Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
VIRUSES IN WASTEWATER SLUDGES AND IN EFFLUENTS USED FOR IRRIGATION

Edward P. Larkin
Virology Branch, Division of Microbiology, Bureau of Foods, Food and Drug Administration, Department of Health and Human Services, Cincinnati, Ohio 45226, USA

Disposal of sewage wastes is a problem of increasing magnitude in the United States. New control measures enacted by Congress require consideration of land disposal of sludges and effluents. The volume of wastes involved and the possibility of contaminating ground water supplies and vegetation has great public health significance. The effectiveness of the sewage treatment process to remove chemical and biological contaminants and the operational efficiency of many sewage treatment plants are factors that must be given careful consideration before final selection of disposal methodology. The quality of our future water and food supplies will be affected either beneficially or adversely by current management decisions.

Introduction

The population of the industrial countries has increased two- to three-fold in the last 50 years. This increase has contributed significantly to the overutilization of raw materials and to an almost uncontrolled pollution of air, land, and water resources. Alarmed by these problems, many governments have attempted to control the sources of chemical and microbial pollutants by laws and regulations. One of the control measures taken by the Congress of the United States was the Water Pollution Control Act of 1965, to which a number of amendments were passed in 1972. This act specified that by July 1, 1983, all disposal wastes must be confined so as not to cause water or other environmental pollution. In 1977 the Administrator of the Environmental Protection Agency indicated in a policy statement that attention should be given to waste treatment processes that renovate and reuse water as well as those that recycle organic matter and nutrients in a more efficient manner.

As a result of this legislation, municipal and industrial waste treatment management must use the best practical technology to decrease environmental pollution. Because of economic considerations and time restrictions, land disposal of sludges and effluents appears to be an attractive alternative, if suitable disposal sites are available. The efficiency of a land disposal process is dependent on the use of a cover crop, preferably one that can be harvested several times a year. An added inducement is the possibility of financial return, if the crop used is of market value.

The history of the use of sewage wastes to irrigate/fertilize agricultural crops in the United States is scanty. However, on one occasion in Colorado, the United States Public Health Service invoked an embargo on the shipment of vegetable crops grown on sewage-irrigated land. Bacterial pathogens were detected in the irrigation water and on the crops irrigated by wastewater from a primary sewage treatment plant (South Platte River Basin, 1966). In many parts of the world sewage wastes are used in crop production. Mexico and India have areas in which food and fodder crops are intensively irrigated with raw sewage wastes from large cities. Storage before use on these agricultural lands is the only treatment process used. In some countries large quantities of human and animal wastes defecated on the ground are washed into agricultural fields during periods of heavy rain. Such practices expose the farmers and consumers of such crops to a variety of pathogenic bacteria, viruses, and parasitic animals. Even if such foods are cooked, the potential for cross contamination is high.

Land disposal of sludges by small sewage plant operations has been an ongoing activity in the United
States for a number of years, but few of these uses have been related to agriculture because of the small quantities or volumes involved. The effluent, which has been disposed into streams or other bodies of water by small communities, probably will be disposed on land in the future, if possible, because of the lower expense involved in meeting the new, more rigid requirements. The middle-sized and larger municipalities will have an increased sludge disposal problem but effluents, because of the high fluid volumes, will still be disposed in a water carriage system. In states where water shortages occur, land disposal of effluents may soon become a necessity for irrigation, for groundwater recharge to increase water resources, and for preventing salt water infiltration. The microbiological, chemical, geological, and hydrological long- and short-range impact of such activities on the environment should be assessed carefully.

About 80% of the treatment plants in the United States are small—less than 2.5 MGD. Such plants usually have primary settling tanks, sludge digesters or drying beds and possibly lagoons for short-term effluent storage. Most plants are operated by part-time, poorly trained personnel. The larger cities have treatment plants of varying complexity designed to remove particulates and other organics to reduce the biochemical oxygen demand. Some of these processes reduce microbial pathogens in the sludges and effluents, but viruses, parasites, and other pathogens have been consistently detected in the treated wastes of the few plants that have been investigated.

