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**Progress Report
Beneficial Uses Program
Period Ending December 31, 1979**

Applied Biology and Isotope Utilization Division

Prepared by Sandia Laboratories, Albuquerque, New Mexico 87185
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Applied Biology and Isotope Utilization Division 4535
Sandia National Laboratories
Albuquerque, NM 87185

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PROGRESS REPORT
BENEFICIAL USES PROGRAM
PERIOD ENDING DECEMBER 31, 1979

INTRODUCTION

This Progress Report describes activities in the Beneficial Uses Program during the quarter ending December 31, 1979.

The Preliminary Safety Analysis Report for the Sandia Irradiator for Dried Sewage Solids (SAND78-1366) was printed in late December. Typing of the Final Safety Analysis Report (FSAR) has begun and the first draft should be completed in the next quarter. Forty-eight hundred pounds of grapefruit were irradiated for the U. S. Department of Agriculture and in November 12 000 pounds of digested and raw sludge were irradiated for New Mexico State University. Static and time-averaged gamma field measurements were made in SIDSS by Far West Technology.

Phase I of the Technology Transfer tasks were completed by CH2M Hill. The work encompassed development of site selection criteria for demonstration plants, identification and screening of sites, and reduction of potential sites to 16. These were then prioritized for further sludge management studies.

Six representative species of bacteria were thoroughly studied to determine the effects of dewatering by evaporation on their survivability and on their D_{10} -values for radiation inactivation. The results of this investigation show that the effects of dewatering on the survival of enteric bacteria and on the specific D_{10} -values of these bacteria is somewhat different for each of the bacterial species. However, in no case did any species display unusual resistance to inactivation by ionizing radiation. It appears therefore that radiation treatment can be used effectively to inactivate enteric bacteria in dewatered as well as liquid sludges.

A second experiment in cattle-feed supplementation has been completed. Cattle fed ad libitum for 84 days consumed a ration for growing-finishing which resulted in a daily consumption of two to three pounds per day of dried raw irradiated sewage solids. Results showed that buildup of heavy metals and refractory organic residues was not beyond ranges reported for conventionally fed beef cattle.

Eighty-seven ewes are in three dietary treatment groups: (1) Negative control (basal), (2) Positive control (cottonseed meal), and (3) Experimental (sludge) supplemented rations. These ewes were bred between October and December and the real drain on maternal nutrients will occur during late gestation and early lactation. Results in February and March should prove informative.

Sixty-four bred ewes were divided into three groups on September 1 and the rations described above were fed to the three groups. Lambing data from December 1979 suggests that a ration containing 7% sludge is adequate to sustain normal fetal growth and development.

Results of the summer greenhouse study are complete and strengthen earlier evaluations of sludge as an important source of nutrients for sorghum. Iron and zinc deficient soils responded well to sludge addition. An additional important observation was made with respect to beneficial effects of sludge. Although all pots were uniformly fertilized with P, the greatest P uptake was always associated with sludge-amended soils. Sewage sludge may be an alternative, or a desirable additive, to commercial fertilizer in solving P-deficiency problems where correction is resisted with commercial fertilizers, as commonly occurs in New Mexican soils.

This quarter's virology report describes a comparative study on the effects of the anionic detergent sodium dodecyl sulfate (SDS) and the chelating agent ethylenediaminetetraacetate (EDTA) on purified rotavirus SA-11 particles. Both chemicals readily inactivated rotavirus at quite low concentrations and under very mild conditions. In addition, both agents modified the viral capsid and prevented the adsorption of inactivated virions to cells. Capsid damage by EDTA caused a shift in the densities of rotavirions from about 1.35 to about 1.37 g/mL and a reduction in their sedimentation coefficients. SDS, on the other hand, did not detectably alter either of these physical properties of rotavirions. Both agents caused some alteration of the isoelectric points of the virions. Finally, analysis of rotavirus proteins showed that EDTA caused the loss of two protein peaks from the electrophoretic pattern of virions but SDS caused the loss of only one of these same protein peaks.

WASTE RESOURCES UTILIZATION PROGRAM

Sandia Irradiator for Dried Sewage Solids (SIDSS)

Documentation

The Preliminary Safety Analysis Report (PSAR) for the SIDSS was resubmitted for final typing October 10, 1979, and was printed December 21, 1979. Preparation of the technical illustrations delayed the printing. The Final Safety Analysis Report (FSAR) was dependent on the original typing of the PSAR, and thus had to await printing of that report. Typing of the FSAR will be completed next quarter and the draft will be sent to Sandia management for approval.

Modifications

The lead shutter automatic lubrication system was completed and installed this quarter. A bucket location display was designed and the prototype circuit completed December 7. Display driver integrated circuits have been ordered and are scheduled to arrive next quarter. Final wiring of the bucket location display circuit will begin in February.

Plant engineering is beginning modifications to the SIDSS that will include: (1) sidewalks, (2) a sink, (3) housings for hydraulic lines, (4) covers for the conveyor, and (5) a new air handling system.

Operations

On November 29, 1979, 4800 pounds of grapefruit were irradiated for the USDA/SEA (Animal and Plant Health Inspection Service) and returned by air freight November 30.

Approximately 2 000 pounds of digested sludge was irradiated for NMSU on December 7. Approximately 10 000 pounds of raw sludge was irradiated for NMSU from December 10 through 19. The digested sludge is being used for field plot studies, and the raw sludge for animal feeding trials.

Dosimetry

Final arrangements were made for Far West Technology of Santa Barbara, CA to make dosimetry measurements on the SIDSS using photochromic dosimeters calibrated by the National Bureau of Standards. The measurements were made October 29 through 31. The final results were received on December 6. The principal field measurements made were the static gamma measurements around the source and time-averaged measurements with compost, water, and grapefruit. The data is being condensed and compared with field calculations.

Technology Transfer Program

During this quarter, Phase I of the Technology Transfer Program conducted with CH2M Hill has been completed. The tasks specified in the contract were described last quarter and only the results of the effort will be presented here. CH2M Hill has (1) developed criteria for selecting good demonstration sites for sewage sludge irradiation, and for identifying potential irradiation sites, (2) identified over 100 potential sites, eliminated the ones with low potential and considered 30 sites for more detailed analysis, (3) performed an individual analysis on each of the sites to reduce the list to 16, (4) made visits to each of the 16 sites and criticized them according to the criteria developed in (1), finally, (5) prioritized these 16 sites selecting four to either help with or do sludge management studies. These studies will examine in detail the use of sewage sludge irradiation (Table 1).

Table 1
Rating Summary for Potential Sludge Irradiation Sites

Sites	Criteria							Total
	Sludge Production	Sludge Quality	Dewatering Capability	Need for New Disposal Options	Capability to Operate Irradiator	Interest	Funding Potential	
1. Albuquerque, New Mexico	10	9	10	10	10	10	10	69
2. Central Kitsap, Washington	0	10	7	8	5	10	5	45
3. Deer Park, Texas	10	5	0	0	3	0	2	20
4. El Paso, Texas	5	10	10	11	10	7	5	55
5. Golden, Colorado and Adolph Coors	10	10	10	5	10	8	8	61
6. Occoquan, Virginia	7	8	8	7	10	7	2	49
7. Odessa, Texas	7	5	0	0	5	0	2	19
8. Provo, Utah	5	10	5	8	10	10	5	53
9. Redding, California	7	10	8	8	10	10	2	55
10. St. Petersburg, Florida	7	10	0	10	10	10	8	55
11. Salt Lake City, Utah and Diversa-Cycle Products	10	7	5	8	10	10	5	55
12. Seattle, Washington	10	3	8	2	10	7	5	45
13. Spokane, Washington	10	0	0	10	10	5	5	40
14. Tacoma, Washington	7	5	0	0	10	2	5	29
15. Tampa, Florida	10	6	5	5	10	7	5	47
16. Yakima, Washington	6	10	5	7	5	7	5	45

The criteria developed for identification of potential sites is shown below.

1. The plant is of adequate size to produce a minimum of 10 000 pounds dry solids per day.
2. Suitable sludge handling equipment exists to concentrate the sludge to at least 35% to 40% solids.
3. The concentration of heavy metals and toxic materials in the sludge does not exceed limits for safe sludge reuse.

4. The attitude and level of skill among operating and laboratory personnel is appropriate for this demonstration project.
5. The existing sludge disposal option is inadequate; a new option is needed.
6. Local management has historically shown a favorable attitude towards innovative processes and local reaction to nuclear technology has been favorable.
7. The political entity responsible for the sewage treatment plant is in a favorable funding position as demonstrated by the state priority list and the entity's political posture.

