate on fungi count agar (Martin 1950). These were incubated at 20-26°C and counted 5 to 10 days later.

2.5. **Actinomyces Population**

Triplicate aliquants of 0.1 ml from each dilution bottle were plated on a medium prepared by adding glycerol, 10 ml; CaCO$_3$, 3 g; K$_2$HPO$_4$, 1 g; DL-aspartic acid, 1 g; and agar, 20 g to 1 liter water and adjusting the pH to 7.0. The medium was sterilized at 121°C for 15 minutes. Spread plates were incubated at 20-26°C for 10 days and then typical actinomycete colonies were counted.

2.6. **Nitrifying Bacteria (Nitrosomonas)**

A medium was prepared by mixing (NH$_4$)$_2$SO$_4$, 2.0 g; K$_2$HPO$_4$, 1.0 g; NaCl, g; MgSO$_4$, 0.5 g; CaCO$_3$, 2.0 g;
MnSO₄, 1 mg; Fe₂(SO₄)₃, 1 mg in 1 liter of water and dispensing in 4 ml amounts into large test tubes. These were sterilized and 5 tubes inoculated with 1 ml of each soil dilution prepared. The tubes were incubated at 20-26°C in a slanting position for 5 days. Populations of Nitrosomonas were estimated by a previously reported method (Vela 1969). Since it was seemed impractical to obtain accurate population estimates of the nitrifying bacteria in soil, the "index" employed by Coppier and Barjac (1952) was used. This index refers to the nitrifying capacity of a soil and reflects the size of the population of nitrifying bacteria therein. The presence of nitrifying bacteria was confirmed by the changes of turbidity, the presence of NO₂⁻, and the absence of NH₄⁺. The presence of ammonia was checked by mixing one drop of culture with one drop of Nessler's Reagent (Hartmen, Leddon Company, Philadelphia, Pa.): a deep yellow color indicated the existence of ammonia. The presence of nitrite was proven by the appearance of a deep reddish purple color when 0.5 ml of sulfanilic acid solution (sulfanilic acid 0.5 g; acetic acid 33%, 150 ml) and 0.5 ml of naphthylamine reagent (naphthylamine 0.1 g; boiling distilled water 20 ml; acetic acid 33%, 150 ml) were added to the culture tubes.
2.7. **Nitrogen Fixing Bacteria**

The populations of *Azotobacter* were determined by a previously described method (Bela and Wyss 1965) using an incubation temperature of 20-26 C; the "index" was used to compare the size of the population. The chemical change (\(\text{N}_2 \rightarrow \text{NH}_4^+\)) was monitored by adding 1 ml of Nessler's Reagent to 5 ml culture; a yellowish color indicated the presence of ammonia.


Microorganisms used for this experiment were cultured in Nutrient Broth (Difco) or other suitable liquid medium and harvested during maximal growth phase. Two ml of these cultures were centrifuged for 30 minutes at 1500 rpm. The supernatant was discarded and the precipitate was washed with sterilized distilled water three times. A coating solution (10% skim milk and 5% lactose) was added, followed by lyophilization in a Virtis dry vacuum machine (Gardiher, New York) until it was pulverized and afterwards kept at 4 C. The pulverized sample was considered the "dry" state and the "wet" samples were those to which water was added after lyophilization. The microorganisms used were the following:
Azotobacter chroococcum and Serratia marcescens from the North Texas State University stock culture collection; Escherichia coli ATCC 11303, Pseudomonas aeruginosa ATCC 10145, Staphylococcus aureus ATCC 6020, Bacillus cereus 629, Salmonella typhimurium B from the collection of Dr. J. B. McBryde. Bacillus cereus ATCC 629 was harvested from seven-hour old cultures to prevent formation of spores. Spores were obtained after a 10 minute boiling water treatment of seven-day old Bacillus cereus ATCC 629. The Yeast (Fleischmann's Active Dry yeast, made by Standard Brands Inc., N.Y.) containing 7% water content were dried by lyophilization to obtain the dry yeast cells. Bdellovibrio bacteriovorus was isolated from Denton City Sewage Pond by using Pseudomonas aeruginosa ATCC 10145 and E. coli as hosts. The identification was made by following the 8th edition of Bergey's Manual of Determinative Bacteriology.

Bacteriophages were also isolated from Denton City Sewage Pond by using E. coli K12 as host organism. Twenty ml of raw sewage were placed in 250 ml of a modified nutrient broth (Difco Nutrient Broth, 8 g; MgSO$_4$·7H$_2$O, 160 mg; CaCl$_2$·2H$_2$O, 100 mg; and distilled water, 1000 ml). This culture was centrifuged at 3,500 rpm for 15 min after
24 hours, and the precipitate discarded. Ten ml of the filtrate obtained by passing the supernatant through membrane filters (millipore, HAWG 04700) was then transferred to 150 ml of an E. coli broth culture. The cultures, at maximal growth, were concentrated by centrifugation at 34,000 rpm for 1 hour. Two ml of coating solution (10% skim milk and 12% sucrose) were added to the sediment before lyophilization. The count of bacteriophages was made by classical soft agar method using the host organism employed for isolation.

4. The Effect of Moisture On Microorganisms Exposed to Microwave Radiation.

Three aluminium weighing pans, each containing 10 g of soil, were kept in each of 5 desiccators with fresh anhydrous calcium chloride. The amount of moisture in the soil was tested after 1, 5, 10, 15 and 30 days of drying. The results were 6.5%, 4.28%, 1.90%, 1.13% and 0.56% respectively. The moisture content of lyophilized soil was found to be no more than 0.01%. The soil samples in the desiccators had more moisture than the lyophilized soil due to the limited drying capacity of the desiccators.