The probability is high that viruses are present in any sewage sludge or effluent. The volume of sludges collected by a plant vary according to the type of process and whether chemical flocculating and precipitating agents are used. The volume of effluent in a plant varies little regardless of the treatment process. Sludges are pumped onto drying beds, into digesters, recycled through the plant, pumped into tank trucks, etc., while the effluent is discharged into ponds, lagoons, lakes, streams or onto the land. Recently, EPA abolished the regulation for postchlorination of effluents because of reported carcinogen production from chlorine reaction with pollutants. Therefore, sludges and effluents are receiving little, if any, germicidal treatment.

**Potential Problems of Land Disposal**

Once the decision is made to consider land disposal of sewage sludges or effluents an effort must be made to determine the potential problems related to the decision, other than costs. Will land disposal solve the problems of stream or other pollution by shifting the pollution to the land? Examination of the sewage wastes composed of complex biological and chemical contaminants suggests otherwise. Little consideration has been given to such concerns in the past, for once the wastes were discharged into the water carriage systems, they were quickly removed from sight and were the problem of other communities or future generations. Waste disposal on land within the community results in immediate adverse reaction from abutting landholds and carries with it the potential for continuous pollution of soil, ground water, and surface vegetation.

Analysis of the biological contaminants alone shows that at any time sewage may contain bacteria, yeasts, molds, and other fungi, algae, chlamydiae, rickettsiae, viruses, animal parasites including protozoa, nematodes, and other larger parasites with their infectious eggs and cysts. The types and variety of contaminants present depend on the human, animal, and other contributors to the disposal system. Only the viruses will be discussed in this paper, but any land disposal decision must include consideration of all potential biological contaminants.

Only a few scientists have investigated the viral content of raw sewage, sludges, and effluents. Detection methods and economic limitations have restricted most studies to the types and numbers of human enteroviruses in the waste treatment system. Some additional information is available on reoviruses and adenoviruses. The viruses that have been most successfully detected are polioviruses (types 1-3) because of the cell culture systems used and the continuous immunization programs in the communities tested. Many of the group A coxsackieviruses (types 1-24) are detectable only in animal systems, although some will propagate in certain cell culture systems. Detection of the group B coxsackieviruses (types 1-6) and echoviruses (types 1-34) are restricted by the methodology and culture systems used. Thus, any report pertaining to the enterovirus content of raw sewage or wastes from the sewage treatment plant is necessarily an underestimate of the actual enterovirus content.

Of even greater importance is the unknown quantity of other viruses present in sewage. The viruses of hepatitis, which produce millions of clinical cases yearly throughout the world, cannot be demonstrated readily in cell culture. Human rotaviruses, probably the most common cause of gastrointestinal disease in children, can be detected in cell cultures but do not appear to replicate. Electron microscopists after examination of feces of humans with and without disease symptoms have reported the presence of coronaviruses, duesto viruses, calcivirus, orbivirus, parvovirus-like agents, entero virus-like agents, reovirus-like agents, papo virus-like agents, etc., many of which could not propagate in the animal and cell cultures tested. In addition, clinical virologists have detected adenoviruses, coxsackieviruses, polioviruses, echoviruses, reoviruses, cytomegaloviruses, herpesviruses, rhinoviruses, measles, rubella, influenza, hepatitis, vaccinia, encephalitis viruses, and human polyoma viruses in urine of patients with and without disease symptoms.
Sewage Treatment Effectiveness

What is the removal efficiency of sewage treatment processes on this variety of viruses? In the past, sewage treatment processes were designed to remove visible particulate materials, reduce the biochemical oxygen demand, and discharge what was left into a nearby stream or other body of water. Virus removal by such systems was small. Today some engineering consultants recommend that discharges from primary sewage plants could be disposed effectively on land. If this assumption is true, there would be no need for a secondary or tertiary treatment process and expenditures for sewage treatment plants could be considerably reduced. Other communities without available land disposal sites are using or considering a variety of secondary and tertiary treatment processes.

