Broadcast of Microbial Aerosols by Stacks of Sewage Treatment Plants and Effects of Ozonation on Bacteria in the Gaseous Effluent

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THE PURPOSE of the investigation was to demonstrate that microbial pollution of the air by sewage treatment plants is an environmental factor which deserves more intensive study. The data derived from this study should be taken into account in planning and locating sewage treatment plants, since these facilities are often constructed in or near residential areas.

Kenline *(1)*, Adams and Spendlove *(2)*, Goff and co-workers *(3)*, and others have shown that microbial aerosols originating from sewage treatment installations do indeed contaminate the atmosphere downwind from these facilities. Coliform bacteria have been collected up to 1.2 km (0.8 miles) downwind from a sewage treatment plant's trickling filter system *(2)*. Ledbetter and Randall *(4,5)* concluded, after studying the aerosolization of bacteria from an activated sludge unit, that the number of micro-organisms in the air after passage over the aeration basins increased markedly and persisted for a considerable time and distance. They found enteric pathogens, especially *Klebsiella,* in large numbers in these aerosols. Dixon and McCabe *(6)* listed the following micro-organisms that have been found in sewage: *Salmonella, Shigella, Leptospira, Mycobacterium tuberculosis, Ascaris lumbricoides, Entamoeba histolytica,* and Coxsackie, poliomyelitis, and infectious hepatitis viruses. Grinstein and co-workers *(7)* probed a stream receiving chlorinated effluent from a sewage treatment plant for virus contamination; virulent strains of poliomyelitis and ECHO viruses were shown to survive the chlorination process.

Woodcock *(8)* and Blanchard and Syzdek *(9)* demonstrated that the spreading of micro-organisms in air is produced by bubbles breaking at the air-water interface of a body of water, where some micro-organisms tend to concentrate. We hypothesized that virulent viruses surviving in chlorinated sewage effluent may be aerosolized, as are bacteria, by this water-to-air transfer mechanism. That viruses can be transported by air currents indoors and that at least some of them remain infectious is well documented. An example of this transfer under nonlaboratory conditions occurred in an outbreak of airborne smallpox in a West German hospital *(10)*. Hemmes and co-workers *(11)*, Mayhew and Hahon *(12)*, and many other investigators have noted that the decay rate under various environmental conditions varies with the individual virus; that is, those conditions favorable to the survival of one virus may be detrimental to that of another and vice versa. Attempted studies of the effects of open air conditions on the infectivity of viruses by Benbough and Hood *(13)* and Berendt and Dorsey *(14)* have not yielded definitive results. In any case, as is pointed out by Zeterberg *(15,16)*, there is a strong probability that bacteria and viruses act synergistically in the causation of respiratory disease; only one virus particle lodged in the proper niche of the respiratory tree is required for infection; viruses need not retain their infectivity to cause deleterious effects on hypersensitive persons.

The sewage treatment plant in our study is one of the few in New York City where odor control is being attempted by mixing ozone with the gaseous effluent. This process is applied only at the building housing the thickening tanks (see chart). In the future, this system of odor control is slated for application to all sewage treatment plants in New York City (oral statement by Norman Nash, deputy director of plants, Bureau of Water Pollution Control, Department of Water Resources of the City of New York). Since our resources were limited, our research was intended to be only a

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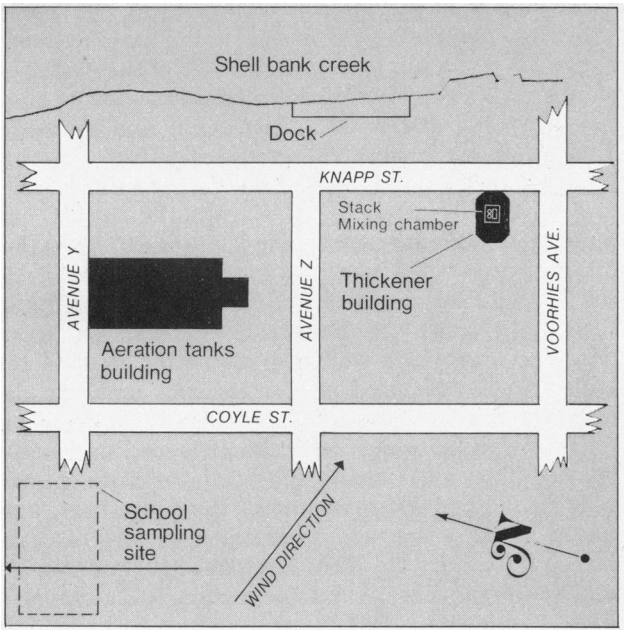
Sites of aerosol studies of sewage treatment plant



qualitative pilot study, and we have not attempted to draw quantitative conclusions.

