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GRADUATE COLLEGE

COLIFORM CONCENTRATIONS IN VEGETABLES IRRIGATED WITH SEWAGE

A DISSERTATION

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BY

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Norman, Oklahoma

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COLIFORM CONCENTRATIONS IN VEGETABLES

IRRIGATED WITH SEWAGE

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COLIFORM CONCENTRATIONS IN VEGETABLES IRRIGATED WITH SEWAGE

INTRODUCTION AND PROBLEM DEFINITION

The use of raw, and partially treated sewage for irrigation, has been a common practice for many years. One of the potential hazards which may be associated with the use of sewage effluents for agricultural irrigation, is the possibility of infecting crops such as vegetables which can be a direct human consumption product. There are many different diseases which can easily be transmitted in this fashion, but it is extremely difficult to attempt individual analysis for these pathogenic microorganisms. For this reason, indicator organisms are of great value to the sanitary engineer, because they help to obtain an index of the amount of pollution or contamination in a given sample.

Juarez, Mexico, a border city with an estimated population of well over 600,000 and no sewage treatment facilities, was selected for the present study because it presented a natural laboratory to study different levels of contamination and pollution due to the use of domestic wastes for agricultural irrigation. The sewer lines from the city discharge into an open channel which transports the sewage to farms along the Juarez Valley, where the principal crops include cotton, alfalfa, wheat, sorghum, and some vegetables. Since the vegetables are normally

a direct human consumption product, it was decided to utilize selected areas where vegetables are normally grown to study biological and chemical parameters in water, soil, and plants.

Due to the lay-out of the collection system and irrigation patterns, it was possible to find farming plots where raw, undiluted sewage was the primary source of irrigation water, and also the opposite extreme, where well water was the only source.

Sampling points were selected to yield the following broad categories: (1) Raw sewage, (2) Intermediate sewage, where some oxidation has occurred, and (3), Well water. Irrigation water, soil, and, ultimately, vegetables were collected at different times and locations throughout the duration of the study which covered approximately 24 months.

Tomatoes were chosen as the representative vegetable since they are normally consumed in the uncooked state, and it was easier to locate tomato fields within the prescribed sampling areas. The primary objective of the study was to establish the degree of bacterial contamination that existed in fields where domestic wastes were normally being used as irrigation sources, and to compare this contamination with amounts found in areas where unpolluted well water was the only source for irrigation. The ultimate goal was to define, by means of an indicator organism, the possible health hazards that might be associated with the human consumption of such vegetables.

It was necessary to choose an indicator organism that would reflect at least partially the contamination levels of the water, soil, and vegetables in question, from a public health point of view. Perhaps the

most important microorganism of public health significance in the area was the dysenteric amoeba. After careful considerations, it was decided not to use this particular microorganism due to the difficulties in identification and quantification, since most of the analyses require direct microscopic examinations (1). Coliphages were also considered as possible indicators of enteric viruses, and preliminary studies were conducted to obtain a familiarization with the quantification techniques (2). Again, after an appropriate routine analysis was selected, it was determined that the procedures were too time consuming and laborious to allow sufficient replication of samples in the time available.

It was then decided to focus on the coliform group of bacteria, which has been extensively used as an indicator of fecal pollution in water. Some members of this group are normally found in the soil, but one member in particular, Escherichia coli, stands out as a well known inhabitant of the human intestine (3). Escherichia coli was then selected as an indicator that would reveal the possibilities of finding other pathogenic bacteria or microorganisms of enteric origin in the same given habitat. The membrane filter technique which will be fully described in Chapter III, was selected due to its accuracy and speed. Total plate counts were utilized to better understand the bacterial populations.

Some chemical parameters were monitored in the water and soil to be able to identify and quantify the different areas in question and to detect the changes which occurred as the raw sewage moved downstream and away from the city. The principal parameters studied were: biochemical oxygen demand, chemical oxygen demand, pH, solids, nitrates,

and phosphates. Although the emphasis of the study was on coliform counts, these chemical analyses helped to correlate pollution levels with contamination levels.

The city of Juarez has very little industry, so industrial wastes were relatively insignificant. The averages for one year of BOD and COD along the open channel helped to establish this, since the BOD comprised approximately 80 percent of the COD. Since the raw sewage in the open channel traveled several miles before being used for irrigation, the organic matter was already in the process of being oxidized and biodegraded. This caused a change in BOD with distance which presented the possibility of comparing BOD values with coliform concentrations and perhaps even suggesting minimum treatment levels which should be practiced to improve the quality of the vegetables.

Laboratory models were disigned for the purpose of testing specific procedures. One of these models consisted of a glass column packed with typical soil samples where translocation movements of bacteria were studied. Another such experiment involved the cultivation of tomatoes, under controlled laboratory conditions, which were watered with samples of domestic sewage from Juarez, Mexico. In this case, the main objective was to learn more about bacterial densities and dilution factors. These and other preliminary studies will be outlined in more detail in Chapter III.

All laboratory space and equipment utilized in the study were provided by the Civil Engineering Department of the University of Texas at El Paso, located just across the border from Juarez, Mexico. Public health officials from both countries were contacted in regard to the

research intended, and the proper permits and approvals were secured. In the case of the United States Public Health Service, written permission to bring domestic wastes from Mexico was granted by the office of Communicable Disease Center in Atlanta, Georgia. A similar permit was obtained from the United States Department of Agriculture to bring soil samples from Mexico. Mexican health and water resources officials supplied valuable information and data pertinent to the irrigation systems of the region and also expressed interest in the research since it was the first study of this nature in the area.

LITERATURE SURVEY

Due to the action of state and local health authorities, the practice of using raw sewage for the irrigation of crops has been greatly discontinued or at least discouraged in Mexico. This practice was quite common in some parts of the country, and in some localities like the Valley of Juarez, it is still being done. Tanner (4) and Melick (5) produced evidence to indicate that health hazards were indeed associated with the use of polluted waters for the irrigation of some vegetables. Of course Mexican officials operate on this basic assumption. The extent of the health hazards is not clearly defined in the available literature and by the same token, there are very few guidelines or safety limits a given farmer could observe if he attempted to operate under such conditions.

In the arid parts of Mexico, farmers still make use of this practice to cope with the water shortage problem. This problem will, no doubt, become more acute with the present rate of population increase and industrial development, unless effective water pollution abatement programs are enforced immediately. At the present time, Mexico has initiated programs and studies to define water qualities and to prevent further contamination of already polluted waters. Unfortunately, the lack of sufficient funds for adequate research makes progress slow.

Previous investigations have indicated that most pathogenic bacteria found in practically all domestic wastes will persist for many days in soils irrigated with sewage, as well as upon the outer skins and leaves of vegetation grown under these conditions. The work of Felsenfield (6), and also the research conducted by Tanner (4), have substantiated this. It appears possible that field workers who cultivate and harvest the produce, food handlers, and, ultimately, the consumer, would be subject to infection in connection with the handling and consumption of fruits and vegetables that had been irrigated with polluted waters. The conclusion of Tanner (4), in 1935, was that "Good epidemiological evidence seems to exist in the literature to the effect that fresh vegetables may cause and have caused communicable diseases and that the sanitary condition of the soil from which they are harvested is important."

The problem of aesthetics as a result of the practices noted before is often mentioned in technical papers, but, at least as far as Juarez is concerned, aesthetic considerations are important only inasmuch as they may affect the market for produce.

Penfold (7) studied a different aspect of the problem. He worked with cattle that had access to fields irrigated with polluted water. During his study, he reported two confirmed cases of cattle being infected with Cysticercus bovis in Melbourne, Australia. He actually traced these parasitic worms to the humans who consumed the beef. Hutchins (8) performed similar studies in Arizona, and concluded there was a strong possibility that the mentioned infection of Cysticercus bovis, came from fields irrigated with partially settled sewage, or from the direct drinking of this liquid which the cattle seemed to

prefer over fresh water. These studies also focused on the strong possibility that the udders of dairy cows could have been exposed to bacterial contamination from the irrigation ditches while grazing in these areas. Infection from this source could easily be transferred to the ultimate consumer via unpasteurized milk.

Wilcox (9) worked with the toxicity of certain chemical compounds and the impact of these on various vegetables and cattle exposed to them by means of polluted waters. Since the objective of the current study is aimed at bacterial populations, the toxicology of the different chemical compounds was beyond the scope of the project, but it is important to mention that some of these toxic compounds may influence the concentrations and survival times of bacterial cells.

Hamlin (10) reported some general studies conducted in South Africa, tracing waterborne diseases from vegetables irrigated with polluted waters to humans and cattle. Several reports indicated that a large portion of the native population in South Africa was infested with intestinal parasites, and that the numbers of ova and cysts that reach the sewer systems from this human source was very large and constituted a serious public health problem. Hamlin (10) discussed specific research programs which were established in the city of Johannesburg, South Africa to determine the actual extent of this problem and at the same time determine the most practical and efficient type of treatment that should be employed in solving these problems. The sewage plants in the city were monitored and, after the completion of the study the Chief Medical Officer of the Union Health Department was presented with the following report (10):

(1) "The cysts of Entoamoeba histolytica, eggs of Ascaris lumbricoides,of Taenia saginata, and of ancyclostomata, were frequently present in Johannesburg sewage as it arrived at the works.

(2) Cysts and eggs were found in screened sewage, primary filter, and humus tank effluent, and also in the effluent from activated sludge plants.

(3) In the samples examined, no eggs of cysts were found to have passed secondary sand filters or undrained land filtration areas."

The results of these researchers appear to indicate that as far as South Africa is concerned, the secondary sand filter will be in South Africa a compulsory adjunct to any sewage plant from which the effluents are to be used for irrigation or simply discharged into streams. In the case of Mexico where sewage treatment facilities are quite scarce, the problem is somewhat more acute and the aim at the present time is at minimum treatment rather than adjuncts or secondary units (11).

There is a vast quantity of information in the literature regarding methods and techniques that can be utilized in quantifying contamination levels in water, soil, and vegetables that have been exposed to pathogenic microorganisms. Dunlop et al.(12) worked directly with enteric microorganisms and isolated different species of Salmonella from soils and vegetables. He also found considerable numbers of Endoamoeba coli cysts and Ascaris ova on vegetables using the "washing" technique where the vegetable was actually washed with clean, sterile water and, later, a routine bacteriological analysis was performed on the known quantity of water used for the washing.

The number of coliform organisms on the surface of a vegetable can be considered as the result of an equilibrium between those being

deposited from the soil or from any other source and those being removed or being killed. At any given time the environmental conditions prevailing will determine the magnitude of this equilibrium value. Of course it is important to keep in mind that the coliform group of bacteria serves only as an indicator of the presence of other enteric organisms, and sometimes correlations of actual pathogens to coliforms are variable. Norman (13) studied these correlations in an investigation that covered three growing seasons. He found that the concentrations of coliform organisms were higher in vegetables irrigated with polluted water than in vegetables which had been grown using uncontaminated water. Nevertheless, he was unable to find a direct or indirect relationship between coliforms and actual Salmonella counts. He also used a rinse technique and was able to isolate both coliforms and Salmonella with routine procedures.

Falk (14) discussed some of the different factors which influence the life span of coliforms on the surface of vegetables. He looked at skin conditions, length of stems, height above the irrigation water, and sunlight. He concluded that the population of coliforms irrigated with polluted water on healthy tomatoes was not significantly higher that that of vegetables irrigated with unpolluted water. However, broken skins and stems as well as development of the fruit close to the ground, contributed to a higher population of coliforms and some enteric forms such as Salmonella. He did not try to establish relationships between Salmonella and coliforms.

Sunlight appears to play an important role in the killing of bacteria on vegetables. In the case of Escherichia coli, studies were

conducted by the same investigator by spraying known concentrations of cells on the surface of tomatoes. The average survival time was checked at 30 days. A similar experiment for Salmonella was conducted and this time the result was only seven days. According to this investigator, the condition of a vegetable is apparently important in determining the average coliform concentration on its surface. Regardless of whether the fruit was grown on soil being irrigated during the growing season, on soil which had received previous but not concurrent sewage irrigation, or on soil which had never received sewage irrigation, the tomatoes with normal stem ends showed lower average contamination levels than those with stems characterized by splits, cracks, and crevices developed during growth. These areas act as harboring places for the contaminating bacteria. Since such tomato portions are not particularly appetizing to the human consumer, it is likely that they would be cut away before consumption.

Falk (14) also noted that direct splashing of contaminants on the fruit from the soil by rain was one of the more likely means of contamination of a vegetable of the tomato type, since the fruit itself develops above the ground. Results showed that there was little relationship between the height of the fruit and its source of coliform concentration. If rain splashing was important, the contamination would be expected to decrease rapidly with the height of the fruit above the ground. Average contamination of the fruit in actual contact with the soil was higher than fruit above the soil in some cases, but these were likely to carry visible particles of soil which could account for these higher numbers. Of course from the public health point of view such visible soil would normally be removed before consumption.

Mills, Bartlett, and Kessel (15) discussed the penetration of bacteria and other microorganisms on fruits and vegetables as well as their resistance. Due to the natural foliage cover of irrigated plants, it would be expected that a protective covering between the soil and the plant would produce fruit of considerably lower contamination than when this covering was absent. However, when such conditions of growth were reproduced this was not found to be the case. It was apparent that although splashing was one of the factors responsible for carrying contamination from the ground to the fruit other factors were of combined greater importance. Among those which were mentioned by these authors were the soil carried by the wind and deposited on the plant surfaces, the soil carried to portions of plant by the movements of insects, and the soil raised during cultivation of crops.

As it was previously mentioned, sunlight is considered to have a destructive effect on intestinal bacteria as a result of dessication, heating, and the action of light of certain wavelengths, especially ultraviolet. Any action which increases the death rate of vegetable contaminants while increasing the rate at which the bacteria reach the surface will decrease the average surface coliform concentration. Hence surface contaminants would be less when the tomatoes were exposed to the sun than when shaded. This phenomena was studied by Felsenfield (6) who also demonstrated that normal tomatoes showed consistently lower average coliform concentration than those which were completely or partially shaded. Abnormal or damaged tomatoes, on the other hand, showed no consistent relationship between coliform concentration and shading. It appears that these tomatoes were able to protect the

bacteria in the cracks and crevices and thereby prevent drying, excessive heating, and the lethal action of ultraviolet rays.

The direct spraying of feces suspensions or Escherichia coli suspensions by Felsenfield (6) upon the surfaces of growing tomatoes was taken as comparable to the contamination which vegetables might receive when night soil was applied directly to the plants. On the basis of the criterion that the consumption of such sprayed plants would be no more dangerous bacteriologically than consumption of the controls when both attained the same average coliform concentration, it appeared that these tomatoes would be safe by the 35th day after spraying had ceased. It must be borne in mind, however, that these statements apply only to test conditions used by Felsenfield (6).

Although the abnormal tomatoes did show higher coliform concentration after 35 days than did the normal tomatoes, the counts on the sprayed fruit were not higher than those of the unsprayed fruit. Apparently, the coliform contaminants added during spraying could not survive the conditions of the field with variations in temperature, moisture, and the action of sunlight. Therefore, when coliform bacteria are used as indicators, it appears that tomatoes sprayed with polluted water would be bacteriologically safe for consumption after about one month. This length of time gives a comparatively broad margin of safety as can be seen from the results of the field experimentation on the survival of Salmonella and Shigella (Felsenfield (6)). These organisms were killed after exposure of a week at the most. They were more sensitive to the external environmental conditions and changes than the coliform organisms under similar conditions. The experiments described

were carried out in a temperate climate. In tropical regions, higher humidity conditions may increase the survival of bacterial contaminants on vegetable surfaces. On the other hand, higher tropical temperatures would tend to decrease bacterial survival. However, the relative humidity within the extremely thin layer next to the vegetable surface in which bacteria are attached is probably near saturation. Furthermore, transpiration would tend to keep the temperature from exceeding values detrimental to the plant. For this reason it is quite likely that the microenvironment of the adhering bacteria on the vegetable surface may be fairly independent of the environment of the plant as a whole. Hence, survival in the tropical regions might be very similar to that in temperate or drier regions. Further experimentation and research along similar lines in tropical habitats would be desirable to complete the picture.

Kozyn (16) investigated field grown vegetables near Moscow, and commonly found intestinal bacteria on the plants, but could not show the presence of typhoid bacteria. It was also noted that the bacteria could not penetrate the unbroken surface of the plant. Remlingen and Nouri (17) also found no pathogenic organisms entering the vascular system of plants. Among their findings, they were able to isolate anthrax and tetanus microorganisms, the former rare in sewage, and the latter harmless on ingestion. Both of these microorganisms were found on the surface of plants from sewage irrigation farms.

Lumsden and Anderson (18) felt, on the other hand, that typhoid fever in Washington, D.C., was possibly caused by the use of green vegetables grown in polluted soil. Creel (19) demonstrated that

S. typhosa could be recovered from the leaftips of plants grown on infected soil. Rain did not completely wash off the infecting material. The organisms were able to survive at least 31 days, a period sufficient for the growth and use of such raw vegetable crops as lettuce and radishes.

Malich (20) noted that typhoid organisms could be readily isolated from vegetables grown in polluted soil. Radishes grown in soil fertilized with typhoid stools were found to be infected with typhoid bacteria in three experiments after 28 to 37 days and on lettuce after 21 days. However, it must be observed that he found positive returns on the vegetables only when the typhoid-stool fertilizers were bolstered by agar-grown organisms from these feces.

The increase of typhoid fever in Paris in the summer months had been thought partially due to the consumption of raw vegetables grown on a Paris sewage farm. Anon (21) mentions that thorough washing of vegetables to which typhoid bacilli had been applied failed to completely remove the organisms. The observation of outbreaks of infection of Salmonella pullorum in hospital patients who had no contact with poultry led Felsenfield and Young (22) to investigate the survival of Salmonella on raw vegetables, as these vegetables may become a source of infection if contaminated with chicken droppings. They studied the following species of Salmonella: pullorum, typhimurium, montevideo, and oranienburg. The vegetables studied were beans, beets, carrots, celery, cucumbers, lettuce, onions, green peppers, green peas, radishes, spinach, tomatoes, and turnips.

These investigators found that S. pullorum survived four to eight

weeks on vegetables in a refrigerator (2^{o} to 4^{o} C.) and two to five weeks at room temperature. On onions the survival time was short due to the antibacterial action of this vegetable, which has been known for some time. It must be pointed out, however, that in this particular case, it was not field survival time that was being investigated, but survival time under fairly constant, controlled laboratory conditions.

As it was previously mentioned, Mills, Bartlett, and Kessel (15) confirmed the results of previous investigators to the effect that bacteria could not penetrate the unbroken skin of fruit and vegetables, but could remain viable on the surface 15 days longer under moist conditions. Furthermore, they found that normal uninjured fruits and vegetables contained no living microorganisms in their tissue, but bacteria pathogenic to man were able to enter injured or decayed parts, and remain alive for 7 to 42 days. Dipping the vegetable in chlorinated water or into boiling water for 10 seconds was found effective in freeing the vegetation from pathogenic bacteria, but not from cysts and ova of higher animal parasites.

Suzuki (23) found that solutions having less than 3 parts per million of chlorine killed the germs of typhoid in less than 15 minutes, of dysentery in 30 minutes, and of cholera in less than 5 minutes. On vegetables, however, not all typhoid bacteria were killed in 40 minutes with 200 parts per million of chlorine. The reason for this was believed to be the inability of the chlorine to reach the deeper parts of the leaves and stems. When curled leaves and stems were removed, 20 parts per million was an effective dosage in a contact time of 5 to 15 minutes. It is also possible that the lower killing power of chlorine in the

presence of vegetables was due to the reducing action of the organic material. Suzuki (23) considered that mass disinfection of vegetables in the food markets of Japan would be ineffective. Lehnder and Nowak (24) produced evidence to support the idea that coliforms can reproduce on green plants and thus extend their viability.

In Colorado, where water conservation and irrigation are practiced, Chapman (25) pointed out that a typhoid rate considerably higher than the national average was present. He attributed this to the absence of laws preventing the sale of foods normally eaten raw, which had been grown under irrigation with contaminated water. In California, he pointed out, such irrigation practices exist, but laws prevent the use of such irrigated vegetables in the raw state, thus resulting in a lower typhoid rate.

One important aspect of the reuse of domestic wastes for agricultural irrigation is the economics from the standpoint of crop yields. Warrington (26) concluded that cotton yields could be improved up to 12.5 percent when irrigated with effluents from an oxidation pond. He concluded that the only harmful effect was the increase in nitrogen, phosphorous and potassium in the soil. Steel and Berg (27) also studied the chemical changes in soil which can be associated with irrigating soils with sewage. They concluded that the major problem was chloride accumulation, but an increase in organic matter was measured. Bower (28) studied ground water recharge with sewage effluents as a possible means of utilizing the waste water not only for agricultural irrigation, but also as a source of recharge to ground water aquifers.

Viruses have also been used in the measurements of contamination

levels. Folliguet et al.(29) discussed viral pollution in waste waters. The transmission of virus through the water route has been given ample investigation by Berg (30), and also by McLean et al.(31) and Clark et al.(32) who studied enteric viruses in particular.