After moisture was measured, the samples were exposed
5. Measurement of the Effect of Microwaves on Microorganisms in Activated Sludge

In order to test the effect of microwave radiation on total bacterial counts and coliform bacteria in sewage effluent, water samples were obtained from the Village Creek waste water treatment plant in Fort Worth, Texas in sterile bottles. These 250 ml samples were agitated and placed in a 500 ml beaker and irradiated. Immediately after irradiation, the samples were placed in an ice bath. Serial dilutions were made and then plated onto Nutrient Agar and EMB plates. The plates were incubated at 37°C and counted after 24 and 48 hours.

The influence of chemical compounds and mixtures of several compounds on microorganisms exposed to microwave radiation: each of various samples of ethyl alcohol, ethylenediaminetetraacetic acid (EDTA) and detergent (Joy, Procter and Gamble, Cincinnati, Ohio) in concentrations (w/v) of 0.01%, 0.1%, 0.5%, 1%, 2% and 4% were mixed with 250 ml sludge and exposed to microwave
radiation. They were then spread on EMB plates and incubated for 24 hours at 37°C and then counted.
RESULTS

1. The Effect of Microwave Radiation on Soil Moisture and Temperature

It will be noted from table 1 that the exposure of the soil sample to microwave radiation was followed by changes of temperature and moisture content. In general, the higher the radiation dose, the greater the temperature increase and the greater the water loss. For example, when total energy was approximately $3.5 \times 10^4$ joules, the soil lost 50% of its moisture content and temperature increased to 49 C. Furthermore, when the time of radiation was increased to 150 seconds (equal to $1.5 \times 10^5$ joules), the temperature was elevated from the initial room temperature to 60 C and moisture content went to zero.

2. The Effect of Microwave Radiation on Soil Microorganisms

Under $4.0 \times 10^4$ joules of radiation, all types of soil microorganisms showed a slight decrease in viable counts (Figs. 3 to 7). As in Fig. 6, total bacterial counts dropped from an initial figure of $3.2 \times 10^7$ to $1.9 \times 10^6$; spore counts dropped from an initial figure of $3.9 \times 10^6$
to $2.6 \times 10^5$. Fungi seemed to be more susceptible to microwave radiation; they were reduced from an initial number of $4.0 \times 10^4$ to $2.7 \times 10^2$. The index of nitrogen-fixing bacteria, showed a decrease of 4.50 to 3.25 when subjected to 40 seconds of radiation. Under the same conditions nitrifying bacteria declined from 4.25 to 2.50. On the fifth day of irradiation (Fig. 4), all microorganisms except spore-forming bacteria showed a slight decrease when radiation intensity was elevated to 40 seconds. Only the spore-forming bacteria appeared to be unaffected. On the tenth day, the spore-forming bacteria seemed to remain in a steady state, whereas other kinds of microorganisms showed a smooth variation under the different doses of radiation. On the fifteenth day after radiation, all viable microorganisms showed decreasing counts and varied with the radiation times. On the thirtieth day, the survival curves of actinomycetes and total bacteria decreased significantly (Fig. 7).

In general, the viable microorganisms tended to show irregular fluctuation and there were no remarkable differences in the count of each group of microorganisms before and after radiation.
3. The Effect of Moisture Content on Soil Microorganisms.

Microorganisms in wet condition were more affected than microorganisms in dry conditions. Fungi were completely killed after 2 minutes of radiation and nitrifying bacteria also showed less resistance to microwave radiation under these conditions. Under prolonged radiation, the differences of the viable cell counts (between wet and dry) were more evident (Fig. 23 and Fig. 24). Fungi seemed to be affected beginning at the threshold of 80 seconds (Fig. 9). When the soil sample was exposed to 4 minutes of radiation in the laboratory, it was found that the soil moisture content had a slight effect on the survival rate of microorganisms, but these effects were more significant when the soil moisture was higher. At 52% moisture content (holding capacity of water), the survival rates of spore-forming bacteria and heterotrophic bacteria declined to less than 0.1%, actinomycetes were reduced to 0.05% and the fungi populations were completely destroyed. When the moisture content in the soil was about 6.5%, both of spore-forming bacteria and heterotrophic bacteria showed a 0.65% survival rate. When the moisture content ranged from 0.5% to 4.3%,
the survival curves seemed to be smoother and to show no discernable differences. After lyophilization, moisture content was reduced to less than 0.1% and the survival rate was found to be 100%.

Weed seeds were devitalized with a treatment of 20 seconds (Fig. 10), whereas most microorganisms survived under the same conditions.

4. Pure Culture Studies

The resistance of microorganisms to microwave radiation varied with species. A dose of 20 seconds was sufficient to kill Azotobacter chroococum (Fig. 11), Azotobacter vinelandii (Fig. 12), Bacillus cereus (Fig. 13), Salmonella typhimurium (Fig. 19) and Serratia marcescens (Fig. 20); Escherichia coli (Fig. 17), Pseudomonas aeruginosa (Fig. 18), Staphylococcus aureus (Fig. 21) and commercial yeasts were completely destroyed on 40 seconds exposure. To the contrary, spore-forming bacteria were shown to have unusual resistance under the wet condition. Dry organisms appeared to have higher resistance than aqueous suspensions and higher doses of microwave radiation only caused a slight reduction in the populations.

