CHEMICAL, BIOLOGICAL, AND ENVIRONMENTAL FACTORS RESPONSIBLE FOR THE EARTHY ODOR IN THE AUBURN CITY WATER SUPPLY

AGRICULTURAL EXPERIMENT STATION/AUBURN UNIVERSITY
R. DENNIS ROUSE, DIRECTOR AUBURN, ALABAMA
BULLETIN 490 JULY 1977

CONTENTS

FIRST PRINTING 3M, JULY 1977

ACKNOWLEDGEMENTS

The authors wish to thank the Auburn Water Board for the financial support of this project, the Auburn Water Treatment Plant for supplying TON values, Dr. John Lawrence for use of the carbon analyzer, and Sandy Williams, Frank Seesock, and Susan Brownlee for technical assistance.

CHEMICAL, BIOLOGICAL, and ENVIRONMENTAL FACTORS RESPONSIBLE for the EARTHY ODOR in the AUBURN CITY WATER SUPPLY

J. D. WEETE, W. T. BLEVINS, G. R. WILT, and D. DURHAM[1]

INTRODUCTION

Background On Odors Of Biological Origin in Water

THE OCCURRENCE of objectionable odors in water used for drinking and commercial purposes is well documented in certain regions of the United States (1, 4, 12, 15) and various other parts of the world. (1, 8, 9, 11). Odors in surface waters may originate from industrial and municipal sewage effluents or from biological activities of algae or heterotrophic microorganisms. Odors of biological origin may be due to decomposition of organisms or to the production of microbial metabolites. These odors, which in severe cases render the water unpalatable, are most often described as earthy, musty, wood-like, potato-bin, camphoric, leathery, and fishy. The problem is of increasing concern to municipal water boards; however, the problem extends into the fresh water fish industry. Fish living in water having certain odorous chemicals may absorb and accumulate the agents responsible for the odor.

The most frequently reported odor in water used for drinking is described as "earthy." The chemical agent responsible for the most widely occurring earthy odor has been identified as *trans*-1,10-dimethyl-*trans*-9-decalol and named geosmin (5,6) and has an extremely low odor threshold of 0.2 ppm.

[1] Associate Professor, Assistant Professor, Associate Professor, and Research Associate, Department of Botany and Microbiology.

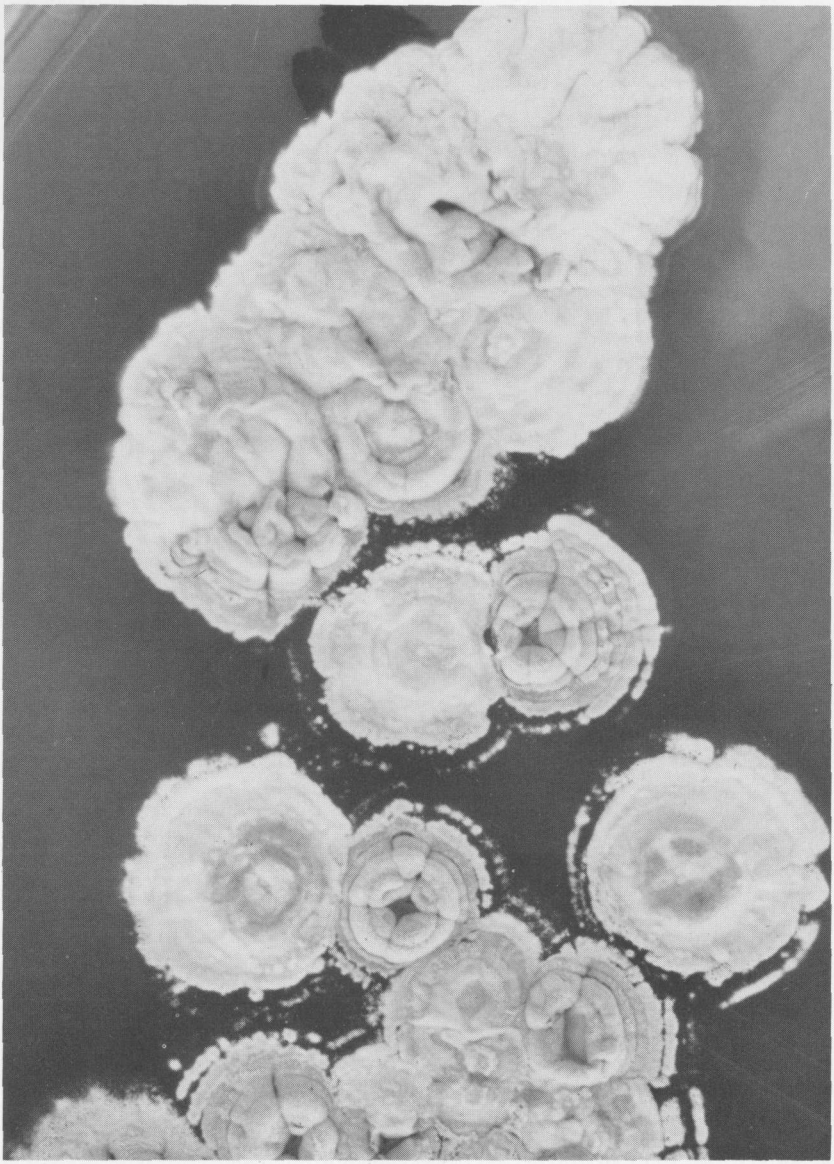


FIG. 1. Typical aquatic actinomycete, (Streptomyces sp.) colonies from Chewacla Creek.

This compound has been isolated from water having the characteristic earthy odor *(13)*.

Geosmin is produced by aquatic actinomycetes and blue-green algae, two microbial inhabitants of soil and water. Actinomycetes are branched, fila-

mentous, heterotrophic bacteria mainly comprised of the genera *Micromonospora, Nocardia,* and *Streptomyces* which comprise a substantial part of the microbial flora in fresh water, especially the shallow bottom muds of lakes and streams. There are over 400 species in the genus *Streptomyces,* and many produce a variety of volatile metabolites. Members of this genus have slender hyphae, 0.5 to 2.0 μm in diameter, with aerial mycelia that form chains of spores at maturity, Figure 1.

Blue-green algae are autotrophic procaryotic microorganisms that may be unicellular or filamentous and inhabit aquatic and soil environments. They are usually more noticeable as "algal blooms" which are large masses of algal cells often floating on the surface of still water. Certain species of the blue-green algae *Anabaena, Aphanizomenon, Oscillatoria, Symploca,* and *Microcystis* can also produce geosmin.

In most untreated water, the microbial population is heterogeneous, well-balanced, and interdependent. However, the influx of extraneous nutrients from fertilizers, agricultural runoff, and domestic animal and human wastes can cause fluctuations in the organic and inorganic content of water. These chemical factors can stimulate microbial growth and may cause an increase in the production of odorous metabolites by these organisms. In addition, such factors as pH, temperature, and dissolved oxygen may be critical for optimal growth of the microorganisms and production of odor compounds.

Odor in the Auburn Water

Like many other towns in Alabama, the Auburn city water supply has had annual episodes of an earthy odor for many years. These odor episodes usually appear in early to late spring, persist for 2 to 4 weeks, and are of low to moderate intensity. In the spring of 1974, however, the odor appeared in late January and persisted for 4 or 5 months. This prompted a study to define the chemical, biological, and environmental factors responsible for the recurring earthy odor. The results are reported in this bulletin.

PROCEDURES

Samples for analysis were taken from both Chewacla Creek (S) and Lake Ogletree (L) according to the map in Figure 2. Collection sites were selected on the basis of potential sources for substances that may serve as substrates for the growth of odor-producing microorganisms. All creek sites and lake sites were sampled at regular intervals over a 2-year period. L4, L5, L6, and L7 were usually sampled from about April to July each year, periods when the water level was high enough to make these sites accessible by boat. L1, L2, L3 were sampled year round. LS8 is in the shallow end of the lake at the mouth of Chewacla Creek and was also sampled year round. One water and one mud

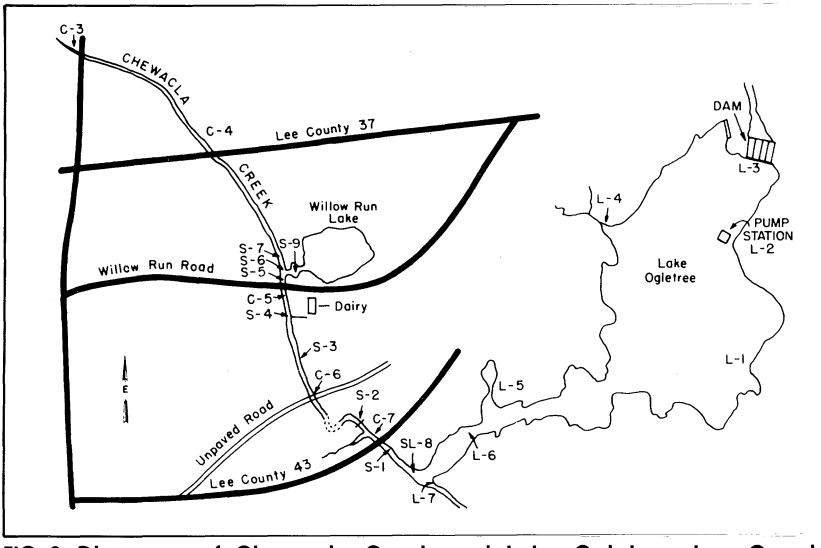


FIG. 2. Diagrams of Chewacla Creek and Lake Ogletree, Lee County Alabama, showing sample collecting sites.

sample were collected at each site for microbial determinations and chemical analysis. Oxygen and temperature readings were made at each site.

Orthophosphate, nitrate, and ammonia in the water samples were determined by the methods given in the 13th edition (1971) of *Standard Methods for the Examination of Water and Waste Water* (19). Total carbon was determined by using a Beckman Infrared Analyzer—Model IR 315.

Fecal coliform and fecal streptococci numbers were determined by procedures described in *Standard Methods* (19) *and Biological Analysis of Water and Wastewater,* Millipore Application Manual AM302.

Actinomycetes from creek and lake mud and water samples were screened for odor-production. The screening was conducted between July, 1974 and June, 1975, until it was decided that most species from the collection sites had been encountered. Species differentiation was based primarily on colony appearance, pigmentation, and media discoloration after incubation. Isolates were transferred from plates for counting to a yeast-extract dextrose (YD) medium, and incubated for 5 days at 25 C. The cultures were grown in inverted petri plates with a depression slide or specially prepared glass reservoir containing dibutyl phthalate. The volatile metabolites produced by the actinomycetes condensed into the dibutyl phthalate which could be injected directly into the gas chromatograph and mass spectrometer for analysis.

Laboratory studies were conducted to determine the effects of various chemical and physical factors on the growth and odor-production by actinomycetes. In all cases, *Streptomyces* isolate 33 was used in these studies. The effects of pH, temperature, carbon source, phosphorus, nitrogen, and cow manure on

growth and geosmin production by *Streptomyces* isolate 33 were also determined.

RESULTS AND DISCUSSION

Biological Factors

One objective of this project was to identify the biological agent responsible for producing metabolites that impart an earthy odor to the Auburn water supply. Upon identification of the nuisance microorganisms, population trends of these etiological agents as well as other microorganisms which might act synergistically in causing odor episodes were examined. Biological indicators of pollution by animal or human wastes were monitored to reveal possible sources of nutrients favorable for the onset of an odor episode.

Silvey and his associates (15, 18) reported accounts of microbiotic cycles of organisms of the Southwestern United States and their association with the occurrence of earthy odors. Blue-green algae have been reported to produce odorous metabolites, but in a degenerative state they can also serve as a source of nutrition for odor-producing actinomycetes (3,15). Algae reportedly have a high nitrate content that would enhance actinomycete growth (3). Proliferation of green algae can increase water pH by 1-3 units, which can also stimulate actinomycete growth. Actinomycetes, often the principal producers of geosmin, reached maximum numbers shortly after decline of blue-green algae (17). It has also been found that availability of oxygen associated with the growth of algae stimulates metabolic activity of actinomycetes and appears to be essential for production of odor compounds. Although non-actinomycetous bacteria do not produce geosmin, they may play an important role in the natural occurrence of the odorous substance. It was shown that populations of gram-positive bacteria increase soon after the decline of actinomycetes which is correlated with the disappearance of the odor caused by the latter (7). The bacterium *Bacillus cereus* was isolated from the lake water, and it was found that the medium in which it was cultured contained an active agent that catalyzed the breakdown of geosmin (16).

In this study, algae present in significant blooms at various times during this project were grown in the laboratory and none of the isolates screened was capable of producing geosmin under the growth conditions used. A known geosmin producer, *Symploca muscorum,* was used as a control for comparison. During the brief mild odor episodes of 1975 and 1976, no major algal blooms were observed in Lake Ogletree. During warm summer months, several massive algal blooms were observed in Willow Run Lake and the catch basin which feeds into Chewacla Creek, but no earthy odor was noted. Several days after the blooms appeared, however, senescing algae were observed in the shallow upper end of Lake Ogletree at the mouth of Chewacla Creek. These

massive blooms occurring in the Lake Ogletree drainage system could supply large amounts of nutrients to the lake water. Heterocysts and akinetes from the senescing algae could also remain in the bottom muds of Lake Ogletree during the winter months and act as an inoculum for a spring bloom.

Mud and water samples were collected at regular intervals from several sites on Chewacla Creek and Lake Ogletree. Only three sites (L1, L2, and L3) were sampled in the lake during months when the water level was low. When the lake level was raised and the lake depth increased, four additional sites (L4, L5, L6, and L7) made accessible by boat were examined. On Chewacla Creek, samples were taken at eight separate sites. A ninth site was chosen at the Willow Run Lake catch basin. This sampling site was not tested on dates when the catch basin was drained. At each site, the following tests were determined: oxygen concentration, temperature, total bacteria, total Gram-negative bacteria, total actinomycetes, nitrate and ammonia nitrogen, total phosphorus, and total organic carbon. In this section, the results on microbial populations are discussed.

Actinomycetes. Appropriate dilutions of mud and water samples were plated onto Actinomycete Isolation Agar for enumeration of actinomycetes which were identified by typical leathery tough colonies, Figure 1. The *Streptomyces* species were determined on the basis of a chalky appearance of the colonies due to chains of spores produced on aerial hyphae. *Micromonospora* species were also counted, but none capable of producing odorous metabolites were detected under the conditions employed. In general, water samples yielded fewer than 10^3 colony-forming units per ml, while numbers in mud usually ranged from 10^3 to 10^5 depending on the sample site. The average numbers of actinomycetes per site for each sampling date for 1974, 1975, and 1976 are plotted in Figure 3 (refer to Figure 2 for the locations of the sampling sites). The number of actinomycetes in mud samples taken at S7 (Chewacla Creek up stream from Willow Run Lake effluent) each year were very low. S9 (catch basin) had very high numbers for 1975 and 1976, but fewer in 1976. S6 is the junction of the catch basin effluent with Chewacla Creek. Below this site, actinomycete numbers were higher at S5 than at S6 and S7. S4 (drainage ditch from a cattle barn) showed much higher numbers of actinomycetes in 1974 and 1975 in comparison to other creek sites. Numbers were much lower in 1976. S3 (below cattle feeding area) showed a high actinomycete number in 1974, a lower number in 1975, and a still lower number in 1976. Sites below the cattle farm showed fewer actinomycetes with increasing distance from the farm. Sites 1-6 and LS8 showed similar low counts for 1976, which indicate a decrease in nutrient input. It is important to note the relative decrease in overall actinomycete levels from 1974 to 1976. This study was not contracted until after the intense odor episode of 1974, so the actinomycete level during the odor period is not known.