Lund et al. (33) discussed some of the problems that might be encountered in virus isolation from sewage samples. The techniques for isolation were quite elaborate and time consuming. Berg and Berman (34) developed a highly sensitive method for detecting small quantities of virus in large volumes of water. Grigorieva et al. (35) were able to isolate enteric viruses from vegetables which had been irrigated with infected water.

Because fo the small size of viruses, all direct observations had to be made with the aid of an electron microscope. Smith (36) discussed some of the advancements in electron microscopy for viruses. Some of the other authors who have investigated viruses from the water resources and public health point of view include: Shtannikov (37), Burns and Sproul (38), Matossian and Garabedian (39), Joyce and Weisner (40), and Conn and Dimmick (41).

EXPERIMENTAL DESIGN

Before the actual field studies were begun, several preliminary studies were conducted with the principal idea of learning more about adsorption and survival in soil and vegetables of bacterial populations. Laboratory models were designed to stimulate actual field conditions as closely as possible.

The first model consisted of a glass column 2 inches in diameter and 40 inches in length. The column was packed with typical soil collected from one of the farming areas. There were 5 openings throughout the length of the tube besides the top opening. These openings were spaced 1, 2, 5, 10 and 40 inches apart to allow collection of samples at different depths (Figure 1). Stoppers were placed on all openings, and the entire column was autoclaved for a period of 45 minutes at a temperature of 121°C and 115 pounds per square inch of pressure.

Three hundred milliliters of sewage were used to rinse the column. At the end of 24 hours, samples were collected from all openings using a flamed loop. The samples were then placed into sterile tap water to prepare different dilutions. Coliform densities were then estimated using the membrane filter technique, which will be fully described under water bacteriological analyses. Incubation periods were set at 24 hours for all samples. After one complete run was finished, the entire column was sterilized again and the experiment was repeated, until 3 sets of

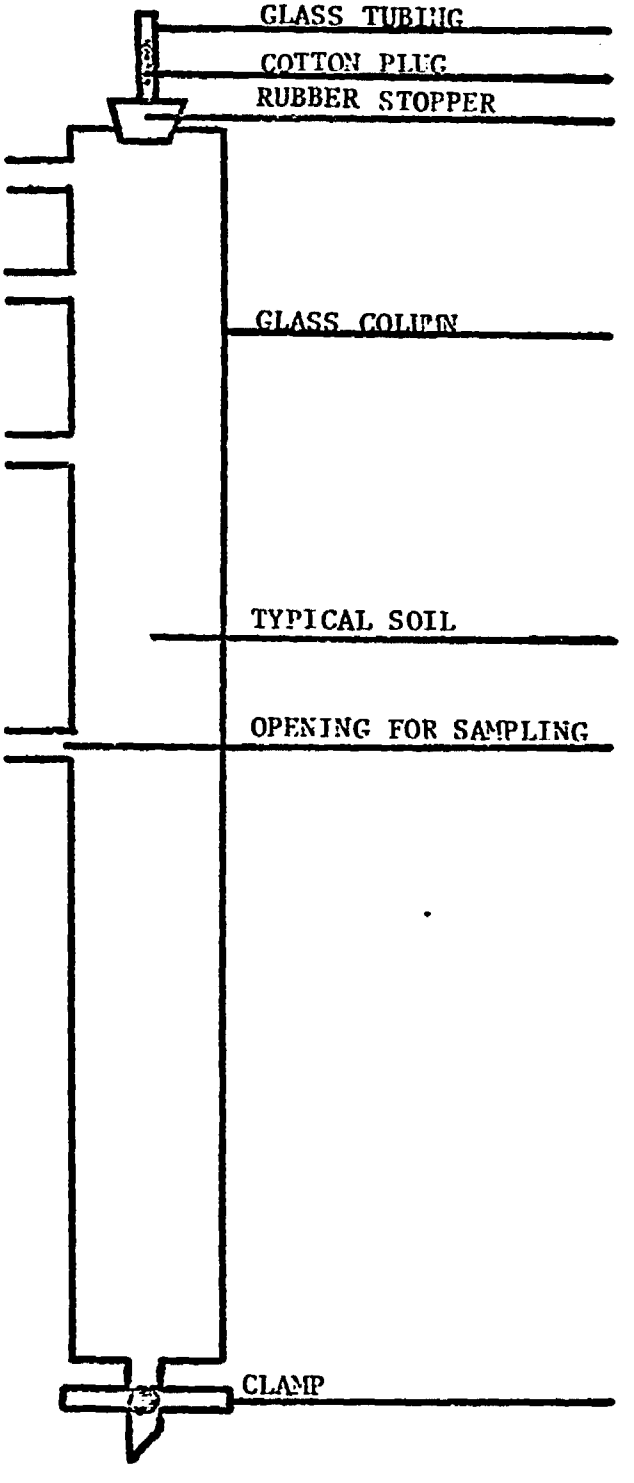


Figure 1. Glass column packed with typical soil.

data were obtained. These results are tabulated in Table I.

The maximum penetration of bacteria was 10 inches below the surface. These experiments which were tailored after the work of Boyd et al. (42), served two purposes: (1) they helped to develop practice and a better understanding of the techniques which would be used later on, and (2) they served to determine the depth at which soil samples should be collected.

A second experiment which was conducted concurrently with the soil column experiment, was to grow tomatoes using a 6 inch bedding of soil collected from the same site as soil used in the packed column. Wooden boxes with approximately 12 square feet of surface area were utilized and once again irrigated with domestic wastes from Juarez. Portions of the plants were collected periodically throughout their development, and, after careful weight determinations, coliform counts were determined by placing the plant pieces in a sterile blender, liquifying them at low speed, and later taking an aliquot from this liquified solution, and diluting for membrane filtration. A diagram of the experimental apparatus is illustrated in Figure 2, and the results are tabulated in Table II.

Again, this preliminary study helped principally in developing and improving the different techniques suggested in the literature and in determining the method which would be used later on the project. The washing technique was also considered, but since previous studies by Mills, Bartlett, and Kessel (15) revealed that there would not be any contamination inside the vascular system of the plant, the blender technique presented more advantages from the practical standpoint.

Since at one time the use of bacteriophages was considered as a

TABLE I

COLIFORM ANALYSES FROM GLASS COLUMN

Depth in inches	Coliforms/gram (dry weight)		
Surface	36,000	117,000	87,000
1	23,000	29,000	12,000
2	1,800	580	4,800
5	0	73	1,200
10	0	0	300
40	0	0	0

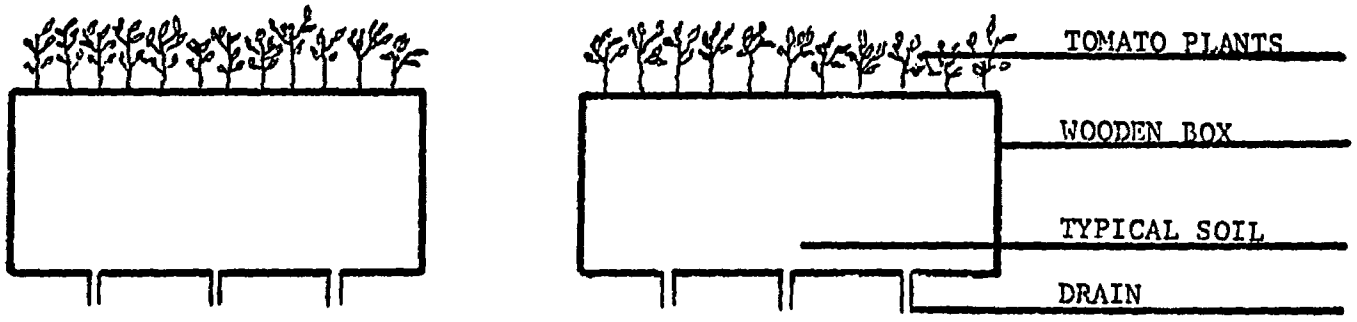


Figure 2. Tomatoes grown in the laboratory.

TABLE II

LABORATORY PLANTS IRRIGATED WITH RAW SEWAGE

PLANT STRUCTURE	NUMBER OF DETERMINATIONS	RANGE (Coliforms/gram)
STEMS	6	920 to 68,000
LEAVES	6	2,500 to 740,000
FRUIT	3	600 to 3,200

different source of indicator organisms, some preliminary studies along this line were also performed. Coliphages in particular, were isolated from sewage samples. This was accomplished by mixing some of the sewage with an enriched solution of tryptone, glucose, and yeast extract, and incubating for 24 hours to allow sufficient virus reproduction. The mixture was then passed through a membrane filter with a pore size of 0.45 microns to separate the bacterial cells from the bacteriophages. The filtrate was then transferred to a lawn of Escherichia coli where the individual phages attacked the bacterial cells. A clear area in the plate indicated the presence of a phage, and the number of clear areas, or plaques, then served as a rough indicator of the titer present in the original sample (Berg (30)).

The process was quite time consuming, and the data that could have been obtained from these analyses were comparable to those of coliforms, i.e., only an indication of pollution, since specific analyses for actual pathogenic viruses were even more involved. Some of the methods investigated by Godbole, Illavia, and Rawal (43), as well as Berg, Berman, Chang, and Clarke (34), and Lund, Hedstrom, and Stannegard (33) were considered, but it was decided that Escherichia coli would be better suited for the study.

Once all the preliminary studies were completed, the field research was actually started by surveying the entire irrigation system and locating sampling points that would represent the different conditions of the domestic wastes. Farming plots where tomatoes were being grown were located adjacent to each of these areas.

Raw sewage samples were periodically drawn from the main collectors

just outside the city limits. More sampling sites were selected between these collectors and the first farming plots. Further downstream, the system becomes an intricate pattern designed to provide as many plots as possible with water. Approximately every 5 miles downstream more sites were located, and ultimately, a small farm high above this irrigation network was selected to obtain the well water samples. At least a fraction of this farm is periodically used for vegetables, so a few samples were available from this site which was considered to be completely free from domestic wastes.

Soil samples were obtained from within the same general areas, i.e., where the different types of water mentioned previously were being used for irrigation. As a final phase of the study, tomatoes from the same location were sampled and analyzed according to the procedures which will be outlined later.

The actual collection of some of the water samples presented no problems or complications, since most of the irrigation ditches and canals are owned and controlled by either state or federal government. The soil and vegetables had to be obtained from private properties, and this was somewhat more complicated. The principal reason for this was that both the state and federal health agencies prohibit the practice of using any kind of waste for the irrigation of crops which have a direct human consumption, as in the case of vegetables. It was evident, then, that most of these individuals were outside the margin of the law, and therefore reluctant to have their products examined for scientific purposes. For this reason, many times it became necessary to obtain some samples, specifically soil, while the owners were not looking, and

the vegetables in the same fashion, or tracing them to the local markets or trucking companies.

Bacteriological Analysis

Water samples were analyzed for total bacteria according to the procedures suggested in Standard Methods for the Examination of Water and Wastewater, 12th Edition (44). Since a total plate count represents only the bacterial population that can reproduce to form colonies at given temperature, oxygen, and nutrient levels, it is obvious that the count will not include 100 percent of the cells present in a given sample (Brock (45)). For this reason a preliminary study of incubation temperatures was performed to select the best growing condition. A series of plates were incubated at 34°C, 35°C, 37°C, and 38°C. A tabulation of the results is presented in Table III. It can be noted from this table that the best yield of colonies was obtained at 35°C. A similar experiment was developed for the coliform organism, which once more proved that 35°C was the best temperature.

Samples were collected in sterile bottles and immediately placed in a chest of crushed ice to prevent multiplication of bacteria in the bottle. Since the sampling sites were relatively close to the laboratory, the elapsed time between sampling and analysis was only a few hours. This eliminated the probability of population alterations due to microbial activity in the samples. Geldreich et al. (46) suggested a delayed incubation period, but this was not necessary for this particualr study due to the relatively prompt analysis.

A series of dilutions was prepared for each sample and plated immediately using strict, aseptic techniques. The media, which consisted

TABLE III

TOTAL PLATE COUNTS USING DIFFRENT INCUBATION TEMPERATURES

(With dilutions of 1:100 and plating volumes of 1 milliliter)

CELLS PER MILLILITER

SAMPLE NUMBER	34°C	35°C	37°C	38°C
1	189	240	210	200
2	176	191	186	190
3	165	172	170	200
4	175	198	175	187
5	167	262	187	175

of tryptone, peptone, glucose, yeast extract and agar, was freshly prepared each time to insure uniform nutrition in all samples. Since coliform counts were the principal scope of this study, it was important to obtain an in-depth picture of the coliform group of bacteria. The literature revealed the abundance and natural occurrence of some members of the coliform group in the soil (47). These bacteria could be easily suspended in irrigation waters once the soil is flooded. With this in mind, the selection of the method for coliform enumeration was a critical aspect of the study. The multiple tube fermentation test was not appropriate since it would not differentiate between members of the coliform group, and it was extremely time consuming and, to a great extent, inaccurate (44).

The use of the membrane filter technique was highly recommended in the literature and proved by far the most adequate technique for this type of study, as recommended by Rawn and Bowerman (48). Forty-five hundreths micron pore size filters were utilized, along with proper funnels, filter holders, incubation dishes and absorbent pads.

Typical coliform colonies were easily observed on the membrane filter, and Escherichia coli colonies in particular assumed the typical green metallic sheen which differentiates them from other members of the coli group. There was one atypical colony type which presented a dark reddish appearance, but after being plated on Eosin Methylene Blue agar, it developed the green sheen. The counts represented then only E. coli.

Soil samples were aseptically collected in plastic, disposable petri dishes. Ten grams (on dry weight basis) from each sample were

aseptically put into 90 milliliters of sterile tap water, to obtain a bacterial suspension (49). From this stock suspension several dilutions were prepared and plated out using the same procedures as for water samples. The 1:100 dilution was utilized for coliform counts using membrane filter techniques. Most of these analyses were performed as suggested by Jensen (49).

Tomato plants were analyzed for coliform bacteria using a combination of membrane filter technique and a low speed blending technique to prepare the dilutions. After investigating several methods and techniques described in the literature, the blender method was selected and developed to yield the most uniform results in repeated samples. Some of the other methods studied included the simple "rinse" method (12) where a vegetable is rinsed with sterile tap water and the water subsequently analyzed for coliforms. This technique presented some difficulties in the reproduction of results. One serious consideration was the surface or skin of the tomato. Crevices and irregularities in the vegetable's surface provided excellent harboring areas for bacterial cells. A simple rinse would not insure an even and consistent removal of these cells. Perhaps atmospheric contamination could have influenced the counts although this would be insignificant at least as far as the coliform group was concerned.

Using a clean, sterile mortar and pestle was another alternative to grind the tomato into small pieces so that the juice could be diluted and analyzed for coliforms. This, once again, introduced serious errors such as non-uniform grinding and uneven distribution of cells within the suspension.

Reproduction of results was difficult and in some cases non-existent. A comparison of results obtained with the mortar vs. the rinse technique are summarized in Table IV, which represents a series of tests performed on tomatoes grown under laboratory conditions. These tomatoes were cultivated and irrigated with raw sewage from the city of Juarez. They were of the same variety as the ones which were being harvested in Juarez, with water allotments similar to actual field conditions.

The use of a blender at low speed produced by far the best results when compared to the rinse and mortar methods. Tomatoes from the same group as those used for previous experiments were analyzed using a blender to liquify the entire fruit. All the equipment that would be in direct contact with the fruit was carefully cleaned and autoclaved. Once the liquification was completed, an aliquot from the contents of the blender container was drawn and diluted in series. One hundred milliliters of the prepared dilution, as well as raw sample were poured through a membrane filter using aseptic techniques and later rolled into a small disposable petri dish which contained an absorbing pad saturated with media (50). In all instances M-Endo Broth for coliform determination was used. The media was rehydrated and prepared daily for more uniform results. These results are also presented in Table IV .

When the actual tomato samples were collected, they were placed immediately in individual sterile paper containers to avoid any secondary contamination. Some of the samples had been handled by humans before, but these were marked to detect any significant variations from the ones picked directly from the field.

The tomatoes handled by humans were normally touched only once

TABLE IV

COLIFORM COUNTS ON TOMATOES

USING DIFFERENT TECHNIQUES

Coliforms/gram of tomato

SAMPLE NUMBER	RINSE	MORTAR	BLENDER
1	300	170	45
2	700	0	95
3	50	0	65
4	0	75	72
5	35	45	85
6	0	0	110
7	0	9	38
8	60	35	95
9	30	42	62
10	0	16	46

prior to the customer handling, that is to say, they were picked from the field and placed in wooden boxes. In these same boxes they were transported via trucks to the local markets.

In the laboratory, the entire tomato was carefully weighed in an analytical balance, and later placed in the sterile blender. A uniform blending time of one minute was used for all samples which proved to be sufficient to completely liquify the entire fruit. It would be expected that some of the bacterial cells would be lysed, ruptured and otherwise destroyed due to the action of the blending blades, but since this method yielded better cell recovery than the rinse or mortar methods, it can be safely assumed that the number of destroyed cells was less than the number of cells that were retained in crevices and irregularities of the fruit's skin (51).

To investigate the effect of rinsing of tomatoes as would be done by a housewife, a series of tomatoes was collected in duplicate. One set was analyzed as it was picked from the field, while the other set was rinsed in 500 milliliters of tap water before analysis. Tomatoes from the raw sewage region had a residual of 25 to 50 cells per gram of tomato, while tomatoes from the intermediate and well water region were negative for the most part, with a few samples ranging from 1 to 5 cells per 100 grams of toma

Chemical Analyses

To obtain a generalized view of chemical characteristics of the irrigation waters, a few selected tests were performed. Most of the analyses were conducted following the procedures and methods outlined in Standard Methods for the Analysis of Water and Wastewater, 12th

edition (44). Perhaps the principal analysis was the 5-day biochemical oxygen demand which was utilized to define the different organic strengths of the sewage along the canal. Samples were collected and promptly prepared the same day using 10 percent, 20 percent, and 100 percent concentrations respectively for raw sewage, intermediate waters, and well water.

Some BOD measurements were also obtained from two wells which constituted the unpolluted type of water. The particular location of these wells insured the absence of contamination from the sewage canal. BOD values in these wells were very low or non-existent. At first, it was believed that the low numbers were a result of an improper seed or the lack of it (52). A bacteriological seed was prepared from raw sewage and 5-day BOD's were again analyzed. It was learned that the seed made little or no difference in these samples. These comparisons are represented in Table V.

Other analyses included pH measurements, alkalinity, hardness, conductivity and some mineral analyses of potassium, calcium, and sodium. These analyses helped in supporting the consistency of the waste and aided in the evaluation of bacterial counts to account for possible variations from the established patterns of microbial activity.

pH was determined by means of a Sargent Model LS pH meter which was periodically recalibrated with the proper buffer solutions. Both alkalinity and hardness determinations were obtained by using the procedures and recommendations of Standard Methods for the Examination of Water and Wastewater (44). A Wheatstone conductivity bridge was utilized in making conductivity determinations as well as total dissolved solids approximations.

TABLE V

VALUES OF 5-DAY BOD FROM WELL WATER

SAMPLES USING SEED AND USING NO SEED

BOD ppm

Sample No.	Seed	No Seed
1	18.0	17.0
2	9.0	9.0
3	7.0	7.5
4	11.0	11.0
5	6.0	5.7
6	0.0	0.0
7	4.0	5.1
8	6.4	6.1
9	5.0	5.0
10	6.4	6.2

Using a La-Motte soils kit, simple colorimetric determinations of a few soil samples were obtained. pH, nitrogen, phosphorous, percent organic matter, and calcium were monitored in a few selected areas which included all three categories of pollution levels. The purpose was simply to distinguish some of the chemical characteristics of the different soils where the vegetables were grown and later sampled. There was very little difference in the overall constitution and chemical composition of these soils at least from the standpoint of the analyses performed (52). Perhaps the only variation was the organic matter content of the soils adjacent to the raw sewage canals which appeared to be somewhat higher than the rest.

Correlation of Coliforms to Pathogens

A correlation of coliforms to actual pathogens was attempted as a final phase of the study to learn the existing ratio of coliforms to pathogens. Of course it was quite difficult to test all the possible water borne pathogens so Salmonella sp. was selected as the representative pathogen.

To insure good results it was decided to obtain a new set of coliform counts data along with the Salmonella counts so that both coliform and Salmonella samples would be collected at the same time and under the same conditions. The samples were drawn from the open channel where the Salmonella counts would be expected to be higher.

The membrane filter technique was utilized for the Salmonella analyses using some modifications in the media and incubation procedures. After the routine filtration was completed, the filters were placed in disposable

petri dishes containing pads saturated with Bacto m-Tetrathionate broth base and incubated for 3 hours at 35°C. The filters were then transferred to pads saturated with Bacto m-Brilliant Green Bile broth and incubated for 16 to 24 hours at the same temperature. A final transfer was made to pads containing Bacto m-Urease Test Reagent where diffusion of the reagent occurred around the colonies in 15 to 20 minutes (50).

As suggested by the literature, purple colonies were urease positive, lactose negative, while yellow colonies were urease negative, lactose positive. Red colonies were urease negative, lactose negative and likely to have been Salmonella spp.