The criteria that were developed to identify site characteristics important for a good demonstration project are those shown here:

1. Visibility
2. Product Utility - Benefit to the Community
3. Local Enthusiasm for the Technology
4. Market Types and Location
5. Site Access for Visitors - Tours
6. Scientific Facilities in the Area
7. Operation Flexibility
8. Continuity
9. High Energy Cost in Area - (Energy Benefit)
10. Situation Should Not Be Unique
11. Can Easily Demonstrate Cost Effectiveness
12. Low Operation and Maintenance Cost
13. High Safety Visibility - Good History
14. Well Operated/Maintained Site
15. Fits in With Plant - Alternate Sludge Scheme
16. Transportation to Market
17. Local Government Support for Program
18. Develop Many Diverse Consumers for Product

The final sites selected for further study are divided into their appropriate categories:

- A. Preliminary Target Sites
 1. Albuquerque, New Mexico
 2. Golden and Adolph Coors, Colorado
 3. Provo, Utah

B. Additional Target Sites Contingent Upon Funding

1. Redding, California
2. Salt Lake City and Diversa-Cycle Products, Utah

C. Sites Requiring Additional Investigation

1. Livermore, California
2. Milwaukee, Wisconsin
3. St. Paul, Minnesota

Phase II of CH2M Hill's Technology Transfer Program has been defined and the contract written and negotiated for the remainder of the fiscal year. The major tasks are listed as:

1. Write or help write sludge management studies for final sites
2. Write generic environmental assessments
3. Prepare updated O&M costs
4. Conduct a seminar on sewage sludge irradiation
5. Write articles for wastewater trade journals
6. Update brochure for demonstration plant
7. Prepare a media packet for press releases.

In addition to this national effort, support was provided for the State of Washington's site selection program. J. Woods, the head of the State Department of Energy for the State of Washington, organized a study committee to analyze possible sites in the State of Washington and to make recommendations to Governor Dixie Lee Ray. Site selections and recommendations are to be completed by February 20.

In addition to the above programs, the EPA in Cincinnati is being contracted to develop a Capsule Report and an Executive Briefing Document on sludge irradiation. The contract is now being negotiated.

Biological Studies

Virology

The quarterly report for the period ending in June 1979 (SAND79-1727) described a study on the effects of wastewater sludge on rotavirus stability. There it was shown that ionic detergents in sludge destabilize this important enteric pathogen and reduce the heat required for its inactivation. One detergent shown to be especially active in this regard is sodium dodecyl sulfate (SDS).

It was also shown in previous reports¹⁻³ that slight modification of the structures of detergents and the environments in which they are placed can greatly alter their effects on rotavirus and other enteric viruses. To understand why detergents affect viruses differently, we must first determine what effects they have on viruses. Knowledge concerning the state of a virus particle following inactivation by a detergent should provide a basis for understanding the mechanism of inactivation by that detergent. The study reported here examines inactivation of rotavirus SA-11 by SDS. For purposes of comparison, a simultaneous study was performed on rotavirus particles inactivated by the chelating agent EDTA.

Background -- Infectious rotaviruses from a number of species have been shown by several investigators⁴⁻¹⁰ to have densities of 1.35 to 1.36 g/mL. It is well established that these particles have a double protein shell.¹¹ Rotavirus particles are also routinely isolated from infected cells and from the feces of infected animals and humans which have densities of 1.37 to 1.38 g/mL. These particles lack the outer protein shell and, in addition, are apparently noninfectious. Purified rotavirus particles from SA-11 infected CV-1 cells were also obtained here with densities of about 1.35 and 1.37 g/mL (Figure 1). In agreement with the results of other investigators, only the 1.35 g/mL particles were found to be infectious.

Figure 1. CsCl gradient centrifugation of rotaviruses tagged with either [³H]-uridine (A) or ¹⁴C-labeled protein hydrolysate (B) as final purification procedure

It has also been reported that the lower density rotavirus particles can be readily converted to the higher density particles by treatment with EDTA.^{12,13} This treatment causes the activation of a virion-associated RNA polymerase and the apparent removal of the outer shell proteins and loss of infectivity. Because it has already been shown that SDS causes loss of poliovirus infectivity by disruption of virion proteins,² it is expected that the protein capsid will also be the rotavirus component affected by SDS that causes the loss of infectivity of this virus. Therefore, for this study the effects of SDS and EDTA on the state of rotavirus particles were studied simultaneously.

It was shown in the previous report³ that treatment of a diluted lysate of rotavirus with 0.1% SDS for 60 min at 21°C caused a reduction of infectivity of greater than 3 orders of magnitude. A similar result was found when the purified 1.35 g/mL rotavirus particles obtained after CsCl gradient centrifugation were treated under the same conditions. Likewise, treatment of these purified virions with 10 mM EDTA for 20 min at 37°C caused their infectivities to be reduced more than two orders of magnitude (results not shown). These treatment conditions were used during the remainder of this study.

CsCl Gradient Analysis of Rotaviruses After SDS and EDTA Treatments -- As mentioned, the treatment of rotavirus particles of 1.35 to 1.36 g/mL density with EDTA causes their conversion to a density of 1.37 to 1.38 g/mL.^{12,13} Therefore, the first physical measurement of the effect of SDS on 1.35 g/mL particles of SA-11 was to determine their densities after treatment. SDS was found to cause no observable increase in the densities of these 1.35 g/mL particles (Figure 2). In contrast, EDTA caused the expected increase in the densities of these particles to 1.374 g/mL.

Many of the purified rotavirus particles shown in Figure 2 were found at the top of the gradient, even without treatment with SDS or EDTA. This indicates that their densities were no longer 1.35 g/mL but 1.34 g/mL or less. Treatment with SDS but not EDTA caused a significantly greater number of the 1.35 g/mL particles to shift to this lower density.

The presence of lower density particles in rotavirus preparations have been reported by other investigators.^{10,14} Their results suggest that this lower density material may be empty protein capsids (both double- and single-shelled) or particles associated with less dense debris. Because the rotavirus particles used in this study were extensively purified, it is highly unlikely that this type of debris will be present. In addition, because these particles were labeled in their RNA component, the low-density materials cannot simply be empty capsids. It is most likely that a portion of the purified 1.35 g/mL particles are altered in some manner during storage at 4°C. This alteration, in turn, may cause the density of the particles to be modified either before or during treatment with high concentrations of CsCl, an effect that could be enhanced by pre-treatment of these virions with SDS.

Figure 2

Analysis by CsCl gradient centrifugation of the effects of SDS and EDTA on the densities of 1.35 g/mL rotavirus particles labeled with [^3H]-uridine. (A) Untreated virions; (B) SDS-treated virions; and (C) EDTA-treated virions.

Analysis of Rotaviruses by Glycerol Gradient Centrifugation -- Next the states of rotavirus particles after SDS and EDTA treatments were examined by an analysis of the sedimentation rates of these particles in glycerol gradients. For this study both 1.35 and 1.37 g/mL purified rotavirus particles labeled with [^3H]-uridine were treated and analyzed. Untreated ^{14}C -labeled viruses of 1.35 g/mL density were used as markers. SDS had no effect on the sedimentation velocities of 1.35 g/mL particles but EDTA caused these particles to sediment at a reduced rate (Figure 3). The rate of sedimentation of the EDTA-treated virions was not detectably different from that of untreated 1.37 g/mL rotavirus particles. This result shows that EDTA, but not SDS, modifies the sedimentation coefficients of 1.35 g/mL rotavirus particles.

Effects of SDS and EDTA on the Adsorptive Properties and Isoelectric Points of Rotaviruses -- The results presented to this point, indicate that rotavirus inactivation by SDS has little effect on the densities or sedimentation coefficients of the viral particles. Therefore, other methods that can be used to detect alterations in the capsid components of virions were investigated.

The initial step in the infection of a cell by a virus is the adsorption of the virus to receptors on the surface of the cell. Factors that prevent viral adsorption normally result in loss of infectivity. The portion of a non-membranous virus involved in adsorption to cellular receptors is its capsid. Therefore, alterations of the capsid could result in loss of viral adsorptive ability and, consequently, loss of infectivity.

Figure 3

Analysis by glycerol gradient centrifugation of the effects of SDS and EDTA on the sedimentation rates of 1.35 and 1.37 g/mL rotavirus particles labeled with [^3H]-uridine. (A) Untreated 1.35 g/mL particles; (B) SDS-treated 1.35 g/mL particles; (C) EDTA-treated 1.35 g/mL particles; and (D) untreated 1.37 g/mL particles. Arrows mark the positions of untreated, ^{14}C -labeled rotavirus particles mixed with the treated samples just before centrifugation. Direction of centrifugation is from right to left.

