Chemical and Biological Phenomena Observed with Sewage Sludges in Simulated Soil Trenches

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ABSTRACT

Chemical and biological observations were made on sewage sludge in a simulated trenching system in the greenhouse. Limed and unlimed raw and digested sludges were placed in simulated trenches in soil profile boxes in which corn (Zea mays L.) was sown. During the 160-day growth period, roots penetrated entrenched digested sludge and root growth proliferated throughout the sludge. Root penetration into the raw sludges was severely restricted. Gas analysis showed that anaerobic conditions prevailed for extensive periods during the 160 days in raw sludge. Methane and CO₂ levels reached 45 and 25%, respectively. The levels of CH₄ and CO₂ were <3 and 21%, respectively, in the digested sludge boxes. Nitrate nitrogen, adjacent to and beneath the sludge, was generally higher with digested than with raw sludge. Zinc and copper did not move from the sludge into the surrounding soil. The increase of these metals in corn leaves was relatively low, reaching only 131 μg/g Zn and 5.9 μg/g Cu as compared with 79 μg/g Zn and 3.8 μg/g Cu in the controls. Although low levels of fecal coliforms survived in the sludge, none were found in the soil surrounding the sludge. Total coliform numbers in the sludge after 160 days were negatively correlated with NH₃-N concentrations, suggesting that NH₃, generated on dissociation on NH₄⁺, may be important in reduction of human pathogens.

Additional Index Words: waste, sludge entrenchment, methane, carbon dioxide, nitrate, ammonia, zinc, copper, total coliforms, fecal coliforms.

Sludge production in the United States is presently estimated at 5 million dry metric tons per year. Waste water treatment is expected to become more complete and advanced, and by 1985 sludge production is estimated to exceed 10 million dry metric tons. Environmental restrictions placed upon ocean dumping, the high costs of energy for incineration, and the increasing cost of land for landfills have caused municipalities to seek sewage waste disposal methods that will accommodate high application rates. A land application system should be able to accommodate large amounts of sludge without causing environmental pollution, while utilizing sludge for its organic matter content and plant nutrients.

In 1972, a field experiment was initiated in which undigested and digested sludges were placed in trenches and covered with overburden (12). This concept offered an approach to disposal of large quantities of sewage sludge in an environmentally acceptable manner resembling landfilling, while improving marginal soils and making nutrients available for plants. Possible benefits

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2 Plant Physiologist, Soil Scientist, Soil Microbiologist, Plant Physiologist, Soil Microbiologist, and Research Chemist, respectively.
included odor control, destruction of pathogens, improvement of water retention and transmission characteristics of soil, and enhancement of nutrient and water relations for crop production. Concurrent with the field studies, a greenhouse experiment was initiated to provide data on soil aeration, root proliferation, nitrogen transformations, metal movement, and pathogen survival under simulated field conditions.

MATERIALS AND METHODS

SOIL BOXES

Five soil profile boxes (60 cm wide, 120 cm high, and 15 cm deep) were constructed with their backs, sides, and bottoms made of sheet aluminum, and their fronts made of Plexiglas. The boxes were filled with soil and sludge to simulate a vertical cross section through a 60-cm-wide trench from the midpoint of the trench to the midpoint of the 60-cm interval between trenches (Fig. 1). The Plexiglass fronts were covered to exclude light except during periods of observation.

TREATMENTS

The treatments studied were: (i) soil alone (control) fertilized according to Maryland Agricultural Extension Service recommendations for corn; (ii) limed raw sludge with a high pH (11.2); (iii) raw sludge with a low pH (6.9); (iv) anaerobically digested sludge with a high pH (9.9); and (v) anaerobically digested sludge with a low pH (5.3). The raw sludges were obtained from the Lower Potomac, Virginia, treatment plant and the anaerobically digested sludges came from the Blue Plains Wastewater Treatment plant in Washington, D.C. The oxygenated raw sludges were mixtures of primary and secondary treatment with lime and FeCl3. Digested sludges were also treated with FeCl3 and lime. All pH values were determined on a 1:1 (wt/vol) ratio of sludge (20% solids) or soil to water. The soil, a Galestown-Evesboro loamy sandy, (Typic Quartzipsamments), was treated with FeCl3 and lime. The three equivalent points on each side of the sludge.