EXPERIMENTAL RESULTS

Bacteriological Analyses

The first samples were collected from the raw sewage effluents from the main collectors and the canal. As was previously mentioned, there was some biological activity and degradation of organic matter as the waste flowed downstream and into the farming areas. To represent average coliform concentration along the channel, a graph was prepared showing coliform vs. distance (Figure 3). Figure 4 represents average total bacterial counts along the same channel.

There were several sampling sites which were considered to contain raw sewage. However, some of these canals were sometimes dry due to irrigation activities, which prevented the collection of continuous samples during the study. For this reason, only four sites in the raw sewage region were sampled consistently.

Coliforms, as well as total bacteria, were monitored and the fluctuations in bacterial counts for one of these raw sewage sites can be observed in Figures 5 and 6.

There were some drastic differences in the population numbers, but these were possibly normal, sporadic and localized bacterial population due to excess nutrients and more favorable growing conditions.

The intermediate downstream sites were spaced approximately 5 miles apart, and there were enough of them to cover the 40 mile strip of canal

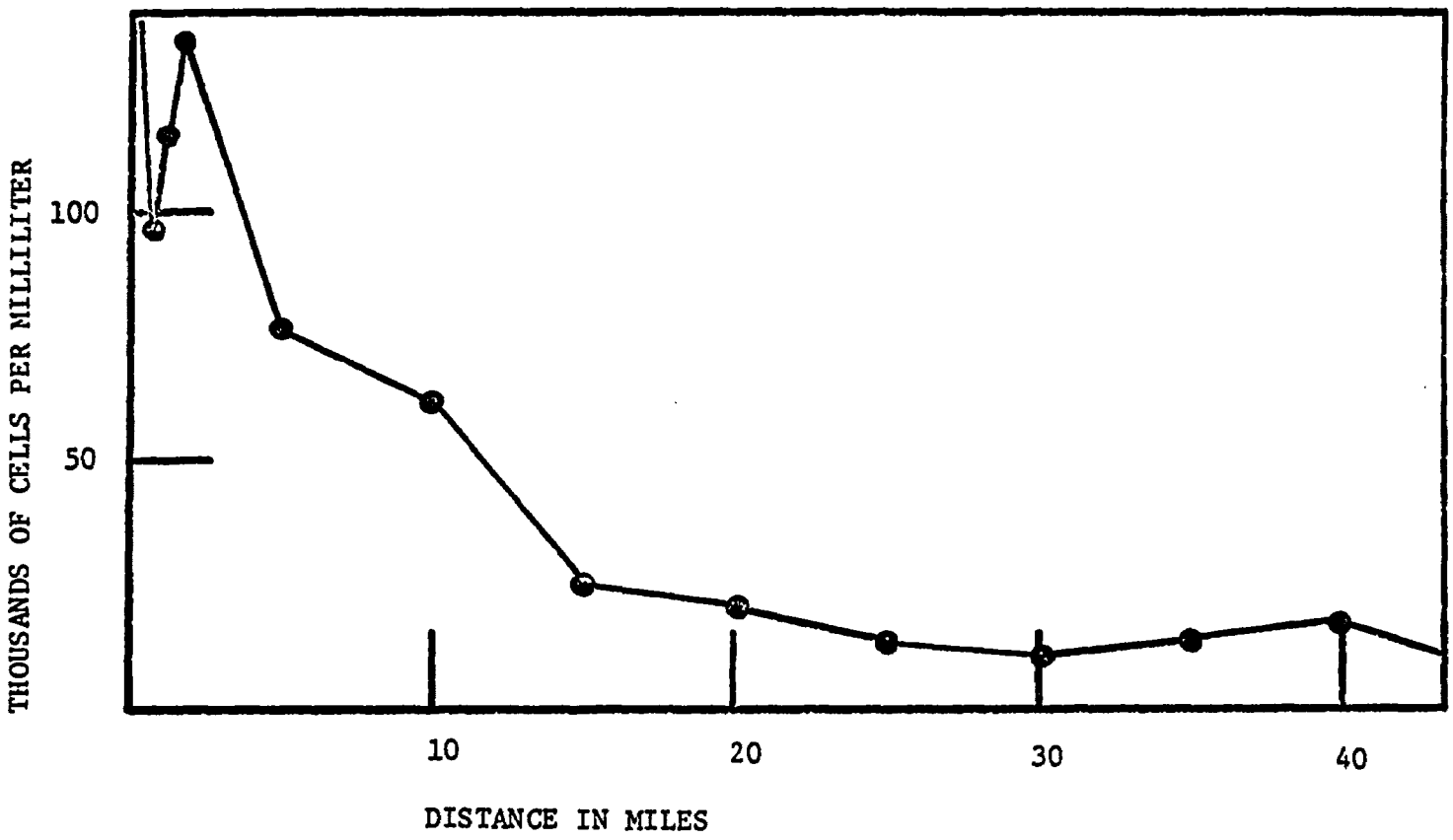


Figure 3. Average Coliform Concentrations Along the Canal.

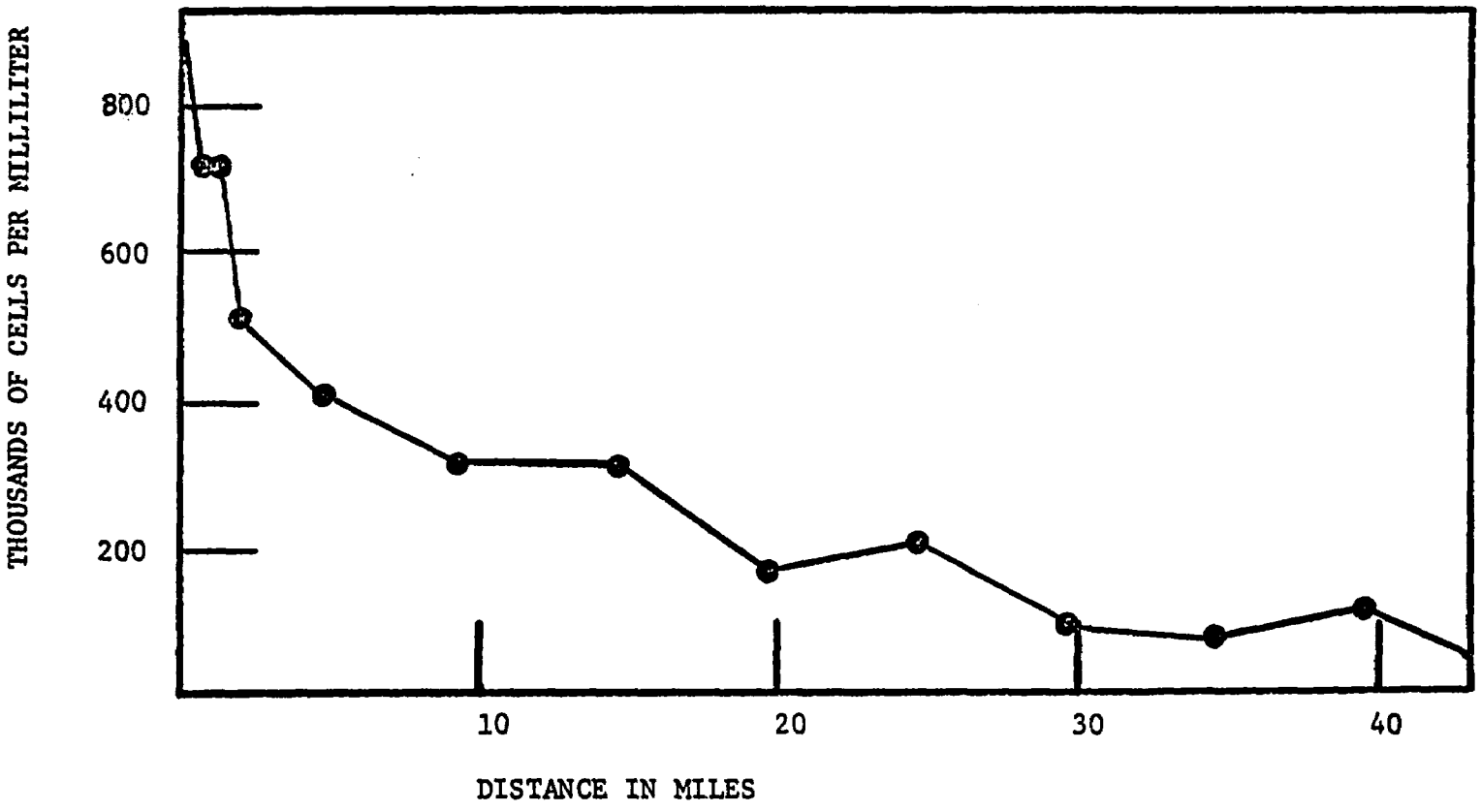


Figure 4. Average Total Bacterial Counts Along the Canal

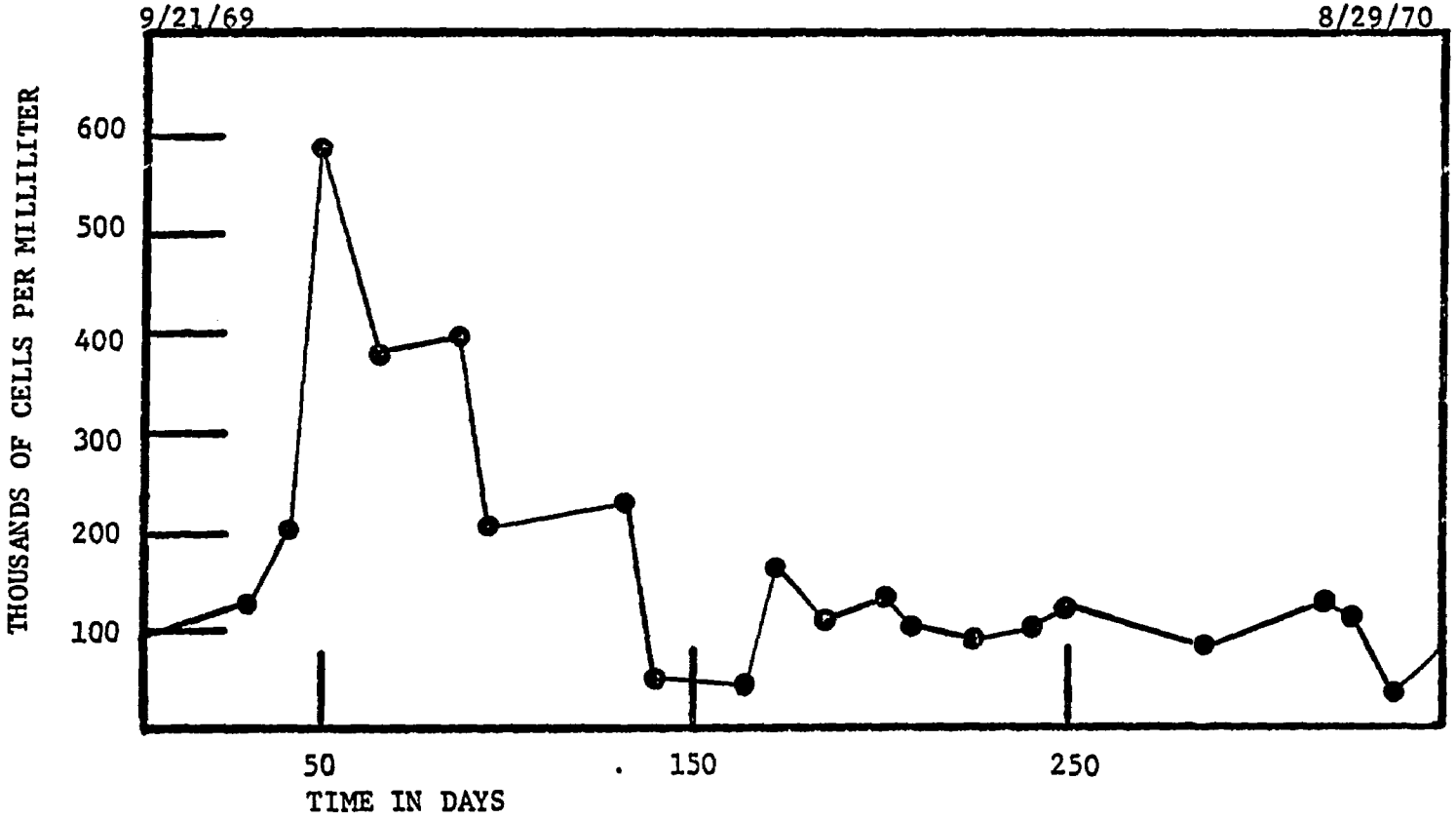


Figure 5. Yearly Variations of Coliform Counts in the Raw Sewage Region.

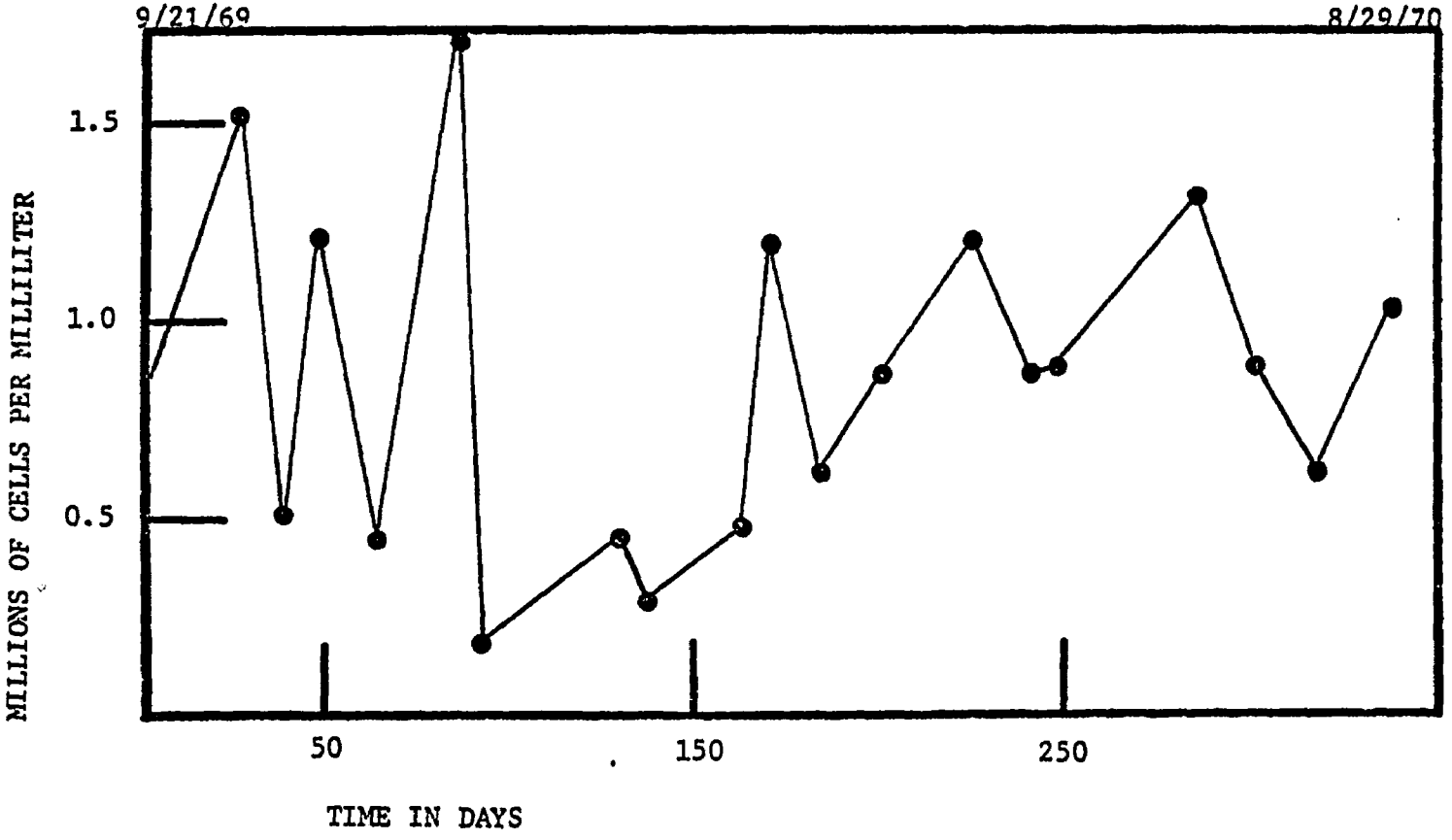


Figure 6. Yearly Variations of Total Bacterial Counts in the Raw Sewage Region.

under investigation. At these sites, most of the heavy solids had settled and the BOD was somewhat reduced.

Individual site concentrations of coliforms as well as concentrations of coliforms vs distance graphs were prepared. These graphs express the changes in bacterial numbers, both coliform and total, which occurred in selected sections of the canal. Figures 7 and 8 represent the local variation on the canal, which is typical of the partially oxidized waste. Figures 9 and 10 represent the yearly variations corresponding to the most remote sampling location.

Irrigation was sometimes carried further downstream, but since no vegetables were located being grown close to this area, it was not pertinent to obtain water samples from this section of the canal.

Well water was sampled from a small agricultural farm located about 45 miles east of Juarez, Mexico. Since this farm was located at a much higher elevation, there was no practical way to utilize domestic wastes in this area, consequently, both the water and the vegetables were considered to be free from sewage contamination. Figures 11 and 12 show the average bacterial concentrations that were present in the well water once it was discharged into the irrigation canal.

Soil samples were collected from plots adjacent to some of the water sampling sites. The bacterial concentrations that existed in these localities were investigated by analyzing both coliforms and total bacteria.

Again, variations which occurred in the raw sewage region were plotted against time as seen in Figures 13 and 14 . These yearly variations were also plotted for the opposite extreme, i.e., the well water

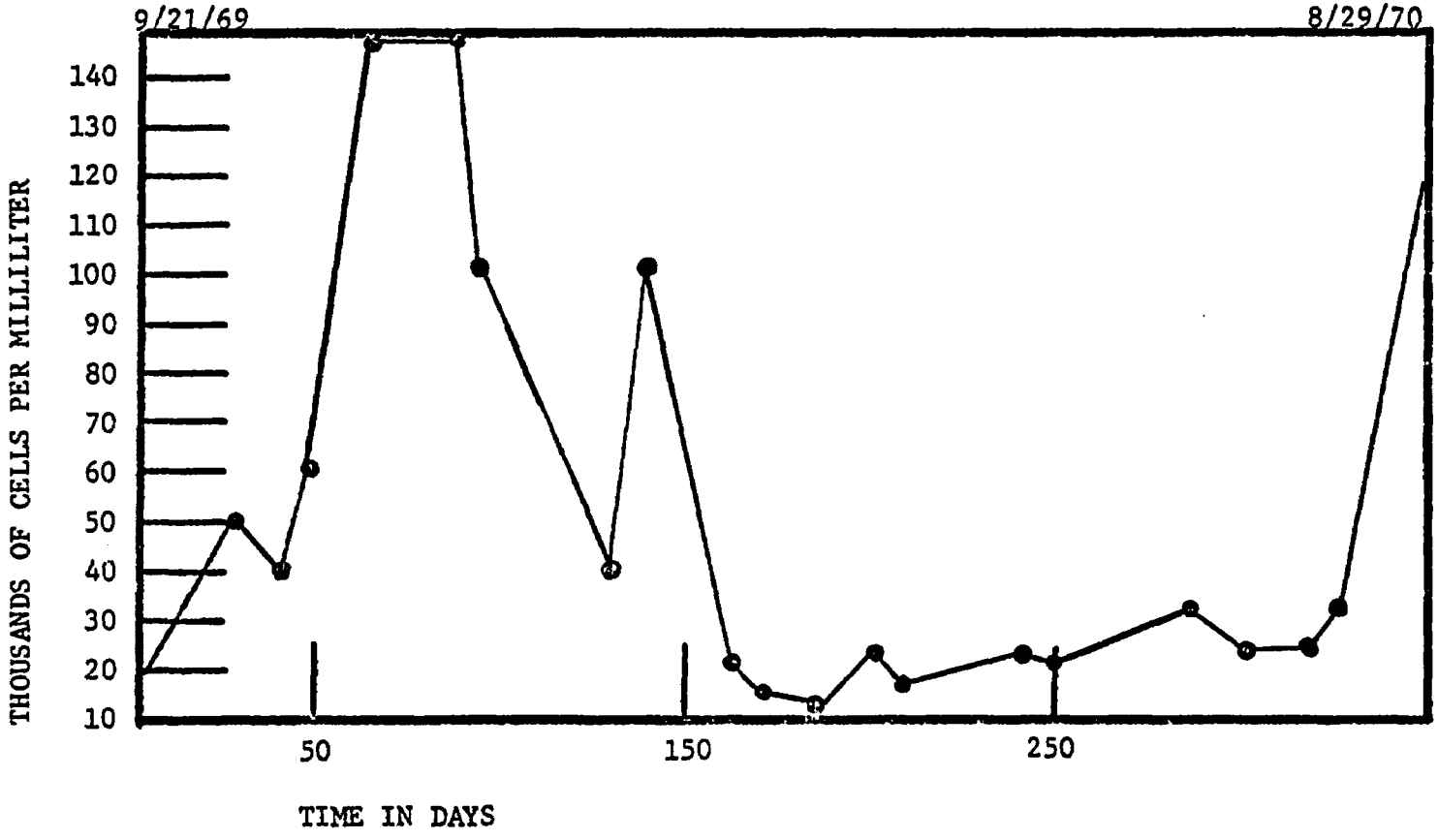


Figure 7. Yearly Variations of Coliform Counts in the Intermediate Section.