Treatment of rotavirus with SDS caused complete loss of ability to adsorb to CV-1 cells (Table 2). EDTA treatment also caused nearly complete loss of adsorptive ability. Therefore, it is clear that both treatments cause the viral capsids to be altered in some fashion which prevents the virions from attaching to cells. This result indicates that capsid damage could be responsible for loss of infectivity in both cases.

Alterations of the capsid of rotavirus by SDS and EDTA were also studied by isoelectric focusing of treated virions. SDS caused some modification of the isoelectric points of 1.35 g/mL rotavirus particles but the effect was not large (Figure 4). EDTA caused a somewhat larger shift in these isoelectric points. The isoelectric points of EDTA-treated 1.35 g/mL particles were essentially identical to untreated 1.37 g/mL particles. This result supports the previous suggestion that both EDTA and SDS affect the capsids of rotavirus particles but the effect of SDS is not as great as that of EDTA.

Table 2

Effect of SDS and EDTA Treatment on the Abilities of
[³H]-Uridine Labeled Rotaviruses to Adsorb to CV-1 Cells*

Sample	cpm Added	cpm Bound	% cpm Bound	% of Control
Untreated	705	104	14.8	-
SDS-treated	744	0	0.0	0.0
SDS-control	755	63	8.3	-
EDTA-treated	715	7	1.0	8.3
EDTA-control	680	82	12.1	-

*[³H]-uridine labeled rotaviruses were treated with either 0.1% SDS for 60 min at 21°C or 0.01 M EDTA for 20 min at 37°C and diluted 50-fold. These samples along with control samples of the infectious virus at the same dilution in either SDS (0.002%) or EDTA (0.2 mM) were added to confluent monolayers of CV-1 cells. Radioactivity added and bound were determined and percentages bound were calculated. All samples were run in triplicate.

Figure 4

Isoelectric focusing of SDS and EDTA treated rotaviruses labeled with [³H]-uridine. (A) Untreated 1.35 g/mL particles; (B) SDS-treated 1.35 g/mL particles; (C) EDTA-treated 1.35 g/mL particles; and (D) untreated 1.37 g/mL particles.

Analysis of Proteins in Rotaviruses Treated With SDS and EDTA by Gel Electrophoresis --

A final determination of the effects of SDS on infectious rotavirus particles was made through an analysis of the proteins in treated virions by SDS-polyacrylamide gel electrophoresis. A number of studies have been reported concerning the proteins in the inner and outer shells of rotavirus particles.^{4, 9, 10, 14-19} In general, these studies indicate that rotavirus from several different species contain about 4 to 6 proteins in their inner shells and 3 to 4 proteins in their outer shells. There is some disparity in the results regarding the amounts of these proteins and their molecular weights.

The electrophoretic pattern of the protein from 1.35 g/mL particles found here (Figure 5a) is quite similar to that found by Todd and McNulty with lamb rotavirus.¹⁹ Although these and other investigators have resolved between 8 and 10 protein bands in their gel patterns, only 5 peaks were clearly resolved in the gel pattern shown here. These major peaks are numbered 1 to 5 but is assumed that they camouflage a number of minor peaks and, therefore, consist of more than 5 viral proteins. Treatment with EDTA (Figure 5b) caused the loss of almost all radioactivity from peaks 3 and 5. This finding agrees with the results of other investigators that suggest rotavirus proteins of this approximate size and quantity are outer shell proteins. When 1.35 g/mL rotavirus particles were inactivated by SDS, only proteins banding in peak 3 were removed from the particles (Figure 5c). This result strongly suggests that some capsid alternation occurs upon treatment of infectious rotavirus with SDS but this alteration is less extensive than that caused by EDTA.

The finding that proteins in peak 3 but not those in peak 5 are removed by SDS from rotavirus particles shows that only a portion of the proteins in the outer shell are removed by this detergent. This finding leads to the suggestion that the protein(s) in peak 3 is located on the extreme outside of the viral particle and is, therefore, most easily removed. It is interesting that the loss of this protein did not detectably alter the density or sedimentation value of the rotavirus particle but that removal of the proteins in both peaks 3 and 5 by EDTA caused a significant change in both. The quantity of proteins in peak 3 is apparently about 43% of that in peak 5. On this basis alone the loss of peak 3 protein should have partially altered the density and sedimentation properties of rotavirus. It is unclear why this was not observed.

The difference in the effects of EDTA and SDS on the outer shell proteins of rotavirus also leads to the suggestion that the bonding through which the different outer-shell proteins associate with other components of the virion are significantly different. Apparently, it is the properties of these bonds that can determine the susceptibility of the virus to a particular detergent or other chemical compound. It may be possible to understand certain properties of these bonds through further studies on the nature of the chemicals that can cause these bonds to be broken.

The results of this study indicate that the physical properties of rotavirus SA-11 are not significantly altered through inactivation by SDS, and some if not most outer shell proteins remain associated with the virions after this treatment. If other rotavirus strains are affected in a similar fashion, inactivation by SDS may be an excellent method to prepare killed virus vaccines of this virus.

Figure 5

Electrophoretic analysis of ^{14}C -labeled proteins retained in rotavirus particles after EDTA and SDS treatments. (A) Untreated 1.35 g/mL particles; (B) EDTA-treated 1.35 g/mL particles; and (C) SDS-treated 1.35 g/mL particles. Direction of electrophoresis is from left to right.

Bacteriology

This section reports the effects of moisture on the inactivation of enteric bacteria in wastewater sludge by ionizing radiation.

Disinfection with ionizing radiation has been investigated with many different organisms in a variety of media. Inactivation of enteric bacteria by radiation in sewage²⁰⁻²⁵ and wastewater sludges²³⁻²⁸ has also been studied by a number of investigators. These reports show that pathogenic, enteric bacteria are generally quite easily inactivated by ionizing radiation in these aqueous environments. A preliminary report from this laboratory suggested, however, that this may not be true in dewatered sludges.²⁹ That report showed that the apparent D_{10} -value (the amount of irradiation required to inactivate 90% of a population of a particular organism or group of organisms) for total coliforms in dried, raw sludge varied from 17 to 353 krads. However, in liquid sludge the D_{10} -value for coliforms was consistently 20 to 30 krads.

Because the use of ionizing radiation to disinfect dried sludges is being given serious consideration, it is imperative to predict the effects of the drying process and gamma radiation on the

survival of enteric bacteria. Therefore, a thorough study was carried out with six representative species in sludge to determine the effects of dewatering by evaporation on their survival as well as on their D_{10} -values. To determine the general applicability of these results, a comparative study was made with the indigenous enteric bacteria in raw sludge.

The results of this investigation show that the effects of dewatering on the survival of enteric bacteria and on the specific D_{10} -values of these bacteria in sludge is somewhat different for each bacterial species. However, in no case did any species display unusual resistance to inactivation by ionizing radiation. It appears, therefore, that radiation treatment can be effectively used to inactivate enteric bacteria in dewatered as well as in liquid sludges.

Materials and Methods Used in This Study -- Cultures of Klebsiella sp., Enterobacter sp. and Proteus mirabilis used in these experiments were provided by Dr. John Uhlrich (Dept of Microbiology, University of New Mexico School of Medicine, Albuquerque). Representative strains of Escherichia coli and Streptococcus faecalis were isolated from raw sludge and the strain of Salmonella typhimurium was obtained from the American Type Culture Collection.

Growth of Bacteria in Sludge. Raw sludge containing about 5% solids was obtained from the Albuquerque Sewage Treatment Plant and sterilized with gamma irradiation (6 megarads). This material was seeded with about 2×10^7 colony forming units (cfu) of a specific bacterium (grown in Trypticase Soy broth) per millilitre of sludge and incubated at 37°C for 48 hr. Bacterial cfu were then measured by standard colony counting procedures as specified for each bacterial type. After diluting the sludge in saline solution, 0.1 mL samples containing Klebsiella sp., Enterobacter sp. or E. coli were streaked on pre-poured M-coliform agar plates and enumerated after 24 hr of incubation at 37°C . Samples (0.1 mL) of S. typhimurium or P. mirabilis were enumerated by the same method on Hektoen-Enteric (HE) agar, and 1.0 mL samples containing S. faecalis were suspended in KF Streptococcus agar and counted after 48 hr at 37°C .