Materials and Methods

Aerosol studies. We sampled the air in and around a New York City sewage treatment plant during the period from December 1970 to September 1971. Andersen samplers *(17)* were used to collect aerosols. The pumps of these samplers were calibrated to draw 0.028 cubic meters of air per minute (1 cubic foot per minute). The stages of the samplers were loaded with petri dishes containing either tryptic soy agar (TSA) or sheep blood agar (SBA), as we did not wish our collections to be subjected to an experimental bias *(18)*.

The aeration building of the plant measures about 120 meters in length, 30 meters in width, and 15 meters in height. In the north wall of the building are eight fans; each one draws in 300 cubic meters of air per minute. At the southern end of the building are two identical exhaust stacks, each with a diameter of 1.62 meters and each with two fans in parallel: together they exhaust 2,100 cubic meters of air per minute (see chart). The effect of all the fans is to draw the air inside the building over the aeration tanks and force it up the stacks and out into the air.

Sampling the open air in the vicinity of the plant from December 1970 to January 1971 was done equidistantly (about 300 meters from the aeration building) upwind at a neighborhood school and downwind at dockside on Shell Bank Creek (see chart). These stations were probably not the best locations from a maximum aerosol coverage standpoint, but they were the most accessible.

The air inside the aeration building was sampled during December 1970 and January and March 1971. The internal atmosphere of one exhaust stack was sampled during March 1971. The number of samplings for each location is shown in table 1. Colonies for identification were chosen at random. Smears were made, gram-stained, observed microscopically, and identified as to morphology. They were then cultured in the appropriate differential media. Further identification was based on biochemical reactions in these media.

Table 1. Average counts of bacterial colony-forming units in the atmosphere of a sewage treatment plant

Sampling point	*Number of samplings*	*Average number of colonies per cubic meter*
300 meters upwind from aeration building	3	17
Inside aeration building	4	21,800
Inside stack of aeration building	3	890
300 meters downwind from aeration building	5	48

NOTE: Atmospheric conditions during sampling period were as follows: −10°C to 10°C temperature, wind westerly 2.2 to 22 meters per second, zero precipitation, relative humidity of 30 to 50 percent. All sampling was done during daylight hours. The sky varied from clear to overcast.

Sewage liquor studies. Four liquid samples were taken at random from one aeration tank. These were subjected to routine bacteriological analysis for the identification of bacteria. The chance finding of acid-fast bacilli led to the following sampling procedure.

Stack lumen studies for acid-fast bacilli. Sterile swabs moistened with glycerine were inserted into the sampling port of the stack located about 6 meters above the roof of the aeration building, the top of the stack being about 9 meters above roof level and about 22 meters above ground level. Exact measurements of stack and building heights were not available to us; therefore, only estimates have been used. After being allowed to remain in the stack lumen for about 15 minutes, the swabs were digested with trisodium phosphate and the concentrate inoculated into tubes of Petragnani's medium and incubated at 37°C. These cultures were inspected periodically for suspicious colonies. Smears of these colonies were made and stained by the Ziehl-Neelsen technique. The growth from those cultures whose smears, upon microscopic inspection, showed acid-fast bacilli, was then scraped off the surface of the medium and emulsified in normal saline. Next, 0.2 ml. of the suspension was injected intraperitoneally into four female guinea pigs which had been previously tuberculin tested and found negative. After 6 weeks, the animals were killed, and autopsies were performed. Their lungs, livers, and spleens were examined for gross lesions. Impression smears and histological sections of the organs were made, stained by the Ziehl-Neelsen technique, and examined microscopically.

Disinfecting properties of ozone intended for odor control. Although ozone is intended for odor control only,

it was deemed propitious to test the disinfecting properties of this gas in air, as it is sometimes used to disinfect liquid effluents. The ability of ozone to disinfect the gaseous effluent of the building housing the thickening tanks (see chart) was tested in August and September 1971. Ozone generated inside this building is injected at various concentrations into the stack at the level of the twin, horizontally oriented exhaust fans. Andersen samplers loaded with either TSA or SBA, depending on the sampling run (tables 2 and 3) were placed on the floor of the stack's mixing chamber (see chart), this being the most practical location. Samples were taken under conditions of no ozone production, minimal (approximately 2.3 kg per day), and maximal (approximately 4.5 kg per day).