In laboratory studies, the moisture content affected the
survival rates of yeast (Fig. 22). Temperature in aqueous suspensions depended upon radiation doses, whereas temperature in dry samples were independent of radiation doses (Fig. 12).

5. The Effect of Microwave Radiation on Microorganisms in Activated Sludge.

Most of the microorganisms in activated sludge will be partially killed by 40 seconds of exposure (Fig. 7); higher doses are necessary to destroy them completely. *Bdellovibrio bacteriovorus* and *Escherichia coli* K12 bacteriophage were completely killed with 40 seconds of radiation when these were suspended in water. When *E. coli* (isolated from activated sludge) was exposed to 20 seconds of radiation in the presence of EDTA, it was found to be more susceptible to microwaves than when alcohol or detergent (Joy) were used. Higher concentrations were necessary to achieve sterilization (Fig. 25).

6. Field Studies

In the application of 2,450 MHz of microwave radiation to an agricultural field for weed seed control, it was determined that microorganisms at different depths showed
no significant differences in survival rate (Table 3, Table 4). Microorganisms in three portions, 0-2.5 cm, 2.5-5.0 cm and 5.0-10.0 cm depth show an irregular survival rate of 0.7 to 1.1. Other independent variables such as field location, the kind of soil, and the moisture content of the soil apparently affect the survival rate.
TABLE 1

EFFECT OF 2,450 MHz RADIATION ON TEMPERATURE AND MOISTURE CONTENT IN POWDERED SOILS IRRADIATED IN THE LABORATORY

<table>
<thead>
<tr>
<th>Radiation; Joules (x 10^3)</th>
<th>Temperature Immediately After, °C</th>
<th>% Loss of Moisture Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>47</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>20</td>
<td>68</td>
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<td>120</td>
<td>78</td>
<td>99</td>
</tr>
<tr>
<td>150</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>Condition</td>
<td>Joules (x 10^4)</td>
<td>Total Count</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Dry</td>
<td>0</td>
<td>1.3 x 10⁶</td>
</tr>
<tr>
<td>Wet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td>6</td>
<td>1.6 x 10⁵</td>
</tr>
<tr>
<td>Wet</td>
<td>6</td>
<td>1.1 x 10⁵</td>
</tr>
<tr>
<td>Dry</td>
<td>12</td>
<td>3.2 x 10⁴</td>
</tr>
<tr>
<td>Wet</td>
<td>12</td>
<td>2.2 x 10⁴</td>
</tr>
<tr>
<td>Dry</td>
<td>24</td>
<td>1.2 x 10⁴</td>
</tr>
<tr>
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<tr>
<td>Wet</td>
<td>48</td>
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*Reflects the number of nitrogen-fixing or nitrifying (NH₄⁺ → NO₂⁻) bacteria per gram of soil; 5.00, the population in untreated soil; 0, the entire population destroyed (Vela and Wyss 1965; Vela 1969).
# TABLE 3

**EFFECT OF MICROWAVE RADIATION ON MICROORGANISMS AT DIFFERENT SOIL DEPTHS IN A FIELD OF LUFKIN SANDY LOAM**

<table>
<thead>
<tr>
<th>Run number</th>
<th>Radiation, Joules/sec/cm²</th>
<th>Soil depth, cm</th>
<th>Total Spores</th>
<th>Actinomycetes</th>
<th>Fungi</th>
<th>N₂-fixation index</th>
<th>Nitri- fication index</th>
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*Survival rate* = \( \frac{\text{number after irradiation}}{\text{number before irradiation}} \)
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<th>Fungi</th>
<th>N₂-Fix.</th>
<th>Nitrifi. bact.</th>
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Fig. 3. The effect of herbicidal UHF fields on soil microorganisms immediately after irradiation in the laboratory. The dashed line indicates temperature in °C and is read by multiplying the number of the ordinate by 10. Nitrogen-fixing and nitrifying bacteria are designated by the "index" described in the text.
Fig. 4. The effect of herbicidal UHF fields on soil microorganisms on the fifth day after irradiation in the laboratory. Nitrogen-fixing and nitrifying bacteria are designated by the "index" described in the text.
Fig. 5. The effect of herbicidal UHF fields on soil microorganisms on the tenth day after irradiation in the laboratory. Nitrogen-fixing and nitrifying bacteria are designated by the "index" described in the text.
Fig. 6. The effect of herbicidal UHF fields on soil microorganisms on the fifteenth day after irradiation in the laboratory. Nitrogen-fixing and nitrifying bacteria are designated by the "index" described in the text.
Fig. 7. The effect of herbicidal UHF fields on soil microorganisms on the thirtieth day after irradiation in the laboratory. Nitrogen-fixing and nitrifying bacteria are designated by the "index" described in the text.
Fig. 8. The effect of UHF fields on microbial populations in activated sludge. The dashed line indicates temperature in °C and is read by multiplying the value of the ordinate by 10.
Fig. 9. The effect of moisture on soils receiving small doses of radiation. Each point is the average of triplicate measurements. Wet soil indicates water holding capacity for a period of 36 hours.
Fig. 10. Comparative survival rates of soil microorganisms and weed seeds irradiated in the laboratory.
Fig. 11. The effect of UHF fields on *Azotobacter chroococcum*. Dry organisms are the organisms after lyophilization; wet were irradiated in aqueous suspension.
Fig. 12. The effect of UHF fields on Azotobacter vinelandii. The dashed line indicates temperature and solid line indicates the log number of viable organisms per milliliter.
Fig. 13. The effect of UHF fields on *Bacillus cereus* and its spores. The dashed line indicates organisms irradiated in aqueous solution.
Fig. 14. The effect of UHF fields on *Bdellovibrio* bacteriovorus (*Escherichia coli* K12 as host). The dashed line indicates organisms irradiated in aqueous and the solid line in dry condition.
Fig. 15. The effect of UHF fields on Bdellovibrio bacteriovorus (Pseudomonas aeruginosa ATCC 10145 as host). The dashed line indicates organisms irradiated in aqueous solution.
Fig. 16. The effect of UHF fields on *Escherichia coli* Kl2 bacteriophage. The dashed line indicates organisms irradiated in aqueous solution.
Fig. 17. The effect of UHF fields on dry and wet cells of *Escherichia coli*. Each point is the average of triplicate measurements.
Fig. 18. The effect of UHF fields on dry and wet cells of *Pseudomonas aeruginosa*.
Fig. 19. The effect of UHF fields on dry and wet cells of _Salmonella typhimurium_.