Dewatering of Sludge by Evaporation. Either sterilized raw sludge containing specific individual bacterial species grown to saturation levels, or fresh, untreated raw sludge with indigenous organisms was poured to a depth of about 1 cm in glass-drying pans and dewatered by natural evaporation at 21°C . Samples taken at different moisture levels were adjusted to their original moisture content (about 5% solids) by blending with calculated volumes of water and assayed immediately for viable organisms to determine the effects of dewatering. Loss of water was essentially complete in all the drying pans after about 7 days when the dried sludge contained approximately 95% solids.

Gamma Irradiation of Dewatered Sludge Samples. Samples of sludge held at 21°C without dewatering, and dewatered samples containing about 50% and 95% solids, respectively, were irradiated with cobalt-60 at a dose rate of 26 krad/min. Survival of organisms in sludge containing specific bacterial species was monitored by plating on the media described above. Viability of microorganisms in sludge monitored for indigenous species was determined by plate

count with nutrient agar (total cfu), KF Streptococcus agar (*S. faecalis* cfu), and HE agar (lactose-fermenting cfu, H₂S-producing cfu, and other non-lactose-fermenting cfu).

It should be noted that thorough decontamination of the blenders used to reconstitute the sludge samples in these experiments required a treatment of more than 30 min in concentrated hypochlorite solution (bleach) or autoclaving. Lesser decontamination procedures resulted in survival of significant numbers of residual viable bacteria in the blenders.

Results -- The most direct method to determine the effects of dewatering on the survival of enteric bacteria and on their rates of inactivation by ionizing radiation in raw sludge is probably to dewater the sludge by evaporation and measure the survival and D₁₀-values of the indigenous organisms in the dewatered samples. Because of the technical difficulties encountered in determining the survival of individual bacterial species by this procedure, it is less than satisfactory. Problems associated with the identification of bacteria in sludge can be avoided if the sludge is populated with only one viable bacterial type. Therefore, the following procedure was chosen for this study:

1. Colonize sterilized, raw sludge containing about 5% solids with only one bacterial species;
2. Dewater the sludge by evaporation after colonization;
3. Measure the effects of dewatering on bacterial survival; and
4. Determine the inactivation rates of the viable organisms in the dewatered samples by ionizing radiation.

This procedure eliminates problems associated with identifying individual organisms in the sludge. At the same time, because the particular bacterial type to be examined was grown in sludge, it should be associated with the sludge in a manner quite similar to indigenous microbial species and, therefore, respond to dewatering and irradiation. To confirm that the behavior of individual bacterial species in seeded sludge are a close approximation to the behavior of indigenous enteric bacteria in sludge, the effects of dewatering and irradiation on the indigenous organisms in fresh raw sludge were also measured.

A temperature of 21°C was selected for dewatering in this study because it is high enough to allow efficient evaporation but is low enough to be an insignificant factor in bacterial death over the time scale in which dewatering occurred. The bacterial count in samples taken at different moisture levels during the drying procedure did not change significantly during the remainder of the drying period when the samples were held at 21°C without further dewatering.

Effect of Dewatering on the Survival of Enteric Bacteria in Sludge. To determine the effect of dewatering by evaporation on the survival of enteric bacteria in sludge, sterilized raw sludge containing about 5% solids was seeded with one of the six model strains of enteric bacteria

(*E. coli*, *Klebsiella* sp., *Enterobacter* sp., *S. typhimurium*, *P. mirabilis* and *S. faecalis*) at a level of about 2×10^7 /mL. After 48 hr of incubation at 37°C , each organism reached a density of about 2×10^8 /mL. This result shows that raw sludge can support the growth of all six bacterial strains. However, the cell density is significantly less than that attained in broth under the same conditions which is typically about 2×10^9 /mL for these bacterial species.

When the recolonized raw sludge samples were dewatered at 21°C , bacterial colony forming units were generally found to increase during the initial stages of dewatering (Figure 6). However, for all enteric bacteria studied, the initial increase in bacterial numbers observed after partial dewatering was consistently followed by a significant reduction in bacterial colony forming units with further dewatering. The size of this reduction ranged from less than one order of magnitude per gram of dry matter (*S. faecalis*) to about four orders of magnitude (*P. mirabilis*).

Figure 6. Effect of dewatering by evaporation on colony forming units (cfu) of several enteric bacteria in sludge. Raw sludge (5% solids) containing about 4×10^9 of a specific enteric bacterium per gram of solids was dewatered by natural evaporation at 21°C . Samples were taken at different moisture levels and assayed immediately for cfu. The surviving fraction of cfu was calculated relative to a sample held at 21°C without loss of water for the same period of time. Symbols: ●, *S. faecalis*; Δ, *E. coli*; ○, *Klebsiella* sp.; ▲, *Enterobacter* sp.; □, *S. typhimurium*; ■, *P. mirabilis*.

We then determined the effect of dewatering on the numbers of indigenous enteric bacteria in fresh raw sludge (Figure 7). The results were quite similar to those found with the seeded sludge except that there was a more rapid growth of most classes of organisms with partial dewatering. Further dewatering, however, again consistently reduced these numbers below those present in the original raw sludge.

Figure 7

Effect of dewatering by evaporation on colony forming units (cfu) of several groups of indigenous enteric bacteria in raw sludge. Raw sludge (4% solids) was dewatered by natural evaporation at 21°C and assayed for recoverable cfu with nutrient agar, HE agar, and KF Streptococcus agar when samples reached 44 and 95% solids. Symbols: ○, total cfu (nutrient-agar), □, *S. faecalis* cfu (KF Streptococcus agar); ●, lactose-fermenters (HE agar); ■, H₂S-producers (HE agar); ▲, other non-lactose fermenters (HE agar).

The increase in the numbers of enteric bacteria in sludge observed after partial dewatering may be due to a number of factors. For example, dewatering may increase the concentration of nutrients available for bacterial growth. A more likely explanation is that aeration is improved upon dewatering and the facultative organisms in sludge are able to grow to higher numbers. Other explanations are also possible.

It should be mentioned that the initial increase in bacterial numbers found after the partial dewatering of raw sludge is not observed in these dewatered samples after the sludge has been retained for several weeks at 21°C without further loss of water. These results will be presented and discussed in the next quarterly report. At this time, it is enough to say that partial dewatering of sludge in itself was not found to reduce the number of viable enteric bacteria, but that thorough dewatering by evaporation does have this effect.

Moisture Effects on Bacterial Inactivation Rates With Ionizing Radiation. Next we examined the inactivation rates of enteric bacteria with ionizing radiation in dewatered sludge. Samples chosen for this study were (1) the original sludge (5% solids) included as a control, (2) partially-dewatered sludge (about 50% solids) included to represent sludge obtained after such processes as vacuum filtration, centrifugation, and composting, and (3) dried sludge (about 95% solids) included to represent material obtained from a drying bed.

Dewatering sludge affected the radiation inactivation rates of typical lactose-fermenting Enterobacteriaceae (E. coli, Klebsiella sp. and Enterobacter sp.) differently. The radiation inactivation rate of the strain of E. coli used was not detectably altered by dewatering (Figure 8). However, the Klebsiella sp. was protected in partially-dewatered but not by fully-dewatered sludge (Figure 9), and the Enterobacter sp. was slightly protected against radiation in sludge by the loss of water (Figure 10).

The effect observed with the Enterobacter sp. was much more pronounced with S. typhimurium where the D_{10} -value observed in liquid sludge more than doubled in the dried state (Figure 11). P. mirabilis, the other H_2S producer studied, was extremely sensitive to ionizing radiation at all moisture levels (Figure 12). Finally it was found that the strain of S. faecalis used here was the most radiation resistant enteric bacterium at all moisture levels and was slightly but not greatly protected by dewatering sludge (Figure 13).

Figure 8. Effect of moisture content on the inactivation rates of E. coli by ionizing radiation in raw sludge. Dewatered samples of raw sludge containing E. coli were irradiated and assayed for recoverable colony forming units (cfu). The percentages of solids and D_{10} -values (based on the lines drawn) for each set of samples is shown. Samples containing 5 and 47% solids were treated and assayed in triplicate while a series of six samples containing 95% solids were tested at each dose specified.

Figure 9. Effect of moisture content on D_{10} -values of *Klebsiella* sp. in raw sludge. A series of six samples were tested at each dose at every moisture level (percentage of solids).

Figure 10. Effect of moisture content on D_{10} -values of *Enterobacter* sp. in raw sludge. Samples at 5 and 57% solids were studied in triplicate at each dose and a series of six samples were studied at each dose at 95% solids.

Figure 11. Effect of moisture content on D_{10} -values of *S. typhimurium* in raw sludge. A series of six samples were tested at each dose at every moisture level (percentage of solids).