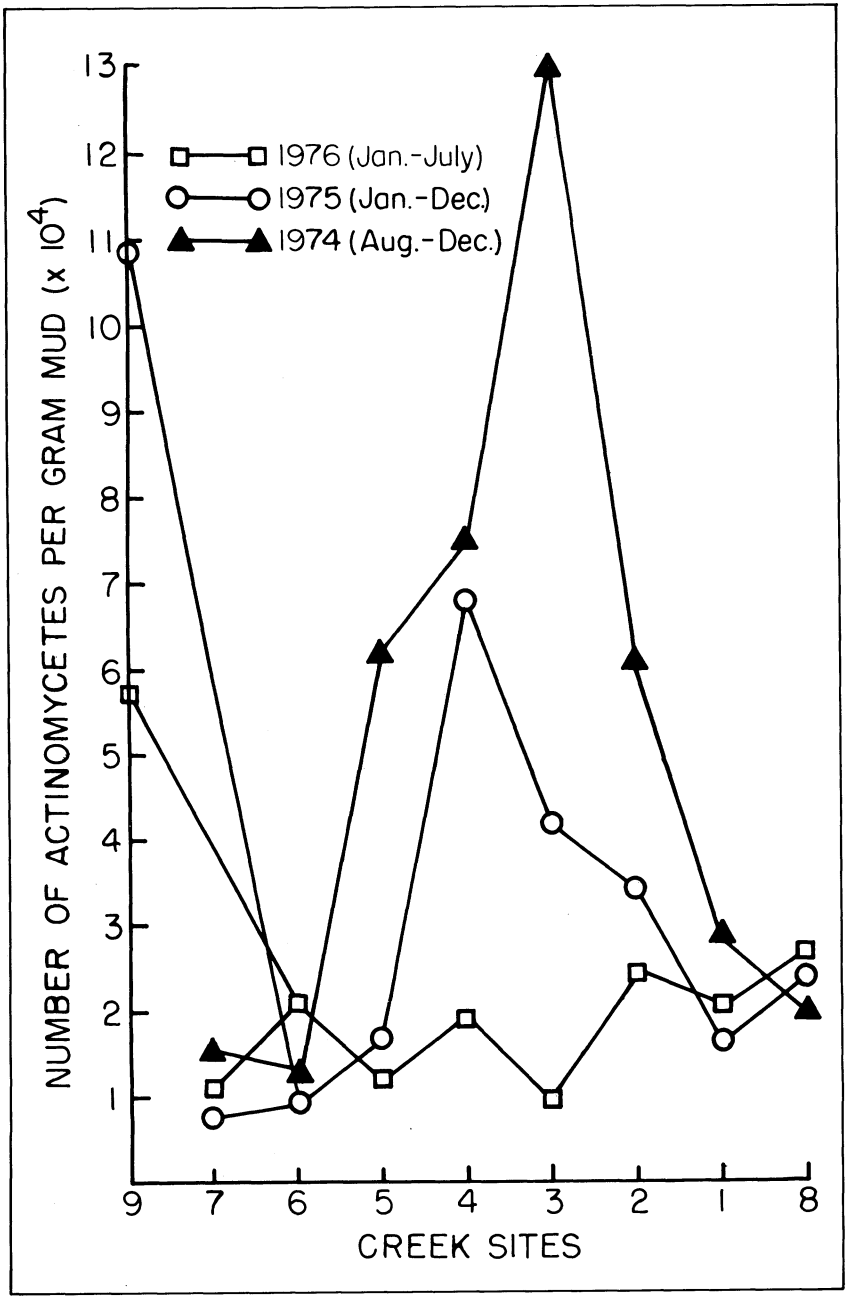


FIG. 3. Average numbers of actinomycetes in mud samples from sites on Chewacla Creek during 1974, 1975, and 1976.

Sites in the shallow end of Lake Ogletree (sites L6, L7, and LS8), shown in Figure 4, did not indicate major differences in actinomycete numbers when compared with sites near the dam (L1, L2, and L3). However, samples at L1-3 were taken in shallow water, possibly accounting for the observed similarities. Maximum yearly averages for lake sites were about 3.5 x 10^4, whereas the maximum number for 1975 at Site 4 in Chewacla Creek was about 7 x 10^4. Although actinomycete numbers were lower in the lake and creek in 1976, creek sites contained about twice the number of actinomycetes as lake sites. An analysis of the average monthly counts of actinomycetes at each creek site indicates that, in general, the numbers of actinomycetes peaked to a maximum in the fall (August to November) and again in the late winter or early spring, February to April, see figures 5-12. At most sites, maximum average numbers were present in October and in March. This may indicate periods of maximum sporulation of *Streptomyces* species, which would increase the number of

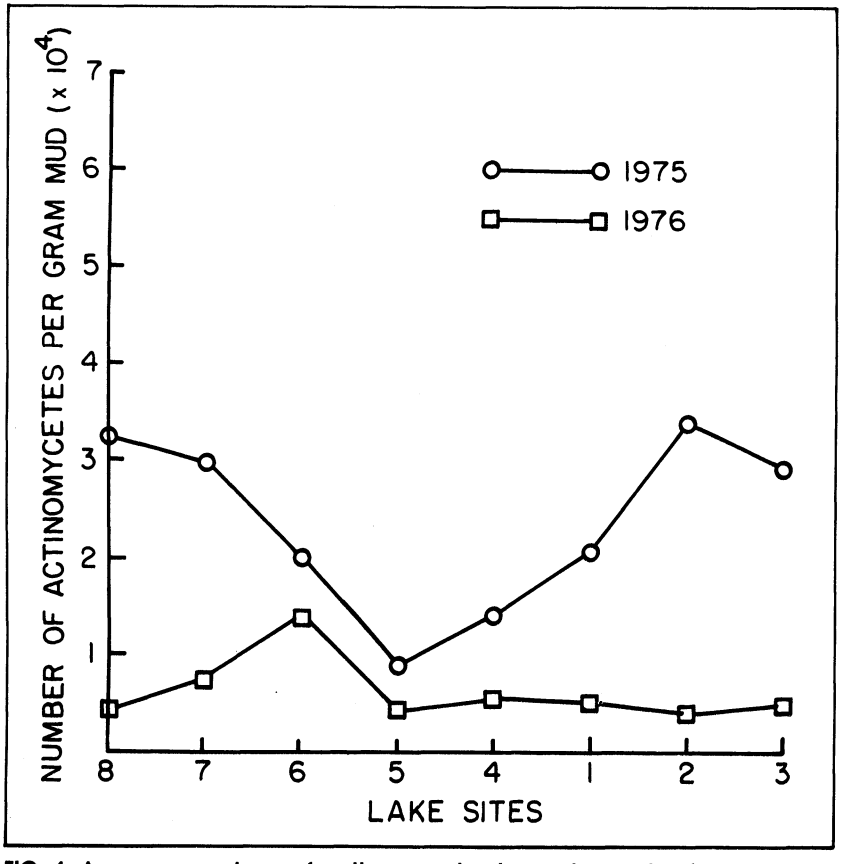


FIG. 4. Average numbers of actinomycetes in mud samples from sites on Lake Ogletree in 1975 and 1976.

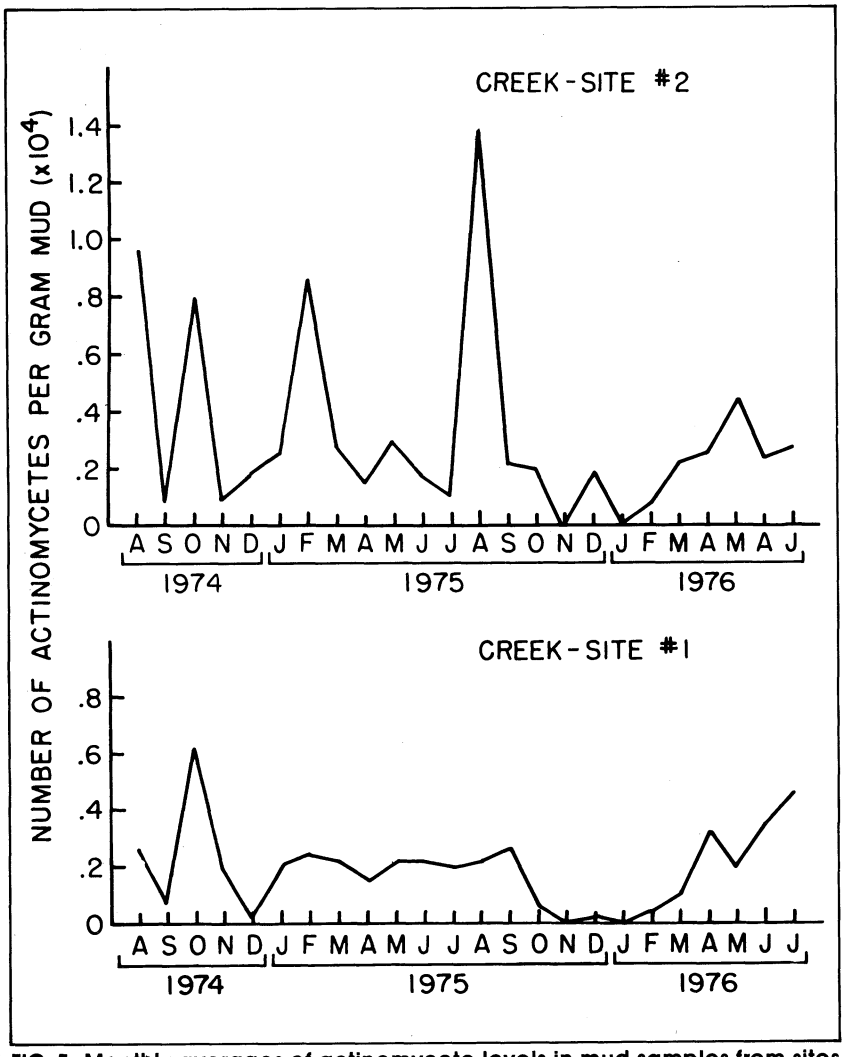


FIG. 5. Monthly averages of actinomycete levels in mud samples from sites No. 1(a) and No. 2(b) on Chewacla Creek.

propagules. The increase in the fall may be attributed to the increase in organic matter in streams from foliage and other vegetation, and reduced water flow, which could promote growth of actinomycetes. The lowest numbers of actinomycetes were present in mid-summer (May through July) and early winter (November to January). In December and January, water temperatures were low (5-10 C) which would not favor growth of actinomycetes. At S9 (Willow Run catch basin), a high actinomycete number was encountered in all months except January and February, 1976, when compared to other sites.

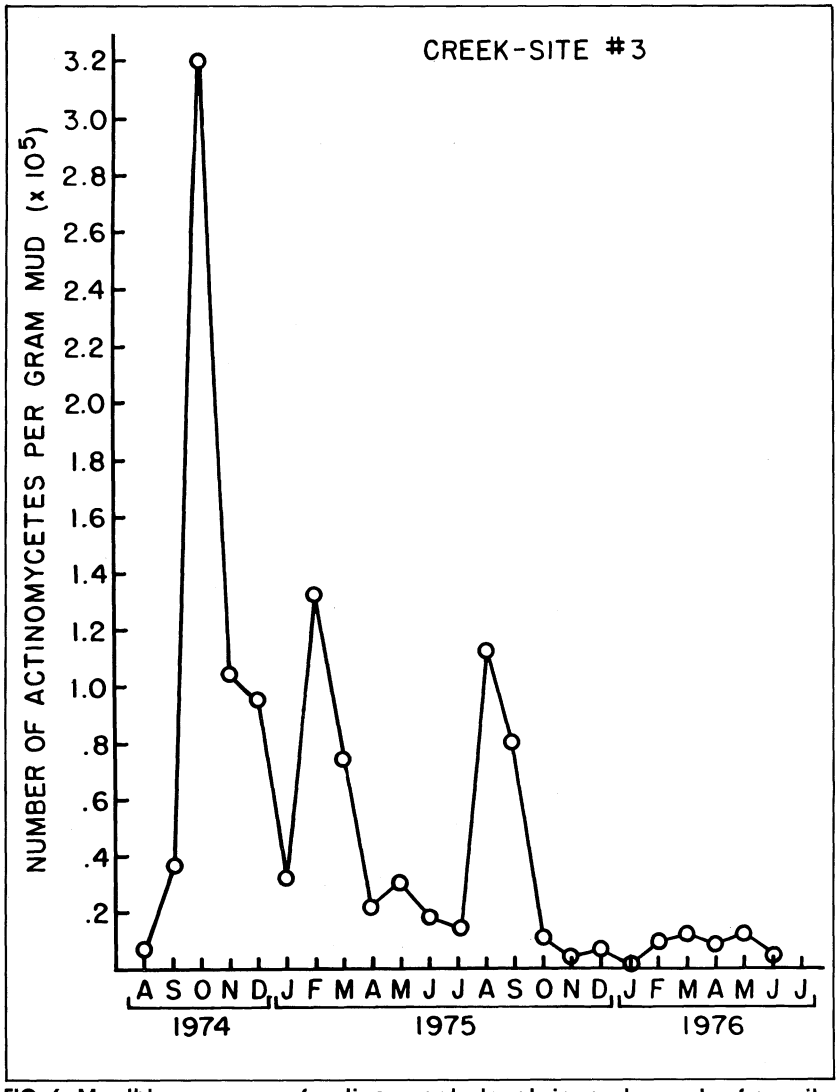


FIG. 6. Monthly averages of actinomycete levels in mud samples from site No. 3 on Chewacla Creek.

It is interesting to note from temperature plots, that the temperature of the water during the periods of highest actinomycete numbers was consistently greater than 10 C, usually between 12-20 C. The relation of temperature and actinomycete numbers suggests that rapid increases in temperature may stimulate sporulation and thus increase the number of propagules. Increases in actinomycete numbers during the spring warming trend would enhance odor production if sufficient nutrients and other favorable conditions were present.

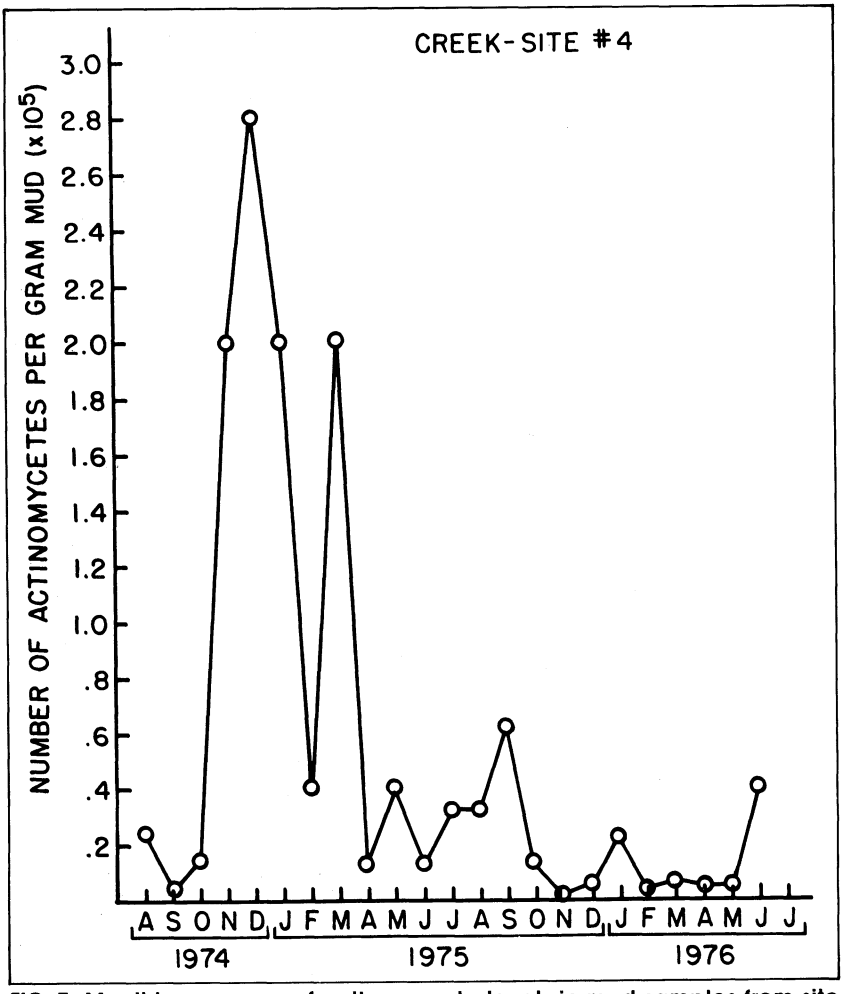
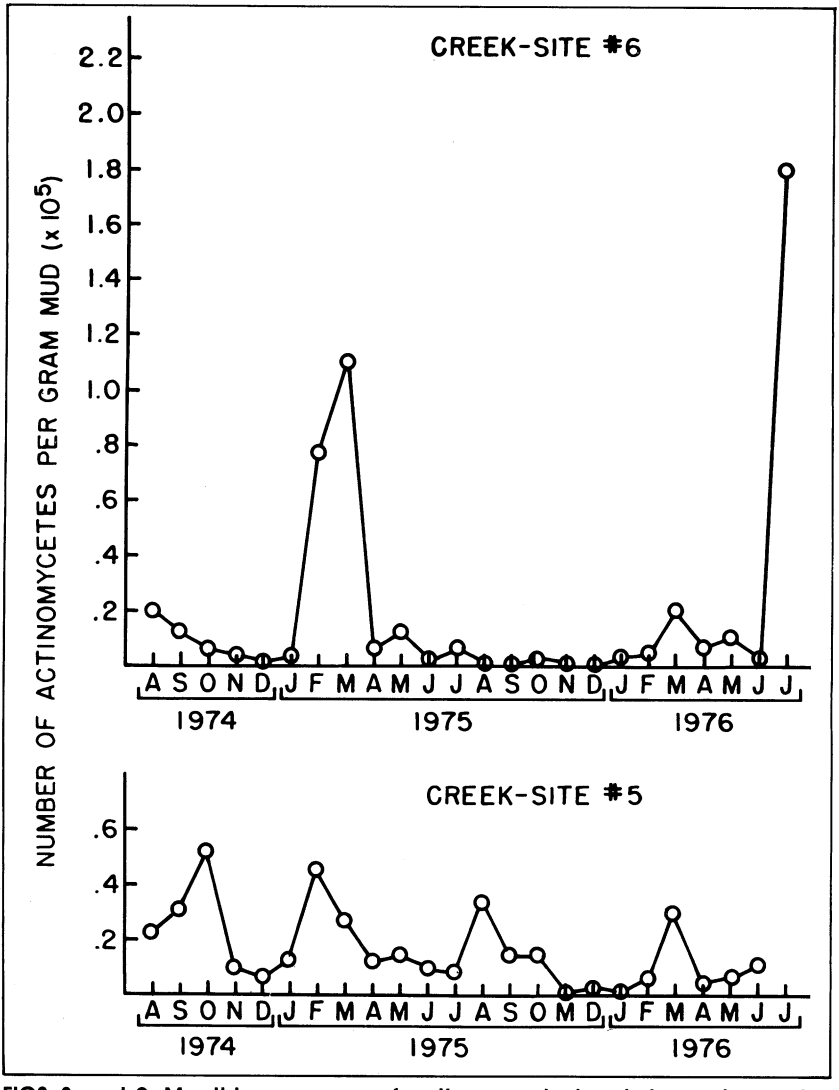


FIG. 7. Monthly averages of actinomycete levels in mud samples from site No. 4 on Chewacla Creek.