*Sal. typhimurium*
Fig. 20. The effect of UHF fields on dry and wet cells of *Serratia marcescens*. 
Fig. 21. The effect of UHF fields on dry and wet cells of *Staphylococcus aureus*.
Fig. 22. The effect of moisture on commercial yeast receiving high doses of radiation. Each point is the average of triplicate measurements. Wet indicates a slurry of yeast cells in sterile distilled water. The solid line indicates yeast cells irradiated immediately after removing from the package and the dashed line cells allowed to rehydrate by exposure to room air.
Fig. 23. The effect of moisture on soil spores and actinomycetes cultured in the laboratory and receiving $2.4 \times 10^5$ joules of radiation. Ordinate indicates the survival fraction (ratio of irradiated to unirradiated).
Fig. 24. The effect of moisture on soil heterotrophic bacteria and fungi cultured in the laboratory and receiving $2.4 \times 10^5$ joules of radiation. Ordinate indicates the survival fraction (ratio of irradiated to unirradiated).
Fig. 25. The effect of alcohol, EDTA and detergent on *Escherichia coli* receiving $2 \times 10^4$ joules of radiation.
DISCUSSION

1. The Effect of Microwave Radiation on Soil Moisture and Temperature.

In the absence of vegetation, when the soil surface is subjected to 2,450 MHz microwave radiation, evaporation occurs directly and entirely from the soil. Then it depletes the moisture of the surface soil and this can involve very considerable losses of water. When the soil is relatively wet, and of relatively high conductivity, its ability to deliver water to the evaporation zone at the soil surface is not likely to be limiting. Therefore, the radiation intensity if $25 \times 10^3$ joules with an accompanying increase in temperature will cause 50% loss of moisture content and $150 \times 10^3$ joules of radiation will completely deplete all of the moisture in it (Table 1).

The soils used were from Bryan, Denton, Decatur, College Station, Weslaco, Denison and San Antonio, Texas, and they were obtained from different sites; i.e., Denton soils were obtained from flower gardens, corn fields, cotton field, sorghum field, and wheat field. The soils used were
of different types and included sandy loam, sand, red clay, and black clay. Since individual experiments showed no discernible differences in the results obtained from different soils, no attempt was made here to show that the results obtained depended on soil type and locality. It is assumed that these have little or no effect on the inactivation of soil microorganisms by 2,450 MHz radiation.

Soil moisture characteristics are strongly influenced by soil texture; the greater the clay content, in general, the greater the water content. Sandy soils contain much less water than clay soils (Hillel 1971). Since soil temperature remained at 80°C or below in all the experiments performed with powdered soils, and microorganisms in the soil were not subjected to the same amount of radiation, it was assumed that the energy transfer characteristics of the soil changed as water evaporated. In order to test this hypothesis, soils were moistened with varying amounts of water, allowed to stand for 36 hours, and then irradiated. The results of 3 experiments are essentially the same as those shown in table 2. Similar results were obtained from sandy soil, and a sample of clay loam. These results indicate that large doses of radiation were required to
inactivate a significant portion of the microbial population in the soil. This "heat shock" effect was not observed in the other microbial populations under study. It is interpreted as heat-shock activation of bacterial and fungal spores. This finding may become critical if pathogenic or undesirable spore-forming organisms are involved because the spores may be activated by irradiation of moist soils if the extent of hydration approaches that used in the laboratory studies.

2. Effect on Soil Microorganisms

2.1. Heterotrophic Bacteria

Irradiation is known to cause certain alternations in the microbial population of soils. High frequency waves have been found to have bactericidal effects (Williams and Ganies 1930) and, at that time, it appeared that the kinetics of inactivation was the same as those observed for UV radiation. It was also assumed that since it was possible to induce mutations in bacteria by irradiation with wavelengths in the ultraviolet spectrum, it could be that microwaves also produced mutations. Luckiesh (1946) suggested that the long-wave limit of germicidal effectiveness may depend somewhat on the type of organism but particularly
upon energy transfer and experimental techniques. The sensitivity of the different segments of the microflora to radiation was found to decrease in the following order: non-sporulating bacteria, fungi, yeast and spore-forming bacteria (Bridges et al. 1956, Davis et al. 1956, Dunn et al. 1948, Gunter and Kohn 1956, Koh et al. 1956) reported that gram-negative bacteria were more sensitive to cathode rays than gram-positive bacteria. Luckiesh (1946) found certain pathogenic bacteria had a resistivity at least 10 times that of E. coli. Bacteria that possess pigments are more resistant to radiation than non-pigmented bacteria (Doetsch and Cook 1973). In the present studies, we discovered that soil plays a surprising role in the protection of heterotrophic bacteria exposed to microwave radiation; some bacteria were affected only with high doses of radiation \(2.4 \times 10^5\) joules).