Figure 12. Effect of moisture content on D_{10} -values of *P. mirabilis* in raw sludge. A series of six samples was tested at each dose at every moisture level (percentage of solids).

Figure 13. Effect of moisture content on the D_{10} -values of *S. faecalis* in raw sludge. A series of six samples was tested at each dose at every moisture level (percentage of solids).

In some cases there was considerable variation between samples in these experiments, presumably due to differences in the numbers of viable bacteria contained in different 0.25-g samples used for the study. These differences coupled with an apparent departure from a single, first-order, inactivation rate constant in some instances make the straight line used to determine the D_{10} -values only an approximation. However, in no instance was the departure from the designated D_{10} -value very large. Furthermore, and perhaps the most significant feature of these results, no sample of any bacterium showed an excessively large D_{10} -value. This is in contrast to the results reported earlier by Brandon and Neuhauser.²⁹ It is likely that their findings came as a result of the use of contaminated blenders, a problem which was recognized and carefully avoided in the present study.

The radiation inactivation rates found for individual enteric bacteria in dewatered raw sludge were next compared with those of several classes of indigenous microorganisms. The results are shown in Figure 14 and summarized in Table 3. Lactose-fermenting bacteria detected on HE agar, which would include *E. coli*, *Klebsiella* sp. and *Enterobacter* sp., had D_{10} -values in both liquid and dewatered sludges of less than 50 krads. H_2S producers found in this same agar, which would include *S. typhimurium* and *P. mirabilis*, were present in relatively low numbers in all samples. Even so, it is apparent that the D_{10} -value for this class of organisms was not unusually high. Other non-lactose fermenters detected on HE agar had D_{10} -values which appeared to be no greater than those of the lactose fermenters.

Figure 14. Effect of moisture content on the inactivation rates of several classes of indigenous enteric bacteria by ionizing radiation in raw sludge. Raw sludge containing 5, 44, or 95% solids with indigenous bacteria was treated with ionizing radiation and assayed for total colony forming units (■, nutrient agar), *S. faecalis* (□, KF Streptococcus agar), lactose-fermenters (○, HE agar), H_2S producers (◇, HE agar), and other non-lactose producers (△, HE agar). A series of six samples was tested at each dose and at every moisture level (percentage of solids). The values given bracket the range of results.

Table 3

D_{10} -Values for Indigenous Microorganisms in Liquid and Dewatered Raw Sludge*

Sludge (% solids)	D_{10} -Value (krads)			
	<u>Streptococcus faecalis</u>	<u>Lactose Fermenters</u>	<u>H₂S Producers</u>	<u>Other Non- Lactose Fermenters</u>
3.5	177	30	< 60	< 40
44	160	30	< 40	< 30
95	95	< 50	< 85	< 60

* Summary of data shown in Figure 14.

The effects of radiation on indigenous S. faecalis were quite similar to those observed with the seeded strain of this organism. In this case, however, no protection was observed in the dewatered samples.

Finally, the inactivation rates for total indigenous colony forming units observed after plating on nutrient agar was consistent with the fact that various types of bacteria are detected on this media. Because the number of bacterial colonies found in KF Streptococcus agar was sometimes greater than that found on nutrient agar, the latter agar clearly does not detect all viable bacteria. It should be noted that a few survivors were found on nutrient agar after high doses of radiation were administered at all moisture contents. The nature and radiation resistance of these survivors is being further examined.

The existence of radiation resistant bacteria is well documented³⁰ and the presence of such organisms in sludge is not unexpected. However, we are aware of no reports concerning the existence of naturally-occurring, pathogenic, enteric bacteria that are unusually resistant to ionizing radiation. For this reason and because of the results found in the present study, it appears that ionizing radiation should be a feasible method of ridding wastewater sludge of bacterial pathogens.

New Mexico State University (NMSU) Agronomic and Refeeding Studies

Animal Experimentation

Heavy Metals and "Refractory Organic Residues" in Cattle Fed Sewage Solids (NMSU-Clayton Exp. 78-79) -- We have completed a second cattle experiment in which assessment of risk from heavy metals and/or refractory organic compounds in sewage solids was the primary objective. Dried, gamma-irradiated sewage solids (LCSS-78/79-1) were incorporated into an otherwise conventional diet for growing-finishing cattle and fed ad libitum for 84 days, at which time representative animals from "control" (conventional diet) and "experimental" (sewage solids diet) groups were slaughtered. Samples of livers and kidneys were tested for a whole spectrum of elements including heavy metals. Also, samples of adipose tissue ("kidney fat") were tested for 22 refractory organic compounds ("pesticide residues").

Results show that feeding the sewage product at levels of two to three pounds per head per day during an 84-day period caused no buildup of heavy metals in livers or kidneys, nor any buildup of refractory organic residues in adipose tissues, beyond ranges that are reported for beef cattle produced conventionally in the U. S. A. (Tables 4, 5, and 6).

Table 4
Effects of Dried, Gamma-Irradiated Sewage Solids on Feeding Performance of
Growing Beef Cattle*

	Treatment Groups			
	Controls		Experimentals**	
	Steers (n = 5)	Heifers (n = 17)	Steers (n = 5)	Heifers (n = 17)
Initial Body Weight, pounds	727	644	732	637
Performance, First 84 Days: Daily Feed, pounds	27.13	21.35	22.80 (-15%)	19.43 (-9%)
Daily Gain, pounds	2.54	1.97	1.95 (-23%)	1.63 (-17%)
Feed/Gain, pounds	10.7	10.8	11.7 (+9%)	11.9 (+10%)
Average Daily Consumption of Sewage Solids, pounds (84 days)	None	None	2.74	2.35
Performance, Last 50 Days: Daily Feed, pounds	-	16.29	-	20.74 (+27%)
Daily Gain, pounds	-	2.60	-	2.71 (+4%)
Feed/Gain, pounds	-	6.27	-	7.65 (+22%)

* NMSU/CLRC Exp. #2, 1978-79

** Sewage solids from Las Cruces Treatment Plant (LCSS-78/79a) were incorporated into a conventional diet: at 5% of diet during first week; at 10% second week; at 15% next seven days and at 20% of diet during final 22 days of initial 84-day phase. During first 84 days, the conventional diet was "Clayton mixture 75 M." During subsequent 50 days ALL cattle (heifers) received a conventional, finishing diet, "Clayton mixture 85C." Thus sewage solids were fed to experimental heifers for 84 days after which these same heifers received the conventional diet, without sewage solids.

Table 5

Element Content^a of Livers and Kidneys From Cattle Fed Conventionally
or Fed Dried, Gamma-Irradiated Sewage Solids^b at About Twelve Percent
of Diet for 84 Days^{c, d}

Elements	Tissues ^e							
	Livers ^f				Kidneys ^f			
	Controls (n = 4)		Experimentals (n = 5)		Controls (n = 4)		Experimentals (n = 5)	
Ag	36.1	(7.8)	34.1	(5.3)	2.4	(.3)	2.8	(.7)
Ca	125	(43)	122	(19)	379	(44)	400	(61)
Cd	.202	(.10)	.136	(.07)	.348	(.07)	.532	(.23)
Cr	3.6	(3.5)	3.0	(2.6)	9.9	(4.4)	9.4	(1.8)
Cu	211	(54)	183	(43)	139	(18)	151	(32)
Fe	190	(39)	172	(37)	172	(49)	210	(45)
K	9131	(203)	8977	(534)	8060	(991)	8554	(1393)
Mg	614	(61)	557	(37)	606	(86)	626	(128)
Mn	7.95	(1.20)	6.44	(.65)	3.15	(.36)	3.38	(.60)
Na	2125	(163)	1705	(228)	2631	(609)	2545	(694)
Ni	1.2	(1.0)	.8	(.4)	4.6	(2.3)	4.2	(.6)
P	10270	(500)	10120	(535)	7690	(1245)	7907	(1448)
Pb	< .8	-	< .8	-	< .8	-	< .8	-
Zn	115	(15)	95	(15)	50	(21)	60	(11)

^aSpecimens were "wet ashed" by nitric acid predigestion and perchloric acid digestion. Element content of resulting solutions were measured by atomic absorption (emission) spectrophotometry in a Perkins-Elmer 403 instrument.

^bSewage solids from Las Cruces Treatment Plant (LCSS-78/79a).

^cCattle were slaughtered on day 85 or 86 without any "withdrawal."

^dThis is the NMSU/CLRC Exp. 2, performed between 1978 and 1979.

^eTissue content, $\mu\text{g/g}$ of dry matter.

^fValues shown are means ($\pm$ standard deviations, $\sqrt{\Sigma x^2/n - 1}$).