Total Bacteria. Total bacterial numbers were determined by plating appropriate dilutions of mud and water samples onto Actinomycete Isolation Agar plates. Yearly averages of bacterial numbers for each creek site (mud) for 1975 and 1976 are shown in Figure 13. High numbers (3 to 4.7 x 10^6 per gram of mud) were consistently present at S9 (catch basin), with the highest numbers occurring in 1975. S7 (above Willow Run Lake) consistently had lower numbers, 2.5 x 10^4 to 7 x 10^5. Total bacteria in Chewacla Creek increased below S6 in 1975, peaking at S3 below the cattle feeding area (2 x 10^6). LS8, in the lake, showed lower numbers, 6 x 10^5 to 1.4 x 10^6. These data indicate that cattle wastes are contributing to the total microbial flora in mud



FIGS. 8 and 9. Monthly averages of actinomycete levels in mud samples from sites 5 (below) and 6 (above) on Chewacla Creek.

samples from the creek sites. Total bacterial numbers were determined only in the last half of 1975 and the first half of 1976. In general, higher numbers were present in the summer months, with lower numbers normally being found in the period between October and December when water temperatures approached a minimum. At S3, S5, S6, and especially S4, however, there was a sharp rise in bacterial numbers during a period beginning in November or December and ending in February. These sites are adjacent to or just below a

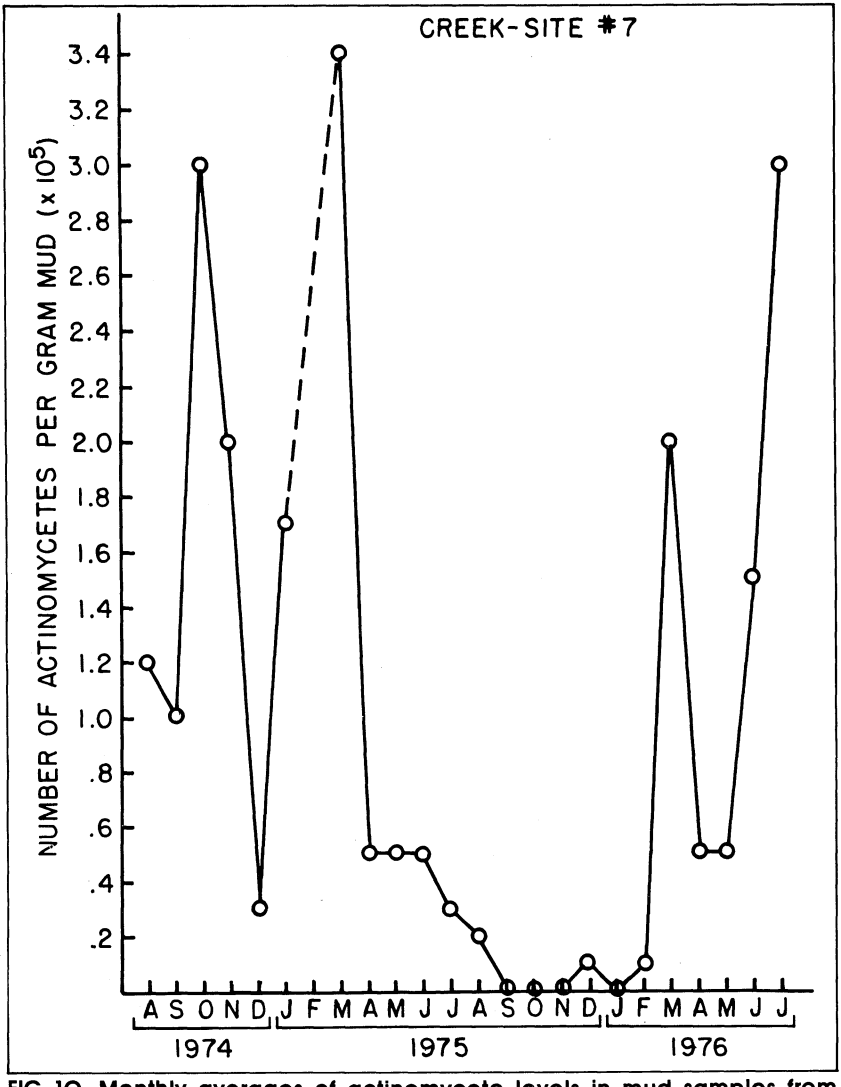
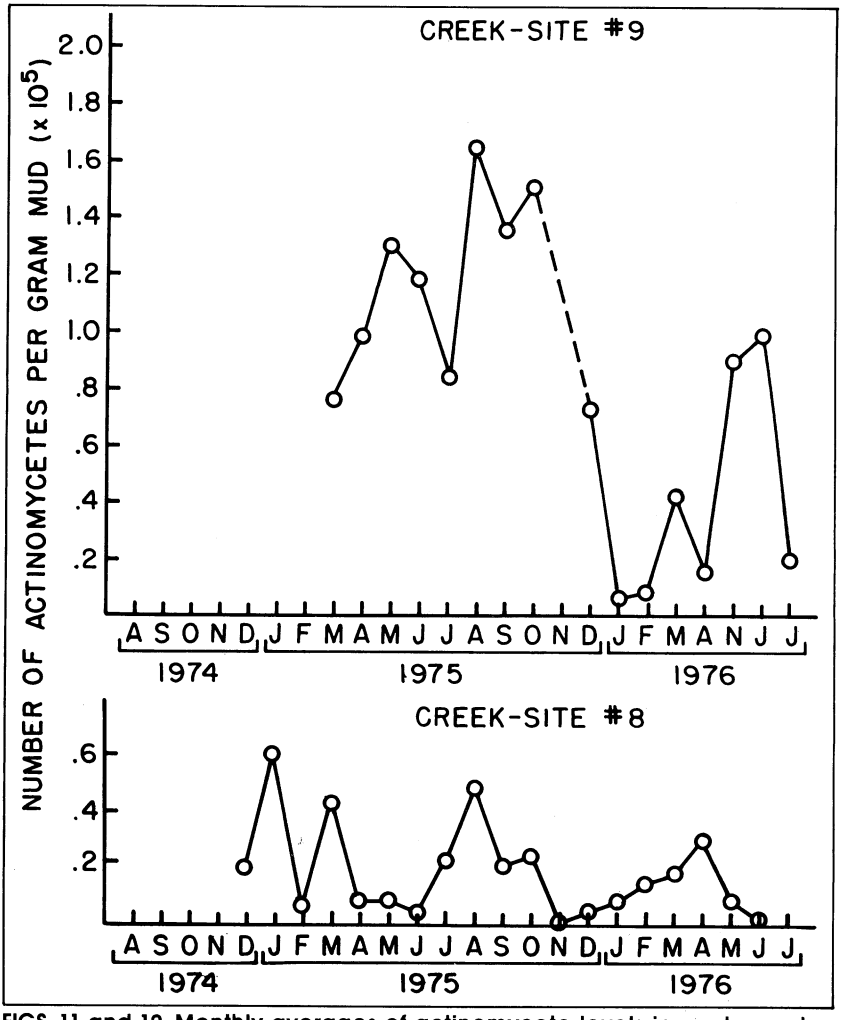


FIG. 10. Monthly averages of actinomycete levels in mud samples from site No. 7 on Chewacla Creek.

privately owned dairy operation. S9 (catch basin) showed consistently higher numbers at all times of the year.

In general, lake sites 4-8 in the upper end of Lake Ogletree showed higher numbers of total bacteria than sites near the dam, Figure 14. Numbers were typically higher in late spring and early summer.

Bacterial numbers in water samples taken from each creek and lake site in 1975 and 1976 were determined. The number of bacteria in the water samples



FIGS. 11 and 12. Monthly averages of actinomycete levels in mud samples from sites No. 8 (below) and No. 9 (above) on Chewacla Creek.

was generally 2 to 3 orders of magnitude lower than in the mud samples. The yearly averages at each site are plotted in figures 15 and 16. S9 consistently contained high numbers of bacteria in water samples with an average of 4.1 x 10^3 bacteria per ml of sample, Figure 15. In 1975, S7 showed the lowest average number at 2 x 10^3/ml. S4 in 1975 showed a relatively low average number of bacteria, 2.4 x 10^3/ml, but at S3, just below the dairy cattle feeding area, the average was up to 3.45 x 10^3/ml. Each year, the number of bacteria in water samples decreased with increasing distance downstream from the dairy farm. Bacterial levels in general were higher in 1976 than in 1975. Sites

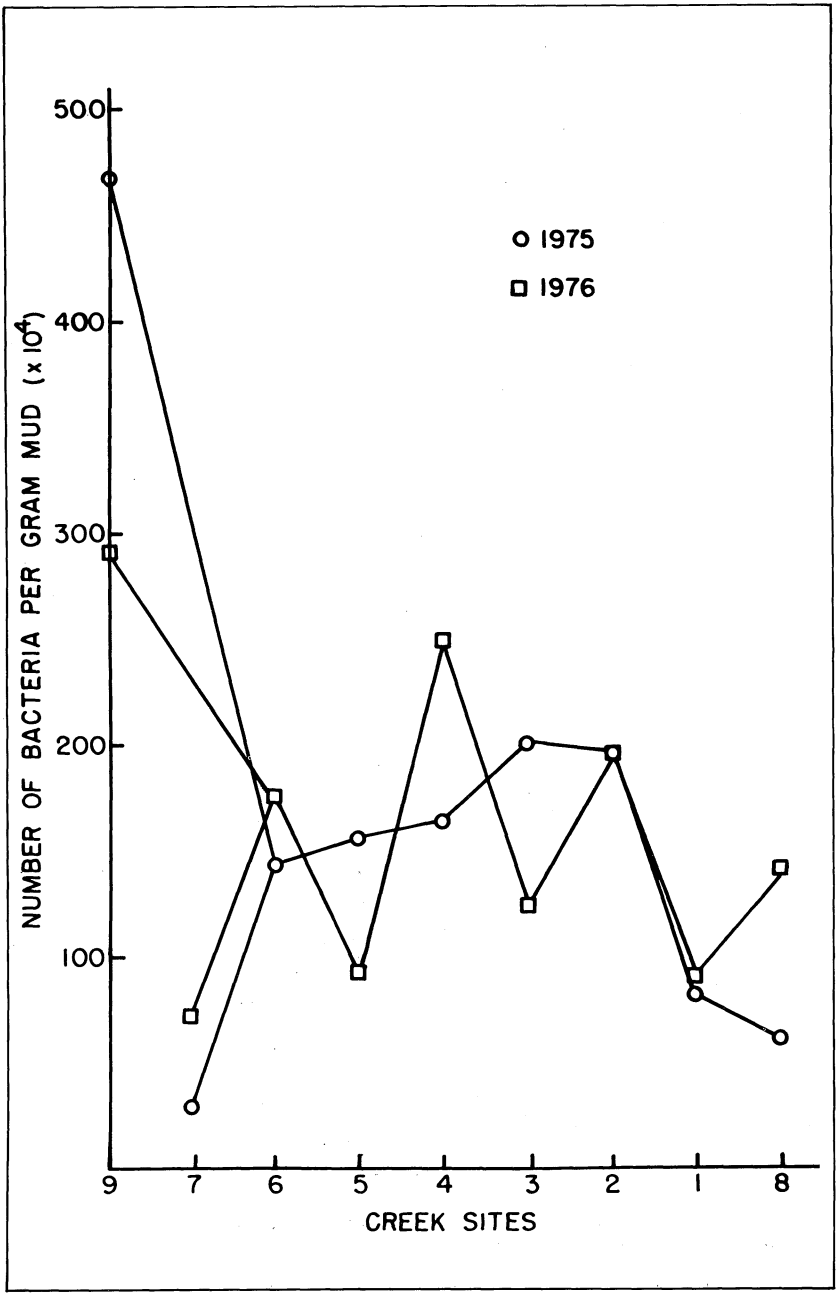


FIG. 13. Yearly averages of non-actinomycetous bacterial levels at sampling sites on Chewacla Creek in 1975 and 1976.

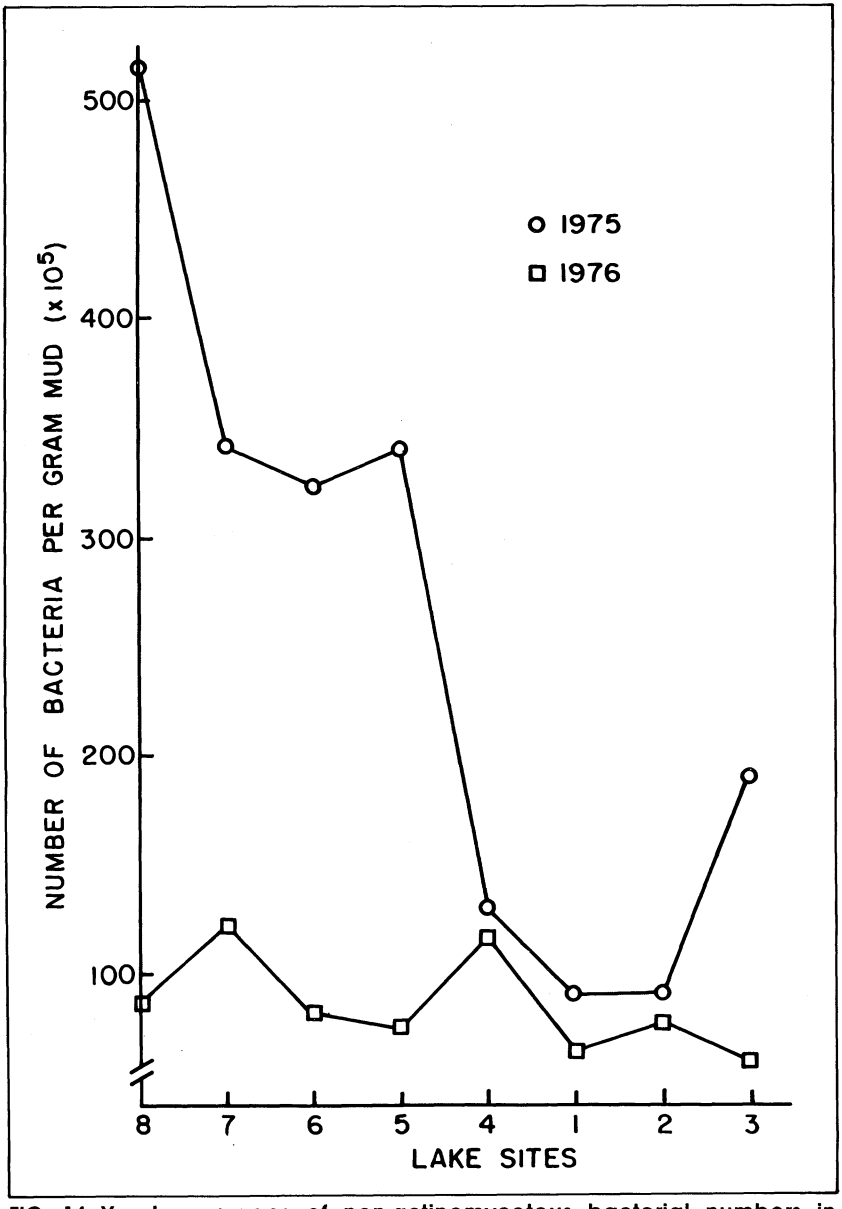


FIG. 14. Yearly averages of non-actinomycetous bacterial numbers in samples from sites from Lake Ogletree.