This differential resistance corresponds to the observations by Davis et al. (1956) and suggests that if an entire segment of the population is not eliminated, the population will return in time to the equilibrium situation.

2.2. Spore-forming Bacteria

Microwave radiation could either activate the
germination or decrease the survival of spores. The germination of spores in the soil is affected by environmental factors such as temperature, pH, nutrients, etc. Some bacteria, such as *Bacillus stearothermophilus* are extremely dormant and have to be exposed to a temperature above 100 °C for activation (Keynan et al. 1965) while others germinate without heat shock. In like manners, the heat resistance of spores varied with species (Tarpley et al. 1953), culture history, irradiation conditions, and post-irradiation treatment.

Some bacterial spores are unusually resistant to microwave radiation. Again, the spores are more susceptible when wet than when dry and it is assumed that water serves as the agent for transferring energy to the spore itself. This resistance of bacterial spores to microwave radiation may correspond to the resistance of any deleterious effect mediated by water; including ultraviolet light, ionizing radiation, and chemical substances.

2.3. Actinomycetes

Actinomycetes have been widely used in experiments with high frequency intensities of radiation (Kelner 1948, Newcombe 1953). The primary effect of microwaves is reduction of the
number of viable organisms. Gier (1937), however, indicated that the application of 666.67 MHz radiation to Actinomyces scabies for 49 hours had no effect. Demerce and Latarjet (1946) found Streptomyces flaveolus was apparently far more resistant to ultraviolet irradiation than was Escherichia coli. Actinomycetes in situ in the soil show similar responses to microwave radiation. The $4 \times 10^4$ joules/cm$^2$ dose of radiation employed in this experiment had little effect on the actinomycete populations which developed following irradiation and incubation (Fig. 3-7). Much higher doses evidently are required for a complete kill, especially in dry soil. The effect of radiation undoubtedly was mitigated by the absorption of radiation energy by the soil.

Although the optimal growth temperature for actinomycetes is about 25 C and few of them grow at 42 C, 5% of spores survive 100 C (Silvey 1953, Roach and Silvey 1958). Therefore, viable spores and radiation-resistant cells could rapidly re-establish the soil population if a complete kill had not been obtained.

2.4. Fungi

Microwave radiation greatly reduced the number of fungi
under the different experimental conditions (Goldblith and Wang 1967, Fleming 1944). The survival rate depended on the radiation dose. Popenoe and Eno (1962) indicated that bacteria and fungi had about the same order of sensitivity to ionizing radiation; but microwave radiation applied directly to soil samples revealed that fungi were more sensitive, especially when wet. Usually $6.0 \times 10^4$ joules of radiation were required to destroy fungal populations in wet soil. This was quite different from the data presented by Luckiesh (1946), he found that some yeast cells appeared to require exposure to as much as 50 times that necessary to kill *E. coli*. The study of the biological effects of ultraviolet radiation on fungi concerned itself for many years with toxic effects. Different molecular structures are responsible for absorption in different regions of the spectrum and the data show specific effects for specific radiations. In the case of microwaves, the effect is totally nonspecific.

*Alternaria solani* was irradiated with 666.67 MHz up to 72 hours with no effects, and on those exposed over 72 hours showed only a slight stunting; but the effect may have been caused by other factors (Gier 1937). This was different
from the data reported by Goring and Clark (1952); using radioactive phosphorus they found that fungal populations increased eight-fold as a result of the radiation while the total number of microorganisms decreased to about one-third after 3 weeks.

2.5. **Nitrifying Bacteria**

Different kinds of radiation cause dissimilar effects on nitrifying bacteria. Low levels of gamma and neutron radiation reduced the rate of nitrification by two-thirds after 2 weeks of incubation and by one-half after 4 weeks of incubation (Stanovick et al. 1961). On the other hand, Popenoe and Eno (1962) proved that nitrification and sulfate production are inhibited by ionization radiation.

The evidence presented in this report shows that soil microorganisms are susceptible to microwave radiation under certain condition. Although the soil plays a significant role in protecting the microorganisms from microwave radiation, $6.0 \times 10^4$ joules of radiation will eliminate the vast majority of the soil microflora. This observation corresponds to Vella's (1969) report using ionizing radiation: The ability of soil microorganisms to carry out nitrification in irradiated soils diminished
as a function of radiation dose and returns to the level observed in unirradiated soils as a function of time.

2.6. **Nitrogen-fixing Bacteria**

The nitrogen-fixing bacteria are more susceptible to gamma and neutron radiation than any other group of soil microorganisms (Rubenchik 1963). It is evident that nitrogen-fixing bacteria survive relatively high doses of microwave radiation (Table 2), and from a previous study (Vela and Wyss 1965) it is assumed that only organisms in situ in the soil are resistant. The assumption was tested by isolating *Azotobacter* from the cultures used in Table 2 and irradiating these in freshly prepared buffer solutions. The data (Fig. 11, 12) show that while these bacteria survive exposure to 2,450 MHz for 8 minutes (4.8 x 10^5 joules) in dry soils, they are inactivated by exposure of only 20 seconds (2.0 x 10^4 joules) in laboratory cultures. Temperatures of soil-water mixtures or laboratory media did not exceed 90 C in these experiments. The results are similar to those obtained with ionizing radiation (Vela and Wyss 1965) and also in other comparable situations (Vela 1974, Vela and Eubanks 1973). These data plainly show that
soil organisms in laboratory cultures are destroyed at temperatures lower than 80 C. By contrast, mixed cultures of bacteria normally found in water are somewhat more resistant (Fig. 8).