Table 6

Content of "Refractory Organic Compounds" in Adipose Tissue of Beef Heifers Slaughtered After Consuming Conventional or Experimental^a Diets for 84 Days, or After Consuming Conventional Diet for 50 Days Following 84 Days on Experimental^a Diet^b

Refractory Organic Residues ^c							
Animal Tag No.	p,p'DDE	αBHC	βBHC	Dieldrin	Oxychlordane	Heptachlor-Epoxide	PCB as AR-1254
Parts per billion in fresh tissue (ppb)							
<u>Controls (Conventional Diet, 84 days: immediate slaughter)</u>							
4150	< 10	10	0	70	< 10	40	< 50
4159	10	10	0	70	< 10	40	< 50
4412	10	10	0	60	< 10	40	< 50
5417	< 10	10	0	70	< 10	40	< 50
$\bar{X} \pm$ standard deviation	< 10	10	0	67 ± 5	< 10	40	< 50
<u>Experimentals (LCSS-78, 79 @ approx. 12% of Diet, 84 days: immediate slaughter)</u>							
4167	50	< 10	< 20	80	10	40	200
4408	50	10	< 20	70	10	40	160
4413	50	10	< 20	80	10	40	190
5414	60	< 10	< 20	60	10	30	150
$\bar{X} \pm$ standard deviation	53 ± 5	< 10	< 20	73 ± 10	10	38 ± 5	175 ± 24
<u>Experimental-Withdrawals (LCSS-78, 79 @ 12% of Diet, 84 days, followed by Conventional Diet, 50 days)</u>							
3303	40	20	0	190	10	90	110
4308	40	20	0	180	20	90	120
4403	30	20	< 20	170	10	80	90
5411	30	20	0	160	10	70	90
$\bar{X} \pm$ standard deviation	35 ± 6	20	< 20	175 ± 13	12 ± 5	83 ± 10	103 ± 15

^a Experimental diet was same as conventional diet except sewage solids were substituted at levels averaging about 12% over an 84-day period. Sewage solids were collected at Las Cruces, NM (LCSS-78/79a).

^b This represents the NMSU/CLBC Exp. 2, performed between 1978 and 1979.

^c Analysis of adipose tissue for 22 refractory organic compounds was conducted by Colorado Epidemiologic Pesticide Laboratory, Colorado State University. TNC, HCB, and pp DDT were detected, at limits of detection (20 ppb) in one or more individuals, but no trends were seen due to treatments. Compounds not detected were: o,p'-DDT, o,p'-DDE, p,p'-DDD, o,p'-DDD, Chlordane, Decthal, Heptachlor, Endrin, Aldrin, Lindane, Mirex, and Methoxychlor.

Cattle Breeding Experiment at USDA/SEA Jornada Experimental Range --

Blood Composition. Specimens of jugular blood were first collected from representative cows in the herd at the USDA/SEA Jornada Experimental Range in April 1979, and later in early June 1979, before supplementation had begun for the 1979 season. The last specimens were collected in late July after supplementation had been accomplished for the 1979 season. Results are summarized in Table 7. As expected, there appear to be some mineral nutrients that are low in the blood of these cattle. Specifically, Cu, Mn, and Zn appear to be in the low range of normal values in the early samplings, becoming "borderline" or deficient by late July. None of the heavy metals were present at levels that would indicate any cause for concern.

Breeding Performance. A summarization of data from the breeding herd was submitted for publication in Transactions, American Nuclear Society (1980). A copy is attached as an appendix to this report. The results tend to confirm the hypothesis that products derived from "raw" (primary) sludge could be recycled as supplemental feeds for ruminants subsisting on poor-quality, roughage feeds, at substantial nutritive benefits without necessarily incurring undue risk from heavy metals or other toxicants such as halogenated hydrocarbons.

Sheep Breeding Experiment -- Since the October 1979 Quarterly Report, work has progressed rapidly on the Sheep Breeding Trial associated with the Sewage Recycling Project. All sheep are currently housed at the research site. Shades, additional pens, and feed-storage areas will be completed in the near future.

Phase I. Eighty-seven fine-wool ewes (four of the original 91 died) are in three dietary treatment groups including: (1) negative control (basal) which will not fully meet the protein requirement of ewes during late gestation and early lactation; (2) positive control (CSM) containing additional protein in the form of 3.5% cottonseed meal and (3) experimental diet (sludge) which contains 7% irradiated sewage sludge. Treatments were imposed ~ 50 days prior to the breeding season and will continue through two successive lambings. Daily blood samples were collected from ten animals in each group throughout an estrous cycle prior to breeding. Serum progesterone and estradiol-17 β will be quantified by radioimmunoassay in these and subsequent weekly samples.

Two Debouillet rams fitted with marking harnesses were placed with each group of females on October 20, 1979, for a 51-day period ending on December 15, 1979. Rams were rotated at three day intervals and breeding dates were obtained by twice-daily observations. Although definitive conception data await lambing, preliminary results obtained by visual observation are summarized in Table 8. As expected, most ewes were bred within the first 17 days of the breeding season. Animals consuming the basal diet appeared to have some difficulty conceiving while the sludge group exhibited conception patterns very similar to those obtaining additional cottonseed meal. Lambing should begin in mid-March 1980, at which time a more critical evaluation will be made.

Table 7

Element Content of Jugular Whole Blood From Mother Cows at the USDA/SEA Jornada Experimental Range, Las Cruces, NM, as Affected by Dietary Supplementation With Cottonseed Meal (CSM) or Experimental Supplement Containing 62% Dried, Gamma-Irradiated Sewage Solids (RS #2)

Number of Cows Sampled	Date of Sampling*				
	4/79	6/79	7/79		
	12	11	Treatment Groups		
			Controls	CSM	RS #2
Elements			6	5	6
Ag	< .004	< .004	.015 (.011)	.021 (.017)	.009 (.009)
Ca	28.8 (4.0)	13.7 (3.5)	13.3 (1.0)	15.1 (3.3)	17.0 (8.0)
Cd	.025 (.031)	< .03	.091 (.110)	.105 (.112)	.097 (.145)
Cr	.20 (.19)	< .04	.058 (.022)	.061 (.031)	.073 (.082)
Cu	.698 (.08)	.520 (.06)	.402 (.08)	.434 (.06)	.565 (.17)
Fe	471 (54)	415 (94)	294 (64)	319 (53)	373 (120)
K	338 (125)	283 (77)	224 (44)	236 (150)	225 (91)
Mg	19.3 (5.4)	13.3 (1.4)	16.4 (2.3)	14.3 (.6)	21.9 (8.3)
Mn	.038 (.01)	< .004	.010 (.008)	.011 (.015)	.018 (.020)
Na	1875 (285)	2053 (223)	1685 (233)	1437 (773)	1750 (494)
Ni	.060 (.027)	< .11	.334 (.267)	.404 (.354)	.429 (.558)
P	184 (12)	185 (27)	144 (24)	158 (13)	188 (68)
Pb	.018 (.022)	< .004	< .01	< .01	< .01
Zn	3.31 (.43)	2.27 (.51)	1.61 (.47)	2.01 (.32)	2.21 (.61)

* Content, $\mu\text{g/mL}$ (ppm), whole blood

Table 8

Conception Data^a of Ewes Fed Basal, Cottonseed Meal or
Sludge Diets Throughout the 51-Day Fall Breeding Season

Item	Treatment		
	Basal (n = 29) (%)	Cottonseed Meal (n = 27) (%)	Sludge (n = 31) (%)
Ewes serviced during first third of season	82.8	96.3	100.0
Ewes serviced during second third of season	20.7	7.4	3.2
Ewes serviced during last third of season	3.4	0	0
Ewes requiring two breedings to conceive	17.2	7.4	6.4
Ewes not bred during breeding season	6.9	3.7	0

^aData obtained by observing mating activity using rams equipped with marking harnesses.

Ewes were also weighed at monthly intervals and weights at the beginning and end of the breeding season are shown in Table 9. Animal weight was not adversely affected at either of these time periods by any of the experimental rations. However, the real drain on maternal nutrients occurs during late gestation and early lactation. Body weight measurements in February and March 1980, may therefore, prove more informative.

Table 9

Breeding Season (Fall) Weights (kg) of Ewes Fed Basal,
CSM, or Sludge Diets^{a, b}

Treatment	Onset of Breeding Season	End of Breeding Season
Basal	57.7 ± .9	64.0 ± 1.3
Cottonseed Meal	56.0 ± 1.1	64.4 ± 1.3
Sludge	56.9 ± 1.1	62.0 ± 1.4

^aData are Mean ± S. E.