SOIL ANALYSIS

At the end of the experiment, soil and sludge sample cores were removed from the trench boxes and analyzed for ammonium (NH4+, N), nitrate (NO3-, N), total Kjeldahl nitrogen (N), DPTA extractable zinc (Zn) and copper (Cu), pH, and total and fecal coliforms. The box fronts were removed and sample cores (7.5 cm diam) were taken horizontally from 15 locations within each box using a sterile aluminum pipe. Samples were taken at five sampling depths (15, 45, 75, 99, and 115 cm) along each of three vertical planes, spaced 15 cm from each side of the box and 15 cm apart. Cores from the center plane were sampled with the nearest edge of the sampler in the soil, 5 cm from the side of the sludge trench.

GAS ANALYSES

Sampling ports in a 7-cm grid pattern were equipped with injectable septa and installed in the rear sides of the boxes. Gas was sampled from nine ports on the grid. Three sampling points were on each of three vertical planes. The planes were spaced vertically at intervals; one was centered in the sludge mass, the next was at the soil interface of the trenched sludge, and the third was centered in the soil mass adjacent to the trenched sludge. The three equivalent points on each plane were: in the soil above the trench, in the center of the trench, and below the center of the trench. Gas samples were withdrawn by use of disposable syringes equipped with "Mininet" teflon valves

CHEMICAL ANALYSES

Total N was obtained by the Kjeldahl method (3). Ammonium-nitrogen and NO3-N were determined by selective ion electrodes after extraction of 10 g of soil with 200 ml of 0.5N K2SO4 and 0.01N Al2(SO4)3 (8). Nitrite was determined in the extract by Shinn's procedures (9). The NO3-N concentration was corrected for Cl- interference using the equation described by Mahendraper (7). Zinc and Cu were extracted by DTPA-TEA (6) and analyzed by atomic absorption. A 1:2 (wt/vol) sludge/solution ratio and a 1:50 (wt/vol) sludge/solution ratio were used for the 2-hour extractions.

BACTERIAL ANALYSIS

To determine total and fecal coliforms a 50-g sample of soil or sludge was diluted 1:10 (wt/vol) with sterile distilled water and shaken vigorously for 15 min. A serial tenfold dilution was made from the suspension for coliform determinations by the most probable number (MPN) method (1). Lactose broth was used for the presumptive test, brilliant green lactose bile broth for the confirmatory test, and EC medium to determine fecal coliforms.

RESULTS AND DISCUSSION

Gases in Soil Atmosphere

The gas data used in this section are from single locations 8-10 cm below the lower soil-sludge interface of each treatment box and from a sampling port at the same depth in the control box. After reviewing data from all sampling ports, these data were selected as representative of the trends in gaseous composition of
the soil atmosphere. At this depth extensive fluctuations which occurred after irrigation of the boxes were minimal.

The composition of the soil atmosphere differed markedly between the digested and raw sludges (Table 1, Fig. 2). Methane levels in the raw sludge treatments increased after 61 days from <1.0 to 35% in the low pH sludge, and to 45% in the high pH sludge. Similar increases in CO₂ content with a corresponding decrease in O₂ level were evident in these treatments (Table 1).

In both digested sludge boxes only trace amounts of methane were found (Fig. 2). No CH₄ was found in the control box. Carbon dioxide levels were <13% in the high pH digested-sludge box. Though CO₂ levels were initially low in the high pH digested-sludge box, O₂ levels did not show a normal inverse relationship. Nitrogen levels increased, ranging from a high of 88.7% on day 13 to near normal atmospheric level of 79.6% on day 62 (Table 1). This increase in N₂ percentage above the 78-80% found in normal surface atmospheric analysis has been observed in other soil atmospheric studies in this laboratory. This increase in N₂ percentage may result from rapid utilization of O₂ and the greater solubility of CO₂ than of N₂ into water held by the soil and sludge.

**Root Growth**

Corn roots in the control box proliferated throughout the soil mass. By 160 days corn roots had penetrated and ramified throughout the high pH digested sludge (Fig. 3). Early in the growing period, a major amount of visible fungal development occurred at the soil-sludge interface. During the growth period, the color of the sludge changed from black to orange-brown, probably indicating a change to oxidized conditions. The low pH (5.3) digested sludge was dewatered by root penetration and growth but to a lesser extent than with the high pH (9.9) digested sludge. In the low pH digested sludge, fungal growth was virtually absent.

