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Reliability Problems With Indicator Organisms for Monitoring Overland Flow Treated Waste Water Effluent[1]

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ABSTRACT

A 2-year study (1975–1976) on the response of indicator organisms in facultative lagoon waste water treated by overland flow was conducted in Utica, Mississippi. Overland flow plots of 4.55 by 45.5 m on 2% slope were established on a Grenada silty clay loam with an artificially established hard pan at 15 cm. The vegetation was a grass mixture dominated by reed canary grass (*Phalaris arundinacea* L.). Undisinfected waste water from a facultative lagoon was applied to the plots at a rate of 1.25 cm in 6 hours on 5 days a week. Fecal streptococci and fecal coliforms were enumerated in the waste water before it was applied to the plots, as it flowed down slope, and as it was discharged from the plots. Fecal streptococci were found to be poor indicators of the sanitary condition of effluent after overland flow treatment of waste water. Their numbers were consistently higher in the discharged effluent than in the waste water, even when only tap water was applied to the treatment plots. Fecal coliforms were questionable indicators of sanitary conditions in the discharged effluent during the summer when fecal coliform numbers were considerably higher in the discharged treated effluent than in the waste water coming directly from the lagoon. The extent of actual pathogen removal or growth on the soil surface during overland flow treatment was not assessed during this study. However, the use of fecal streptococci or fecal coliforms as indicators of pathogen removal by overland flow during the summer is seriously questioned. Disinfection after treatment by overland flow, at least during the summer, is suggested.

Additional Index Words: **fecal coliform, fecal streptococci, disinfection, growth, seasonal effect.**

A primary goal of a waste water treatment system should be to produce an effluent that is safe for discharge into the environment. This goal is relatively simple to achieve when the waste water treatment system is self-contained and a satisfactory means of disinfection is available. However, in land treatment systems, the waste water is not treated in an enclosed and self-contained process. The spray application of the waste water for land treatment has generated a considerable amount of concern and scientific interest. Hickey and Reist (1975) reviewed the health significance of airborne microorganisms from waste water treatment processes. Two conditions were found to be common in most of their studies: the presence of large numbers of human pathogenic microorganisms in untreated waste waters, and the survival of airborne microorganisms for considerable distances during wind dispersion.

When waste water is treated by overland flow, it can be applied by surface application at the top of the treatment slopes. Surface application nearly eliminates the problem of aerosol disease transmission. Bacterial infection, even with spray distribution, would not be a problem if disinfected waste water was applied. However, disinfection of a facultative lagoon waste water presents special problems. It contains a large amount of organic matter, primarily algal cells. A large amount of chlorine would, therefore, be required to overcome the protecting effect afforded the microorganisms by this organic matter. Furthermore, facultative pond effluents are often alkaline, with pH values frequently as high as 10 or 11 during periods of active photosynthesis. At such pH levels, hypochlorous acid is very ineffective as a disinfecting agent.

Significant transmission of bacterial pathogens through the heavy-textured soils with the extensive surface organic debris that is normally present on an overland flow system would be of low probability (Carlson et al., 1974; Krone et al., 1958). Thus, significant bacterial disease transmission from overland flow systems would probably be limited to the discharged effluent. Indicator organisms, such as total coliforms, fecal coliforms, and fecal streptococci, as well as *Salmonella*, have frequently been used to assess the potential of disease transmission in effluents (Gallagher and Spino, 1968; Geldreich, 1970; Geldreich et al., 1968; Geldreich and Kenner, 1969; Rudolfs et al., 1950). Problems of specificity, survival, and the significance of the relative abundance or ratios between indicator organisms have been reported (Van Donsel et al., 1967; Geldreich, 1970). Reduction in the number of indicator organisms, e.g., fecal coliforms, to lower levels is often either required or felt to be highly desirable in a waste water treatment system. Information on the fate of indicator organisms in waste water treated by overland flow systems is needed. This study was undertaken to determine the changes in indicator organism numbers during overland flow and to thereby assess the need for disinfection of a facultative lagoon waste water following overland flow treatment.