It is assumed then, that habitat and native environment are of great importance in resistance to microwave radiation. That is, the organisms in situ in their native habitat are more resistant than in any other environment.

3. Pure Culture Studies.

3.1. *Azotobacter*

The high resistance of *Azotobacter* cells to radiation is indicated by the fact that this bacterium may survive for several days in a culture medium containing 80 mc of beta ray radiation per liter medium. Cells suspended in water were less affected by irradiation than those suspended in a nutrient medium (Rubenchik 1963). This group of microorganisms, as exemplified by *Azotobacter vinelandii* (Fig. 12) and *Azotobacter chroococcum* (Fig. 11) were completely killed by 20 seconds of radiation when they were suspended in aqueous media. To the contrary, dry organisms survived much higher doses of the same microwave
radiation. This resistance difference is due primarily to the thermal effect.

3.2. *Bacillus cereus* and its spores

*Bacillus cereus* suspended in aqueous media is greatly reduced or destroyed by microwave radiation. Powers (1962) found that the survival rates of *Bacillus megaterium* spores irradiated in liquid suspension depended on the gaseous atmosphere in which the spores were irradiated. Since *Clostridium sporogenes* (Tarpley et al. 1953) appeared to be the organism most difficult to kill, it is suggested that the spores of this organism are particularly resistant to ionizing radiation. In the same way, spores of *Bacillus cereus* survive high doses of microwave radiation; however, the mere fact that certain bacteria are spore producers does not necessarily mean that the vegetative cells also possess resistance to radiation.

3.3. *Bdellovibrio bacteriovorus*

Two strains of *Bdellovibrio bacteriovorus* were isolated from a sewage pond, one using *E. coli* Kl2 as host and the other using *Pseudomonas aeruginosa*. Dry organisms of both strains were resistant to higher doses of radiation, but
all the organisms were killed after 40 seconds of radiation at a rate of $10^3$ joules per sec.

3.4. *Escherichia coli* Kl2 Phage

The effects of UV and visible lights on bacteriophages have been reviewed by Pollard (1954) and Kleczkowski (1957). Viruses do not absorb visible light and so are not affected directly by it to any appreciable extent. However, they may be affected indirectly when colored materials are present in the media. The action spectra of UV on different viruses (Gates 1934, Hollaender and Oliphant 1944, Rivers and Gates 1928, Zelle and Hollaender 1954) resemble the absorption spectra of nucleic acids. It appears that the nucleic acids in these viruses are involved in the mechanisms of inactivation by UV. In the present study, the wet coli-phages were completely killed with $4.0 \times 10^4$ joules of microwave radiation; on the contrary, the dry phages were nearly unaffected after an irradiation of $2.4 \times 10^5$ joules. From these data I concluded that the heating effect was the major factor in phage inactivation.

D'Herelle (1926) noted that several phages were inactivated by heating at 75°C for 30 minutes, whereas some survived and some did not after heating at 70°C. According
to Chang et al. (1950), the survival rates of coliphage suspended for 1 minute at temperatures ranging from 65 to 70°C were 84% to 54.5% respectively. In comparative terms, the survival rate observed in these studies was 77.1% after 10 seconds of microwave radiation at the rate of $10^3$ joules.

3.5. *Escherichia coli*

This microorganism was found to be easily inactivated by microwave radiation (Goldblith and Wang 1967). The resistance of this microorganism to radiation depends on the culture phase at time of exposure. It was relatively insensitive during the lag phase (nonproliferative growth) and followed by a marked increase in sensitivity during the exponential (log) phase of growth. Maximal sensitivity was reached at the end of the exponential phase and then decreased to the level characteristic of the lag phase (Casarett 1968). This microorganism was inactivated by microwave radiation at doses of $4 \times 10^4$ joules. When dry, the organism was unaffected by radiation doses up to $2.4 \times 10^5$ joules. The strain of *E. coli* isolated from activated sludge possessed more resistance to microwaves than the laboratory strain (Figs. 7, 8, 25).
3.6. *Pseudomonas aeruginosa*

According to Tarpley et al. (1953), this microorganism was inactivated by relatively low radiation doses while other species of *Pseudomonas* required greater dose. This microorganism in aqueous suspension was killed by exposing it to 40 seconds of radiation, but in the dry conditions it was shown to be resistant to 240 seconds of radiation (Fig. 18) at the same intensity: 1 x 10³ joules per cm² per sec.

3.7. *Salmonella typhimurium*

Different *Salmonella* species were found to vary widely in sensitivity to ionizing radiation (Novak et al. 1967). *Salmonella typhimurium* in dry form was significantly more resistant to 2.4 x 10⁵ joules of microwave radiation than were the moist cells. However, it must be noted that prolonged exposure will begin to injure the cells (Fig. 19).