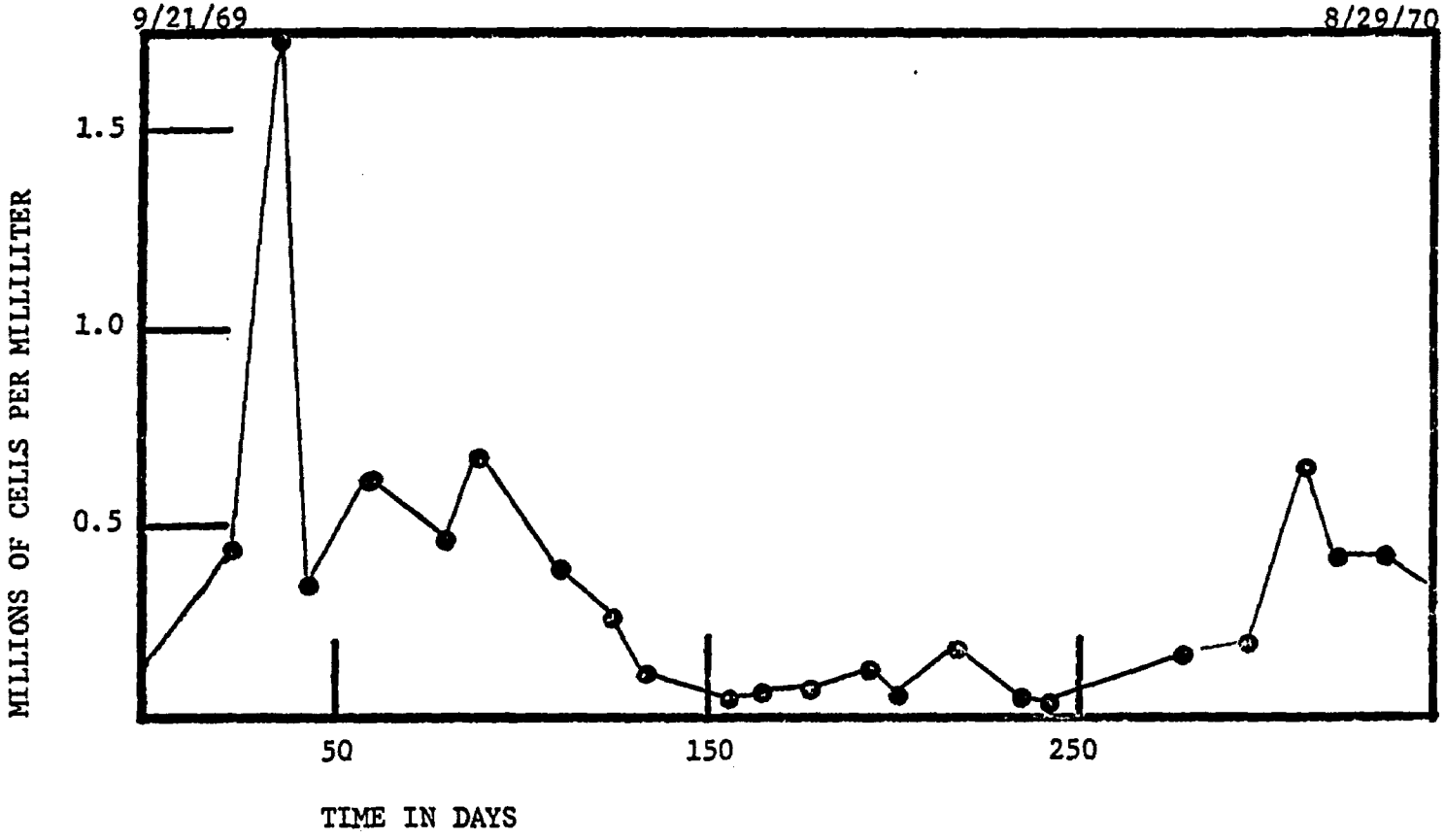


Figure 8 . Yearly Variations of Total Bacterial Counts in the Intermediate Section.

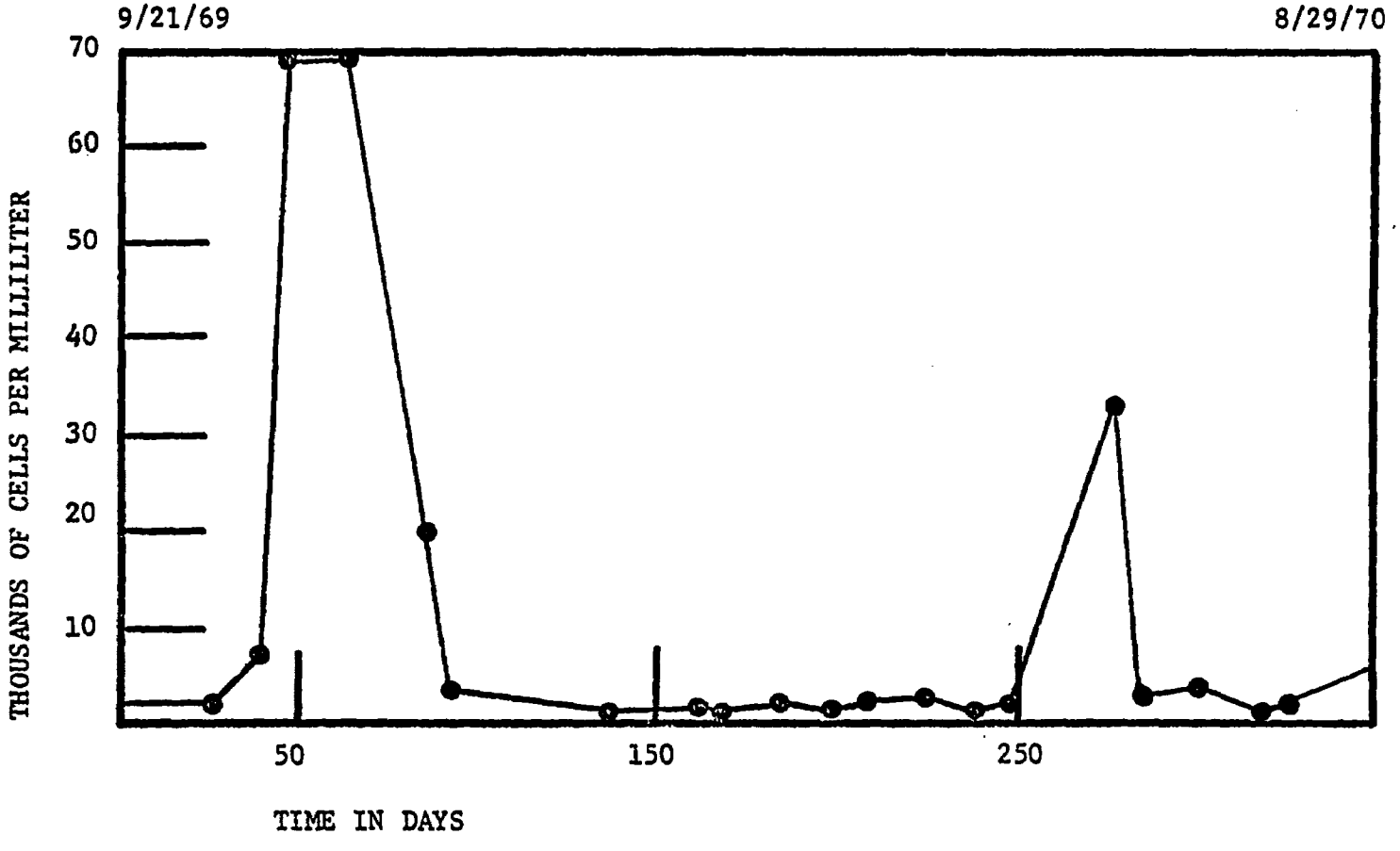


Figure 9. Yearly Variations of Coliform Counts in the Most Oxidized Section.

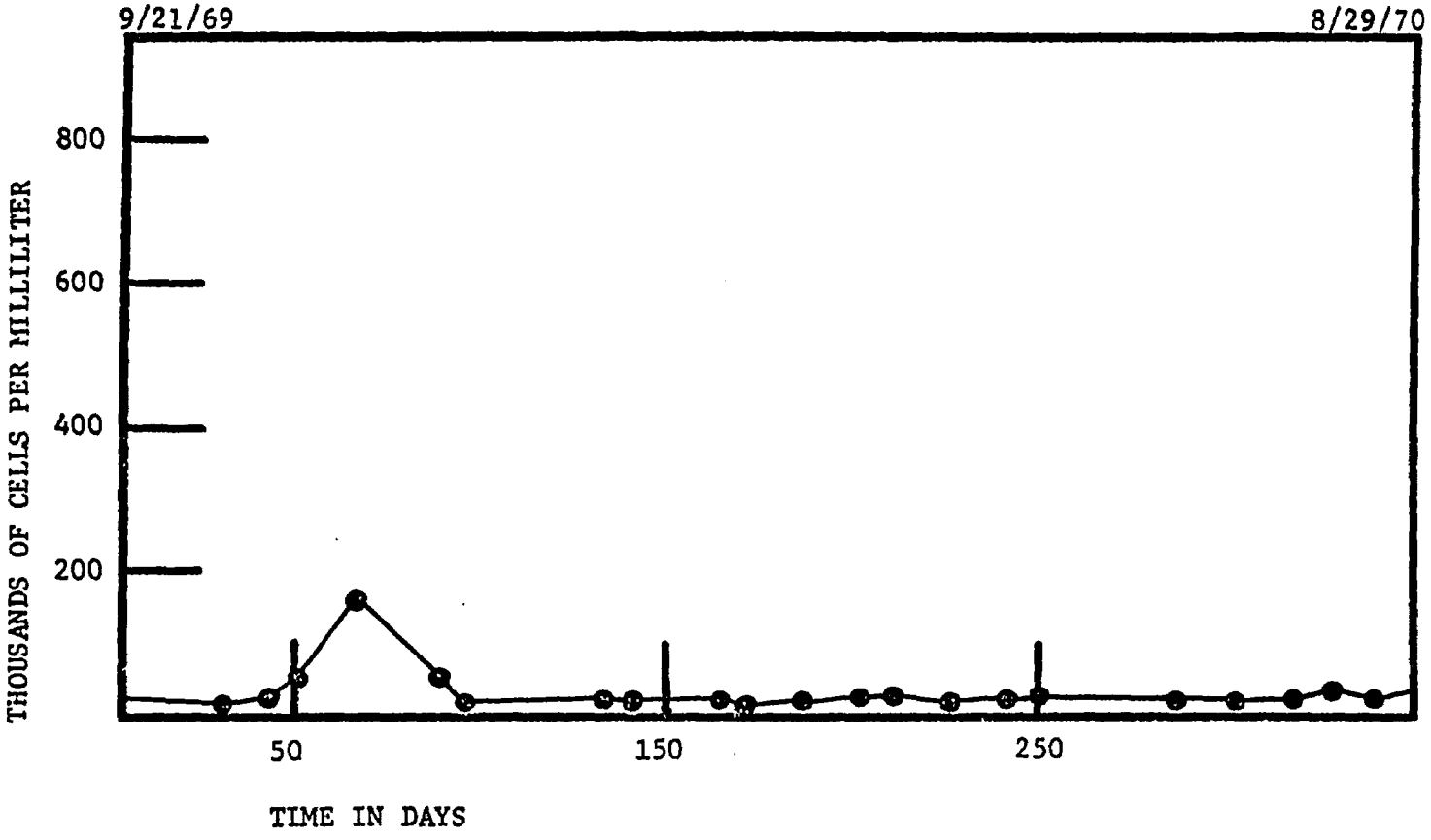


Figure 10. Yearly Variations of Total Bacterial Counts in the Most Oxidized Section.

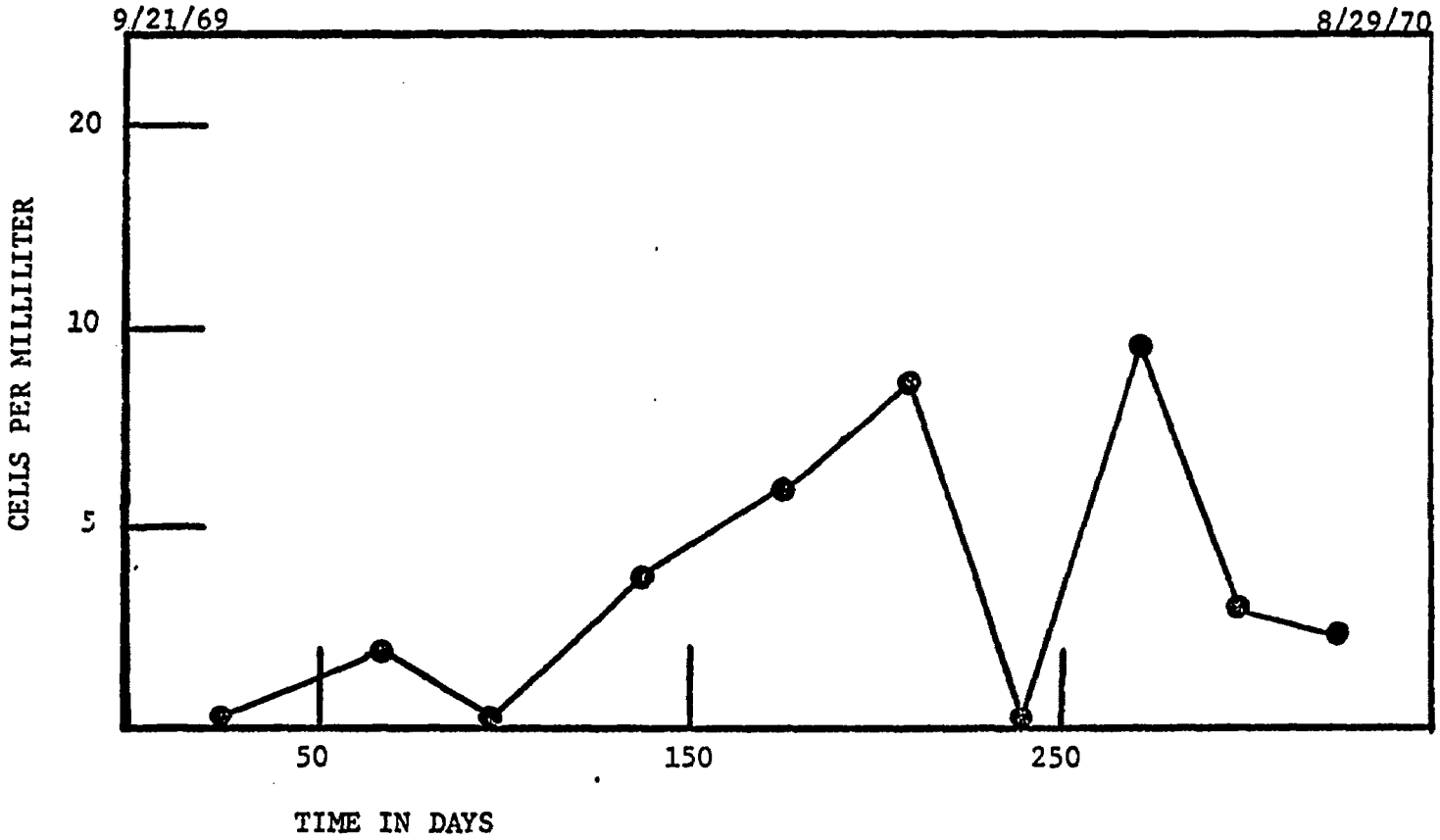


Figure 11. Yearly Variations of Coliform Counts in Well Water After it Was Discharged into the Irrigation Canal.

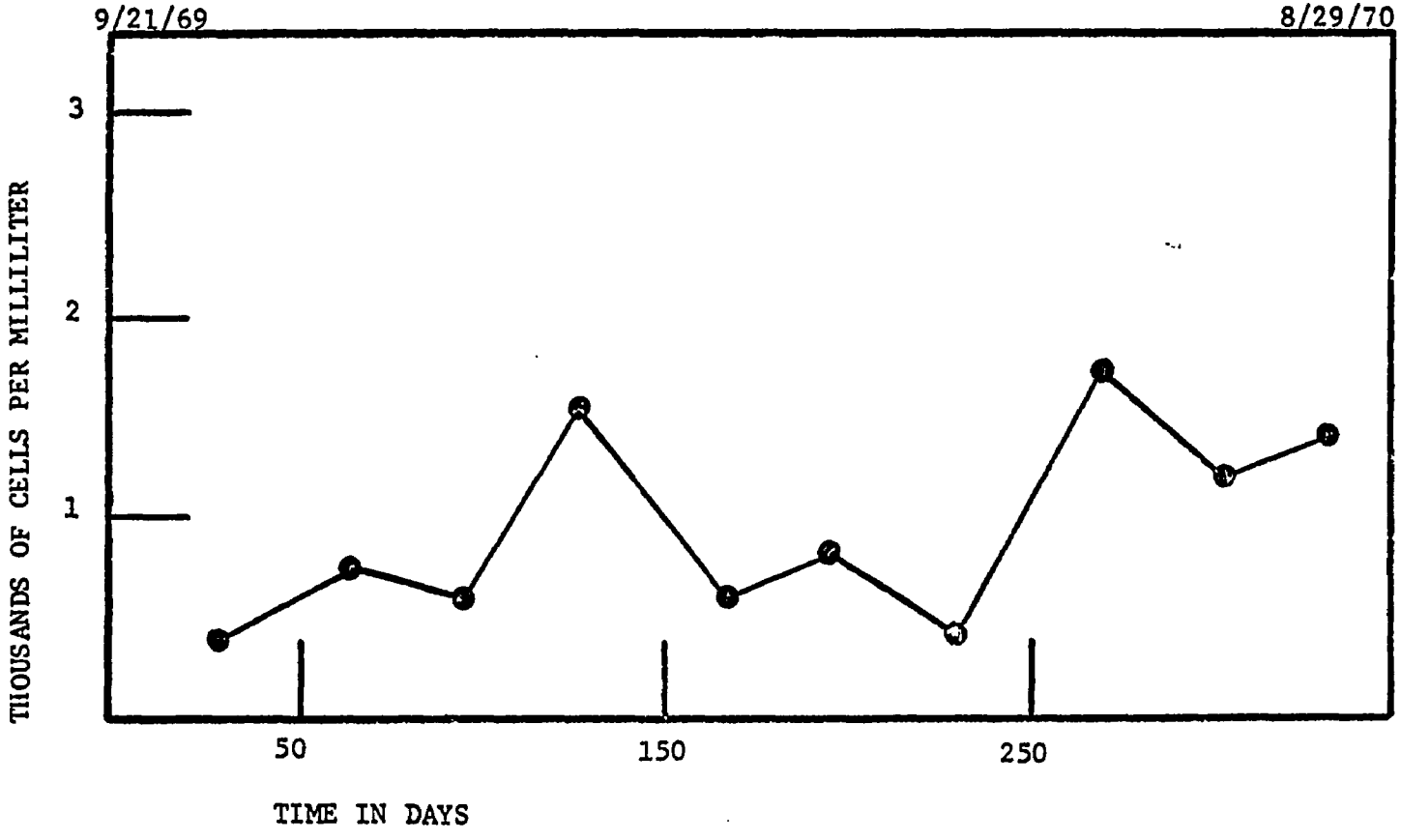


Figure 12. Yearly Variations of Total Bacterial Counts in Well Water.