Root penetration in the high (11.2) and low (6.9) pH raw sludge was greatly restricted (Fig. 4). A fungal mat

![Fig. 2—Percent methane gas in soil 8–10 cm below the lower soil-sludge interface in simulated sludge trench boxes 12-160 days after entrenchment. Sludges: △—△ raw high pH; □—□ raw low pH; ○—○ digested high pH; ●—● digested low pH.]

![Fig. 3—Corn root distribution in the high pH digested sludge box after 160 days.]

<table>
<thead>
<tr>
<th>Table 1—Percent gas in soil 8–10 cm below the lower soil-sludge interface in simulated sludge trench boxes and control box.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Raw sludge</strong></td>
</tr>
<tr>
<td><strong>High pH</strong></td>
</tr>
<tr>
<td><strong>Days</strong></td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>25</td>
</tr>
<tr>
<td>34</td>
</tr>
<tr>
<td>46</td>
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<tr>
<td>54</td>
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<tr>
<td>61</td>
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<td>67</td>
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<tr>
<td>76</td>
</tr>
<tr>
<td>89</td>
</tr>
<tr>
<td>99</td>
</tr>
<tr>
<td>132</td>
</tr>
<tr>
<td>159</td>
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</tbody>
</table>

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developed at the soil-sludge interface. Above the fungal mat was a 5- to 8-cm zone into which the corn roots did not penetrate. The root tips in close proximity to this zone became flaccid, brown, and stopped growing. Root penetration into the low pH raw sludge was more restricted than for the other sludges. In both raw sludge treatments, little dewatering was visible in the sludge, even after 160 days.

Moisture sealing of soil pores near the sludge and the resulting lack of direct atmospheric exchange may have lowered O\textsubscript{2} tension, thus permitting an increase in level of toxic gases by anaerobic digestion of the undigested raw sludges. Certain gaseous compounds such as NH\textsubscript{3} and CH\textsubscript{4} may have reached phytotoxic levels (5, 13), preventing root penetration into the raw sludges and subsequent dewatering. Furthermore, Smith and Russel (10) indicated that under anaerobic conditions, ethylene may reach concentrations inhibitory to root elongation. We did not establish the presence of ethylene. However, both NH\textsubscript{3} and CH\textsubscript{4} were found in elevated levels in the raw sludge boxes (Fig. 2 and 5).

**Nitrogenous Constituents in Sludge and Soil**

The organic nitrogen level in the sludge solids remained high as indicated by total Kjeldahl N values of 1.8 and 3.0% in the high and low pH digested sludges, respectively, and 2.7 and 4.5% in the raw high and low pH sludges, respectively. In the digested sludge treatments, total N in the soil below the sludge ranged from 348 to 565 µg/g; whereas, in the raw sludge treatments, total N ranged from 554 to 1,400 µg/g. The differences in total nitrogen in the soil profile are primarily the result of the difference in ammonia (Fig. 5). In the control box, total N was near 300 µg/g, NO\textsubscript{3}-N was low, ranging from 2-8 µg/g, and NH\textsubscript{4}-N averaged 2 µg/g. Samples from all boxes were negative for nitrate.

After 160 days, NO\textsubscript{3}--N levels in the raw sludge were considerably higher than those in the digested sludges (Fig. 5). This accumulation of NO\textsubscript{3}--N in the raw sludge indicated that nitrification had occurred. Gas samples from the center of the high pH raw-sludge mass showed O\textsubscript{2} levels > 10% and CH\textsubscript{4} levels < 1% during the sampling period. In the low pH raw sludge, O\textsubscript{2} levels were lower, > 5%, and CH\textsubscript{4} levels higher, up to 34%. The O\textsubscript{2} levels in the raw sludges were apparently sufficient to support nitrification. The differences in NO\textsubscript{3}--N levels between the raw high pH and the raw low pH sludges may have resulted from initial inhibition of nitrification by the high pH (11.2) of the high pH sludge. In contrast, generally higher NO\textsubscript{3}--N levels were found in the soil adjacent to and beneath the sludge in the high pH digested sludge box than in either of the raw sludge boxes. Lower
O₂ levels and higher CH₃ levels below the raw sludges could have resulted in denitrification (Table 1 and Fig. 2). Similar results were obtained in the field and were attributed to denitrification occurring beneath raw sludge treatments (12). In the field studies, higher chemical oxygen demands were found beneath the raw sludge trenches which reduced O₂ concentrations and, in time, resulted in denitrification. Similar levels of COD should have been present in our sludges because the sludge sources were the same.