Table 2. Average counts of colony forming units (CFU) in a series of tests in the ozone mixing chamber, using sheep blood agar

Stages of Andersen sampler	Control - no ozone (CFU)	Minimal ozone (CFU)	Maximal ozone (CFU,
I	85	25	45
II	85	29	40
III	213	71	103
IV	213	107	205
V	175	61	97
VI	19	10	16
Totals	790	303	506

Results

The average numbers of colonies per cubic meter of air sampled inside and outside the sewage treatment plant are listed in table 1. The air inside the aeration building contained 21,800 viable bacterial colony forming units (CFU) per cubic meter. This number diminished to 890 in the stack of the building, but about 300 meters downwind from the aeration building, that quantity dropped to 48 CFU per cubic meter. At a similar distance upwind, the average count was 17 per cubic meter.

Table 4 lists the organisms identified in samples taken around the plant; the majority of corresponding particles were in the pulmonary retention range 2.0 − 5.5 μm (stages III and IV of the Andersen sampler). Those collected downwind included *Bacillus, Flavobacterium, Micrococcus, Streptococcus,* and *Klebsiella.* Klebsiellae were also collected inside the aeration building, inside the stack, and downwind as well, while haemolytic streptococci were captured inside the building in addition to downwind. *Aeromonas, Moraxella,* and *Alcaligenes* were identified in the atmosphere inside the building. Salmonellae were identified in the liquid samples from the aeration basins, as were mycobacteria, which were also discovered in the stack lumen. When cultured on Petragnani's medium, the mycobacteria grew as irregular, rough, buff-colored colonies within 2 weeks. Subcultures in vivo for 6 weeks injected into four female tuberculin-negative guinea pigs resulted in gross lesions of the lung, liver, and spleen of these animals. Impression smears of these organs stained by the Ziehl-Neelsen technique showed acid-fast organisms, as did the histological sections.

Effect of ozone. The effect of ozone on counts of airborne bacteria is outlined in table 2, which shows averages of several tests. Apparently, viability is not diminished significantly. The highest viable counts, both with and without ozone present, were found in stages III and IV of the Andersen sampler. This result indicates that these particles were in the 2.0 − 5.5 μm range and therefore may be retained in the lower respiratory tract. The data shown in table 3 reinforce the results in table 2. Additionally, the relative survival of bacteria collected on enriched and nonenriched media is compared in table 3.

Discussion

The data on *Klebsiella* (table 4), the most ubiquitous organism found in this study, indicate that these bacteria are carried from the air above the aeration basins to the stacks and thence to the outside atmosphere via air currents generated by the fans inside

Table 3. Average counts of colony forming units (CFU) in a series of tests in the ozone mixing chamber, using two media for comparison

Stages of Andersen sampler	No ozone		Minimal ozone		Maximal ozone	
	TSA (CFU)	SBA (CFU)	TSA (CFU)	SBA (CFU)	TSA (CFU)	SBA (CFU)
I	33	64	16	45	45	52
II	23	72	28	38	12	45
III	50	153	47	105	55	160
IV	58	153	70	190	65	130
V	23	55	35	40	50	71
VI	5	5	3	17	11	13
Total	192	502	199	435	238	471

Note: TSA—tryptic soy agar, SBA—sheep blood agar

Table 4. Micro-organisms identified in the atmosphere in and around a sewage treatment plant

Organism	Sewage liquor	Inside aeration building	Inside aeration building stack	Downwind from aeration building[1]
Mycobacterium ...	+	−	+	−
Klebsiella	−	+	+	+
Salmonella	+	−	−	−
Bacillus	−	−	−	+
Flavobacterium ...	−	−	−	+
Aeromonas	−	+	−	−
Moraxella	−	+	−	−
Alcaligenes	−	+	−	−
Streptococcus	−	+	−	+
Micrococcus	−	−	−	+

[1]No organisms identified upwind of the aeration building.
Note: − no organisms present, + organisms identified.

the aeration building. Our observations are in agreement with those of Randall and Ledbetter *(4,5)* that Klebsiellae are the best indicators of bacterial air pollution from sewage sources. These investigators correctly observe that there exists a definite possibility of airborne infection from activated sludge plants, where aeration basins are the most important source of pathogens.

Although we have no direct data to support our hypothesis, we believe that Klebsiellae, among many other micro-organisms in the aeration tanks, are being aerosolized by the mechanism described by Woodcock *(8)* and Blanchard and Syzdek *(9)* via bubbles at the air-water interface of a body of water where some micro-organisms are concentrated. The fact that the air inside the aeration building contained an average of 21,800 CFU per cubic meter tends to confirm our belief.

The smaller number of viable bacteria collected in the stack could be explained by the settling of particles. This tendency would be enhanced by the high relative humidity within the building, which could hydrate the particles. The possibility that some bacteria adhere to the stack walls cannot be excluded.

The difference between the average numbers of CFU per cubic meter of air sampled upwind and downwind is most probably due to microbes being carried downwind from the sewage treatment plant, a finding in agreement with the observations of Goff and co-workers *(3)* on the effects of meteorological conditions. We believe that the atmospheric conditions under which we sampled, specifically temperature and sunlight, account for the lower numbers of bacteria collected outside the plant.