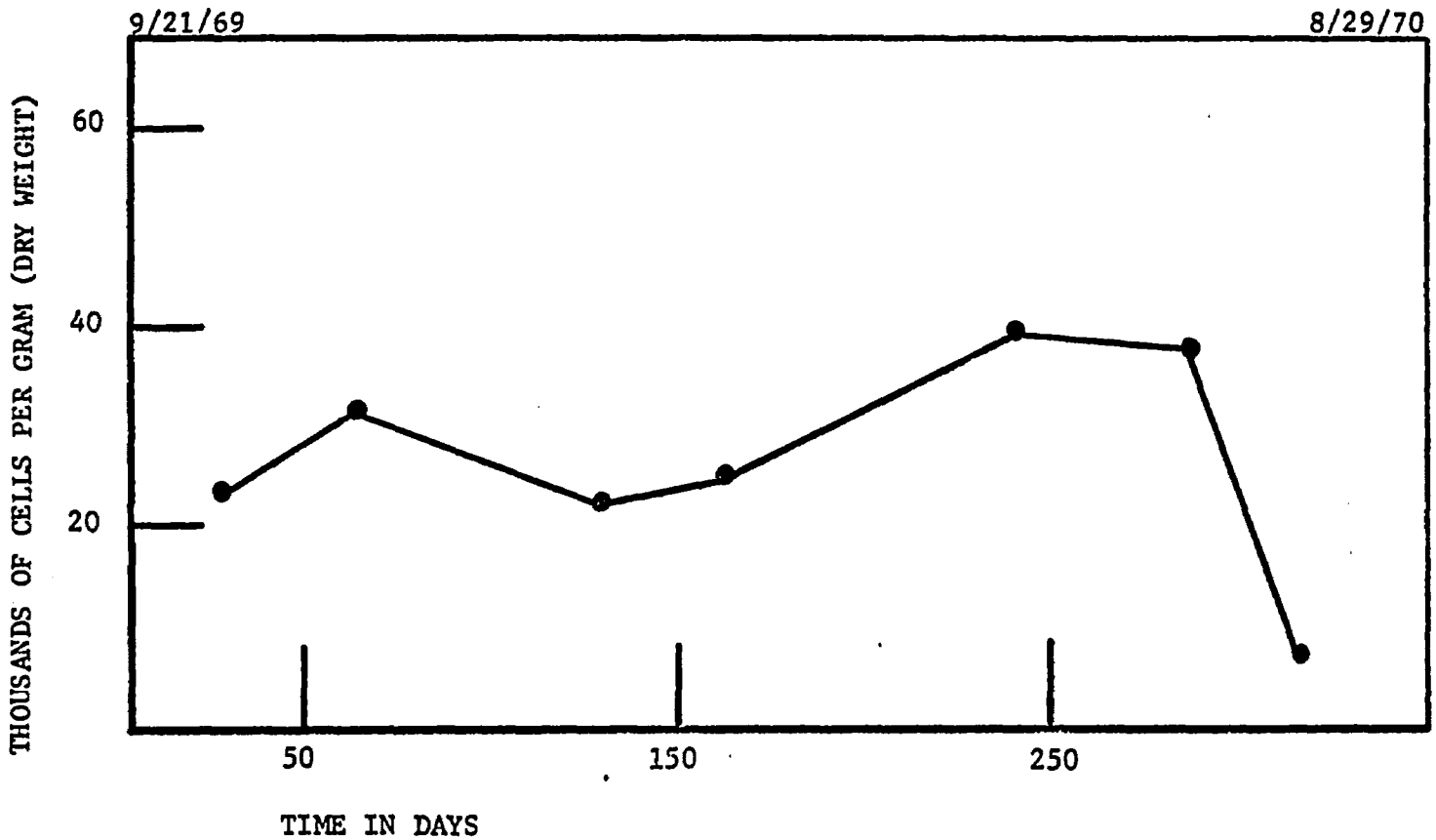


Figure 13. Yearly Variations of Soil Coliforms in the Raw Sewage Region.

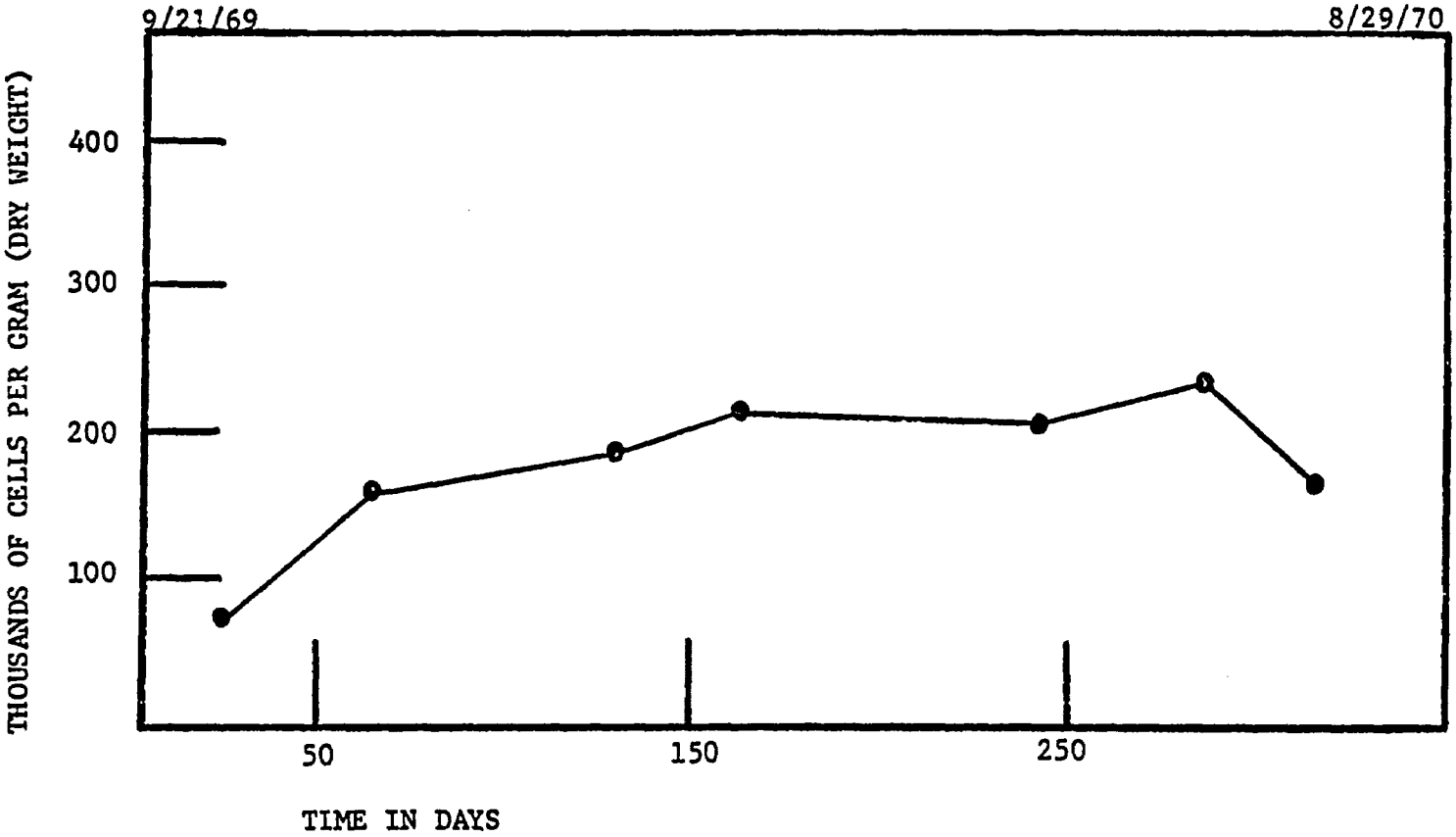


Figure 14. Yearly Variations of Total Bacteria in Soil Samples from the Raw Sewage Region.

irrigated site, as presented in Figures 15 and 16 . The average for one year was calculated for each site, and plotted against distance, as can be observed in Figure 17 , which represents coliform counts. It should be noted that all soil samples were collected after irrigation periods. Usually the sample was obtained 24 to 48 hours after irrigation had ceased. For this reason, the total number of soil samples was less than the number of water samples.

No anaerobic counts were attempted, since all the soil samples were collected from the surface.

Tomatoes were collected from fields where well water was the only source of irrigation water and the soils had never been exposed to any type of waste. The opposite extreme, in terms of pollution, was located in the outskirts of the city where some plots were regularly being irrigated with raw sewage and the soils had previous exposure to the contamination. As was discussed in the literature review, there is evidence to support the idea that most of the actual plant contamination was in the form of air-borne dust particles containing the microorganisms (14). For this reason it was quite important to know the history of the soil being used for the crops.

The irrigation system being practiced in the area was furrow type irrigation with approximately 60-centimeter beds and plants located only in one side of the bed. The average field received approximately nine complete irrigation allotments throughout the season. Since water was scarce and expensive for the average farmer, drainage systems were non-existent in the area. They used a simple saturation method which, on the average delivered about 12,500 cubic meters per hectare.

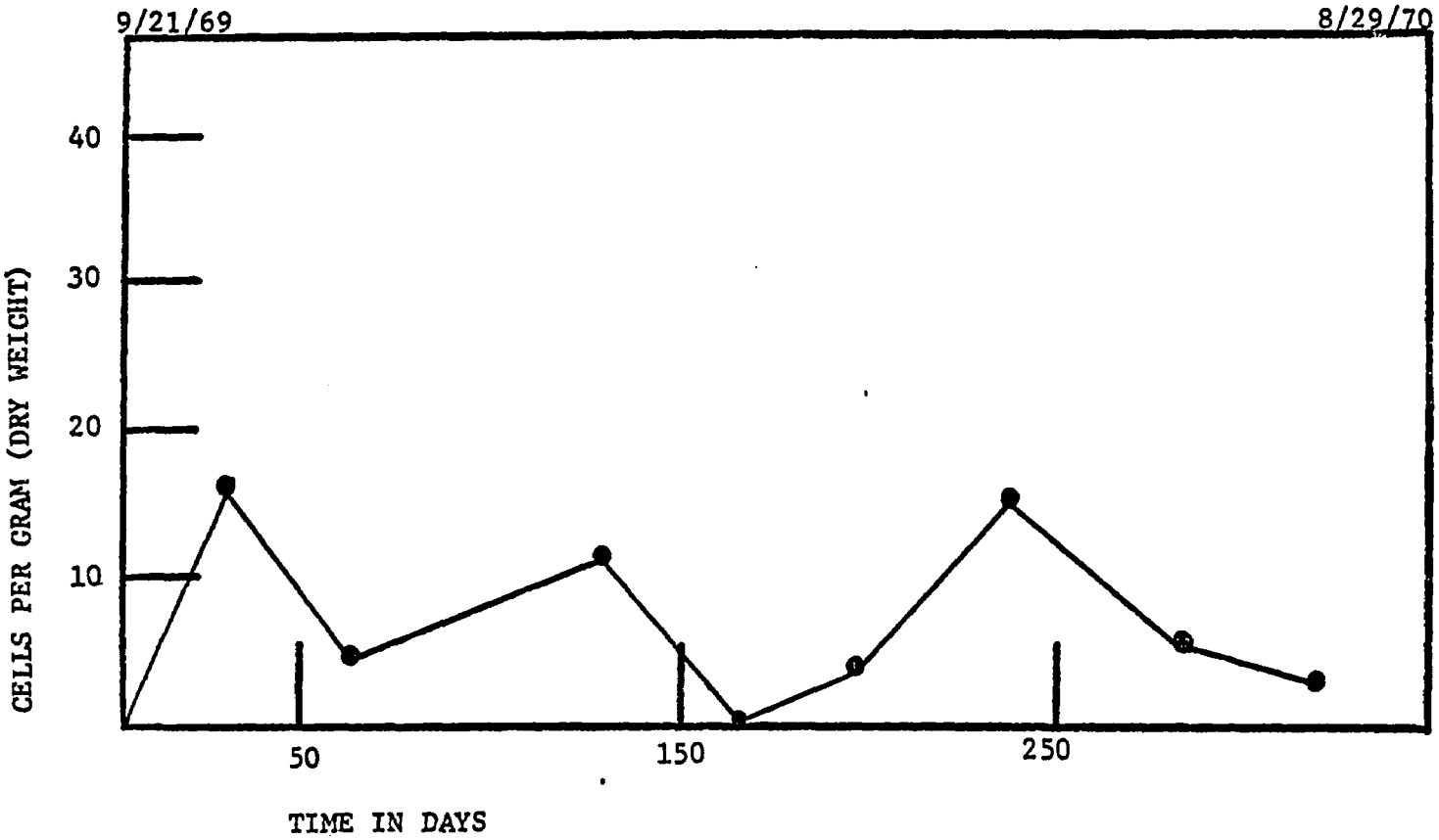


Figure 15. Yearly Variations of Soil Coliforms in the Well Water Region.

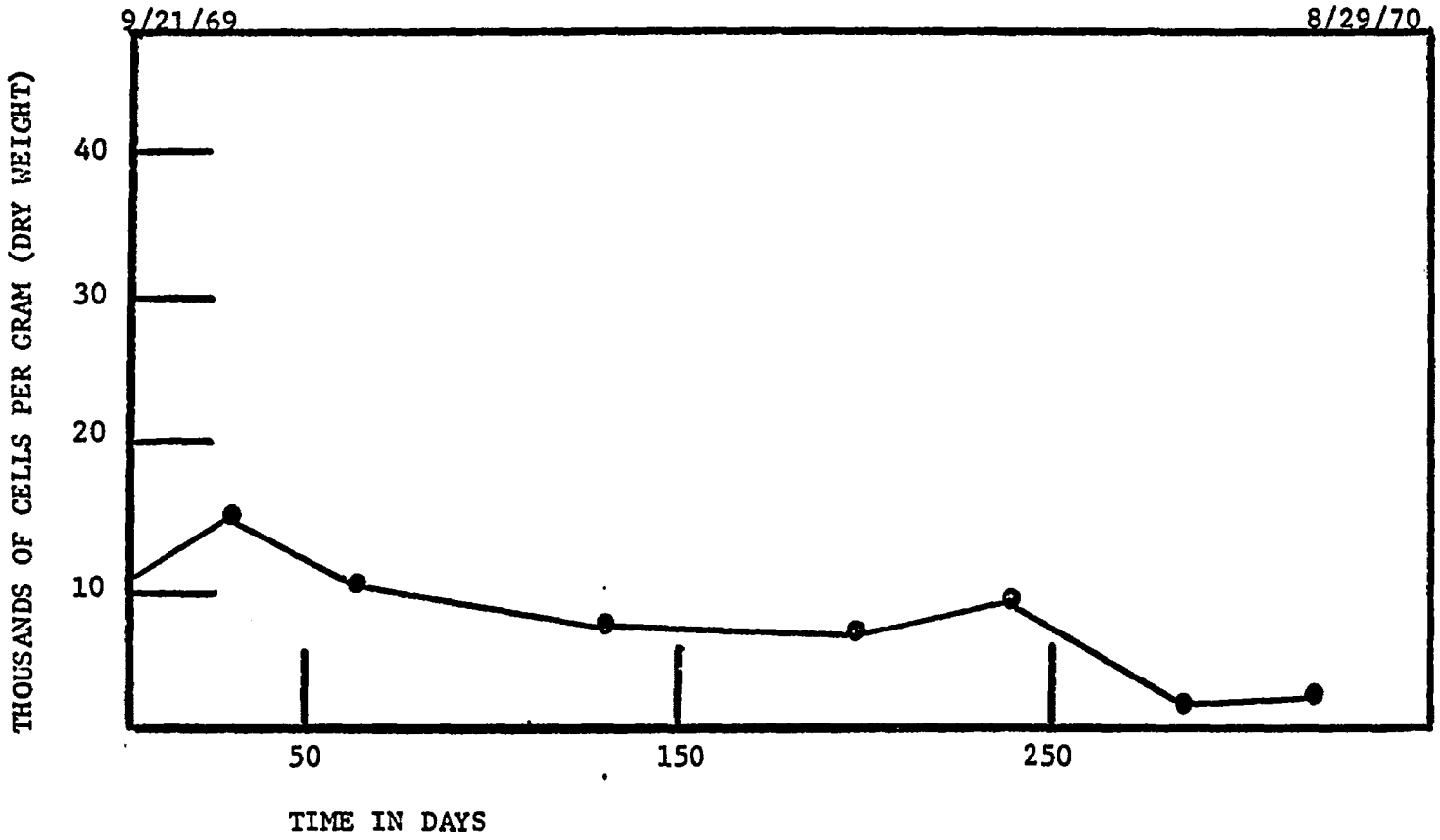


Figure 16. Yearly Variations of Total Bacteria in Soil Samples from the Well Water Region.

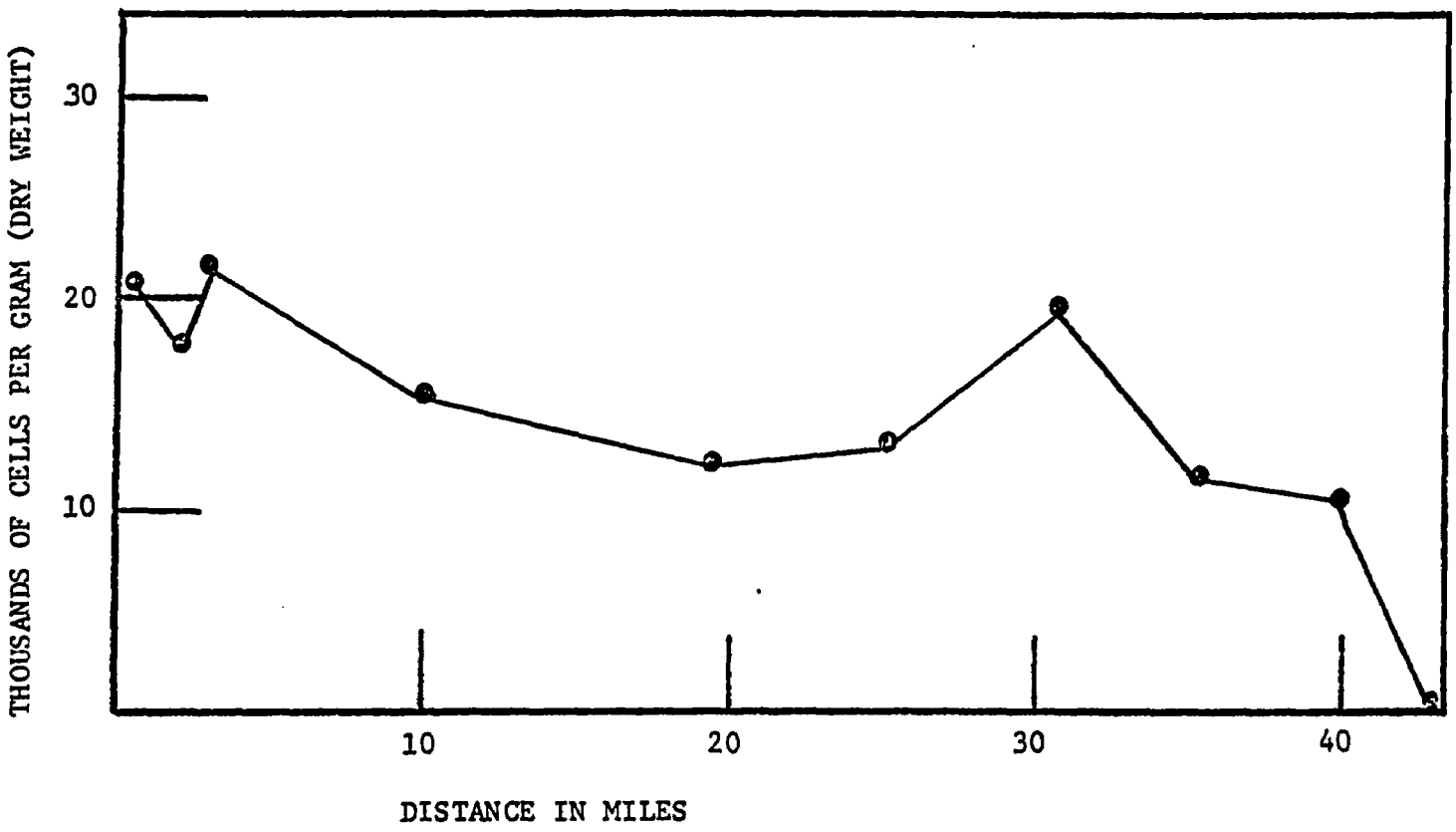


Figure 17. Average Soil Coliforms Downstream from the City.

Other plots were located further downstream where the raw sewage had undergone more biological activity. Some of these plots were often supplemented with well water. Consequently, this particular region was not as constant in composition as the two extremes previously mentioned. However, it served the purpose of presenting an intermediate level of contamination. The samples collected represent one complete growing season.

As was mentioned before, full cooperation from the individual farmers was difficult to obtain, and some of the samples had to be procured from trucking companies or directly from the retailer at the local markets. From personal observations, the average tomato was touched only once or twice by a human hand. To learn the possible alterations this factor might have introduced, a few random samples were collected from households where tomatoes had been handled freely by housewives prior to the normal rinsing or cleaning procedures usually practiced on such products. The average bacterial content of these tomatoes was not appreciably higher than the field collected ones as can be seen in Table VI.

The results from all the vegetable analyses are presented in Tables VII, VIII, and IX . Table VII shows raw data obtained from tomatoes grown mainly with raw sewage. Cell numbers include a wide range of values, but while figures are far from being constant they do show a tendency towards a normal concentration.