2-4 contained high bacterial levels, 3.9 to 4.8 x 10^3/ ml. There was an increase in total bacterial numbers below a source of animal waste in 1976. The increases in total bacteria at the creek sites adjacent to or below the dairy farm

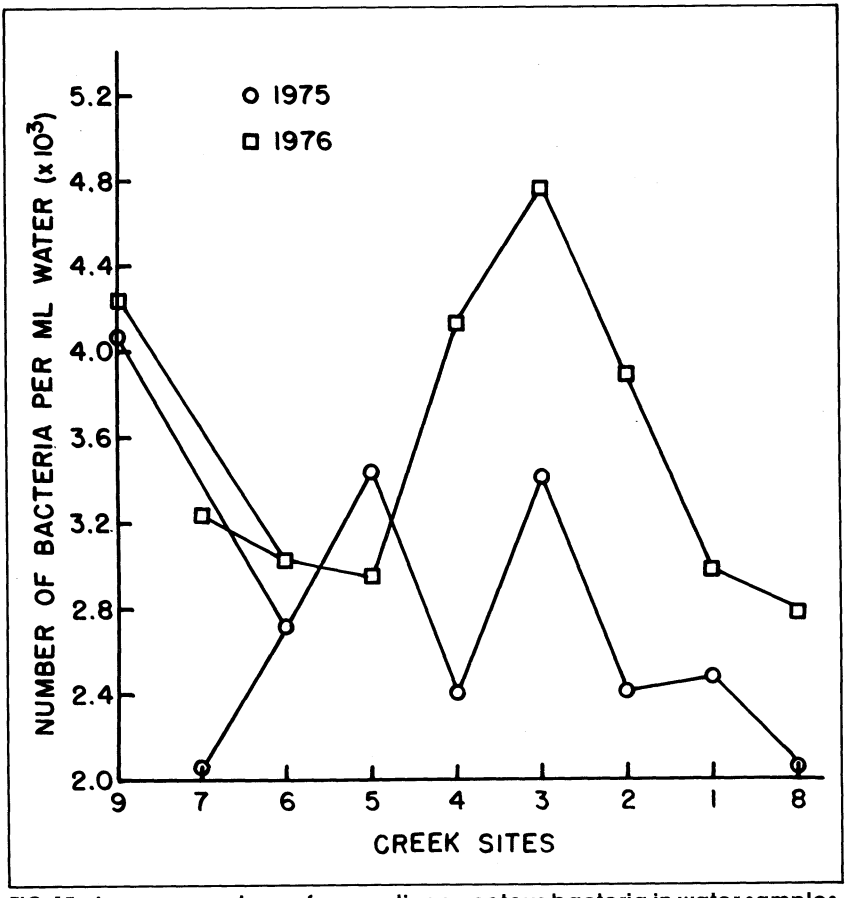


FIG. 15. Average numbers of non-actinomycetous bacteria in water samples from sites on Chewacla Creek in 1975 and 1976.

is another indicator that organic pollution was occurring at these sites and contributes to the total nutrient input to the Auburn water source.

Average bacterial levels in water samples taken from the lake were variable, Figure 16, with overall levels in 1976 being higher than in 1975. Values for 1975 ranged from 9.8 x 10^2/ ml to 1.5 x 10^3/ml with the exception of S7, which showed a higher average number of 5.7 x 10^3/ml. In 1976, bacterial levels at sites nearest the Lake Ogletree dam (L1, L2, L3) were as high or higher than at those sites in the shallow upper end of the lake. L1-3 ranged from 3.3 to 6.5 x 10^3/ml whereas L4-7 ranged from 1.6 to 3.8 x 10^3/ml. The highest average for the sampling period was at the dam, 6.5 x 10^3/ml at L3, in 1976.

The number of bacteria decreased with increasing distance from the dairy farm on Chewacla Creek to yearly averages of 2.8 x 10^3/ml in 1976 and 2 x

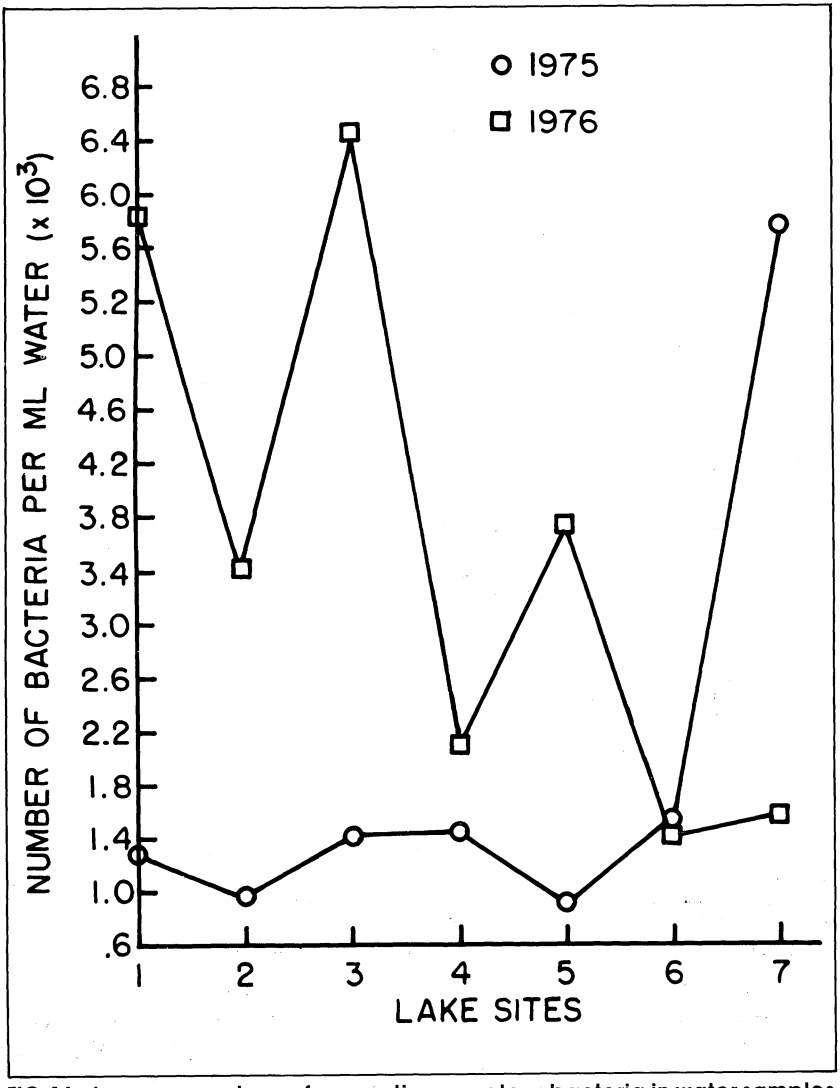


FIG. 16. Average numbers of non-actinomycetous bacteria in water samples from sites on Lake Ogletree in 1975 and 1976.

10^3/ml in 1975 at LS8 where the creek flows into Lake Ogletree Figure 15; however, the averages for Lake Ogletree reached 6.5 x 10^3, Figure 16. This suggests that favorable conditions for bacterial growth and multiplication exist in the lake or that significant additional contamination of the lake occurred, possibly by runoff. The total number of bacteria in lake mud samples taken in 1975 and 1976 also decreased with increasing distance from a source of pollution.

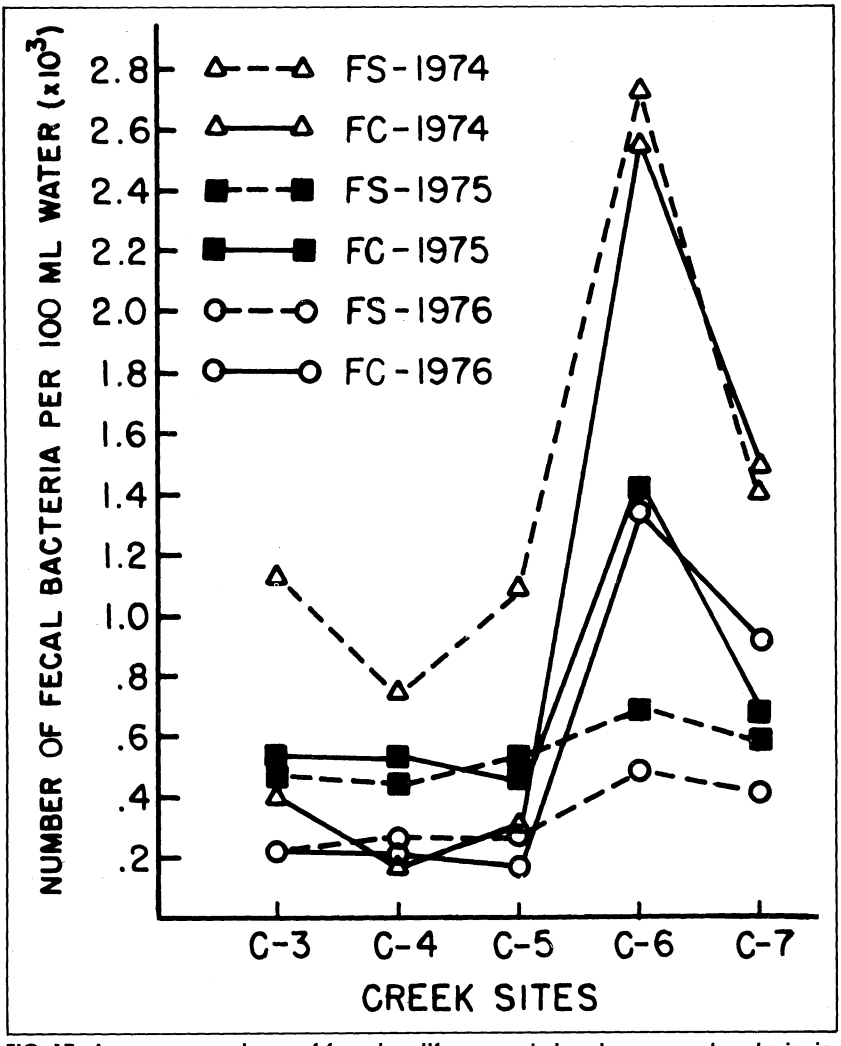


FIG. 17. Average numbers of fecal coliform and streptococcus bacteria in water samples from sites on Chewacla Creek in 1974, 1975, and 1976.

Fecal Coliforms *(FC)* and Fecal Streptococci *(FS)*. Five sites on Chewacla Creek were chosen for analysis and are geographically located as follows: the bridge on Lee County Highway 12 *(C3)*, the bridge on Lee County Highway 37 *(C4)*, the bridge on Willow Run Road, below Willow Run Lake *(C5)*, the bridge at an unpaved path below a private dairy farm *(C6)*, and the bridge on Society Hill Road *(C7)* (refer to Figure 1*)*. Yearly averages for fecal coliforms and fecal streptococci at each site in 1974, 1975, and 1976 are plotted in Figure 17. These data indicate that FC numbers were higher in 1974

when the odor was most intense than in 1975 and 1976. The 1974 samples were taken soon after the intense odor episode. The FS levels were also higher in 1974. It is important to note that coliform counts were very much higher below the private dairy farm (C6) compared to sites C3, C4, and C5.

The presence of FC and FS indicate animal waste in Chewacla Creek. These data show that animal waste is gaining access to Chewacla Creek above Lake Ogletree, and a sizable contribution is being made in the vicinity of the private dairy farm. The fecal coliform/ fecal streptococcus ratio (FC/FS) may indicate the type of animal waste pollution, i.e. poultry, livestock, or human. The FC/FS ratios found in this study indicate that pollution at most test sites in Chewacla Creek during 1974 and 1975 was caused by livestock and mixed animal waste. In 1976, these ratios indicate a "grey area," or waste of uncertain origin, animal or human.

The FC counts above site C6 did not vary greatly from 1974 to 1976. However, the counts at site C6 and C7 showed a decrease in 1975 and 1976 compared to 1974. This reduction in fecal coliform numbers may be due to the installation of an animal waste lagoon at the private dairy farm which may have prevented some animal waste from entering Chewacla Creek.

Fecal coliform and fecal streptococcus counts were performed as a part of the project primarily to determine if Chewacla Creek was receiving recent pollution in the form of animal manure, a potential substrate for actinomycete growth and odor production. Also, fecal coliform counts indicate a type of pollution which may have serious consequences to public health.

Chemical Factors

One of the main objectives of this study was to confirm the identity of the compound(s) responsible for the recurring earthy-odor in the Auburn water supply. This study was initiated after the major odor episode in 1974, so raw water containing the responsible odorous compound(s) was not available. This was further complicated by the fact that a significant odor episode did not occur during the study period. Thus, two approaches were taken to confirm earlier reports of the odorous compounds(s) from the Auburn water supply. First, carbon filters used during the 1974 episode was obtained from the Auburn Water Treatment Plant and analyzed for the odorous compound(s). In addition, carbon was placed at collection sites on Chewacla Creek and Lake Ogletree during the spring and early summer of 1976 and subsequently analyzed for odorous compounds. Second, microorganisms were isolated from collection sites and screened for the production of odorous substances.

Diethyl ether extracts of carbon used in filters at the Water Treatment Plant contained a multitude of chemicals as shown in Figure 18, but not all of them were odorous. However, geosmin was identified as a major component of the extract from the carbon.

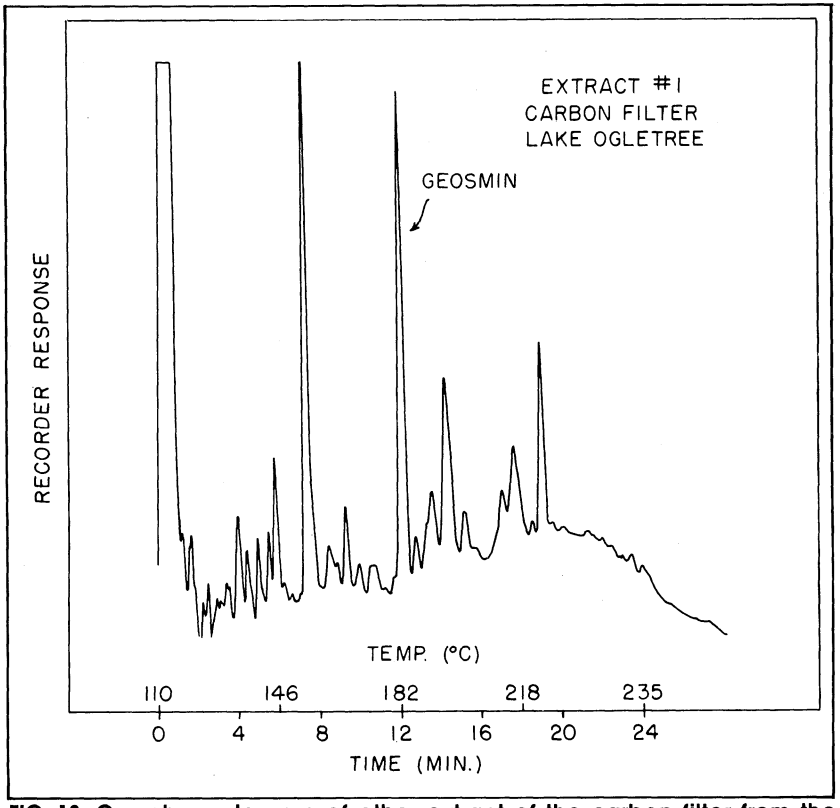


FIG. 18. Gas chromatogram of ether extract of the carbon filter from the Auburn water treatment plant (1974 odor episode) showing the presence of geosmin as a major component.