The data in tables 2 and 3 indicate that ozone causes no practical reduction in the numbers of bacteria. However, the number of samplings was not sufficient to verify this observation statistically. It is of particular interest, in the light of findings of Zelac and co-workers *(19)* that inhaled ozone can cause chromosome breaks in circulating lymphocytes in vivo, that several sewage treatment plants presently under construction in New York City will employ ozone as a deodorant. The roof of one plant will be used for recreational activities, and the entire complex is located in a densely populated residential area *(20)*. Research is urgently needed to determine how far ozone will travel downwind and still be reactive.

The large differences in viable counts between samples collected on SBA compared with TSA (table 3) indicate that the addition of blood to the medium allows the survival of fastidious or injured organisms or both types. This, coupled with the fact that the collected microbes are within the retention ranges of the lower pulmonary tract, indicates that their entry into the human body could cause disease.

Klebsiellae, streptococci, and mycobacteria are well established respiratory pathogens which could be disseminated by these facilities. *Klebsiella* is commonly isolated from patients hospitalized with urinary tract infections *(21)*. Hospitals take no special precautions for the disposal of infected urine from these patients. It is passed directly into the municipal sewage system. Therefore, it is no surprise that these Klebsiellae find their way into the aeration basins of sewage treatment plants, where they are aerosolized. In his review, O'Connor *(22)* cited several studies which show increased susceptibility to *Klebsiella* infection in the presence of gaseous pollutants. Treatment of infection caused by bacteria aerosolized by sewage treatment plants may be compromised by the increasing resistance to antibiotics of fecal coliforms. Sturtevant and co-workers *(23)* have shown that sewage is an ideal environment for the successful episomal transfer of antibiotic resistance genes among enteric bacteria.

Unfortunately, epidemiologic data on the incidence of infectious disease among sewage treatment plant personnel and among residents of nursing homes and children attending schools in the immediate vicinity of sewage treatment plants were not available to us. Correlation of these data with findings such as ours should be undertaken. The results of our preliminary study indicate that a combined quantitative microbiological and epidemiological study should be carried out to clarify further the role played by sewage treatment plants in the airborne dissemination of disease. Such a project could indicate that feasibility studies be undertaken before the selection of prospective sites for the location of sewage treatment plants.

Conclusions

The bubbling of air into aeration tanks causes some of the bacteria concentrated at the liquid-air interface to become airborne. Thus, these bacteria are found in great numbers in the atmosphere of aeration buildings. The air currents created by fans in the wall of the buildings and in the exhaust stacks carry numbers of bacteria into these stacks and from there into the outside air. Among these bacteria are viable potential respiratory pathogens. Ozonation does not appreciably attenuate these aerosols.

Our results point to the existence of a possible health hazard, such as mycobacterial disease, especially for sewage treatment plant workers and for highly susceptible population groups such as young children, the elderly, and the infirm who reside in areas where the atmosphere is contaminated by the gaseous effluent of sewage treatment plants.

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SYNOPSIS

PEREIRA, MARTIN RODRIGUES (Gibbs and Hill, Inc., New York City), and BENJAMINSON, M. AARON: *Broadcast of microbial aerosols by stacks of sewage treatment plants and effects of ozonation on bacteria in the gaseous effluent. Public Health Reports, Vol. 90, May–June 1975, pp. 208–212.*

In the aeration basins of sewage treatment plants, compressed air is supplied to diffusers near the bottom of tanks to aid in the conversion by aerobic bacteria of dissolved and suspended solids of sewage into particles that will settle. Air bubbles breaking at the air-water interface will aerosolize bacteria that concentrate in the uppermost microlayer. The microbiological output of a plant in New York City with such a system was monitored.

Samples of the gaseous effluent were collected inside the aeration building, inside the building's stack, 300 meters upwind (background sampler), and 300 meters downwind (test sampler), using Andersen samplers. Among the genera identified in the atmosphere in and around the plant were *Mycobacterium, Klebsiella,* and *Streptococcus,* all potentially pathogenic.

The disinfection power of ozone, which is generally used for odor control, was also tested. Samples were taken from the ozone mixing chamber in the stack of the thickening tank building. No significant difference in general bacterial counts could be detected at different levels of ozone production. It appears that in the air, ozone is an ineffective bactericidal agent.

Results in this preliminary study demonstrate the need to evaluate the hazard of microbial aerosols generated by sewage treatment plants similar to the one studied. The possibility of such hazards is of special interest where facilities are located upwind of populations especially susceptible to infections, because of age or debility.

Correlations with epidemiologic data are indicated.