3.8. *Serratia marcescens*

It had been shown that this microorganism will follow exponential decrease in colony-forming units over a wide range of ionizing radiation doses (Casarett 1968). Again, an exposure to 2 x 10⁴ joules of 2,450 MHz radiation will completely kill wet cells but much higher doses are necessary to kill the dry cells (Fig. 20).
3.9. *Staphylococcus aureus*

The per cent of *Staphylococcus* killed is found to vary directly with wave length of radiation used (Gates 1929). With regard to microwave radiation, this microorganism was shown to be not significantly affected in the dry condition, but was completely destroyed in the wet condition with much lower doses of radiation (Fig. 21).

3.10. Yeast

The radiosensitivity of yeast cells (*Saccharomyces cerevisiae*) has been shown to be related to ploidy; haploid (n) are most radiosensitive and diploid (2n) are most resistant (Tobias 1958). According to Beckwith and Olson (1932), this microorganism was both killed and its growth rate impaired when it was exposed to ultrasonic radiation. Microwave radiation was also shown to affect it (Fleming 1944). In these studies, the survival rate of yeast increased following decrease in moisture content of the suspending medium (Fig. 22). This suggests that moisture enhances the killing effect of microwave radiation in yeast as it does in the other organisms studied.

It will be noted that the microorganisms studied can be divided into three groups according to their susceptibility
to microwave radiation when the cells are wet. The first group which includes *B. bacteriovorus*, *E. coli*, *P. aeruginose*, yeast and virus are decimated by exposure to $4 \times 10^4$ joules of 2,450 MHz radiation. The second group, which includes *A. chroococcum*, *A. vinelandii*, *B. cereus*, *S. typhimurium* and *S. marcescens* is completely destroyed by exposure to $2 \times 10^4$ joules. Spores, the third group, can survive higher radiation doses.

Most microorganisms are relatively radioresistant, especially in the vegetative stage (Casarett 1968). Radiosensitivity also depends on the stage of the growth cycle of the microorganism at the time of irradiation. The patterns of the survival curves caused by microwave radiation depend greatly on the water content of the suspending medium. For most microorganisms, survival curves follow exponential kinetics; but they vary with or within the species.

4. The Mechanisms of Killing of Microorganisms by Microwave Radiation

When a microwave beam impinges upon a target any one or a combination of three things may occur. It may be absorbed; it may be reflected; or it may pass completely through the target. The actual effect depends on the
frequency, power density, duration of the exposure, dielectric constant and conductivity of suspending medium, geometrical configuration, composition, and the thickness of the target. When the target is a microorganism, the kind of suspending media, and the composition of cells are significant variables in determining the amount of energy absorbed. In general, for the frequency ranges below 1,000 MHz or above 3,000 MHz, approximately 40% of the incident energy is absorbed by tissue. In the 1,000-3,000 MHz range, absorption varies from 20% to 100% depending on the above factors (Schwan and Piersol 1954, 1955). The energy of a microwave quantum, being only about $10^{-5}$ eV, is insufficient to produce ionization and excitation. Since the bond energy of molecule ranges from about 1 to 10 ev, the only possible effects are volume heating due to movement of ions in the electromagnetic field (heating effect) or changes in the magnetic orientation of molecules. This may be observed as a "pearl chain" formation (Liebesny 1938), manifested by the lining up of red and white blood cells or bacteria constrained to travel only parallel or at right angles along the electromagnetic line of force (Teixeira-Pinto et al. 1960), or as dielectric
saturation in which protein side chains align themselves along lines of force. The reported athermal effects can be classified into two categories, physical and chemical. However, microwave radiation at an energy level of one kilowatt and a frequency of 2,450 MHz is not sufficient to cause chemical change. Among the physical effects, the formation of pearl chains (due to the forces acting on particles) was related to a decrease in zeta potential (Heller and Teixeira-Pinto 1959). Apparently, this phenomenon is correlated with the physical process on the surface of the particles and probably no chemical change is involved. Such a decrease of zeta-potential might be explained by absorption on the surface of either ions or molecules, by increase in ionic strength of the medium or by the removal of surface charge group. These changes would probably be lethal to microorganisms.

Studies of enzymatic activity at different radio-frequencies and microwave energies indicate that there are no selective effects (Carroll and Lopez 1969, Kiirend et al. 1974). Many of the examples of altered metabolic function are probably related to changes in cell membranes. For example, yeast can be shown to lose ability to retain
potassium ions following irradiation. This effect has been tentatively attributed to an uncoupling of the oxidative phosphorylation associated with active transport across the cell membrane (Casarett 1968). Moreover, orientation effects on subcellular particles may disturb cell division (Rosen 1972).

Much of the thought on the effect of electromagnetic radiation has been influenced by the idea that cells contain certain non-reductant "target" or "sensitive sites" which might be anything from molecules to organelles, but whose structural integrity is essential for survival (Zimmer 1961). Damage to an appropriate number of these "targets", whether induced by radiation or other agents, is assumed to be lethal. From the microbial studies of pure strains in a dry condition in which the temperature is kept at room level throughout the experiment, most microorganisms are found to produce a temporary temperature rise which would result in the death of the cell. When some critical temperature level is not exceeded, the cell would survive. However, the continued heating effect generated by microwave radiation will evaporate the water molecules and deprive the cell of its essential water (i.e., dehydrate the cytoplasm) or,
perhaps, modify functional chemical groups of the cell. Any of these effects will disturb the physiological balance either inside or outside the cell membrane, and finally lead to irreparable damage. By comparing the temperature and survival curves generated in dry and wet microorganisms, it is evident that bacterial cells do not absorb microwave energy (Fig. 12). Water is a dipolar molecule and a good medium for heat transfer; its existence will produce the heating effect observed on exposure to microwave radiation of microorganisms. Therefore, the moisture content in the suspending media will greatly affect the survival rate. This finding will be exaggerated in sensitive groups of microorganisms, such as fungi.