^b. Column means do not differ ($P > .25$)

Phase II. During their second month of gestation, 64 ewes were divided into three groups on September 1, 1979, and fed the rations described previously to examine the influence of dietary sewage sludge on reproductive function in pregnant ewes. Weekly blood samples obtained during this period and through lactation will be assayed for progesterone and estradiol-17 β . Monthly weights were also obtained and representative values from early and late gestation are shown in Table 10. Neither basal nor sludge diets adversely affected animal weights during either of these periods indicating that all rations were adequate to maintain animal performance during gestation.

Table 10
Gestation Weights (kg) of Fall Lambing Ewes Fed Basal,
Cottonseed Meal or Sludge Diets^{a, b}

<u>Treatment</u>	<u>Early Gestation</u>	<u>Late Gestation</u>
Basal	56.5 $\pm$.8	66.4 $\pm$ 1.1
Cottonseed Meal	56.8 $\pm$ 1.1	66.0 $\pm$ 1.2
Sludge	57.0 $\pm$ 1.4	66.0 $\pm$ 1.5

^a Data are Mean $\pm$ S. E.

^b Column means do not differ ($P > .25$)

Lambing occurred during a two-week interval in December 1979, and a preliminary summary of lamb production is presented in Table 11. Lamb crop did not appear to suffer adversely from either the negative control or sewage sludge diets. It would be difficult to attribute variations in sex ratio of offspring or twinning rate to treatment since ewes were already pregnant prior to being placed on trial. The large number of male lambs produced in the basal and sludge groups and the number of twins obtained in the cottonseed meal group are, however, interesting to note. Lamb birth weights (Table 12) were not altered by feeding sewage sludge to ewes during gestation compared to basal or cottonseed meal diets (5.04 $\pm$.18 vs 5.04 $\pm$.18 vs 4.68 $\pm$.14 kg. respectively; $P > .10$). These results suggest that a ration containing 7% sludge is adequate to sustain normal fetal growth and development.

Table 11
Lambing Data From Ewes Fed Basal, Cottonseed Meal, or
Sludge Diets During Gestation

<u>Item</u>	<u>Treatment</u>		
	<u>Basal</u> (%)	<u>Cottonseed Meal</u> (%)	<u>Sludge</u> (%)
Lamb Crop	109.1	130.4	113.6
Male Lambs	66.7	53.3	72.0
Ewes Bearing Twins	9.1	30.4	18.2

Table 12

Lamb Birth Weights (kg) From Fall Lambing Ewes Fed Basal,
Cottonseed Meal, or Sludge Diets During Gestation^a

Treatment	Males	Females	Combined ^b
Basal	5.14 ± .23	4.86 ± .18	5.04 ± .18
Cottonseed Meal	4.5 ± .23	4.86 ± .18	4.68 ± .14
Sludge	5.14 ± .18	4.86 ± .32	5.04 ± .18

^aData are Mean ± S. E.

^bCombined Column Means do not differ ($P > .10$)

Jugular blood was obtained from seven ewes in each group at parturition and serum was tested for 26 constituents by a commercial laboratory (the laboratory is located at VetPath, on One Malcolm Avenue, in Teterboro, New Jersey). Serum calcium, iron, magnesium, potassium, chloride, phosphorous or sodium were not altered ($P > .10$) by feeding basal or sludge diets compared to cottonseed meal. Similarly, total protein, globulin, blood urea nitrogen, creatinine, uric acid, triglycerides, cholesterol, bilirubin, serum transaminase, and lactic dehydrogenase were not affected ($P > .10$) by the various treatments. As shown in Table 13, however, alkaline phosphatase (Alk. Phos.), gamma glutamyl transpeptidase (G-Glu-Trans), albumin and glucose differed significantly between the various dietary regimens. Alkaline phosphatase and gamma glutamyl transpeptidase were depressed in animals fed the negative-control diet compared to cottonseed meal or sludge. These values were, however, not below the normal reference range and may, therefore, reflect the decrease in protein intake that was designed into this trial for basal ewes. Similarly, serum albumin was decreased by both basal and sludge diets compared to cottonseed meal, again perhaps suggesting a slight dietary protein deficiency. Serum glucose was dramatically elevated at parturition by feeding sewage sludge. Increased blood glucose occurs in several abnormal conditions many of which are endocrine related, but the cause of the increase in sludge-fed ewes is not presently known. Lambs will be weaned in mid-February 1980, at which time a second serum sample from these ewes will be tested for the same constituents.

Table 13

Serum Constituents at Parturition in Ewes Fed Basal,
Cottonseed Meal, or Sludge Diets^a

Item	Treatment		
	Basal	Cottonseed Meal	Sludge
Alk. Phos. (IU/L)	27.0 ± 1.8 ^b	37.3 ± 3.4 ^c	37.0 ± 3.1 ^c
G-Glu-Trans (units/L)	31.1 ± 2.3 ^d	37.4 ± 3.4 ^e	38.1 ± 1.2 ^e
Albumin (g/dL)	2.8 ± .1 ^b	3.0 ± .1 ^c	2.8 ± .1 ^b
Glucose (mg/dL)	74.7 ± 12.8 ^b	83.9 ± 12.9 ^b	136.6 ± 19.4 ^c

^aData are Mean ± S. E.

^{b, c}Row means with different superscripts differ ($P < .05$)

Rat Bioassays of Beef Wholesomeness From Steers Fed Sewage Solids -- Commercial, albino rats have been fed Conventional or Experimental beef as 10%, 20%, or 30% of diet from weaning through maturity and one cycle of reproduction. Neither growth nor numbers of offspring weaned were adversely affected by dietary beef. Weaned offspring from all treatments were submitted to the New Mexico Diagnostic Laboratory for histopathological examinations. Data will be summarized in a subsequent report.

Agronomic Studies (Soil Chemistry)

Greenhouse Studies -- Results of the summer greenhouse study are complete and strengthen earlier evaluations of sludge as a source of important nutrients for sorghum (Table 14). Results of the winter greenhouse studies were reported in the January 1 to June 30, 1979 Progress Report³¹ and appeared to identify sewage sludge as a good source of Fe for sorghum. The summer crop yielded several times as much sorghum as the winter crop and not only more clearly identified earlier trends but identified new ones as well.

Table 14
Sorghum Dry Matter Yields* for Each Treatment on Three Soils

Treatment	Soil (g/pot)		
	Glendale	Lea	Elliott
(1) Sludge 0, Fe 0	22.7 c	5.6 cde	6.9 b
(2) Sludge 0, Fe 10	24.6 c	7.2 cd	6.6 b
(3) Sludge 10, Fe 0	23.7 c	20.2 b	21.0 a
(4) Sludge 10, Fe 10	26.1 abc	18.4 b	20.1 a
(5) Sludge 20, Fe 0	29.1 abc	27.2 a	20.6 a
(6) Sludge 20, Fe 10	26.7 abc	28.1 a	20.5 a
(7) Sludge extract 0, Fe 0	24.7 c	2.8 e	6.7 b
(8) Sludge extract 0, Fe 10	25.3 bc	4.3 de	6.8 b
(9) Sludge extract 10, Fe 0	20.5 abc	4.2 de	5.8 b
(10) Sludge extract 10, Fe 10	25.7 abc	6.1 cde	9.3 b
(11) Sludge extract 20, Fe 0	31.8 ab	6.8 cd	10.6 b
(12) Sludge extract 20, Fe 10	32.1 a	8.0 c	10.3 b

* Average of three replicates

abcde Means in the same column which are followed by the same superscript letter are not different (P > .05)

The Lea soil was selected for study because it is naturally and severely Fe-deficient. Sewage sludge additions to the Lea soil increased DTPA extractable soil Fe and Fe uptake by sorghum. Sorghum yields were well correlated to sludge rates. Soluble sludge extracts were not nearly as effective in improving Fe uptake as sludge. The Fe available to plants appears to have come directly from the sludge. Inorganic Fe additions to the Lea soil did little to improve Fe uptake. Yield increases also correlated with increased DTPA extractable Zn and Zn uptake.

The Elliot soil was initially identified as a Zn-deficient soil but later characterized as only low in "available" (DTPA-extractable) Zn. Sorghum yields in this oil were affected only by the sludge treatments; there was no significant difference among sludge rates. Fe and Zn uptake correlated well with increases in DTPA extractable Fe and Zn values and also correlated well with yields. Sewage sludge is apparently a good source of Fe and Zn for soils such as the Lea and Elliot soils, where micronutrients are in short supply.