An advanced waste treatment plant using an activated sludge process has been reported to remove 99.99% of the viruses present in the raw sewage (Berg, 1973). On occasion, the same plants are affected by unknown chemicals or other contaminants that drastically reduce the effectiveness of their biological systems, and virus removal efficiency drops to less than 50%. Other sewage treatment processes vary in their efficiency from 0% to 99% with little consistency in operations. In addition, mechanical failures and heavy storm water inflows result in the bypass of raw sewage for varying periods of time. The greatest problem affecting plant operation efficiency is the failure of municipalities and industry to pay sufficiently high wages to encourage qualified people to manage the complex biological, chemical, and engineering aspects of a well-operated treatment plant. The huge expenditures in construction grant activity will be of little use without capable people to operate and supervise closely every phase of the sewage treatment process.

The number of enteroviruses estimated to be present in raw sanitary sewage ranges from 5 to 21,000/100 ml, with an estimated average of 200 to 7000/100 ml in the United States (Clarke et al., 1964). In addition, at least an equivalent number of viruses other than the enteroviruses are probably present for an average of 400 to 14,000/100 ml. If virus removal efficiency of 70% is estimated, 120 to 4200/100 ml would be left in the effluent. The other viruses would settle in the sediment which makes up the raw sludge or might be oxidized to some extent in the treatment process during sludge digestion or drying. If the sludge is further processed with an estimated 90% virus reduction, ~ 28 to 980/100 ml of sludge would remain. The virus removal estimates depend on operational efficiency of the treatment plant, which varies considerably with the competence of the personnel and design of the process.

Disposal of such sludges and effluents onto the land automatically introduces virus contaminants in addition to other micro- and macrobiological forms of life. The type of application used and the frequency of application directly influence the numbers of contaminants and their location on the vegetation.

For thousands of years, farmers have applied animal wastes to land. The method of application should provide valuable and useful data for land disposable practices. Usually the manure was spread during periods other than the planting and growing season. In many cases the manure pile had been allowed to ferment for varying periods of time before removal to the fields. Many land disposal projects use irrigation, or are projected for irrigation on a continuous basis, even during the crop growing season.

Virus Persistence

Information on the persistence of viruses on crops irrigated/fertilized by sewage wastes is limited. However, available data showed the poliovirus 1 persisted for ~21 days after the last waste application on lettuce and radishes during the warm months of the year. In one phase of this study, poliovirus 1 was recovered from lettuce 36 days after irrigation had ceased. During the colder months poliovirus 1 was detected in soil for 60 to 90 days after the application of inoculated sludge or effluent (Larkin, 1976; Tierney, 1977). In another study, 2 of 60 field samples of vegetables were found to be contaminated with enteroviruses (Bagdasar'yan, 1964). In addition, 3 of 146 vegetable samples that had been irrigated with sewage wastes were contaminated with coxsackievirus B (Artykov, 1973).

Viruses have been detected in treated sludges and effluents and have been recovered from crops irrigated with sewage wastes. Viruses may persist on crops and in soil longer than the time required to plant, grow, and harvest the crops. When spray systems are used, daily irrigation of crops is a common practice, and this results in an almost continuous exposure of the total vegetation to virus contamination.

The number of viruses in irrigation water that comes in contact with vegetation varies with crop size and density and with the volume of water applied. There are no known quantitative data available to estimate the number of viruses that would be detectable on a lettuce leaf or a blade of grass. However, if a dense field of alfalfa was irrigated, most of a spray-irrigated effluent or sludge would contact the vegetation. The persistence of viruses on crops would be affected by rainfall, solar radiation, and humidity. Therefore, viruses would probably persist longer in densely vegetated fields than in fields with sparse vegetation.