Ammonium-N levels (Fig. 5) were high after 160 days in all treatments, particularly in the raw sludge. In the soil beneath the digested sludge, NH₄⁺-N ranged from 1 to 317 μg/g in contrast to 344 to 1,190 μg/g for the raw sludge treatments. Higher NH₄⁺-N levels in the raw sludge profiles probably resulted from leachate which contained high levels of NH₄⁺-N.

**Zinc and Copper**

Zinc and Cu did not move from the sludge into the soil (Table 2). The lack of downward movement appears to be due to: (i) metals were retained in the sludge mass as metal-organic matter chelates, inorganic precipitates (as carbonates, sulfides, etc.), and other sorbed phases or, (ii) the dissolved metal level was low; hence little movement into the soil could occur. Although the Zn and Cu concentrations of corn leaves (Table 3) increased, these levels were not sufficient to cause phytotoxicity. A foliar Zn concentration > 500 μg/g or a foliar Cu concentration > 20 to 25 μg/g would be necessary to cause metal toxicity in corn (4). Greater uptake of metals by plants might have been expected if the sludge and soil pH had decreased and if roots had penetrated more of the sludge mass.

**Coliform Bacteria**

At the end of 160 days entrenchment, the initially higher numbers of total and fecal coliforms of the low pH raw sludge were reduced to levels considerably lower than those of the low pH digested sludges (Table 4). The much greater production of NH₄⁺ in the low pH raw sludge than in the low pH digested sludge (Fig. 5), and a resulting formation of NH₃ as a result of an increase in pH, could have been the major responsible factors. Recent, unpublished work in this laboratory has shown that NH₃ is virucidal and, although we have found no indications in the literature, we would also expect it to be bactericidal.

As a test of the possible bactericidal effects of NH₃, regression analysis was run to relate the log total coliforms in the sludge of the simulated trenches and NH₃-N.

<table>
<thead>
<tr>
<th>Location</th>
<th>Digested sludge</th>
<th>Raw sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High pH</td>
<td>Low pH</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>Fecal</td>
</tr>
<tr>
<td>In sludge initially</td>
<td>88</td>
<td>6</td>
</tr>
<tr>
<td>160 days</td>
<td>141</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Above sludge 160 days</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Below sludge 160 days</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
</tbody>
</table>

\[ \text{pH} = \text{pK} + \log \left( \frac{[\text{NH}_4^+]}{[\text{NH}_3]} \right). \]  \[1\]

For rigorous applicability, the equation calls for activities. Also, pK is known to vary with ionic concentration and temperature. However, since the concentration of other ions for calculation of activities and the temperature were not known, especially as these factors varied with time, we arbitrarily used N in μg/g for the concentration terms and let pK = 9.4.

Letting \( z = \text{antilog} \left( \text{pH} - \text{pK} \right) \) and \( y = \text{the determined value for NH}_4^+ \), we can write \( \text{NH}_3^+ \) of Eq. [1], in terms of \( \text{NH}_4^+ \) and solve for the \( \text{NH}_3^+ \) concentration \( x \) by

\[ x = \frac{yz}{z + 1}. \]  \[2\]

The log total coliforms surviving was very poorly correlated negatively with NH₄⁺-N or log (NH₄⁺-N + NH₃-N), pH, NH₃-N, and log NH₄⁻-N. The NH₃-N concentrations were calculated using the Henderson-Hasselbalch equation:

\[ \text{pH} = \text{pK} + \log \left( \frac{[\text{NH}_4^+]}{[\text{NH}_3]} \right). \]  \[1\]

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\[ x = \frac{yz}{z + 1}. \]  \[2\]

The log total coliforms surviving was very poorly correlated negatively with NH₄⁺-N or log (NH₄⁺-N + NH₃-N), with \( r^2 \) values of 0.173 and 0.121, respectively. The negative correlation was much better with pH, NH₃-N, and log NH₄⁻-N. Respective \( r^2 \) values were 0.498, 0.523, and 0.631, all being significant at the 5% level (Table 5).