Tables VIII and IX were prepared for the intermediate contamination areas and for the well water samples. In all cases extreme variations were present, but this was typical at least from the

TABLE VI

COLIFORM COUNTS ON TOMATOES FROM THE FIELD

AND FROM AN AVERAGE HOME

Coliforms/gram of tomato

FIELD TOMATOES (Intermediate Sewage)	HOME TOMATOES
14	122
0	0
135	0
0	215
0	62
1450	116
320	500
140	600
--	189
139	800
230	269
Average= 242.8	261.2

TABLE VII

COLIFORM COUNTS ON TOMATOES IRRIGATED WITH RAW SEWAGE

Coliforms/gram of tomato

Sample No.	Collection site			
	2.0 miles from city limits	1.5 miles from city limits	0.5 miles from city limits	City limits
1	150	300	250	22
2	14	128	35	135
3	32	1,700	15,000	1,500
4	150	16,000	37,000	14,000
5	31,500	1,400	1,500	37,000
6	149	179	35	149
7	700	242	150	169
8	8,100	34	890	249
9	89	154	740	108
10	154	3	1,430	2,400
11	---	---	200	34
12	---	---	148	---
Average=	4,103	2,014	4,781	5,074

TABLE VIII

COLIFORM COUNTS ON TOMATOES IRRIGATED WITH INTERMEDIATE SEWAGE

Coliforms/gram of tomato

Sample No.	Collection Site			
	10 miles from city limits	15 miles from city limits	20 miles from city limits	25 miles from city limits
1	38	14	450	134
2	7,400	0	3,240	1,500
3	800	135	1,480	42
4	649	0	14	1
5	769	0	169	0
6	320	1,450	235	305
7	1,100	320	640	149
8	349	15,000	740	0
9	211	140	1,800	1,800
10	14	139	649	148
11	9	230	1,640	---
12	1,200	---	13	---
Average=	1,072.4	242.8	922.5	40.7

TABLE IX

COLIFORM COUNTS ON TOMATOES IRRIGATED WITH WELL WATER

Coliforms/gram of tomato

Sample No.	Collection Site (Yards away from well)			
	200	200	100	100
1	279	14	0	20
2	52	125	25	39
3	0	0	0	152
4	3	12	0	13
5	11	0	35	141
6	12	25	14	10
7	145	0	16	17
8	526	35	167	8
9	210	1,500	0	0
10	12	200	135	0
11	11	15	—	—
12	14	7	—	—
Average=	106.4	161.0	39.2	33.2

standpoint of previous experiments of the same nature (13). Since there were many factors which influenced the deposition of bacterial cells onto the surface of tomatoes, it would be extremely difficult to pinpoint the actual reasons for these variations. Tho factors include foliage, thickness of the plant, height of plant, type of soil, average wind velocity and direction, crevices and irregularities of the plant, and fruit, and other climatological variables.

Chemical Analyses

The average BOD figures for the complete canal are presented in Figure 18 . As in the case of the water and soil samples, the yearly variations for the raw, intermediate and most oxidized sewage are graphically represented in Figures 19 , 20 and 21. In the case of BOD, no important fluctuations occurred, and therefore, it can be safely assumed that the organic components of the waste were uniform throughout the year.

As the BOD vs. distance graph indicates, (Figure 18), BOD values were consistently decreasing, as were coliform counts. Average coliform counts and average BOD values correspond very closely in most sections of the graphs (Figures 3 and 18). Near the end of the canal, or at least near the end of the sampled section, total bacterial counts decreased somewhat faster than BOD values (Figures 4 and 18). Perhaps one of the factors which influenced this change was time. Perhaps the low velocity of the sewage in the canal provided enough retention time to reduce the number of coliforms. As the water became less turbid due to increased sedimentation of solids, there was also a deeper penetration of sunlight and thus more cells were probably eliminated.

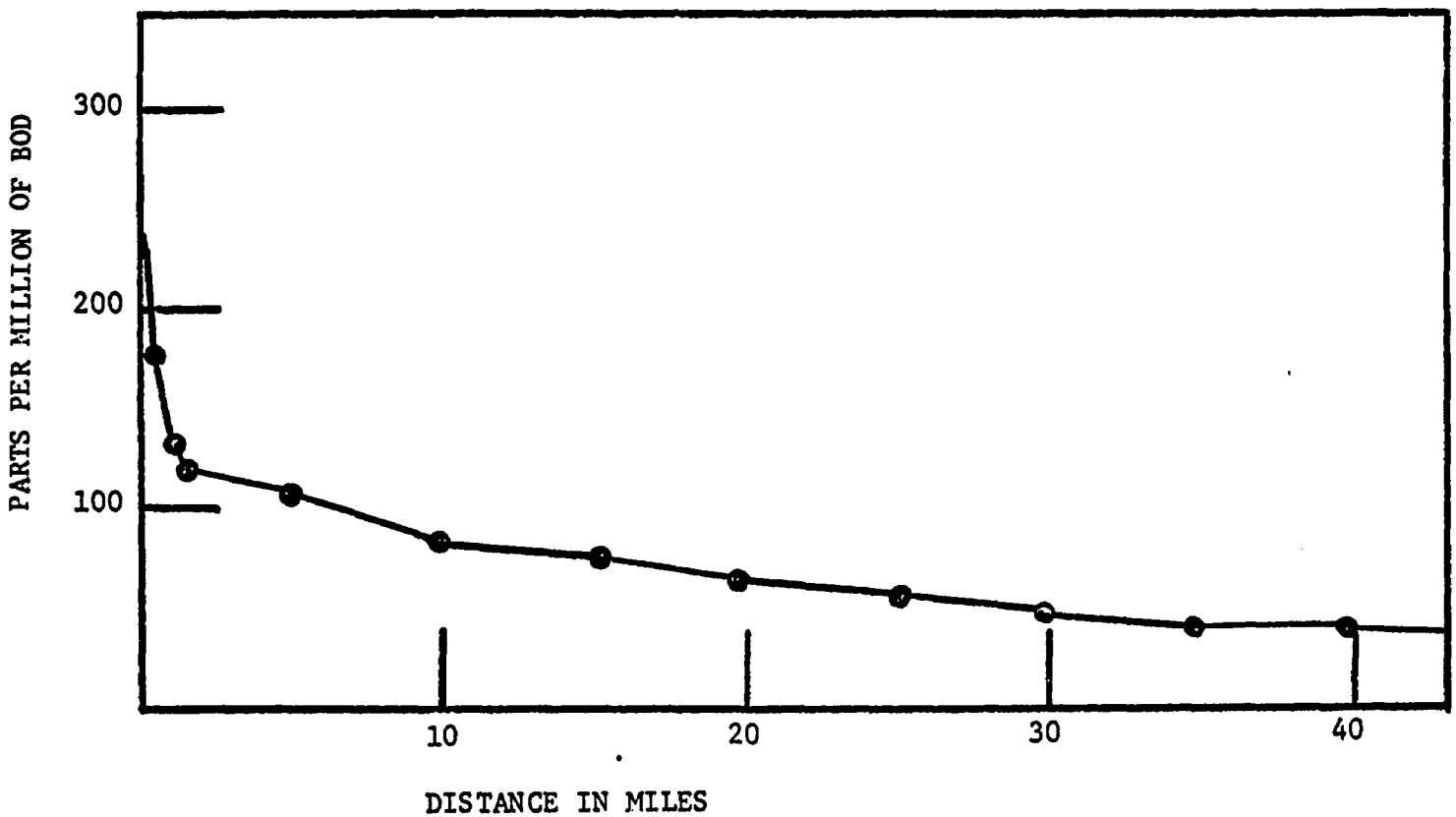


Figure 18. Average BOD Values Along the Open Channel.

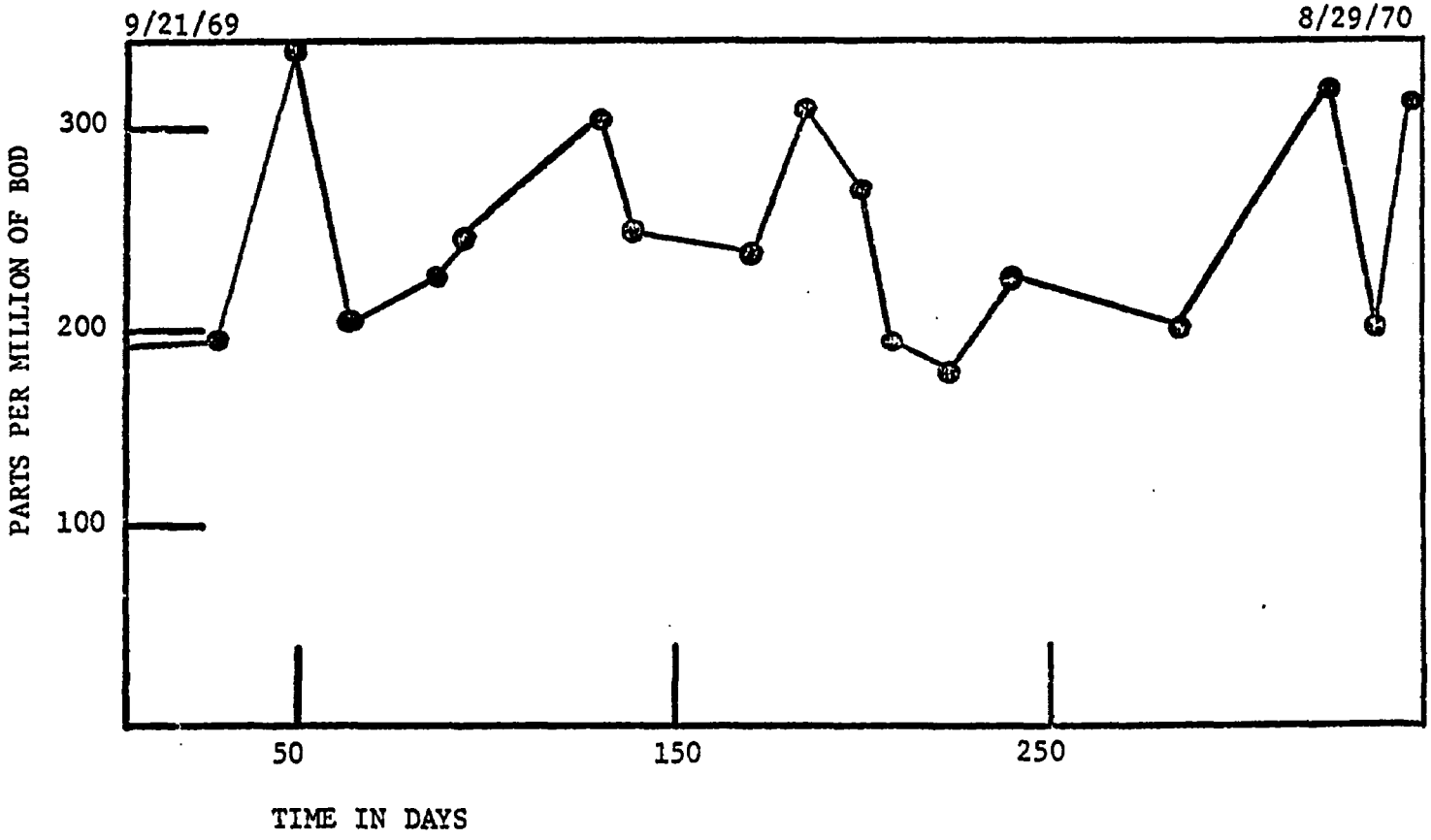


Figure 19. Yearly Variations of BOD Values in the Raw Sewage Region.

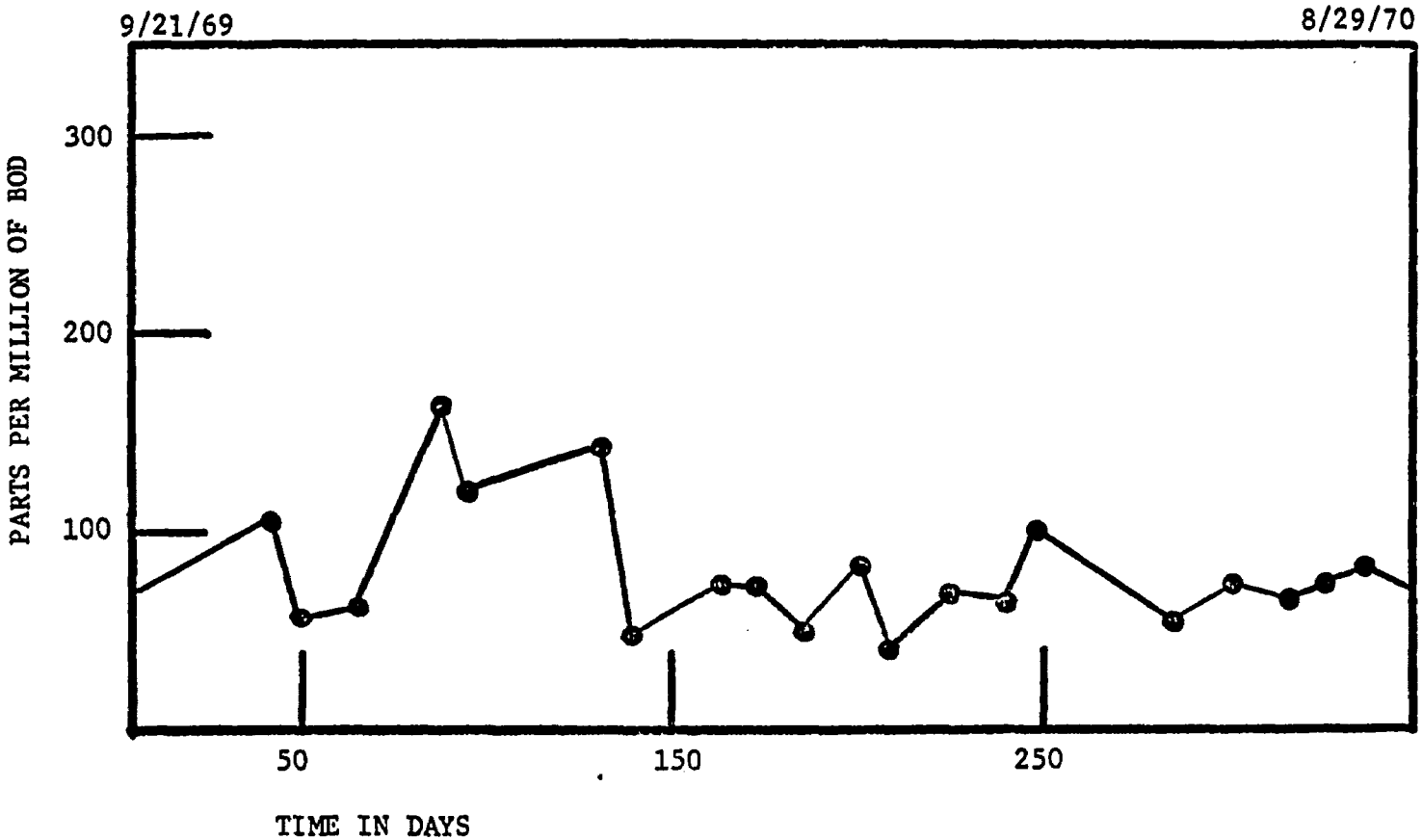


Figure 20. Yearly Variations of BOD Values in the Intermediate Sewage Region.

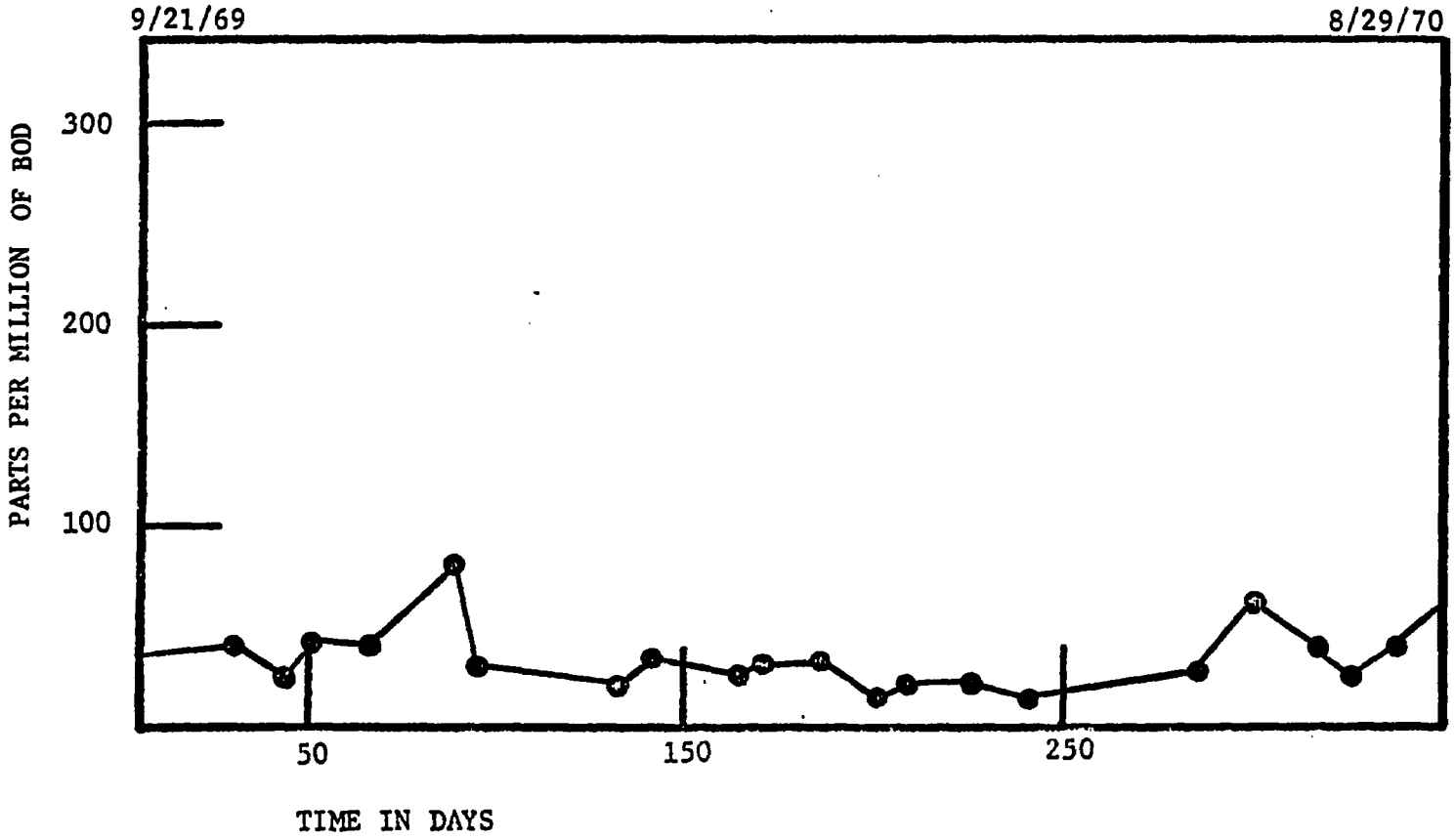


Figure 21. Yearly Variations of BOD Values in the Most Oxidized Region.

Of course, this was not substantiated with any specific data and the argument presented is merely a speculative proposition. Obviously more research needs to be performed and conclusive data obtained to support this idea. Because the scope of the project did not include this specific type of investigation and since time was a limiting factor, it was impossible to obtain an in-depth look at this phenomena.

As was mentioned before, the city had very little industry and thus industrial wastes were not a major problem. Chemical oxygen demand analyses were performed to learn what percent of the total oxidation was due to biological activity, and ultimately, to obtain a better understanding of the waste involved in the study. There were some sporadic high COD values which, perhaps, were the result of some periodic operations in some of the factories. COD values are presented in Figures 22 , 23 , and 24, for the different types of water studied. These graphs show only the yearly variations of these representative sites. The changes of COD against distance are presented in Figure 25 and a comparison of COD and BOD vs. distance is shown in Figure 26.

In an overall average the BOD constituted approximately 77.5 percent of the COD for the raw sewage group, 78.3 percent for the intermediate water and 21 percent for the well water samples.