In 1976, carbon filters were placed at various sites of the creek and at L6 and L7 for 5 weeks (mid-April to late-May) and then analyzed for geosmin. Because of some heavy rains which destroyed a few of the filters, only filters from S3, LS8, L6, and L7 were recovered. Geosmin was detected in carbon only from L6.

Various microorganisms such as algae, actinomycetes, and other bacteria collected from the creek and lake were screened for the production of odorous metabolites. Actinomycetes were the only organisms from the sampling sites that produced geosmin under conditions used in the laboratory. These organisms were screened between August, 1974 and July, 1976. Approximately 90 percent of the aquatic actinomycetes collected from the creek and lake sites produced geosmin. The organisms fell into two categories with respect to the production of principal odorous substances: Group 1, geosmin production with no 2-methyl-isoborneol, see below, Group II, geosmin production with 2-methyl-isoborneol. The latter group produced

geosmin as a minor odorous product relative to 2-methyl-isoborneol and represented approximately 2 percent of the geosmin-producing aquatic actinomycetes screened in this study. The complexity of the volatile samples is illustrated in the gas chromatograms in figures 19, 20, and 21. Figure 19 represents a typical pattern of volatile substances produced by representatives of Group I. Compound No. 4 in this chromatogram is geosmin, which is one of the predominant volatile metabolites of this isolate, *Streptomyces* sp. 33L. Other isolates of this group had almost identical gas chromatographic patterns; however, the relative proportions of the various compounds varied. The identity of geosmin was confirmed using combined gas chromatography-mass spectrometry.

As noted above, a small number of aquatic actinomycetes isolates (Group II) produced another known odorous compound called 2-methyl-isoborneol. A typical gas chromatographic separation pattern is illustrated in Figure 20. Compound *a* in this figure was identified as 2-methyl-isoborneol; identification was confirmed by combined gas chromatography and mass spectrometry. Compound *b* in this figure was identified as geosmin, a minor odorous product of members of this group.

To further confirm this research, a *Streptomyces* sp. known to produce both geosmin and 2-methyl-isoborneol was obtained from Dr. N.N. Gerber at Rutgers University who was the first to characterize these odorous substances (5,6). Geosmin is the major odorous product relative to 2-methyl isoborneol in this species, Figure 21. This suggests that isolates used in this study may be different species than that supplied by Dr. Gerber.

Streptomyces isolate 33L from Lake Ogletree was screened for other odorous metabolites. Depending on who was monitoring the individual compounds as they emerged from the gas chromatographic column, several odors were associated with certain volatile metabolites. Geosmin was the major odorous product, but several of the minor components had odors described as musty, potato-bin, and sweet. These minor odorous compounds were not considered as problems in the Auburn water supply.

Environmental Factors Related to the Growth and Production of Odorous Substances by Actinomycetes

Field Studies. Field studies concerned with environmental factors that influence odor-production included determining the phosphorus, nitrogen (ammonia and nitrate), and the total carbon content of water at the creek and lake sites indicated above. Other factors such as water temperature, pH, oxygen content, rainfall, and air temperature were measured directly or obtained from the Auburn University Environmental Studies Service Center. The data obtained in this study are summarized in Table 1. Data obtained in field studies will be discussed and compared to values considered acceptable

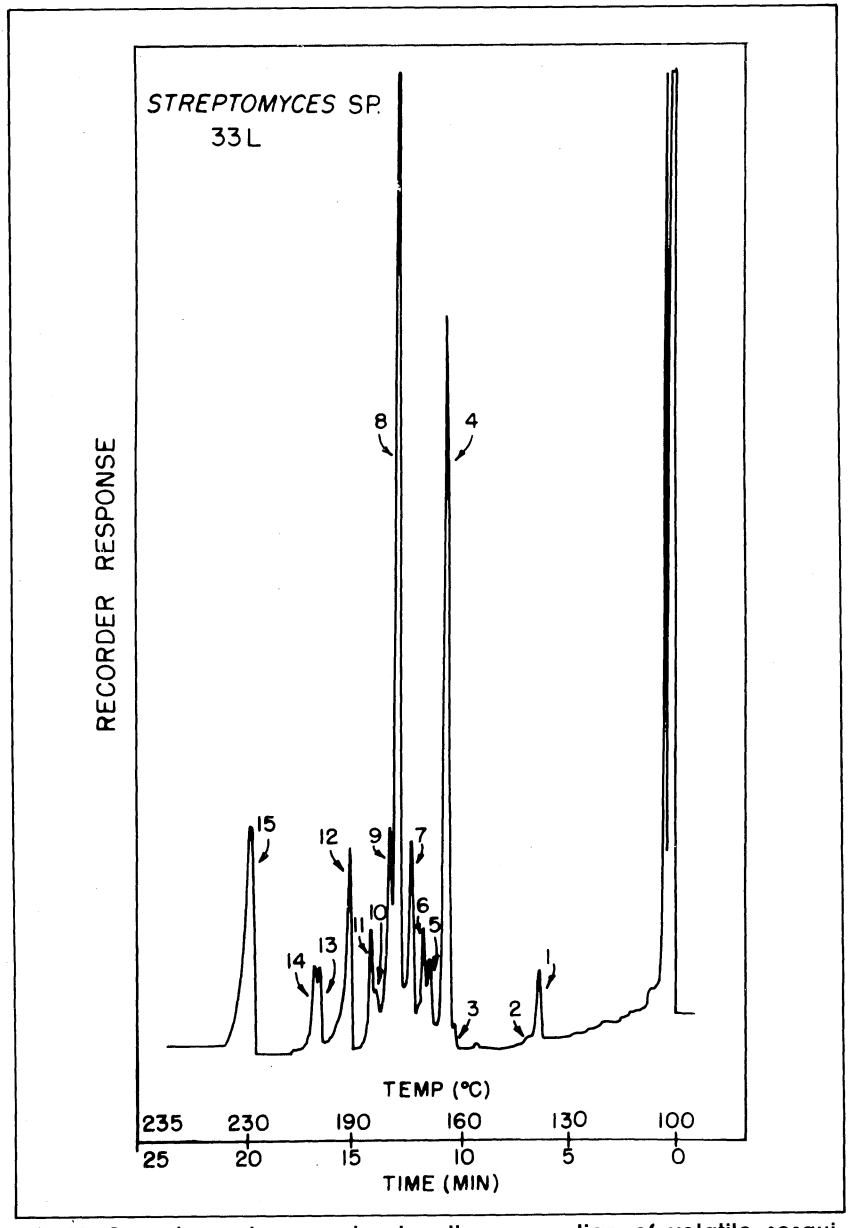


FIG. 19. Gas chromatogram showing the separation of volatile sesquiterpene products from STREPTOMYCES sp. 33L. Gas chromatographic separations of the sesquiterpenes were carried out using a 3 m x 2 mm glass column packed with GE SE-30 (3%) coated on Chromosorb Q (80/120 mesh). Oven temp. programmed from 100 to 235 C at 6 C/min, Injector and detector temp. 250 C.

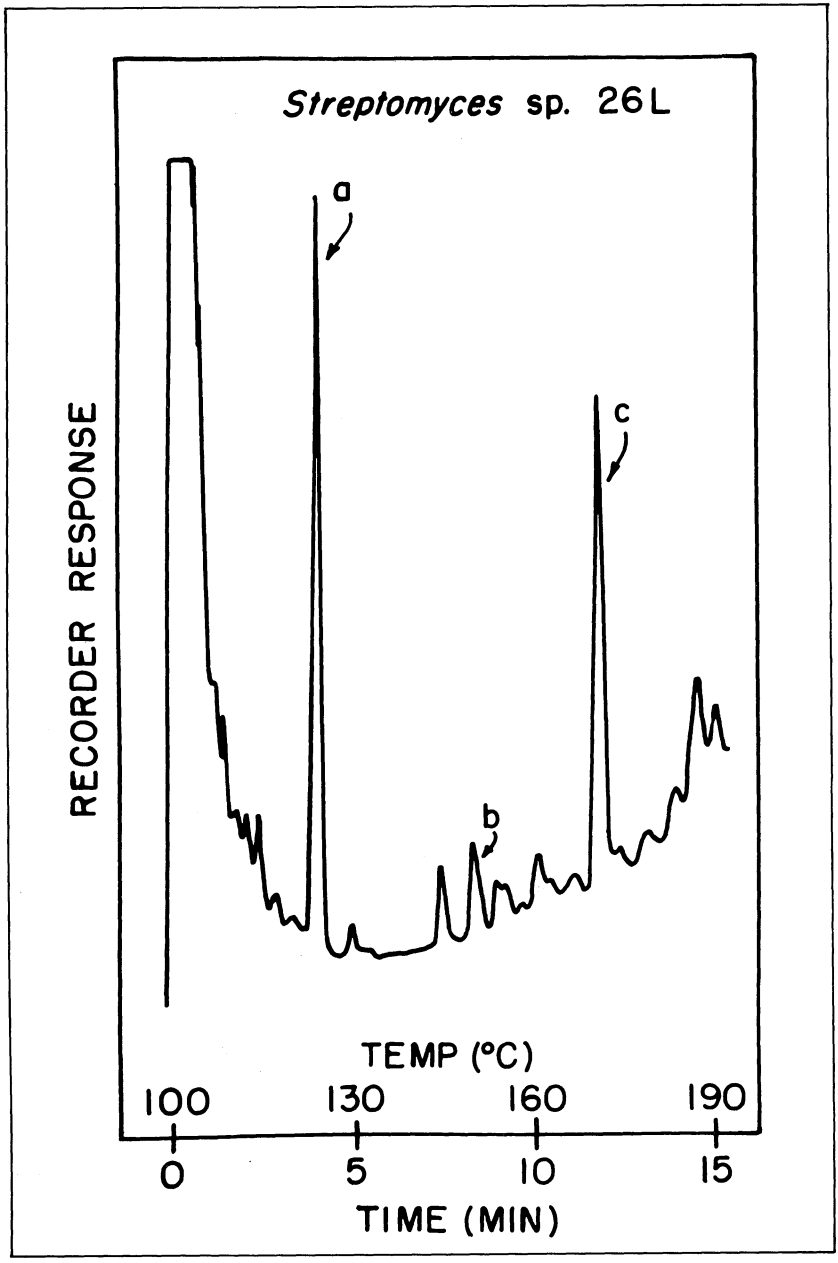


FIG. 20. Gas chromatogram showing the separation of volatile sesquiterpene products of STREPTOMYCES sp. 26L. GLC operating conditions are identical to those described in Fig. 1. a) 2-methyl-isoborneol, b) geosmin, and c) dibutyl phthalate contaminant.

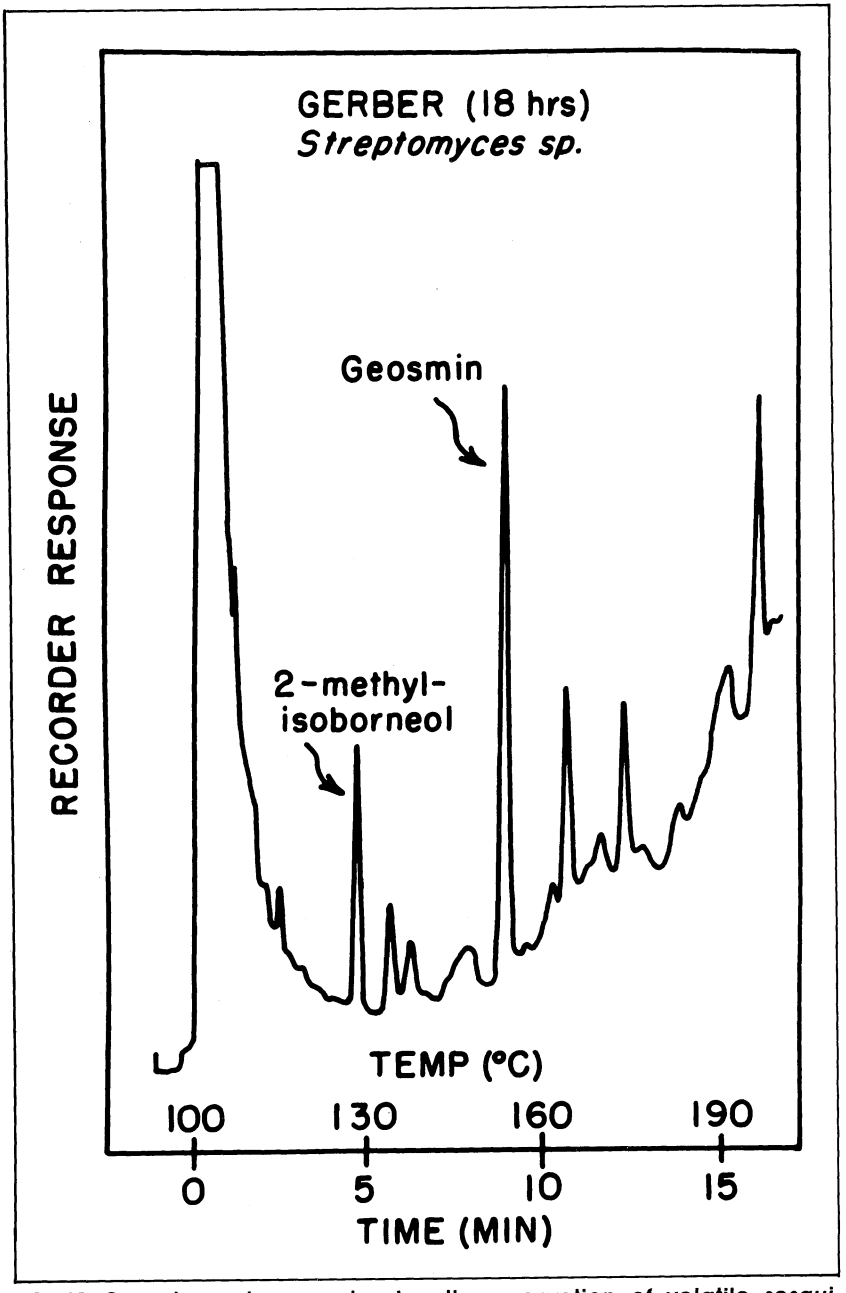


FIG. 21. Gas chromatogram showing the separation of volatile sesquiterpene products of STREPTOMYCES sp. CWW3. GLC operating conditions are identical to those described in Fig. 1.

TABLE 1. SUMMARY OF NITROGEN, PHOSPHORUS, DISSOLVED OXYGEN, AND CARBON DATA FOR WATER SAMPLES TAKEN FROM CHEWACLA CREEK AND LAKE OGLETREE DURING THE STUDY PERIOD

Parameter measured	1975[a]		1976[a]		Average	
	Low	High	Low	High	Low	High
Nitrogen (mg/l)						
Total						
Creek	ND	10.5	ND	5.7	0.89	1.69
Lake	ND	4.5	ND	3.5	0.66	0.99
Ammonia						
Creek	ND	0.10	0.06	0.51	0.03	0.23
Lake	ND	0.37	ND	0.87	0.06	0.20
Nitrate						
Creek	ND	1.22	1.0	3.26	0.17	1.35
Lake	ND	2.06	ND	2.01	0.29	0.83
Phosphorus (mg/l)						
Creek	ND	125.0	ND	167.0	13.6	6.0
Lake	ND	29.0	—[b]	—[b]	11.0	—[b]
Carbon (mg/l)[c]						
Creek	6.8	49.2	—	—	15.6	16.6
Lake	8.2	59.9	—	—	—	—
Dissolved Oxygen (ppm)						
Creek	4.8	12.5	5.5	14.0	8.5	9.1
Lake	5.8	10.0	5.5	11.0	8.5	9.2
pH[d]						
Creek	5.0	8.5	—	—	—	6.8
Lake	—	—	—	—	—	6.9

[a] Values represent monthly averages
[b] Little phosphorus detected
[c] Values for carbon are for samples taken during 1975 and January, 1976
[d] Values represent averages for the study period

(14). Unless otherwise specified, chemical analyses and physical data were collected each month of 1975 and during January through July of 1976.