In one of our experiments lettuce and radish crops covering about 20% of the soil surface were irrigated with inoculated sludge and effluent containing ~ 1 × 10^6 PFU/ml of poliovirus 1. The number of viruses detected varied from 150 to 4400 PFU/g of lettuce or radish leaves 24 h after irrigation with sewage sludge.
On the effluent-irrigated crops, from 25 to 500 PFU/g were detected after 24 h. The virus concentration in the soil varied from $\sim 1 \times 10^4$ to $\sim 1 \times 10^9$/g after 24 h and viruses persisted at higher levels in the soil than on the plants. If these data could be transposed to a natural situation, there probably would not be more than one detectable virus/g of sparse vegetation in a field 24 h after irrigation. On a densely vegetated field the virus concentration would probably be higher.

The method of irrigation affects the probability of virus contact with the vegetative surface. Spray irrigation or flooding enhances the probability of surface contact whereas ditch or drip irrigation methods reduce the probability of contamination, except during storms or high winds when the vegetation could be in contact with the irrigation waters and soil. Crops grown underground present a different set of contamination problems, especially in densely grown crop areas, with viruses persisting at higher levels and for longer times in the soil than on surface crops.

**Current Technology**

It has been assumed in the foregoing that sewage treatment plants are operating efficiently and that the virus concentration data are close to that reported in the literature. There is reason to doubt the virus data. For example, children infected with rotaviruses produced $\sim 1 \times 10^4$ virus particles/g of feces (Konno, 1977). There are also $\sim 35,000$ cases of clinical hepatitis A reported yearly in the United States. Epidemiologists at the Center for Disease Control estimate that there are between 10 and 20 times as many subclinical cases as reported cases. Electron microscopy of feces obtained from hepatitis cases show the presence of virus concentrations of at least $1 \times 10^9$/g. If there are 350,000 cases of hepatitis A and 1 million cases of rotavirus gastroenteritis each year in the United States, patients with these two diseases should be contributing high titers of undetected viruses into the sewage system on a continuing basis.

Few sewage treatment processes are monitored for viruses, pathogenic bacteria, or parasites. In the past, when postchlorination of the effluent was required, limited monitoring for fecal coliforms was performed. At the present time microbiological monitoring of sludges and effluents is nonexistent.

Some epidemiologists believe that no public health problems are associated with biological contaminants of sewage, especially if drinking water processes are effectively controlled. Therefore, sewage disposal on land is an acceptable alternative to them. The basic problem with this is that there is no data base on which to make assumptions. No experience exists relative to large-scale disposal of sewage wastes on the land in the United States. The data obtained from other countries are con-

founded by the fact that water supplies are usually contaminated. Therefore, the role of food and fodder crop contamination in disease incidence is unknown.

On a number of occasions, it has been stated that there is no need to worry about the low level virus contamination that would probably occur if sewage wastes were used to irrigate/fertilize crops. Daily consumption of a few viruses would stimulate the immune mechanism, it has been argued, and thus maintain high levels of protection against disease. Such advocates seldom admit the potential for replication of these viruses and subsequent discharge into the immediate environment of high concentrations of viruses that could result in clinical disease. Also, all consumers have not had previous contact with all types and strains of viruses that may be present, and on ingestion could become victims of clinical disease. Such infections could be foci for continuing contact infection.

Some changes have occurred recently in the basic sewage treatment process in the United States. Plastic granules have replaced stones in some trickling filter beds, oxygen has been substituted for air in secondary processes and a number of physical-chemical treatment processes have been investigated. Batch processes, recycling, and other innovative changes in existing treatment treatment methodology are being investigated (Process development, 1979). Therefore, any disposal systems projected for use in the United States should be of high caliber. Wastes from efficient sewage treatment plants, it has been argued, should not be compared to raw sewage. The argument may be valid quantitatively but not qualitatively. Biologically and chemically, there may be little qualitative difference. The problem may be one of reduced magnitude, but it is not obviated.