MATERIALS AND METHODS

The site was an experimental overland flow treatment system at Utica, Mississippi. The source of waste water was a 14.8-ha facultative lagoon serving the city of Utica, Mississippi. The overall experimental site and its operation were described by Peters and Lee (1978). The soil was a Grenada silty clay loam (fine-silty, mixed, thermic, Glossic Fragiudalf) with a hard pan artifically established at the 15-cm depth by compaction. The plots were 4.5 by 45.5 m, and the slopes were 2 and 8%. The vegetative cover was a grass mixture of reed canary grass (*Phalaris arundinacea* L.), Kentucky 31 variety of tall fescue (*Festuca arundinacea*), perennial ryegrass (*Lolium perenne* L.), and bermudagrass (*Cynodon dactylon* L.). Reed canary grass was the predominant vegetation. A characeristic, 2- to 3-cm mat of organic matter and adventitious roots was present on the soil surface. The waste water was

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pumped from the lagoon and applied to the plots via a distribution trough at the head of the slopes. Waste water flowed over the surface in a film that ranged from a fraction to 3 or 4 cm in depth. The waste water was applied at a rate of 1.25 cm during 6 hours on 5 days/week. Normally, runoff was discharged at the bottom of the slope about 2 hours after waste water application.

Samples were collected on the 2% slope from the waste water applicator, from the soil surface at various points along the treatment slope, and from the discharge point of each treatment plot during 1976 and 1977. Triplicate samples used for analysis were composites of three samplings during the day. Samples were stored at approximately 5°C and were tested within 24 hours.

Samples for the background number of fecal streptococci and fecal coliforms were taken on adjacent plots of the 8% slope when only tap water was applied and before any waste water was applied to these plots. All other measurements were made on the 2% slope plots. Total coliforms, fecal coliforms, and fecal streptococci were measured with millipore-type HA S-pak, 0.45-μm, white, grid-marked filters according to the standard procedure for filtering technique (APHA, 1975). The media used were KF streptococcus medium for the fecal streptococci and M-FC medium for the fecal coliforms. Total coliform counts were made using M-Endo MF broth.

Nutrient analyses were made on waste water and effluents by the use of a Technicon autoanalyzer. Suspended solids and biochemical oxygen demand were determined by standard procedures (APHA, 1975).

RESULTS AND DISCUSSION

Fecal streptococci in the waste water and overland flow treated effluent are presented in Table 1. The number of fecal streptococci increased as the waste water flowed through the overland flow system. The nonenteric origin of the "fecal" streptococci on the overland flow system at Utica, Mississippi, was further substantiated by data in Table 2. When tap water containing O SPC (Standard Plate Counts) fecal coliforms or fecal streptococci per 100 ml was applied to plots of 8% slope, a count of over 3×10^4 SPC/100 ml of fecal streptococci was found in the discharged effluent. Additionally, large numbers of fecal streptococci were found at several other sites when tap water was applied to the surface and sampled as it flowed over the vegetated soil surface (unpublished data, P. G. Hunt). High counts of fecal streptococci in the runoff from these diverse environments strongly suggest that this test is a poor indicator of pathogens in overland flow systems. Yet, these data are not extremely surprising in that certain strains of fecal streptococci have been reported to be rather common (Kibbey et al., 1968; Geldreich and Kenner, 1969). Geldreich and Kenner (1969) also reported *Streptococcus faecalis* var. *liquifacients* to be related to vegetation, insects, and agricultural soil, and less indicative of fecal pollution than *S. bovis* or *S. equinus.* Also, *S. faecalis* var. *liquifaciens* was found to survive considerably longer than the enterococcal species *S. bovis* or *S. equinus* at temperatures of both 10 and 20°C. The ratio of fecal coliforms to fecal streptococci has also been criticized as a poor indicator of pollution sources in most natural environments (Geldreich and Kenner, 1969; McFeters et al., 1974).