The Glendale soil was characterized as Fe and Zn-sufficient by the DTPA soil test and was not expected to respond to sludge additions. Indeed, yields from the control treatments for Glendale soil were quite good. Other yields reflected some statistical trends but were not clearly related to soil test values which reflect sludge treatments. However, the highest yields were observed in the sludge treatment.

Plant tissue from all soils was analyzed for P following the summer crop and identified an additional potentially-important benefit of sewage sludge. Although all pots were uniformly fertilized with P, the greatest P uptake was associated with sludge amended soils in all cases. P-deficiency is common in New Mexican soils and has resisted correction with commercial fertilizer. Sewage sludge may be an alternative, or a desirable additive, to commercial fertilizer in solving P-related fertility problems. This aspect of sewage fertility value will be considered in more detail in subsequent agronomic studies.

Laboratory Studies -- Laboratory studies have concentrated on the effects of sludge addition on herbicide (specifically 2,4-D) behavior in soil. Both adsorption and degradation studies are underway. Results are still preliminary, but indicate an important aspect of sludge studies hereto largely ignored. Our first results with fresh sludge added to soil showed little or no effect on 2,4-D behavior in two soils. Preincubating the sludge with the soil for two months, however, changed results substantially. Apparently, at first the sludge is relatively unreactive towards the herbicide. As the sludge becomes converted to soil humus-type material with aging, it becomes much more reactive. For example, incubated sludge appears to increase 2,4-D adsorption and to increase the rate of herbicide degradation.

Further studies with other soils and other chemicals are necessary in incubated and non-incubated sludge amended systems to verify the preliminary findings. Data collected to date, however, appear to suggest that sludge changes its character with time; such changes cannot be ignored.

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APPENDIX

SEWAGE SOLIDS AS SUPPLEMENTAL FEED
FOR RUMINANTS GRAZING RANGELAND FORAGE

SEWAGE SOLIDS AS SUPPLEMENTAL FEED FOR
RUMINANTS GRAZING RANGELAND FORAGE^a

G. Stanley Smith,^b H. E. Kiesling,^b C. H. Herbel,^c
E. E. Ray,^b Patricia Trujillo,^b Ruy Orcasberro,^b
and J. S. Sivinski^d

New Mexico State University, Las Cruces, NM 88003
and
Sandia National Laboratories, Albuquerque, NM 87185

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^bProfessor, Assistant Professor, Professor, Research Specialist, and Research Assistant, respectively, Department of Animal and Range Sciences, NMSU.

^cResearch Leader, USDA/SEA Jornada Experimental Range, Las Cruces, NM 88001.

^dManager, Beneficial Uses Program, Sandia National Laboratories, Albuquerque, NM 87185.

The solids in raw sewage represent a vast resource of nutrients that are potentially returnable to agriculture where constraints of pathogens and toxic chemicals are not prohibitive. Gamma-irradiation from "waste" radionuclides such as Cesium-137 appears to be feasible as a means of pathogen reduction in large-scale operations.^{1,2} Since 1975 New Mexico State University has evaluated dried, "radicized" solids from raw sewage (primary sludge) as a prospective supplemental feedstuff for ruminants (cattle, sheep) subsisting on poor-quality, roughage feeds. Our published reports^{3,4,5} and unpublished data support the view that products derived from raw sewage could provide substantial nutritive value to ruminant animals, without necessarily incurring intolerable risk to the animals or the human food chain, especially if such products are used only periodically in the maintenance of breeding herds and/or in the early "background" programs of young ruminants destined for slaughter much later.

Objectives of experiments reported here were to demonstrate under practical conditions the benefits from an experimental feed supplement (containing 62% dried, irradiated sewage solids) for gestating-lactating beef cows grazing dormant forage on semi-desert rangeland, and to assess, under extreme conditions, the accumulation of heavy metals and/or refractory organic compounds (toxicants) in tissues of cattle fed sewage solids for almost three months.

Experiment 1. During 1978 and 1979 seventy-four beef cows were managed as a single herd at the USDA Jornada Experimental Range, on semidesert rangeland, near Las Cruces, New Mexico. One-third received no supplemental feed whereas one-third received conventional "supplement" (Cottonseed Meal, CSM) and the other one-third received experimental "supplement" (RS#2, containing 62% sewage solids) for about three months in 1978 and two months in 1979 when cows were grazing dormant grasses while in late gestation and early lactation. Effects of supplemental feeding were measured mainly in terms of calf numbers and body weights at weaning and subsequent rebreeding by cows. Results are summarized in Table 1. Samples of jugular blood, livers (biopsy sampled), and milk from these cows, taken at the end of the supplemental feeding period in each year, showed no differences ($P > .05$) between supplementation treatment groups, in contents of Cd, Hg, or Pb.

Experiment 2. Dried, gamma-irradiated sewage solids were incorporated into an otherwise conventional diet for growing-finishing cattle and fed ad libitum for 84 days in feedlot, at which time representative cattle fed conventionally or experimentally were slaughtered. Others that had been fed experimental diet were changed to conventional diet and fed for 50 additional days and slaughtered. Samples of livers and kidneys from animals slaughtered after 84 days were analyzed by Atomic Absorption (Emission) Spectrophotometry for heavy metals and other elements. Results are summarized in Table 2. Samples of adipose tissue ("kidney fat") from representative animals slaughtered after 84 days and after 84 + 50 days were tested for 22 refractory organic compounds ("pesticide residues") of which only 7 were detected, and none were at concentrations that exceed the range of values reported for beef cattle raised conventionally.

Conclusions

These results support our previous findings and tend to confirm the view that products derived from "raw" sewage (primary sludge) could be recycled as supplemental feeds for ruminants subsisting on poor-quality, roughage feeds, at substantial nutritive benefits, without necessarily incurring undue risk from toxicity to the animals or accumulation of toxicants such as heavy metals or halogenated hydrocarbons ("refractory organic compounds"). Further research is required before feeding of sewage products could be recommended in practical livestock production.

Table 1

Breeding Performance of Beef Cows Given No Supplemental Feed (NONE) or Conventional Supplement (Cottonseed Meal, CSM) or Experimental Supplement (RS#2, containing 62% sewage solids) During 1978 and 1979

Items	Supplemental Feed		
	None	CSM	RS#2
Cows per group, 1978	24	25	25
Calves weaned, 1978	17	22	23
Cows per group, 1979	23	24	24
Calves weaned, 1979	14	19	17
Total Calves/Total Cows	31/47	41/49	40/49
"Calf Crop, " %	66	84	82
Percentage change	-	+27	+24
Total Weight of Calves Weaned, Kg/ # Cows:			
1978	3375/24	4283/25	4304/25
1979	2470/23	3070/24	2550/24
TOTAL	5845/47	7353/49	6854/49
Calf weight/cow in group, Kg	125	150	140
Percentage Change	-	+20	+12
Cows Pregnant (11/30/79), %	61	87.5	87.5
(2/28/79), %	74	96	87.5

Table 2

Element Content of Livers and Kidneys (dry basis) From Cattle Fed Conventionally or Experimentally (in which dried, gamma-irradiated sewage solids comprised about 12% of total diet for 84 days)

Elements	Tissues							
	Livers				Kidneys			
	Conventional Group		Experimental Group		Conventional Group		Experimental Group	
	(n = 4)		(n = 5)		(n = 4)		(n = 5)	
	ppm, dry basis					ppm, dry basis		
	$\bar{x}$	($\pm s$)	$\bar{x}$	($\pm s$)	$\bar{x}$	$\bar{x}$	($\pm s$)	
Ag	36.1	(7.8)	34.1	(5.3)	2.4	2.8	(.7)	
Ca	125	(43)	122	(19)	379	400	(61)	
Cd	.20	(.10)	.14	(.07)	.35	.53	(.23)	
Cr	3.6	(3.5)	3.0	(2.6)	9.9	9.4	(1.8)	
Cu	211	(54)	183	(43)	139	151	(32)	
Fe	190	(39)	172	(37)	172	210	(45)	
Mg	614	(61)	557	(37)	606	626	(128)	
Mn	8.0	(1.2)	6.4	(.6)	3.2	3.4	(.6)	
Ni	1.2	(1.0)	.8	(.4)	4.6	4.2	(.6)	
Pb	< .8		< .8		< .8	< .8		
Zn	115	(15)	95	(15)	50	60	(11)	
Na, %	.212	(.016)	.171	(.023)	.263	.254	(.069)	
K, %	.913	(.020)	.898	(.053)	.806	.855	(.139)	
P, %	1.03	(.05)	1.01	(.05)	.77	.79	(.14)	

* Experimentals do not differ ($P > .05$) from Conventionals in regard to any of the elements measured.

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