Correlations of Salmonella to Coliforms

Salmonella counts were monitored for six weeks during the summer when there was a higher index of enteric disease among the population. These counts were quite low and for the most part negative. However, the data obtained was utilized in estimating the existing ratios of Salmonella

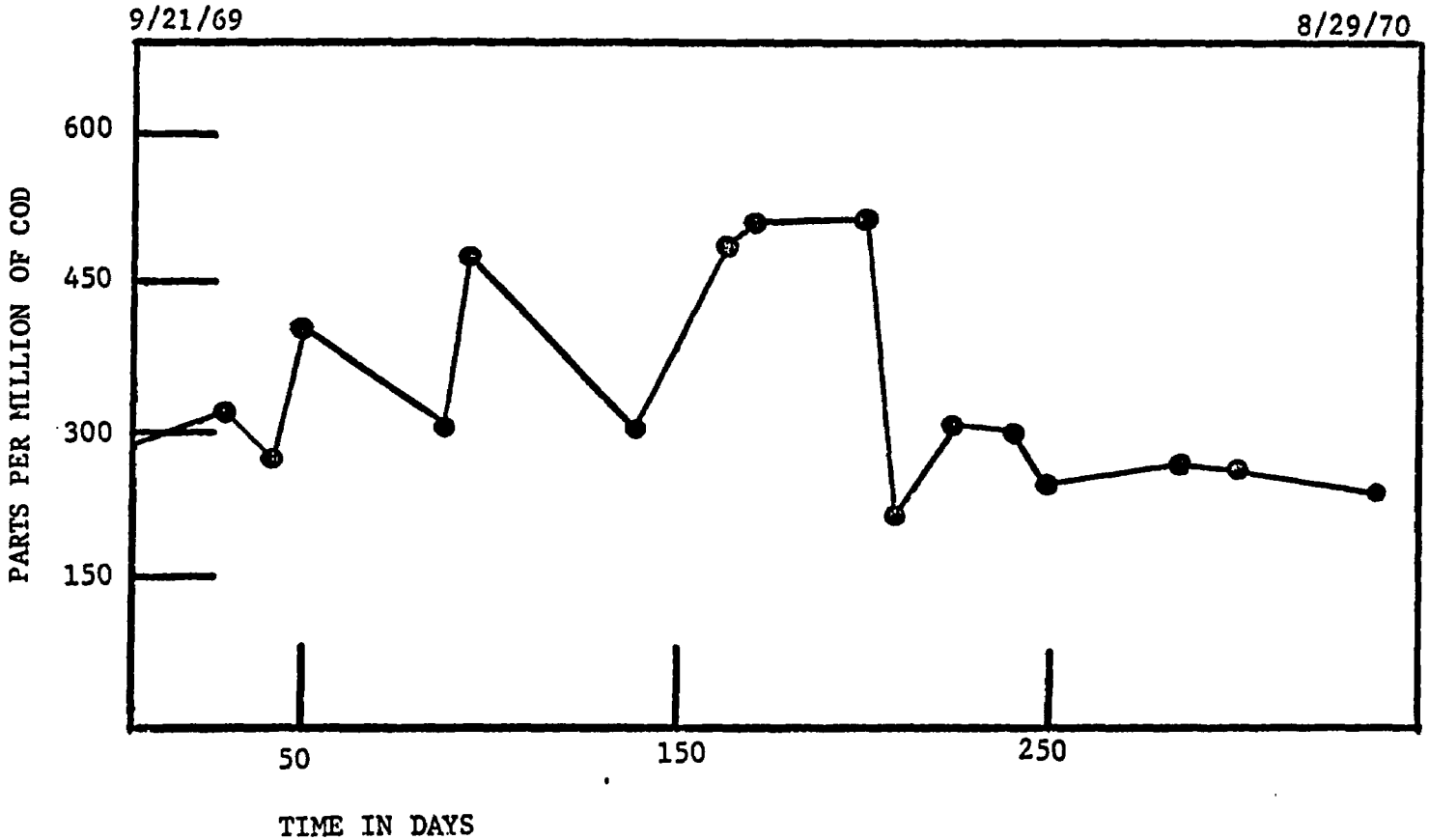


Figure 22. Yearly Variations of COD in the Raw Sewage Region.

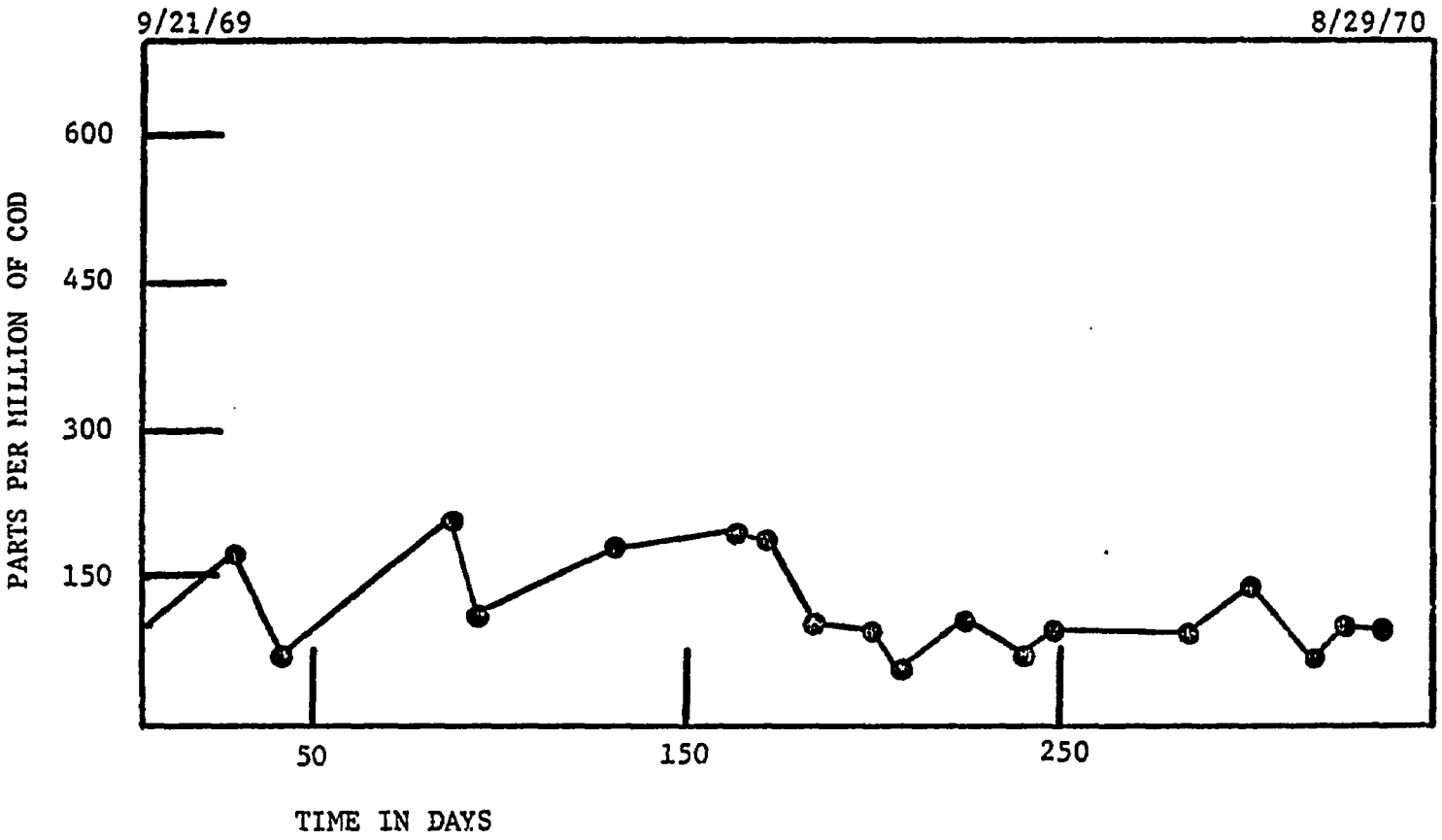


Figure 23. Yearly Variations of COD in the Intermediate Sewage Region.

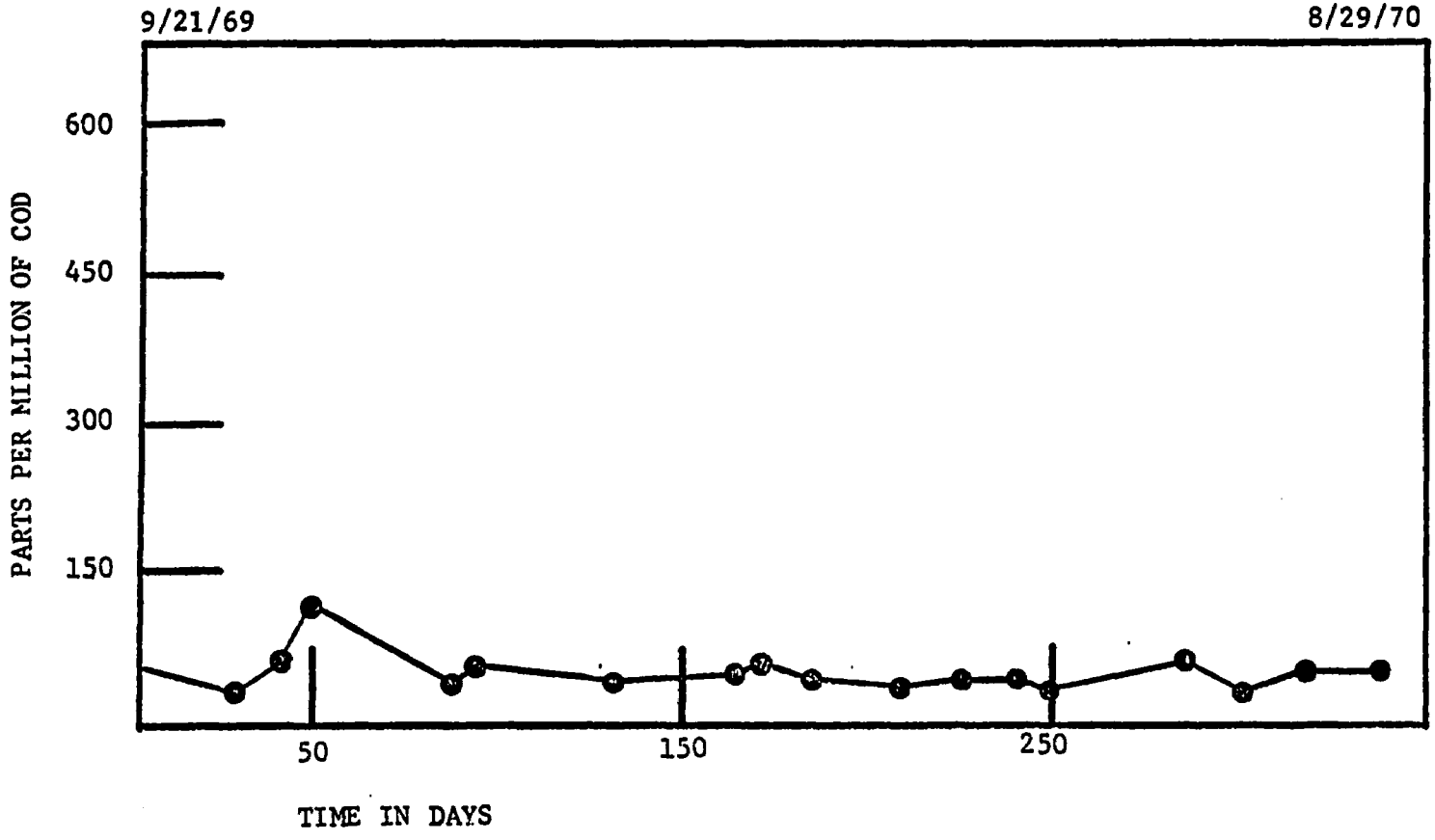


Figure 24. Yearly Variations of COD in the Most Oxidized Region.

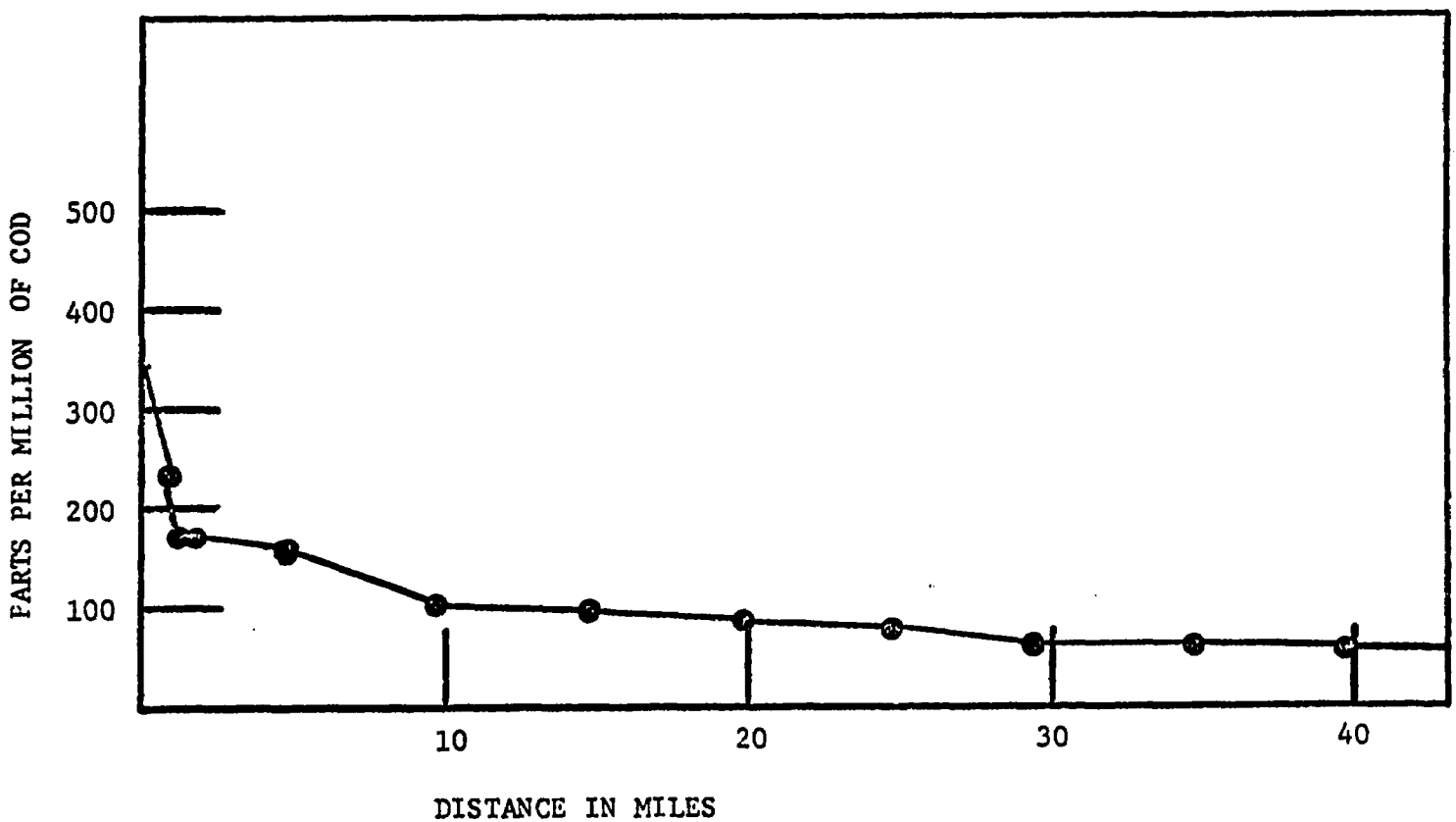


Figure 25. Average COD Values Along the Canal.

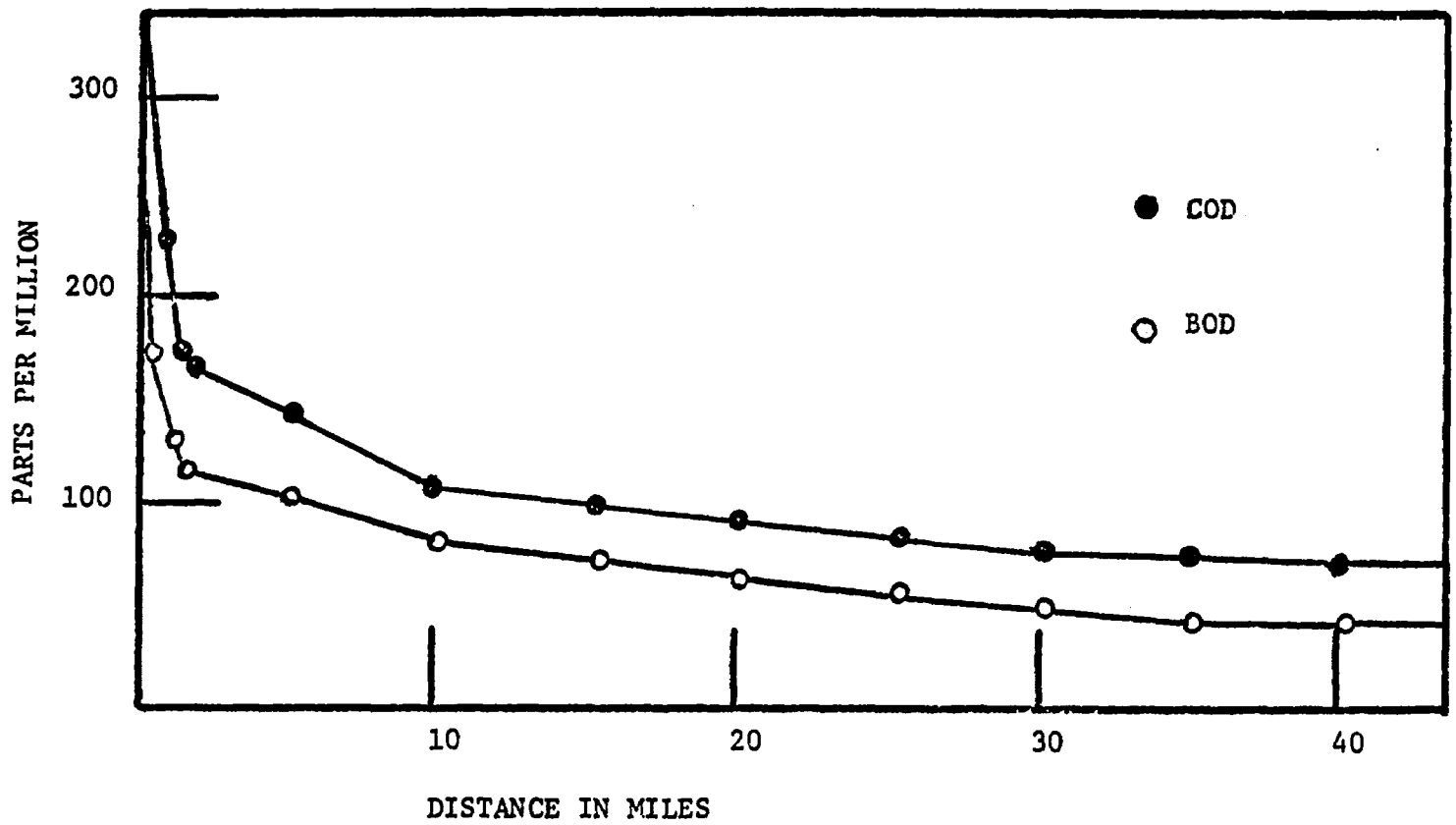


Figure 26. BOD and COD values Along the Open Channel

to coliforms in the open channel. Of course positive results were obtained only in the raw sewage region since the survival time of Salmonella in the open channel would be exceeded further downstream. A summary of the results is presented in Table X.

It can be observed from this table that where positive results were obtained for Salmonella the ratio to coliforms was quite low. In these positive samples the range was between 1 and 7 billion coliforms for every Salmonella cell. This data suggests that as far as vegetables are concerned, the only potential hazard present is that from coliforms themselves, since they could produce pathogenic symptoms if ingested in large quantities.

TABLE X

CORRELATION OF SALMONELLA SP. TO COLIFORM BACTERIA

SAMPLE	SAMPLING DATES		
	8/11/71	8/17/71	9/19/71
	(CELLS PER MILLILITER)		
1	0.03 36,400,000	0.01 42,300,000	0 51,700,000
2	0 8,000,000	0 16,000,000	0 70,000,000
3	0 10,960,000	0 23,800,000	0.005 77,100,000
4	0.016 9,000,000	0 15,700,000	0 9,300,000
5	0 9,640,000	0.006 18,600,000	0.008 24,300,000
6	0 12,860,000	0 19,300,000	0 11,500,000
7	-	0.008 600,000	0.008 1,000,000

CONCLUSIONS AND DISCUSSION OF RESULTS

Although the principal objective of the study was to monitor coliform organisms in vegetables as an index of contamination hazards, other less important parameters were also monitored. This does not mean, however, that all the possible variables were monitored (53). As was previously mentioned, many other factors contributed to the multiplication and distribution of bacterial cells within the water, soil and plant structures. For this reason some basic assumptions had to be made throughout the study. First of all, survival of bacteria was an important aspect which had to be considered in the sampling procedures (54). The tomatoes were collected soon after irrigation, but the lapse of time was not always the same for all samples due to irregular irrigation schedules. Therefore, varying incubation times of bacteria could have caused minor deviations in the results. This factor is probably one of the principal causes of unexplained variations in numbers of coliforms as shown in Tables VII, VIII, and IX.

Another aspect was the wind movement and the resultant translocation of bacterial cells. Whereas the literature points out that this may be an effective means of bacterial distribution within the plant (15), it was not the purpose of this study to correlate the obtained data with numbers of airborne cells, localized wind currents and sporadic

dust storms which happened to be abundant in the area.

The results of the different types of water analyzed revealed that there was a definite pattern of oxidation and biodegradation of the organic compounds as the sewage from the city flowed downstream. This was the result of sedimentation and bacterial action which was helped, at least partially, by the small amount of dissolved oxygen which the open channel was able to obtain along the way. In the same fashion, coliform organisms became less numerous downstream. This was demonstrated to be true not only in raw sewage, but also in the soils that were periodically irrigated with this waste. The numbers of coliform organisms found on tomatoes grown in these conditions were by no means constant, but the averages from the 3 different types of locations studied revealed a definite decrease in organisms downstream (Figure 2).

Although vegetables irrigated with initially uncontaminated well water did contain coliform organisms, these were found to be in relatively small amounts, thus lessening the potential of a health hazard. It should be noted that well water was transported by irrigation canals which passed through an area occupied by farm animals. Well water was sampled as it came out of the well and found to be negative for coliforms in repeated analyses. Therefore, farm animals did contribute to coliforms on tomatoes.