High phosphorus concentrations, together with nitrates and organic carbon are sometimes associated with heavy aquatic plant growth, but these substances may also have other origins such as run-off wastes, etc. It is generally accepted that uncontaminated water contains 10-30 μg/l total phosphorus, but slightly higher concentrations may be encountered. Monthly averages for phosphorus (orthophosphorus phosphate) at Chewacla Creek sites samples in 1975 ranged from N.D. (not detectable) to 125 μg/l. The average phosphorus concentration for all sites for 1975 was 13.6 μg/l. Phosphorus was detected at these sites primarily between January and July, with little phosphorus being detected between August and December of 1975. In 1976 the average monthly phosphorus concentration ranged between N.D. and 167 μg/l with an average of 6 μg/l for the sampling period. Phosphorus was detected less frequently during the sampling period of 1976, compared to the sampling period of 1975. At only four sites in 3 different months did the phosphorus concentration exceed levels of uncontaminated water in 1975 and at only three sites during 2 months were the levels above 30 μg/l in 1976. Occasional high phosphorus readings were encountered, but were not

consistent with respect to sampling date or sites. The Alabama Water Improvement Commission (AWIC) found orthophosphates at 5 μg/l and total phosphorus at 77 μg/l in a survey from April through June, 1974 (2). The phosphorus content of samples taken from sites on Lake Ogletree in 1975 ranged from N.D. to 29.0 μg/l for an average of 11.0 μg/l. Very little phosphorus was detected in samples from lake sites in 1976.

The nitrogen content of water from the creek and lake sites was measured as free ammonia and nitrate. Free ammonia represents the first product of the decomposition of organic matter, and high levels of this form of nitrogen indicates "fresh pollution," although some exceptions do exist (14). Low values for free ammonia content are 0.015 to 0.03 mg/l (ppm) and high values are 0.10 mg/l or above. Since nitrates are the final product of the biochemical oxidation of ammonia, their presence indicates "previous" pollution. Its presence may indicate pollution such as nitrogenous organic matter of animal or possibly plant origin. Manure and fertilizer contain high levels of nitrates. Low levels of nitrate in water are less than 0.1 mg/l and high levels are greater than 1.0 mg/l.

The total nitrogen (ammonia plus nitrate) content of water from both the creek and lake sites taken in this study was generally considered moderate to high, based on established standards for uncontaminated water. For drinking water, an upper level of 45 mg/l nitrate has been set by the EPA (Federal Registrar, EPA, Water Programs, National Interior Primary Drinking Water Regulations). Total nitrogen in water from the creek sites ranged from N.D. to 10.52 mg/l in 1975 and N.D. to 5.71 in 1976. Total nitrogen values for lake samples ranged from N.D. to 4.54 mg/l for 1975 and N.D. to 3.54 mg/l for 1976. The average total nitrogen values for creek sites were 0.89 mg/l in 1975 and 1.69 mg/l in 1976 which is higher than the value of 0.605 mg/l previously reported. (2). For lake samples, average nitrogen values were 0.66 mg/l in 1975 and 0.99 mg/l in 1976. In this study, ammonia content of water contributed a relatively minor amount to the total nitrogen values. Ammonia from creek samples ranged from N.D. to 0.10 mg/l in 1975 and 0.06 to 0.51 mg/l in 1976, for averages of 0.03 and 0.23 mg/l, respectively. For lake samples, the ammonia content ranged from N.D. to 0.37 mg/l in 1975 and N.D. to 0.87 mg/l in 1976, for averages of 0.056 and 0.20 mg/l, respectively. These values are generally higher than that found by the Alabama Water Improvement Commission (AWIC) in samples taken from Lake Ogletree during April through June, 1974 (2). The AWIC found a mean of 0.085 mg/l for ammonia.

Nitrate concentrations for creek samples ranged from N.D. to 1.22 mg/l for 1975 and from 1.0 to 3.26 mg/l for 1976, with average values at 0.17 and 1.35 mg/l, respectively. These values are considerably higher than that for samples from Chewacla Creek reported by the AWIC for April through June,

1974. Lake samples contained between N.D. and 2.06 mg/l for an average of 0.29 in 1975 and between N.D. and 2.01 mg/l for an average of 0.83 mg/l in 1976. These values are lower than the single value of 4.25 mg/l obtained in October, 1975 by the Alabama Environmental Health Administration, Division of Public Water Supplies *(2)*.

It is apparent that the overall nitrogen values were considerably higher in 1976 than 1975, but the difference was only slightly detectable in the odor level during the study period. While the ammonia content of the water, creek, and lake, was relatively high during periods when the odor appeared, it also reached higher levels at periods when the odor was not present. As long as the appropriate nutrients *(see below)* are available, the single most important factor for geosmin production appears to be a particular stage of growth of the actinomycetes. Although ammonia or nitrate *per se* do not appear to be the primary triggering factors for geosmin production by actinomycetes *(see below)*, high levels of these substances suggest contamination of organic material that may stimulate odor production.

Knowledge of the organic carbon content of water is important from the standpoint of its effect on the dissolved oxygen. A high organic carbon content of water corresponds to low dissolved oxygen, since the latter is consumed in the oxidation of organic matter. The organic carbon content of creek and lake samples taken in this study was monitored only during 1975 and January, 1976 *(because the carbon analyzer was not available after the latter date)*. The organic carbon content of Chewacla Creek samples ranged from 6.8 to 49.2 mg/l, and in samples from Ogletree Lake it ranged from 8.2 to 59.9 mg/l for yearly averages of 15.6 and 16.6 mg/l, respectively. The same general trend in the organic carbon content of the water was found in samples from creek and lake sites throughout the sampling period, Figure 22. These values are almost two times greater than that found by the AWIC for samples taken from April through June, 1974 *(2)*. Although the values found in this study fall within the range considered acceptable for untreated waters, the organic carbon content of the water was generally higher during the brief odor period of 1975.

Dissolved oxygen is often used as a measure of water quality because it is required to support aquatic life. Dissolved oxygen levels of 2.5 to 3.0 ppm are required to prevent secondary tastes and odors from developing and to support fish life. Levels of 5.0 ppm are required for game fish reproduction and is the lower limit standard set by the AWIC. In 1975, dissolved oxygen concentrations in Chewacla Creek ranged from 4.8 to 12.5 ppm for an average of 8.5, and 1976 ranged from 5.5 to 14.0 ppm for an average of 9.1. In the lake, dissolved oxygen levels ranged from 5.0 to 10.0 ppm for an average of 8.5 in 1975, and 1976 ranged from 5.5 to 11.0 ppm with an average of 9.2. These values are within the range for raw water and are similar to a previously reported value of 9.0 ppm reported for Chewacla Creek *(2)*. Changes in the

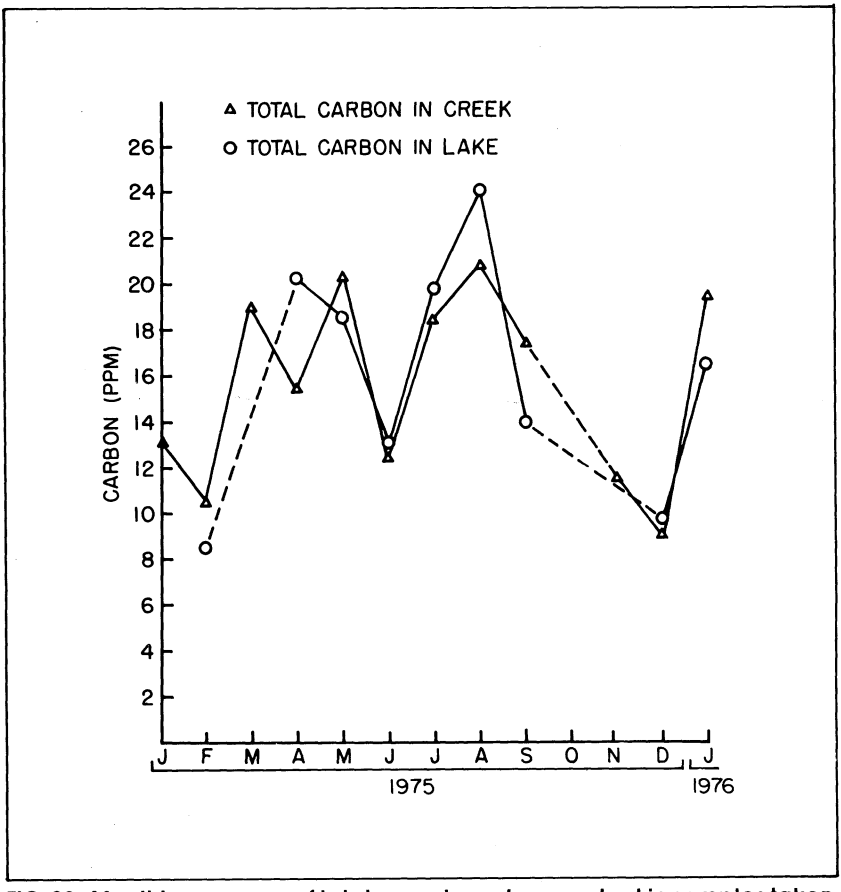


FIG. 22. Monthly averages of total organic carbon content in samples taken from sites on Chewacla Creek and Ogletree Lake.

dissolved oxygen content of the water correlate generally with changes in water temperature, as would be expected, Figure 23. Higher dissolved oxygen values were obtained during the cooler months of December-January each year.

Untreated water ranges between 5.0 and 8.5 in pH (a measure of acidity or alkalinity). Little fluctuation in pH values was noted in either the creek or lake sites of this study which averaged 6.8 and 6.9, respectively. This is consistent with values obtained at the Auburn Water Treatment Plant for raw water and the only other known report of 6.7 reported by the Environmental Health Administration (2).

Water temperature follows natural seasonal cycles and may be one of the most important factors in odor production (see below).

Laboratory Studies. A laboratory study was conducted on the effects of various physical and chemical factors that may be responsible for geosmin

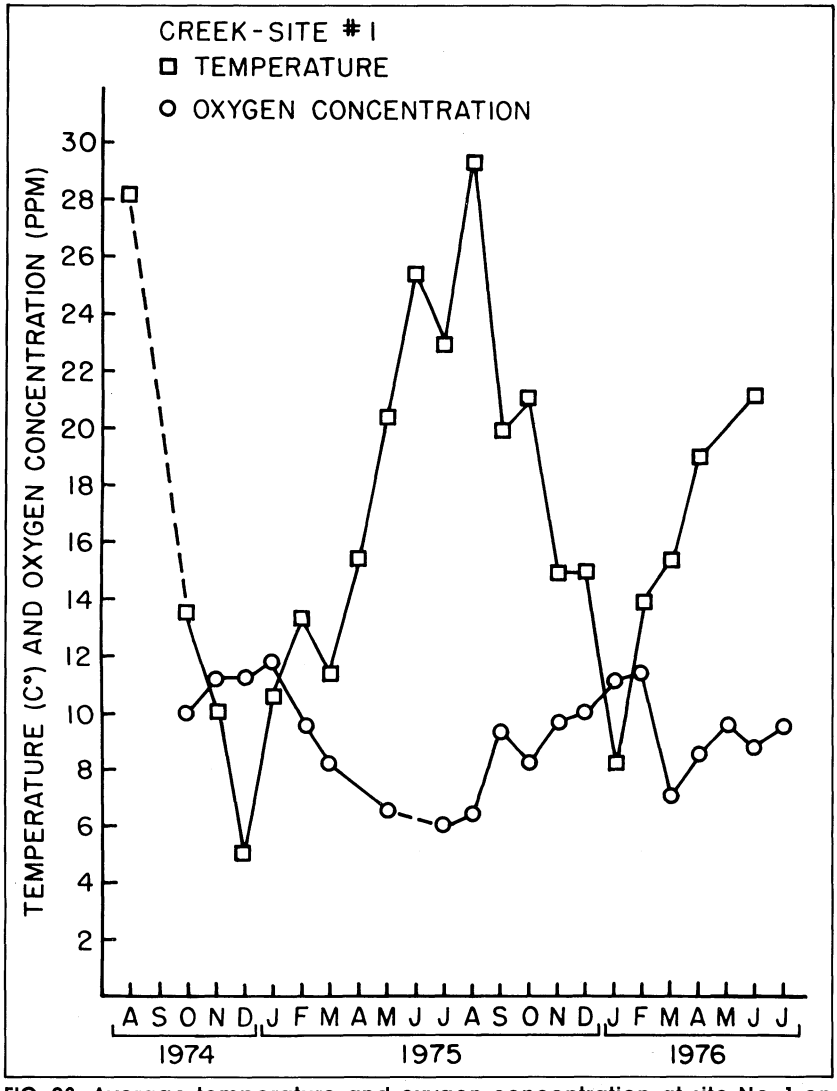


FIG. 23. Average temperature and oxygen concentration at site No. 1 on Chewacla Creek in 1974, 1975, and 1976.

production by actinomycetes in the field. *Streptomyces* isolate 33L was used in this study, and factors tested included temperature, pH, carbon substrate, phosphorus, and nitrogen. In addition, the effect of fresh cow manure on growth and geosmin production was determined.

Optimal temperature for the growth of *Streptomyces* isolate 33L was 30 C, but the organism was capable of growth throughout the 10 to 35 C temperature range tested, Figure 24. This is the temperature range en-

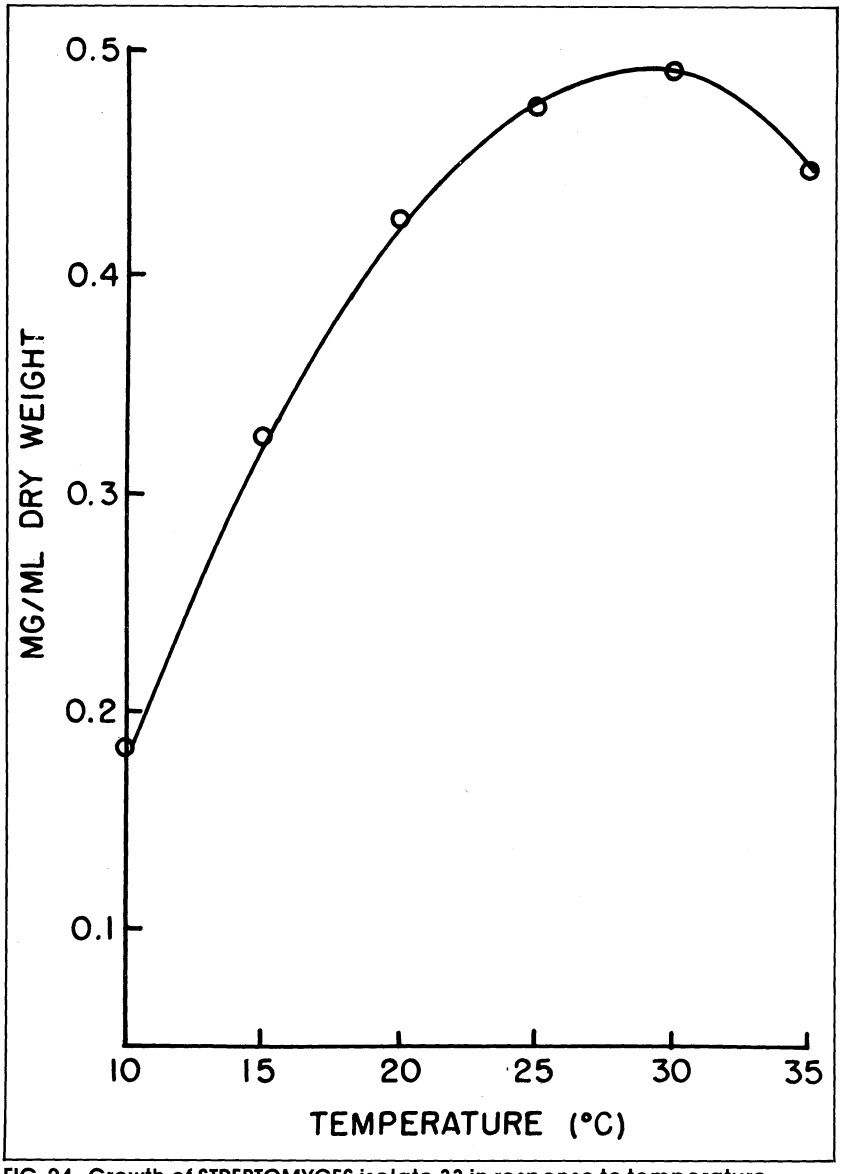


FIG. 24. Growth of STREPTOMYCES isolate 33 in response to temperature.

countered in Chewacla Creek and Lake Ogletree during a typical year. Optimal temperature for geosmin production by this isolate was 25 C; no geosmin was detected when the organism was grown at temperatures below 15 C, Table 2. In addition, sporulation did not occur below 15 C and the degree of sporulation increased as the growth temperature was elevated above 15 C.

TABLE 2. EFFECT OF TEMPERATURE ON GEOSMIN PRODUCTION[a]

TEMPERATURE (C)	μG GEOSMIN/MG DRY WT[b]
10	0.000[a]
15	0.061[b]
20	0.083[b]
25	0.276[c]
30	0.125[d]

[a] Triplicate plates for each temperature were incubated for 5 days and geosmin production was determined as described in Materials and Methods.