If \( \text{NH}_3 \) was a significant factor in controlling the final total coliform populations observed, one would also ex-
Table 5—Regression statistics relating log surviving total coliforms in the sludge of the trench boxes to NH₄-N (i.e., NH₄⁺ + NH₃), log NH₄⁺N, pH, NH₃-N, and Log NH₃-N.

<table>
<thead>
<tr>
<th>Statistic</th>
<th>NH₄⁺N†</th>
<th>Log NH₄⁺N†</th>
<th>pH</th>
<th>NH₃-N</th>
<th>Log NH₃-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>r²</td>
<td>0.173</td>
<td>0.121</td>
<td>0.498*</td>
<td>0.523*</td>
<td>0.631*</td>
</tr>
<tr>
<td>Slope</td>
<td>-4.97x10⁻⁴</td>
<td>-0.908</td>
<td>-1.50</td>
<td>-0.0035</td>
<td>-1.37</td>
</tr>
<tr>
<td>Intercept</td>
<td>2.57</td>
<td>5.49</td>
<td>13.04</td>
<td>2.71</td>
<td>4.61</td>
</tr>
</tbody>
</table>

* Significant at 5% level.
† Value as determined includes NH₃-N.

expect a correlation with pH since it should partially reflect the amount of NH₄⁺ formed and its dissociation to NH₃. The increased negative correlations with use of either the NH₃-N concentration or its log over that for NH₄⁺ + NH₃-N concentration indicates that the increased pH may have resulted from something other than NH₄⁺+NH₃-N accumulation. Since coliforms apparently were affected, one might also expect bacterial pathogens to be susceptible to destruction by NH₃ as its concentration increases in the entrenched sludge.

Although the data indicate that NH₃ is involved in determining the final coliform populations, the evidence is, of course, only circumstantial. The concentrations of other unknown substances or undetermined conditions changing somewhat in parallel with the NH₃ concentration may have been the actual effective agents.

After 160 days of entrenchment, low numbers of total and fecal coliforms (3 to 593 MPN/g) were found in the soil below and above all the sludges (Table 4), but a consistently greater number of total and fecal coliforms appeared above than below the sludges. This indicates a soil environment above the sludge slightly more conducive to growth and survival of the coliforms. Whether the organisms moved from the sludge into the soil by diffusion, growth, or flow with water is not known. Movement of water upward from the sludge would have been by capillary conduction.

These results showing a much greater reduction of coliforms in the low pH raw sludge than in the low pH digested sludge indicate that, although raw sludge is initially more hazardous because of its higher pathogen content, containment in the trench may result in reducing the pathogen level of the low pH raw sludge below that of the low pH digested sludge.

CONCLUSIONS

A greenhouse study with simulated sewage sludge trenches indicated that corn grown in the overburden above digested sludge trenches assists in dewatering the sludge by root penetration. Anaerobic conditions prevailed for extensive periods in raw sludges as shown by low O₂ levels and high levels of CH₄ and CO₂. We believe the high levels of NH₄⁺—N and anaerobic conditions in the raw sludge trenches inhibited plant root growth. We found higher total Kjeldahl-N in the soil below raw sludge trenches than below digested sludge trenches. We attributed this difference to the difference in NH₄⁺-N content. After 160 days, Zn and Cu had not moved into the soil surrounding the entrenched sludge and levels detected in corn leaves were below metal toxicity ranges. Decreases in numbers of total coliform bacteria in the low pH sludges may have been caused by the NH₃ produced upon dissociation of NH₄⁺.

Results of these trench simulation greenhouse studies indicate that precautions are necessary in the field. Unstabilized entrenched sewage sludge may prove initially inhibitory to root growth. Trench site selection should include consideration of soil texture, ground water movement, and depth to water table since nitrogen leaching occurred below simulated trenches. Copper and Zn were not phytotoxic during the 160 days of this study and were not displaced from the trench, but care should be taken with sludges of higher metal concentrations. Long-term monitoring studies are needed to evaluate the "weathering" of entrenched sludge and its effects on plants and ground water.

LITERATURE CITED