However, it would be a mistake to conclude that organisms with a high water content are necessarily the most vulnerable to microwave radiation; biological damage depends both on the temperature rise produced which in turn depends both on the thermal capacity and the ability for heat to be conducted away (Grant 1969).

It is extremely difficult to differentiate and quantitate thermal and non-thermal effects of microwave at the cellular level. From a practical standpoint, the
overall long range effects of radiation upon a population are probably more important than the immediate effect of death, especially in the case of a sublethal dosage to the entire population.

5. The Effect on Microorganisms in Activated Sludge

Microbial populations in activated sludge were found to be reduced by microwave radiation. Most microorganisms in sludged water survived in spite of $4 \times 10^4$ joules of radiation applied (Fig. 8). Whereas, the same dosage of radiation will completely kill all kinds of laboratory cultures of microorganisms. Microorganisms in sludge water were more resistant to radiation than those cultured in the laboratory. Higher doses of radiation are necessary to kill them. Figure 8 indicates that an increase in temperature results in primary damage to the microorganisms.

From these data, it becomes obvious that resistance to microwave radiation is not simply a matter of moisture content. Obviously bacteria suspended in water in situ in their natural habitat are more resistant than those removed from their natural habitat and then suspended in water. Therefore, it appears that a major part of the radiation resistance characteristics of the bacteria are associated
with the ameliorating effect of habitat; these data are in
total agreement with a previous report (Vela and Wyss 1965).

The effect of sensitizers on *Escherichia coli*:
Radiation energy absorbed by materials other than bacteria
affects the bacteria indirectly or the chemical reaction
between cell and sensitizer may yield the cell more
susceptible. The materials may be altered by radiation
so that they may become harmful to the bacteria, or absorbed
energy may be retransferred to bacteria and thus affect it.
With regard to the effect of chemical compounds (alcohol,
ethylenediaminetetraacetic acid) and a detergent on *E. coli*.
Ethylenediaminetetraacetic acid was found to be most
effective in enhancing killing of bacteria. Ethylenediamine-
tetraacetic acid was probably a better energy absorber and
heat transfer agent for microwave radiation but it could
also have the effect of removing essential divalent cations
from the bacterial cell and thereby making the cell more
susceptible: it was without the scope of this work to
determine the exact mechanism of action. In addition, due
to the large concentrations required (Fig. 25), it was
impractical to use these chemical compounds or mixtures
to enhance the sterilization of microorganisms in the
activated sludge.
6. Application to the Agricultural Field

The advantage of the microwave methods of pest control over chemical pesticides is, of course, that the microwave treatment leaves behind no poisonous residues to contaminate the environment. As figure 10 indicates, weed seeds are easily killed by exposure of soil to $1 \times 10^4$ joules/cm$^2$ of microwave radiation energy, while microorganisms show much greater resistance. Microwaves can be used as a good tool in agricultural fields to control weed seeds. The data shown in table 3 were obtained from a sandy loam field in Lufkin, Texas, these data clearly show that the soil microflora were not severely disturbed by exposure to quantities of microwave radiation sufficient to inactivate the vast majority of weed seeds. The lower survival rate soil depths of 2.5-5.0 cm and 5.0-10.0 cm may be associated heat retention deep in the undisturbed field soil. Temperature profiles were not obtained; but repeated measurements showed that surface soils returned to ambient temperature in 1 to 4 hours, while the temperature 10 cm deep in the soil was still 40 to 50°C eighteen hours after irradiation.

While it may appear that lower survival rates result from prolonged heating of the subsurface soil, the greater
body of data does not bear this out. Table 4 shows values obtained from fields in Harlingen and Weslaco, Texas. Since different radiation intensities were used in alternating dry and wet plots, the effects observed in the laboratory probably interact to yield a composite effect.

Microwave radiations leave no residual or lingering effects, since bacterial counts of the soils made 1, 5, 15, 30 days after irradiation were indistinguishable from those obtained on the day of irradiation and the only variation evident was that due to plate counting error (Figs. 3 to 6). The present study indicates that once the initial destruction takes place, there is no further bactericidal activity associated with microwave radiations.

Whatever the nature or extent of the overall effect, it is obvious that field applications of 2,450 MHz radiations effective in the elimination of weed seeds have little effect on the autochthonous microflora.

While designations such as "total bacterial count" and "fungi" may leave questions regarding the effects of microwaves on specific species or even genera within the class designation, there is no ambiguity in the designations "nitrifying" and "nitrogen-fixing" bacteria. By considering
the effect of microwave radiation on organisms that fall into both designations, it is evident that the effect of microwaves on soil microorganisms is not specific or selective but rather that it affects all microorganisms in approximately the same manner. This assertion is in accord with the findings of other investigators of comparable systems (Brown and Morrison 1953, Hamid et al. 1969, Lechowich et al. 1969).

These experiments also showed that radiation doses many times greater than those needed for weed control have no major effect on microbial populations in the soil. On this basis, I claim that microwaves promise all the benefits of the best herbicidal and insecticidal substances without the determental and potentially catastrophic adverse effects of those chemicals.
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