Table 1—Fecal streptococci in waste water and overland flow treated effluent during periods of the year.

	Fecal streptococci*	
Time period (1976)	Waste water	Effluent†
	SPC/100 ml	
1 June–7 July	9,000	26,000 (2)
21 July–28 Sept.	3,600	877,400 (5)
27 Oct.	1,500	16,000 (1)

* Values for time periods differ significantly according to Scheffe linear comparison.
† Values in parentheses are number of sampling dates during 1976.

Table 2—Indicator organism response to tap water on an overland flow system.

Distance	Fecal coliform	Fecal streptococci
m	SPC/100 ml	
0.0	0	0
45.5	6	30,300

These data supported the contention that the fecal streptococci are not good indicators of the sanitary status of discharged effluent from an overland flow system. They also indicated that the fecal coliform to fecal streptococcus ratio of discharged effluent from an overland flow system is not a reliable indicator of waste water source. Since fecal streptococci, particularly the counts of *S. bovis* and *S. equinus,* are more specific for feces, a more detailed study of these organisms would be more meaningful when related to the fecal coliform numbers (Geldreich et al., 1968; Geldreich and Kenner, 1969). However, because of their rapid death rate (McFeters et al., 1974), such tests would be of questionable value, and thus were not pursued in this study.

Thomas et al. (1976) reported a considerable reduction in fecal coliform numbers after treating raw commuted sewage waste water by overland flow. However, the final number of fecal coliforms was $>10^4$/100 ml. They found similar but less effective reductions of total coliform organisms. Our use of treated rather than raw commuted waste water should have lowered the number of pathogens and coliforms applied (Geldreich et al., 1964), and thus should have reduced the number of organisms in the treated effluent. However, we found that total coliform numbers in the discharged effluent were generally quite high, erratic, and unrelated (10^4 inflow and 10^6 outflow) to experimental treatments. We felt that a considerable number of coliforms of nonfecal origin was obtained from vegetation and soils. Our findings agreed with previously published reports (Geldreich et al., 1968).

In our study the background level of fecal coliforms was low (Table 2), and fecal coliforms were removed from waste water during most of the year by overland flow treatment (Fig. 1). We expected fecal coliforms to be reasonably good indicators because these organisms are generally not as common on the soil surface or on vegetation as are fecal streptococci or total coliforms (Kibbey et al., 1978; Geldreich et al., 1968; Bell and Bole, 1978), and they do not survive for long periods on vegetation when exposed to sunlight (Bell, 1976; Bell and Bole, 1976).

However, data obtained during this 2-year study showed considerably higher fecal coliform populations during summer months in the treated effluent than in the applied waste water (Fig. 1). Therefore, growth of fecal coliforms may be significant during the summer periods. Growth of indicator organisms has been re-