[b] Means having the same lower case letter are not significantly different at the 0.05 level of significance.

Growth of heterotrophic procaryotic microorganisms (bacteria and actinomycetes) is favored at higher pH values (greater than 7). *Streptomyces* isolate 33L grew best at pH values between 7 and 9, Figure 25. While geosmin was detected at all pH values tested, greatest production was found at the higher pH values, Table 3.

The effects of phosphorus on growth and geosmin production by *Streptomyces* isolate 33L were determined. Growth increased linearly with increasing phosphorus concentration in the range 0.0258 to 0.774 μg/ml, Figure 26. Geosmin was detected at the phosphorus concentrations tested, but the levels were generally low. Phosphorus seems to have little stimulatory effect on geosmin production.

Effects of various carbon substrates on growth and geosmin production by isolate 33L were determined by substituting the selected substrate for glucose in RS medium containing 0.75 percent yeast extract. Succinate, glycerol, and sucrose were more effective as a substrate for growth than others tested such as glucose, cellobiose, maltose, yeast extract alone, and lactose, Table 4. However, succinate, sucrose, and lactose were more effective as substrates for geosmin production relative to the yeast extract control, Table 5. Yeast extract supported limited cellular growth, but promoted greater geosmin production on a dry weight basis than glucose, cellobiose, maltose, or glycerol.

TABLE 3. EFFECT OF PH ON GEOSMIN PRODUCTION[a]

pH	μg Geosmin/mg dry wt[b]
10	0.281[a]
9	0.212[a]
8	0.185[ab]
7	0.173[bc]
6	0.154[c]
5	0.101[d]

[a] Triplicate plates for each pH were incubated for 5 days and geosmin production was determined as described in Materials and Methods.

[b] Means having the same lower case number are not significantly different at the 0.05 level of significance.

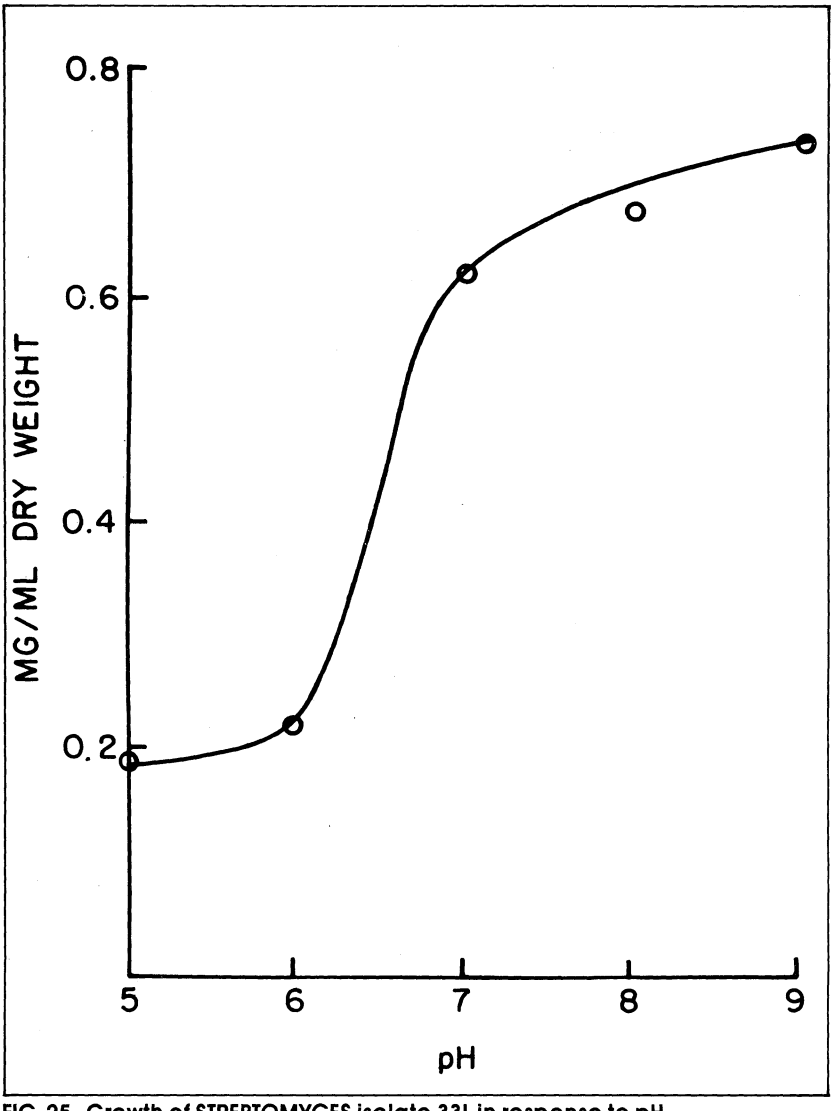


FIG. 25. Growth of STREPTOMYCES isolate 33L in response to pH.

The effects of nitrate (NO_3) and ammonia (NH_3) as sole nitrogen sources in supporting growth are shown in Table 6. Nitrate appears to be the best nitrogen source for growth of isolate 33L. In all cases, geosmin production was greater using both ammonia and nitrate as nitrogen sources and little differences between the two were noted. Generally, lower concentrations of either nitrogen source resulted in greater geosmin production on a dry weight basis.

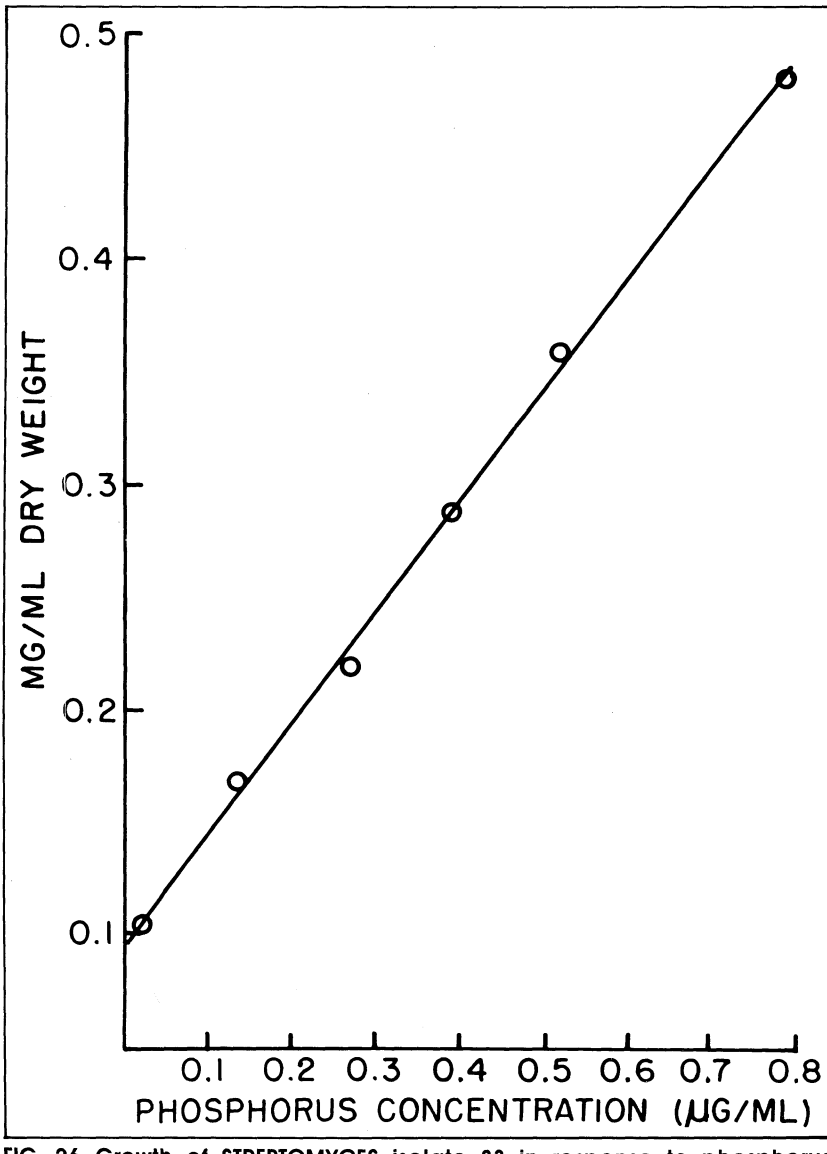


FIG. 26. Growth of STREPTOMYCES isolate 33 in response to phosphorus concentration.

Fresh cow manure used alone (in water agar) and as a supplement to RS medium was tested for its effect on growth and geosmin production by *Streptomyces* isolate 33L. There was considerable geosmin produced by the actinomycete on RS medium supplemented with manure, but there were no significant differences between dilutions (0.5, 1, 2.5, 5, 10, 100 g/l) and the

TABLE 4. EFFECT OF CARBON SOURCES ON GROWTH OF *STREPTOMYCES* ISOLATE 33L

Substrate[a]	Dry weight[b]
Cellobiose	1.48
Glucose	1.30
Glycerol	1.06
Succinate	1.00
Maltose	0.86
Xylose	0.82
Sucrose	0.62
Acetate	0.62
Galactose	0.61
Citrate	0.50
Lactose	0.15
Mannitol	0.09

[a] Substrate added in 50 mM concentration.

[b] Mean of 3 replicates in mg/ml.

TABLE 5. EFFECT OF CARBON SOURCES ON GEOSMIN PRODUCTION BY *STREPTOMYCES* ISOLATE 33L

Substrate[a]	Dry wt[b]	μg Geosmin[c]	μg Geosmin/mg dry wt[d]
Glucose	206	3.82	0.019[a]
Cellobiose	216	4.02	0.019[a]
Maltose	173	4.48	0.030[b]
Glycerol	255	7.68	0.030[b]
Yeast extract	64	2.01	0.031[b]
Lactose	159	5.59	0.035[c]
Sucrose	82	4.41	0.055[d]
Succinate	101	10.81	0.107[e]

[a] Media contained 0.75% yeast extract with carbon sources added at 2% w/v or v/v concentration.

[b] Mg dry wt after 5 days incubation at 26 C.

[c] μg geosmin calculated by comparing peak areas to reference geosmin (20 μg/ml).

[d] Means having the same lower case number are not significantly different at the 0.05 level of significance.

TABLE 6. EFFECT OF NITROGEN SOURCES ON GROWTH OF *STREPTOMYCES* ISOLATE 33L

Nitrogen conc.[a]	Dry wt (nitrate)[b]	Dry wt (ammonium)[b]
0.0655	2.8[a]	2.0[a]
0.131	3.5[b]	2.4[b]
0.262	4.2[c]	2.5[b]
0.524	5.4[d]	3.7[c]
1.31	5.5[d]	4.0[d]
2.64	6.5[e]	5.1[e]
5.24	8.0[f]	6.2[f]

[a] The medium of Romano and Safferman was supplemented with $NaNO_3$ or NH_4Cl. Final nitrogen concentration listed in μg/ml.

[b] Dry weight per ml of medium following 10 days incubation at 26 C. Means having the same lower case letter are not significantly different at the 0.05 level of significance.

TABLE 7. EFFECT OF AMINO ACIDS ON GEOSMIN PRODUCTION BY *STREPTOMYCES* ISOLATE 33L

Medium[a]	Geosmin[b] (μg/g dry wt)
Control (RS medium)	336
Yeast extract	160
Alanine	392
Aspartic acid	636
Asparagine	202
Glutamic acid	226
Glutamine	343

[a]With exception of the control, RS medium contained 0.75% yeast extract or 5mM concentration of the appropriate amino acid.

[b] Each value represents the average of 4 to 6 replications each of which represents 2-3 gas chromatographic analyses.

RS medium or yeast extract control. When isolate 33L was grown on water agar supplemented with fresh cow manure, however, an average of 6.6-fold increase in geosmin production on a cellular dry weight basis was found when compared to the RS medium supplemented with the manure.

In early experiments, the addition of yeast extract to the RS medium resulted in greater geosmin production by *Streptomyces* isolate 33L compared to RS medium used alone. Yeast extract is a rich source of vitamins, amino acids, and other substances that may stimulate geosmin production by actinomycetes. In a preliminary experiment, the following substances were screened for their ability to enhance geosmin production by isolate 33L: p-aminobenzoic acid, nicotinic acid, vitamin B, inositol, riboflavin, pyridoxine, pantothenate, thiamine, and cholesterol. In addition, the following amino acids were tested: glutamine, asparagine, alanine, glutamic acid, and aspartic acid. In the preliminary screening, neither the vitamins nor cholesterol stimulated geosmin production; there was evidence of stimulation only for the amino acids. For this reason, the amino acids, which were selected for their potential as an organic nitrogen source or relationship to organic acid metabolism, were checked again for their effects on geosmin production. The results of this experiment are given in Table 7. In this case, the production of geosmin by *Streptomyces* isolate 33L grown in RS medium supplemented with yeast extract was about one-half that when the organism was grown on RS medium alone. With the exception of aspartic acid, none of the amino acids tested resulted in significant difference in geosmin production over the control (RS medium). Isolate 33L grown on aspartic acid resulted in almost a two-fold increase in geosmin production. Implications of this result with respect to geosmin production in the field are not clear.

Correlation of Rainfall, Temperature, and Threshold Odor Number (TON)

Historically, the period of intense odor experienced in Auburn has always been during the late winter or early spring. In 1974, the odor was particularly intense and lasted from mid-January through April. In 1975 and 1976, the odor was barely detectable and lasted only a short time. In 1975, the odor was detected for only a few days in May; in 1976, the odor was detected in mid-April and lasted only a couple of weeks. Very few Auburn citizens could detect the odor during 1975 and 1976. Tables 8 through 10 show the water

TABLE 8. AVERAGE WEEKLY WATER TEMPERATURE AND AVERAGE THRESHOLD ODOR NUMBER AT AUBURN WATER TREATMENT PLANT FOR ODOR PERIOD OF 1974[a]

Days	December, 1973		January, 1974		February, 1974		March, 1974		April, 1974	
	T	TON	T	TON	T	TON	T	TON	T	TON
1-7	59.7	—	54.6	—	59.6	22.9	58.0	5.7	64.4	5.0
8-14	54.7	—	56.9	—	54.7	19.1	64.3	5.9	63.3	4.7
15-21	50.9	—	56.4	—	56.0	8.7	61.2	4.7	65.9	2.4
22-28	51.0	—	60.4	—	53.9	5.6	59.4	7.0	67.0	3.2[b]
29-31	53.0	—	61.3	33.3	—	—	63.0	4.7	71.0	—

[a] T = Degrees farenheit
TON = Threshold odor number
[b] Last recorded TON test on April 22, 1974.

TABLE 9. AVERAGE WEEKLY WATER TEMPERATURE AND AVERAGE THRESHOLD ODOR NUMBER AT AUBURN WATER TREATMENT PLANT FOR ODOR PERIOD OF 1975

Days	January, 1975		February, 1975		March, 1975		April, 1975		May, 1975	
	T	TON	T	TON	T	TON	T	TON	T	TON
1-7	53.0	—	53.3	—	54.4	—	60.3	1.2	66.4	1.0
8-14	53.0	—	51.4	—	55.6	—	59.6	1.0	66.9	1.0
15-21	50.0	—	55.3	—	55.6	—	61.0	1.0	68.9	1.5
22-28	51.6	—	56.4	—	60.3	—	65.4	1.0	72.6	5.0
29-31	55.7	—	—	—	60.7	—	65.5	1.0	71.3	3.8

TABLE 10. AVERAGE WEEKLY WATER TEMPERATURE AND AVERAGE THRESHOLD ODOR NUMBER AT AUBURN WATER TREATMENT PLANT FOR ODOR PERIOD OF 1976

Days	January, 1976		February, 1976		March, 1976		April, 1976		May, 1976	
	T	TON	T	TON	T	TON	T	TON	T	TON
1-7	48.4	—	49.7	—	61.1	—	63.9	—	68.4	1.0
8-14	45.4	—	51.7	—	60.4	—	65.7	2.0	68.4	1.0
15-21	44.1	—	56.9	—	59.1	—	69.0	1.6	69.1	—
22-28	47.7	—	57.3	—	60.6	—	70.0	1.2	70.0	1.0
29-31	48.7	—	60.0	—	63.0	—	68.0	1.0	71.3	—

temperatures and the threshold odor numbers as measured at the Auburn Water Treatment Plant for the odor periods of 1974, 1975, and 1976, respectively. Each year, the odor was not detected until water temperatures were sustained near 60 F, or about 15 C. A high threshold odor number of 50 was obtained in the last week of January, 1974, with a weekly average of 33.3. This is one of the highest TON readings recorded at the treatment plant. In 1975, the highest TON value was 5.0; and in 1976, the highest TON value was 2.0. These values accounted for only a slight odor in the treated drinking water.