The vegetation chosen to control chemical contamination of the soil in a land disposal project should not enter the human food chain. Viruses, pathogenic bacteria, and parasitic animals survive on crops and in soils for periods ranging from several days to years. Because such organisms may persist longer than the time required for growth and distribution of the crops, consumption of such foods could be a health hazard. Compounding this problem are the known deficiencies in plant operation, mechanical breakdowns, lack of microbiological monitoring, limited or absent germicidal treatment, and the lack of nationwide regulations and policies governing the application of sewage wastes to land (Larkin et al., 1978).

**Conclusion**

The Romans observed an association between the location of their drinking water sources and certain diseases in their cities. This observation led to the isolation of potable water from waste discharge areas. As
this civilization declined, its environmental controls against disease were forgotten and such controls were not resurrected even in more enlightened countries until the eighteenth and nineteenth centuries. During the preceding centuries, bubonic plague, typhus, smallpox, cholera, typhoid, malaria, and uncontrolled infant and juvenile mortality ravaged the human population. Even today, many of these diseases, along with numerous other parasitic, bacterial, and viral infections, plague the Asiatic, African, and South American continents. The knowledge required to control most of these diseases is available, but because of economics and higher priority activities, millions of people are exposed to a daily onslaught of microbial and chemical contaminants.

All countries are effectively polluting the waters of the world. The United States has been fortunate in having available the Atlantic and Pacific Oceans, the Great Lakes, and numerous large and small rivers and streams in which to dump its wastes. In addition, because of relatively limited populations, large land areas are available for waste disposal in some parts of the country. Whether the existence of these areas will effectively reduce water pollution problems in the future without these areas becoming new zones of contamination will depend on the ability of managers of municipal and industrial wastes treatment processes to control the biological and chemical contaminants present in the wastes disposed on the land.

References

Artykov, M. S. (1973) Sanitary-virusological study of the effectiveness of decontamination of wastewater in agricultural irrigation fields, *Gig. Sanit.* 39(4), 110–116.

Bagdasar’yan, G. A. (1964) Sanitary examination of soil and vegetables from irrigation fields for the presence of viruses, *Gig. Sanit.* 11, 37–39.

Berg, G. (1973) Removal of viruses from sewage, effluents, and waters. I. A review, *Bull. WHO* 49, 451–460.

Clarke, N. A., Berg, G., Kabler, P. W., and Chang, S. L. (1964) Human enteric viruses in water: source, survival and removability, in *International Conference on Water Pollution Research*, Vol. 2, pp. 523–536. Pergamon Press, London.

Konno, T., Suzuki, H., Imai, A., and Ishida, N. (1977) Reovirus-like agent in acute epidemic gastroenteritis in Japanese infants: Fecal shedding and serologic response, *J. Infect. Dis.* 135(2), 259–266.

Larkin, E. P., Tierney, J. T., and Sullivan, R. (1976) Persistence of virus on sewage-irrigated vegetables, *J. Environm. Eng. Div.* ASCE 102, 29–35.

Larkin, E. P., Tierney, J. T., Lovett, J., Van Donsel, D., Francis, D. W., and Jackson, G. J. (1978) Land application of sewage wastes: potential for contamination of foodstuffs and agricultural soils by viruses, bacterial pathogens and parasites, in *State of Knowledge in Land Treatment of Wastewater*, Vol. 2, H. L. McKim, Sym. Coord., pp. 215–223. U. S. Army Corps of Engineers, Cold Regions Research and Engineering Laboratory, Hanover, NH.

Process development for municipal wastewater (1979) Internal memo, MERL, ERC, EPA, Cincinnati, OH, pp. 1–15.

Tierney, J. T., Sullivan, R., and Larkin, E. P. (1977) Persistence of poliovirus 1 in soil and on vegetables grown in soil previously flooded with inoculated sewage sludge or effluent, *Appl. Environ. Microbiol.* 33, 109–113.

South Platte River Basin irrigation of vegetables with sewage polluted water, (1966) South Platte River Basin Project, Denver, CO, Fed. Water Pollution Control Admin., pp. 1–27.