Since the general shape of the graphs of BOD vs. distance, and Coliforms vs. distance (Figures 18 and 3) was quite similar, a correlation of BOD to coliforms was attempted. A graph was prepared to represent the similarities of the curves (Figure 27). At approximately 15 miles the slope of both lines approaches zero. A similar graph was prepared to observe the correlation of BOD to coliforms on vegetables (Figure 28).

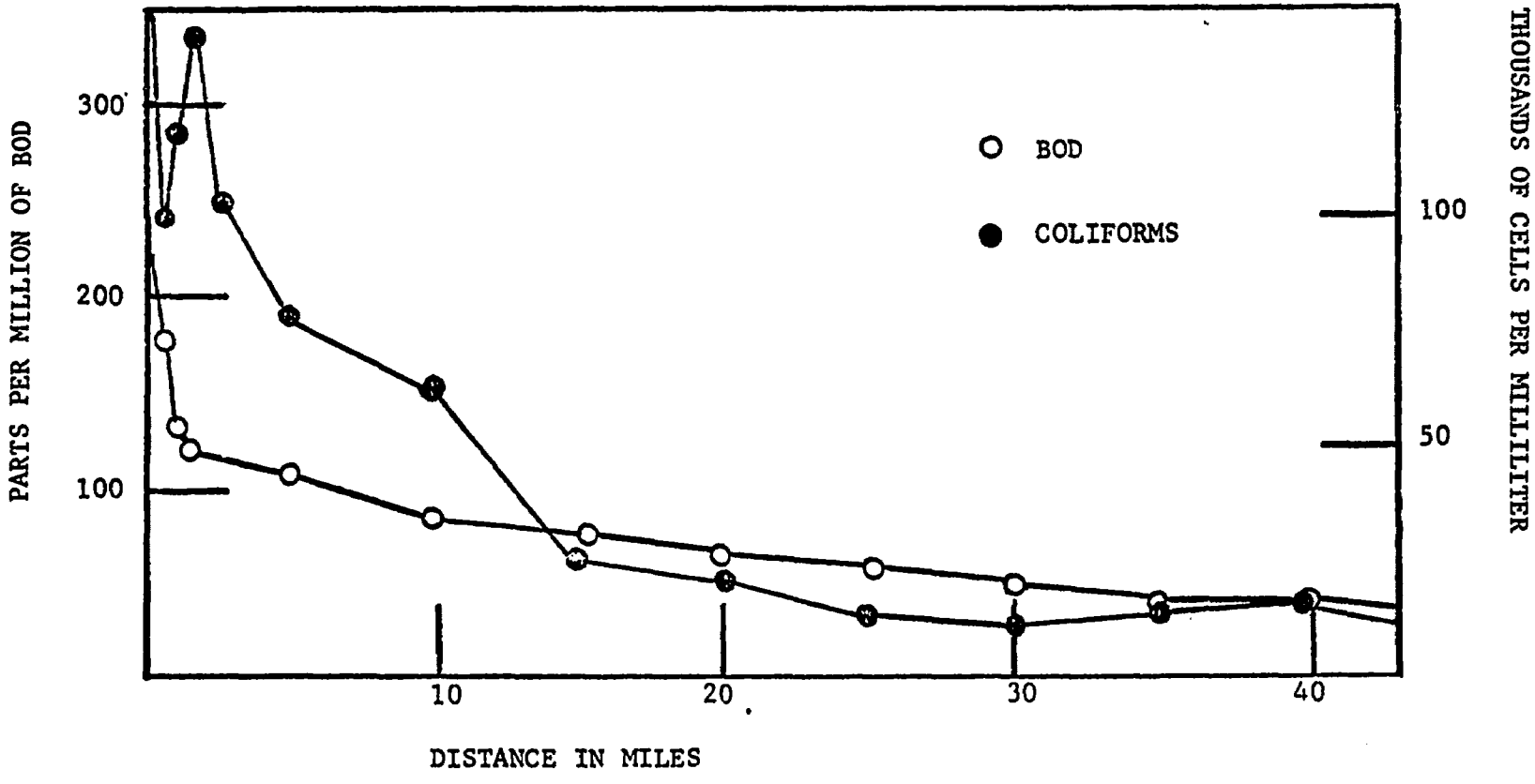


Figure 27. A Comparison of BOD and Water Coliforms Against Distance

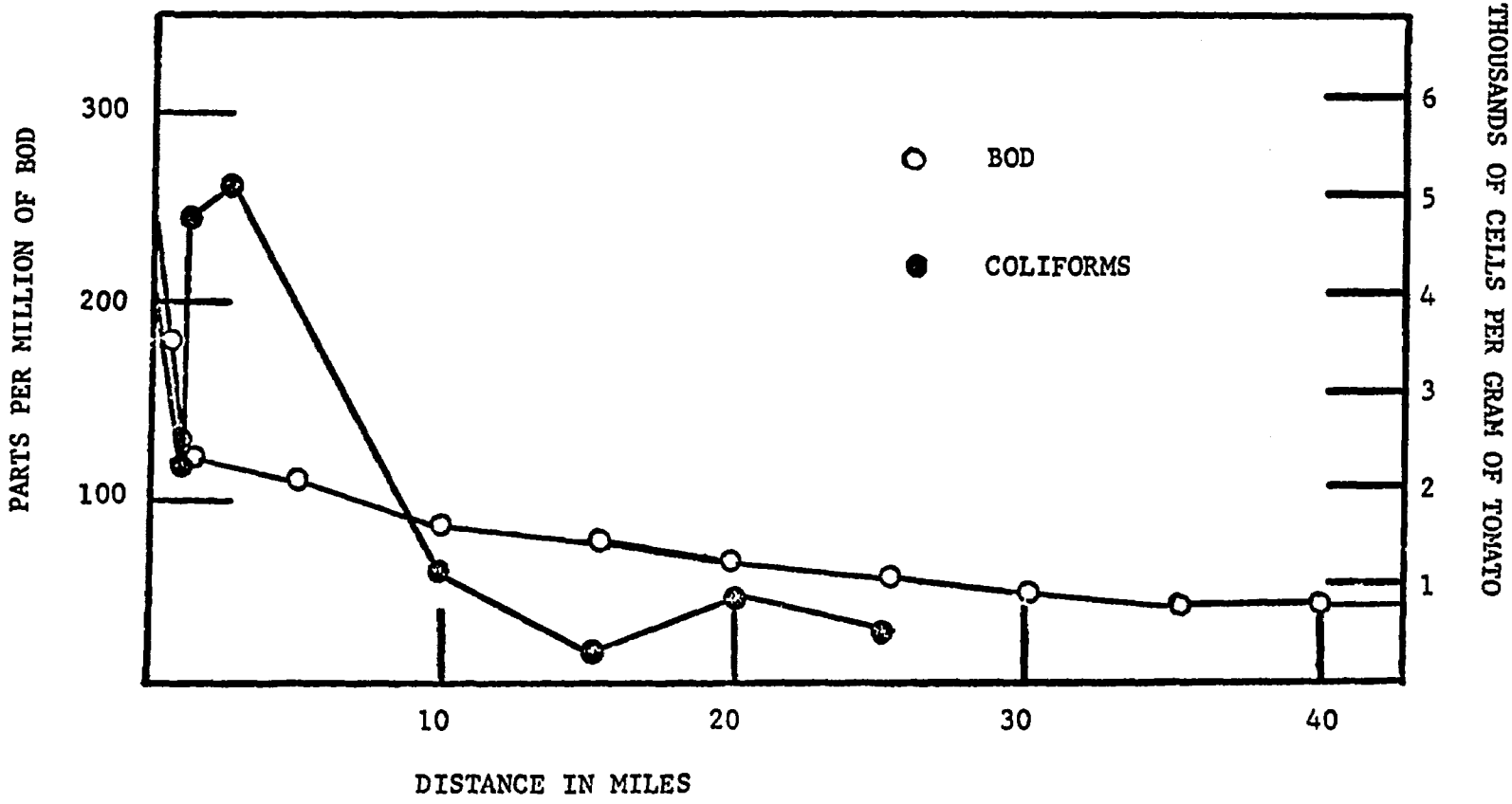


Figure 28. A Comparison of BOD and Tomato Coliform Counts Against Distance.

It appears that when the waste had attained a BOD of less than 60 parts per million, the concentration of coliforms on vegetables was less than 800 cells per gram of tomato. Although these figures were high, there was a good chance that most of these cells would dessicate, or otherwise be eliminated when the tomato was rinsed by the average housewive. This was backed up with the information obtained from the investigation on the rinsing of tomatoes before consumption. The data obtained from these experiments suggested that while there were some residual cells after rinsing on the tomatoes harvested from the raw sewage region, there were almost none in the tomatoes from the intermediate and well water regions. The evaluation of this type of data could very well be used in the criteria for the design of basic treatment of the domestic waste.

There are very few guidelines or public health limits available that could be used in evaluating the coliform counts obtained from the tomato samples. The state of Arizona, for instance, allows the reuse of domestic wastes for irrigation, but enforces strict regulations (55). Arizona demands that treated sewage effluents be chlorinated before they can be used on vegetables or any other crop of direct human consumption. For any other type of crop, they have set a limit of 25 parts per million of BOD. Under these conditions they allow the grazing of animals, except for dairy cattle. The state of Arizona also specifies that there should be a minimum of 15 days elapsed between the last irrigation and harvest time. Based on the literature surveyed, this seemed to be sufficient time to eliminate Salmonella cells since their survival time in irrigation water was 7 days. However, E. coli was shown to survive for periods up to 30 days (14). All irrigation activities are effectively controlled in

Arizona by issuing permits to all farmers making use of sewage wastes.

The problem of correlating vegetable contamination with enteric disease within the city was beyond the scope of this study. However, the correlation of coliforms with waterborne pathogens has already been established (44). A complete study of this nature would require extensive epidemiological work and more specific analyses of pathogenic organisms. The correlation attempted in this study between Salmonella and coliforms was only meant to be supporting data in evaluation of the potential health hazards present. Since Salmonella analyses were negative for the most part, it was quite evident that at least as far as the sections of the canal studied were concerned, this waterborne pathogen presents no immediate problem.

The conclusions of the present study can be summarized as foolows:

(1) Unwashed tomatoes from most of the sewage containing irrigation water would not be bacteriologically acceptable for human consumption based on coliform data obtained in this study.

(2) Simple rinsing of tomatoes with unbroken skins from an area receiving 30 milligrams per liter of BOD and having 900 coliforms per gram of tomato, resulted in a final concentration of only 1 to 5 coliforms per 100 grams of tomato, which is well within comarable standards for bacteriological drinking water quality..

(3) In general, coliform concentrations in irrigated ditches correlated with BOD concentrations, and both decreased with distance from the source.

(4) A rapid, simple method for measuring contamination per gram of tomato was developed, which might prove useful to city health departments in the near future.

Perhaps the idea of minimum treatment could be applied in the future at the individual farmer level in Mexican farming communities. The state could subsidize the design and construction of oxidation lagoons, prior to the time a complete and adequate sewage plant could be made a reality. It should be mentioned that while some of the chemical and/or biological parameters studied in this research were not fully utilized in the preparation of this paper, they will be of great help to the city of Juarez when the time comes to look into the design of a sewage plant. The realization of this study prompted city officials to consider the problem more carefully and, for the first time, ponder upon the importance and need for proper sewage disposal.

The results obtained from this study did not solve any immediate public health problems, but certainly started a new line of thinking and concern among citizens of Juarez, since publications have been presented in local newspapers describing the problems and making people aware of the prevalent situation (56).

BIBLIOGRAPHY

1. Chandler, A. A., and C. P. Read. 1964. Introduction to Parasitology. John Wiley and Sons Inc., New York.

2. Luria, S. E., and J. E. Darnell. 1953. General Virology. John Wiley and Sons, Inc., New York.

3. McKinney, R. E. 1962. Microbiology for Sanitary Engineers. McGraw-Hill Book Co., New York.

4. Tanner, F. W. 1935. Public Health Significance of Sewage Sludge When Used as a Fertilizer. Sewage Works., 7: 611.

5. Melick, C. O. 1917. The Possibility of Typhoid Infection Through Vegetables. J. Infect. Dis., 21: 28.

6. Felsenfield, O. 1948. Contaminacion Artificial de Salmonella en Legumbres. Reporte Tecnico de Agricultura, 37., Diario de Agricultura., Mexico, D.F.

7. Penfold, W. J, 1935. Report on the Apparent Outbreak of Cystecercus Bovis in 1933 at Melbourne and Metropolitan Board of Workers. Farm Weribee. Private File., Department of Agriculture Juarez, Mexico.

8. Hutchins, W. A. 1939. Sewage Irrigation as Practiced in the Western States. Tech. Bull. no. 675, U.S. Dept. of Agri., Washington, D.C.

9. Wilcox, L. V. 1948. Agricultural Uses of Reclaimed Sewage Effluent. J. Sewage Works., 20,1: 24.

10. Hamlin, E. J. 1945. Presidential Address. J. and Proc., Inst. of Sewage Purification., Part 2: 7.

11. Cuadra, M. J. 1967. El Uso del Agua Negra para Riego en los Valles de Mexico y El Mezquital. Memorandum Tecnico 252, Secretaria de Recursos Hidrauilcos., Mexico.

12. Dunlop, S. G., R. M. Twedt, and Weng-Lan Low. 1951. Salmonella

in Irrigation Water. Sewage and Ind. Wastes., 23,9: 1118.

13. Norman, N. M., and P. W. Kabler. 1953. Bacteriological Study of Irrigated Vegetables. Sewage and Ind. Wastes., 25,5: 605.

14. Falk, L. L. 1949. Contamination of Vegetables Grown in Polluted Soil. Am. J. Publ. Health., 39,18: 1338.

15. Mills, R., C. L. Bartlett, and J. F. Kessel. 1925. The Penetrating of Fruits and Vegetables by Bacteria and Other Particulate Matter, and the Resistance of Bacteria, Protozoan Cysts, and Helmenth Ova to Common Dissenfection Methods. Am. J. Hyg., 5: 559.

16. Kozyn, M. B. 1908. Bacteriological Studies of Field Grown Vegetables from Moscow. Vrack (St. Petersburg), 22 (1907); E.S.R., 20: 64.

17. Remlingen, L., and S. Nouri. 1957. Penetracion de Microorganismos en la Epidermis del Fruto. Reporte Tecnico de Agricultura, 38, Diario de Agricultura, (Trans.), Mexico, D.F.

18. Lumsden, L. L., and J. F. Anderson. 1910. The Origin and Prevention of Typhoid in the District of Colombia. Bull. Hyg. Lab., 79.

19. Creel, L. 1957. Recuperacion de Salmonella en Vegetales. Reporte Tecnico de Agricultura., Diario de Agricultura 1: 37., Mexico D.F.

20. Malick, C. O. 1917. Transmision de Infeccion a Traves de Legumbres. (Trans.), Private Files., Secretaria de Agricultura y Ganaderia., Delicias, Mexico.

21. Anon, L. 1923. Typhoid Fever in Paris. J. Am. Med. Assoc., 80: 1628.

22. Felsenfield, O. and V. M. Young. 1945. The Viability of Salmonella on Artificially Contaminated Vegetables. Poultry Sci., 24: 353.

23. Suzuki, L. 1957. Clorinacion de Legumbres. Reporte Tecnico de Agricultura. (Trans.), Instituto de Investigaciones Agricolas. Private Files, C. Estrada., Delicias, Mexico.

24. Lehnder, A. and W. Nowak. 1955. Mikrobial Abt. d Boyer Landesanstalt f. Pflanzenban, Munich. Zur Frage der Zer Weitraumrgen Abwasserverwertung. (On the Problem of Too Extended Waste Water Utilization). Gesundh., Ing. 76: 235.

25. Chapman, H. 1946 El Uso de Aguas Negras y la Prevencion de Epidemias. Publicacion Tecnica de Agricultura del ano de 1946., Mexico, D.F.

26. Warrington, S. L. 1952. Effects of Using Lagooned Sewage

Effluent on Farmland. Sewage and Ind. Wastes., 24,10: 1243.

27. Steel, E. W. and J. M. Berg. 1954. Effect of Sewage Irrigation upon Soils. Sewage and Ind. Wastes., 26,11: 1325.

28. Bower, H. 1961. Returning Wastes to the Land, A New Role for Agriculture. J. Soil and Water Cons., 23: 5.

29. Folliguet, J. M., L. Schwartzbrod, and O. G. Gaudin. 1966. Viral Pollution in Waste Water, Surface and Drinking. Bull. World Health Org. 35: 737.

30. Berg, G. 1967. Transmission of Viruses by the Water Route. Interscience Publishers, New York.

31. McLean, D. M., J. R. Brown, and R. Laak. 1966. Virus Dispersal by Water. JAWWA., 58: 920.

32. Clark, H. F., E. E. Geldriech, H. L. Jeter, and Paul W. Kobler. 1951. The Membrane Filter in Sanitary Bacteriology. Publ. Health Reports., 66,30: 951.

33. Lund, E., C. Hedstrom, and O. Stannegard. 1966. A Comparison Between Virus Isolation from Sewage and Fecal Specimens from Patients. Amer. Jour. Epidemiol., 84: 282.

34. Berg, G., D. Berman, S. Chang, and N. A. Clarke. 1966. A Sensitive Quantitative Method for Detecting Small Quantities of Viruses in Large Volumes of Water. Amer. Jour. Epidemiol., 83: 196.

35. Grigorieva, L. V., A. S. Gorodestsky, T. G. Omelyanets, and L. A. Bogdanenko. 1967. Survival of Bacteria and Viruses on Vegetables Irrigated with Infected Water. Gigiena: San. (USSR) 30: 28., Water Poll. Abs. (Brit.) 40: 14.

36. Smith, K. M. 1959. Recent Work on the Electron Microscopy of Viruses. Nature, 184,4697: 1440.

37. Shtannikov, E. V. 1965. Purification of Virus Contaminated Water by Means of Ion-Exchange Polymers. Gigiena: San (USSR)., 30,11: 29.

38. Burns, R. W., and O. J. Sproul. 1967. Viricidal Effects of Chlorine in Wastewater. JWPCF, 39,11: 1834.

39. Matossian, A. M., and G. A. Garabedian. 1967. Viricidal Action of Sea Water. Amer. Jour. Epidemiol., 85: 1.

40. Joyce, G., and H. H. Weiser. 1967. Survival of Enteroviruses and Bacteriophages in Farm Pond Water. JAWWA., 59: 491.

41. Conn, H. J., and I. Dimmick. 1946. Filters Suitable for

Separating Soil Bacteria from Bacteriophages. J. Bact. 52,4: 489.

42. Boyd, J. W., T. Yoshida, L. E. Vereen, R. L. Cada, and S. M. Morrison. 1969. Bacterial Response to the Soil Enironment. O.W.W.R., Project A-001. University of Colorado., Fort Collins, Colorado.

43. Godlobe, S. H., D. J. Illavia, and B. D. Rawal. 1967. A Method for the Examination of Large Volumes of Water Samples for the Detection of Virus Pollution, Suspended Cell Culture. Environ. Health (India) 8: 70., Water Poll. Abs., (Brit.), 40: 378.

44. Standard Methods for the Examination of Water and Wastewater, 12th. Edition. 1965. APHA, AWWA, and WPCF., New York.

45. Brock, T. B. 1970. Biology of Microorganisms. Prentice Hall Inc., Engleweed-Cliff., New Jersey.

46. Geldriech, E. E., P. W. Kobler, H. L. Jeter, and H. F. Clark. 1955. A Delayed Incubation Membrane Filter Test for Coliform Bacteria in Water. Am. Jour. Publ. Health. 45,11: 1462.

47. McLaren, A. D., and J. Skujins. 1968. The Physical Environment of Microorganisms in the Soil. The Environment of Soil Bacteria. An International Symposium., University of Toronto Press., Toronto, Canada.

48. Rawn, A. M., and F. R. Bowerman. 1956. The Membrane Filter: Advantages and Disadvantages. Water and Sewage Works., 103,1: 36.

49. Jensen, V. 1968. The Plate Count Technique. The Ecology of Soil Bacteria. An International Syposium. University of Toronto Press., Toronto, Canada.

50. Microbiological Analysis of Water. 1969. Millipore Corporation. Application Report AR-81., Bedford, Mass.

51. Rudolf, W. L. L. Falk, and R. Ragotzkie. 1951. Contamination of Vegetables Grown in Polluted Soil (Bacterial Contamination)., Sewage and Ind. Wastes., 23,3: 253.

52. Quimica Agricola. 1964. Publicaciones Salvat., Mexico, D. F.

53. Herskovitz, M. 1967. Utilization of Sewage for Agriculture Purposes. Water and Sewage Works., 5: 181.

54. Gainey, P. L., and T. H. Lord. 1957. Microbiology of Water and Sewage. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

55. Policy Governing Use of Waste Treatment Plant Effluents for Irrigation. 1962. Arizona State Department of Health., Division

of Environmental Health, Phoenix, Arizona.

56. El Fronterizo. 1971. Juarez Morning Newspaper. Cadena Periodistica Garcia Valseca. Marzo de 1971.