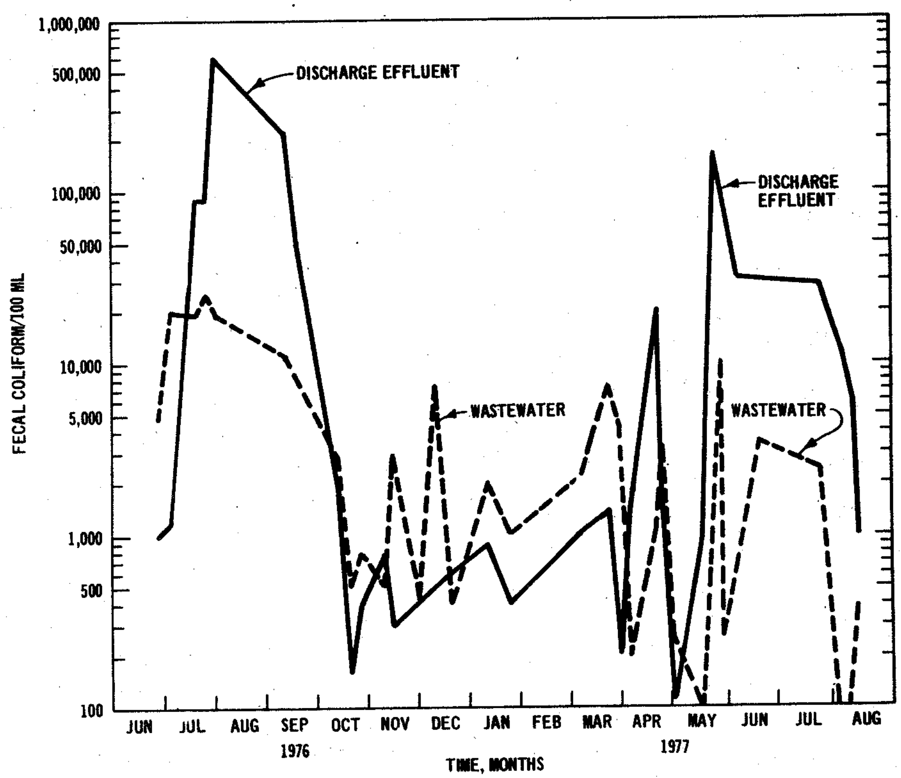

Fig. 1—Fecal coliform responses to influences of season.

ported by Hendricks and Morrison (1967) and Lance and Whisler (1975). This growth was generally associated with conditions of high nutrient status. Sediments of the Poudre River, for example, were reported by Hendricks and Morrison (1967) to promote significantly more growth than rivers or sewage effluents. Lance and Whisler (1975) reported fecal coliform growth in secondary effluents enriched with glucose but not in unamended secondary effluents.

Possibly, growth-promoting factors such as nitrogen, phosphorous, easily biodegradable organic matter, and a suitable environment could be present in the liquid film flowing over the soil surface of the overland flow system. Nutrients could be supplied from the waste water itself, as well as from plant roots and decomposing organic matter on the surface. Data in Table 3 show that both inorganic nutrients and biodegradable materials were abundantly present. In the summer, the liquid

Table 3—Selected constituents in applied waste water and discharge effluent from overland flow plots at Utica, Miss.

	Waste water		Discharge effluent†	
Constituent	Mean	Range	Mean	Range
pH	8.6	7–10	8	7–9
		mg/liter		
Suspended solids	35	8–75	14	5–17
BOD_5	22	6–37	10	5–13
TKN	20	13–26	4	3–6
Total P	10	7–13	7	4–8
Ortho P	9	6–12	6	3–7

† Discharge effluent from 1.27-cm application in 6 hours, 5 days per week.

temperature could be high enough to promote significant growth. Temperatures near 37°C would favor growth. It should be noted that better survival of fecal coliforms has been associated with cooler temperature, <15°C (Mitchell and Starzyk, 1975); so the large increase in numbers during the summer was most probably associated with growth rather than simple survival and accumulation.

The exact cause of this increased fecal coliform count in the overland flow discharged effluent is not clear. However, the fact that fecal coliform numbers increased during two consecutive summers suggest that these organisms, measured by the membrane filter technique (APHA, 1975), are not reliable indicators of sanitary conditions of overland flow discharged effluent, at least during the summer months. The actual sanitary condition would depend upon the response of the pathogenic organism population to these conditions.

Since total coliforms and fecal streptococci are also questionable indicators, systems monitoring is an evident problem. One solution to this problem would be to collect the discharged effluent, disinfect it by some method, and monitor the fecal coliforms as the effluent was discharged from the disinfection chamber. This, of course, would assume a need for disinfection that may or may not already exist. However, this approach has more merit than treating the waste water at the discharge point of a facultative lagoon. Thus, the problems associated with indicator organisms present a significant but not an insurmountable problem to system monitoring. This work also suggests that fecal coliform counts may not be adequate indicators for summer monitoring of nonpoint pollution from areas like pastures, where growth might be stimulated by nutrient release from manure. It further implies that more research on indicator organisms in the environment needs to be conducted.

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