Since rainfall is a means by which excessive nutrients from cattle feedlots, fertilized fields, etc. can be introduced into streams and lakes; it is useful to know the rainfall patterns before and during the odor episodes of each year. Table 11 shows monthly precipitation for the latter part of 1973 and for 1974, 1975, and 1976. In December, 1973 and January, 1974, a total of 20 inches of rain was recorded in Auburn, 9.8 inches above normal. Then in February and March 1974, 7.13 inches were recorded, more than 5 inches below normal. The temperature of the water (at the water plant) reached 15 C in mid-January, and the intense odor closely followed. Since the subsequent rainfall for February and March was very low, a dilution factor due to additional water was not introduced and the odor remained in the water at relatively high levels through April. In contrast, the temperature in 1975 did not reach 15 C until late March. Moreover, the rainfall in January, February, and March was nearly 7 inches above normal, and a slight odor was detected only at the water plant in the first week of April. However, 10.51 inches of rain fell in April, 1975, which was 5.3 inches above normal. This probably had an overall dilution effect on the developing odor. The rainfall was normal in May and the odor was again slightly detectable during mid-May for about 2 weeks. In 1976, the temperature reached 15 C the first of March, but the odor was not detected

TABLE 11. MONTHLY PRECIPITATION FOR 1973, 1974, 1975, 1976[a]

Month	Normal Rainfall	1973 Rainfall	1974 Rainfall	1975 Rainfall	1976 Rainfall
	In.	*In.*	*In.*	*In.*	*In.*
Jan.	4.83	—	8.66	6.65	3.58
Feb.	5.32	—	4.09	8.35	2.30
Mar.	6.93	—	3.04	8.44	7.54
April	5.21	—	5.10	10.51	2.90
May	3.86	—	4.80	4.39	8.00
June	4.25	—	1.86	4.69	5.04
July	5.38	—	2.71	11.39	4.21
Aug.	4.07	—	5.73	6.87	—
Sept.	4.20	2.50	5.87	6.06	—
Oct.	2.51	0.90	1.25	7.75	—
Nov.	3.89	3.00	2.92	3.22	—
Dec.	5.51	11.40	6.95	4.63	—

[a] Obtained from Environmental Study Service Center, Auburn University

until April 14. The rainfall in early 1976 was below normal. However, in March 7.54 inches of rain fell, which was only about 0.6 inches above normal. This could have washed enough nutrients into the watershed to enhance odor production, which was detected in mid-April. April rainfall was more than 2 inches below normal.

There is a very good correlation between the odor episodes and the water temperatures taken in Chewacla Creek. At every creek site, the water temperature reached an average of 15 C in April in 1975, with the maximum TON occurring in early to mid-May. In 1976, the creek site temperatures reached 15 C in March, whereas the maximum TON was reached by mid-April, example, Figure 23. Since the odor was detected about 3-4 weeks following the time when water in Chewacla Creek reached 15 C, these data may be useful information for predicting approximate dates when the odor episodes may be encountered. Lake water reached 15 C about 2 weeks before that in the creek site. Water taken from lake sites was from shallow areas where little or no current existed, however, accounting for an earlier rise in temperature. Lake site readings may also vary with the time of the day.

SUMMARY

In the winter and spring of 1974, drinking water in Auburn, Alabama contained a highly unpleasant earthy odor. While Auburn has a history of such a problem, the odor had never been severe enough to cause difficulty in drinking the water. The Auburn Water Board contracted Auburn University to investigate the chemical, biological, and environmental factors responsible for the recurring earthy odor. Lake Ogletree and a tributary stream Chewacla Creek, the source of Auburn water, were examined at regular intervals over a 2-year period to determine fluctuations in the microbial flora and to search for environmental trends that may promote the production of geosmin. During this 2-year period, no intense odor episode occurred, although very short periods of low intensity odor occurred in both 1975 and 1976.

Untreated water normally contains naturally occurring organic chemicals, some of which may be odorous. In most cases, these substances are present in relatively low levels and are not generally detected by taste or smell. Under certain circumstances, however, organic chemicals, including odorous substances, may accumulate in water due to increased activity of certain types of organisms that make up the natural microflora or industrial effluents. Earthy odors in treated and untreated water are a problem encountered by many cities and towns throughout the world. The principal chemical agent most frequently responsible for the earthy odor is called geosmin.

Like other water sources, Lake Ogletree, the Auburn water supply, contains many organisms, some of which are odorous. It was confirmed in this study that geosmin was the principal chemical agent responsible for the 1974 odor

episode in the Auburn water. However, the odor was only slightly detectable for brief intervals during mid-May, 1975 and the latter part of April, 1976.

The presence of geosmin in higher plant tissues has been recently reported; however, certain blue-green algae and actinomycetes are the only organisms known to produce geosmin at this time. Bacteria, including actinomycetes, and algae in water and mud samples from Chewacla Creek and Lake Ogletree were screened for geosmin production. Only acinomycetes from these sources produced geosmin under the conditions used in our laboratory.

Throughout the year, microbial components of the creek and lake water fluctuate in number and kind. The most visible evidence of this fluctuation are algal blooms, which are the result of a very rapid proliferation of cells when conditions are favorable. Actinomycetes and bacteria also have periods of rapid cellular growth, but this growth is not apparent by superficial observation. No algal blooms were associated with earthy odors in the Auburn water supply during the study period.

Although increase in actinomycete numbers does not necessarily indicate that there will be a corresponding odor episode, the brief odor episodes observed were accompanied by a prior increase in actinomycete numbers. Blue-green algae cannot be ruled out as a potential source of the odor problem; however, actinomycetes *(Streptomyces* sp.*)* are considered the principal geosmin-producing organisms in Chewacla Creek and Lake Ogletree and the primary cause of the earthy odor problem in the Auburn water supply.

Although bacteria, other than actinomycetes, are not responsible for the odor problem in Auburn water, certain species indicate the presence of organic pollution that may provide a source of nutrients for geosmin producers. Hence, total bacteria, coliforms, and streptococci, which are indicators of fecal pollution, were enumerated at various locations along Chewacla Creek between Lee County Highway 12 and the junction of this creek with Lake Ogletree. The highest numbers of total bacteria were present in the summer months and the lowest numbers were isolated between October and December. At sampling sites on Chewacla Creek adjacent to or immediately downstream from a source of animal pollution, a sharp rise in total numbers was evident in December through February. This increase could be indicative of increased nutrient input into the Auburn water supply during a period before onset of the odor episodes.

The presence of fecal coliforms and streptococci demonstrates the presence of animal wastes in Chewacla Creek. The sharp increase in numbers of fecal coliforms below a private dairy farm shows that this serves as a direct source of contamination of Chewacla Creek. The decrease in numbers each year from 1974 to 1976 may indicate a decreasing contribution of this facility to the pollution of the Auburn water supply. The ratios of fecal streptococci/fecal coliform (FS/FC) indicate pollution was due to livestock; however, the ratios in 1976 indicate a decreasing contribution of livestock wastes. This fecal

contamination indicates that materials representing potential nutrients for odor-producing microorganisms are being put into the water supply, a cause for concern to public health.

No correlations were detected between odor production in the creek and lake and the carbon, nitrogen, phosphorus, and oxygen content and pH of the water. Laboratory studies showed a correlation between geosmin production and carbon substrate, and nitrogen. If, indeed, the relationship between these parameters and geosmin production are detectable in nature, it is unlikely that a correlation would have been uncovered in this study since the odor was only slightly detectable for brief periods during this investigation.

There was a correlation between the odor episodes and two environmental parameters, temperature, and rainfall. Data compiled from temperature and TON readings at the Auburn Water Treatment Plant show that the odor was detectable only after the water temperatures reached approximately 15 C. The intensity of the odor appears to be related to amounts of rainfall before and during the odor episodes.

Above normal rainfall can introduce excessive nutrients into Lake Ogletree prior to the 15 C critical temperature (critical for growth and sporulation of actinomycetes). If excessive rainfall continues after the water temperature reaches 15 C, a dilution effect may decrease the intensity of the odor. If low amounts of rainfall occur immediately after the water reaches 15 C, however, an intense and lengthy odor episode may follow.

Since Chewacla Creek reached a sustained temperature (April in 1975; Mid-March in 1976) of 15 C, about 3-4 weeks prior to any detectable odor, the rainfall pattern during this 3-4 week period may have determined the severity of the episode, assuming that excessive rainfall occurred prior to the 15 C critical temperature. In 1975, rainfall in the 3 months prior to the detectable odor was somewhat above normal. Following onset of the odor in April, however, the rainfall was excessive, 5 inches above normal, and resulted in a reduced odor intensity. In 1976, the rainfall prior to odor detection was below normal with the exception of March which showed slightly above normal rainfall. Rainfall during the episode was far above normal, however, again diluting the odor compound and decreasing the intensity. As stated earlier, coliform levels in Chewacla Creek were lower in 1976 than 1975 with the result of lower contaminating nutrient level.

Since actinomycetes are present in the water and mud throughout the year, the question remains as to the mechanism responsible for geosmin production at a certain time of the year. Geosmin production in nature cannot be explained on the basis of a single chemical or environmental factor. Instead, it is the result of precise timing between the occurrence of a particular stage of growth in the actinomycete life cycle and level of available nutrients, particularly organic matter, which are influenced by certain environmental factors or events.

The life cycle of actinomycetes has been studied in the laboratory and several stages of growth have been observed (15). These stages include a primary and secondary mycelium and spores. The primary and secondary mycelium seem to differ at least to some extent in their growth requirements, and odor production is associated with spore release and fragmentation of secondary mycelia. Increases in actinomycete numbers, however, may not necessarily correspond to the occurrence of detectable odor. In those cases, increase in actinomycete numbers may have been due to propagation (vegetative fragmentation) of the organism by a stage of growth not associated with geosmin production.

Temperature seems to be the overriding factor controlling the rate of growth and transition from one growth stage to another. However, in the case of transition from primary to secondary mycelium, temperature may be only indirectly involved through its effects on the oxygen content of water. Higher oxygen content seems to favor the transition from primary to secondary mycelia. The observation that odor episodes usually disappear by mid to late May could be due to oxygen stratification of Lake Ogletree so that even in shallow water (upper end of Lake Ogletree) anaerobic conditions exist in the bottom muds. These anaerobic conditions are unfavorable for growth or odor production or both by actinomycetes. In this geographical region, oxygen stratification in many lakes and ponds usually occurs by June, personal communication, Fisheries and Allied Aquacultures, and typically lasts through October.

REFERENCES

(1) BAYS, L. R., N. P. BURMAN, AND M. W. LEWIS. 1970. Taste and odor in water supplies in Great Britain: A survey of the present position and problems for the future. Water and Treat. Exam. 19:136-160.

(2) CONVERSE J. B. AND CO., INC. AND BETZ ENVIRONMENTAL ENGINEERS INC. 1976. Environmental Impact Study for City of Opelika, AL.

(3) DICKSON, K. L. 1968. Actinomycetes and water quality. J. Amer. Water Works Assoc. 60:379-381.

(4) DOUGHERTY, J. D. AND R. L. MORRIS. 1967. Studies on the removal of actinomycete musty tastes and odors in water supplies. J. Amer. Water Works Assoc. 59:1322-1326.

(5) GERBER, N. N. 1967. Geosmin, an earthy-smelling substance isolated from actinomycetes. Biotechnol. Bioeng. 9:321-327.

(6) GERBER, N. N. 1969. A volatile metabolite of actinomycetes, 2-methyl-isoborneol. J. Antibiotics 22: 508-509.

(7) HOHEN, R. C. 1965. Biological Methods for the Control of Tastes and Odors. Southwest Water Works Journal, 47:3-26.

(8) KIKUCHI, T., MIMVRA, T., MASADA, Y., INOVE, T. 1973. Odorous compounds in water supplies. Identification of geosmin from water of Lake Inbanuma by mass spectrometry combined with gas chromatography. Chem. Pharm. Bull. 21:1847-1848.

(9) LEVENTER, H. AND J. EREN. 1969. Taste and odor in the reservoirs of the Israel National Water System. *In* Developments in Water Quality Research. Proc. Jerusalem International Conference on Water Quality. pp. 19-37.

(10) MORRIS, R. L. 1962. Actinomycetes studied as taste and odor cause. Water and Sewage Works. 109:76-77.

(11) PIET, G. J., B. C. J. ZOETEMAN, AND A. J. A. KRAYEVELD. 1972. Earthy-smelling substances in surface waters of the Netherlands. Water Treat. and Exam. 21:281-286.

(12) ROMANO, A. H. AND R. S. SAFFERMAN. 1963. Studies on actinomycetes and their odors. AWWA 55, 169-176.

(13) ROSEN, A. A., C. I. MASHNI, AND R. S. SAFFERMAN. 1970. Recent developments in the chemistry of odor in water: The cause of earthy/musty odor. Water Treat. and Exam. 19, 106-119.

(14) SALVATO, J. A. 1972. Environmental Engineering and Sanitation (2nd). Wiley-Interscience. New York.

(15) SILVEY, J. K. G. 1974. Determination of nuisance organisms. Proc. AWWA Water Quality Technology Conference. Cincinnati, Ohio, Dec. 3, 4.

(16) SILVEY, J. K. G., D. E. HENLEY, W. J. NUNEZ, AND R. C. HOHEN. 1970. Biological Control. Control of Naturally Occurring Tastes and Odors by Micro-organisms. Proc. Natl. Biological Congress Detroit.

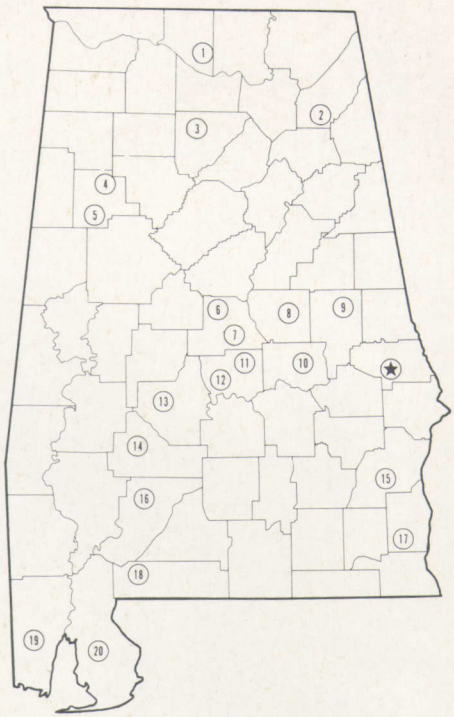
(17) SILVEY, J. K. G., D. E. HENLEY, AND J. T. WYATT. 1972. Planktonic blue-green algae: Growth and odor-production studies. J. Amer. Water Works Assoc. 64:35-39.

(18) SILVEY, J. K. G. AND A. W. ROACH. 1964. Studies on the microbiotic cycles in surface waters. J. Amer. Water Work Assoc. 56:60-72.

(19) STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER. 13th ed. American Public Health Association, Washington, D. C.

Alabama's Agricultural Experiment Station System
AUBURN UNIVERSITY

With an agricultural research unit in every major soil area, Auburn University serves the needs of field crop, livestock, forestry, and horticultural producers in each region in Alabama. Every citizen of the State has a stake in this research program, since any advantage from new and more economical ways of producing and handling farm products directly benefits the consuming public.



Research Unit Identification

★ Main Agricultural Experiment Station, Auburn.

1. Tennessee Valley Substation, Belle Mina.
2. Sand Mountain Substation, Crossville.
3. North Alabama Horticulture Substation, Cullman.
4. Upper Coastal Plain Substation, Winfield.
5. Forestry Unit, Fayette County.
6. Thorsby Foundation Seed Stocks Farm, Thorsby.
7. Chilton Area Horticulture Substation, Clanton.
8. Forestry Unit, Coosa County.
9. Piedmont Substation, Camp Hill.
10. Plant Breeding Unit, Tallassee.
11. Forestry Unit, Autauga County.
12. Prattville Experiment Field, Prattville.
13. Black Belt Substation, Marion Junction.
14. Lower Coastal Plain Substation, Camden.
15. Forestry Unit, Barbour County.
16. Monroeville Experiment Field, Monroeville.
17. Wiregrass Substation, Headland.
18. Brewton Experiment Field, Brewton.
19. Ornamental Horticulture Field Station, Spring Hill.
20. Gulf Coast Substation, Fairhope.