SEWAGE EFFLUENT IRRIGATION: II. MICROBIOLOGICAL COMPOSITION OF INPUTS AND ASSOCIATED CHANGES IN SOIL MICROFLORA

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INTRODUCTION

In 1973 microbiological studies were initiated in support of the Swift Current pilot project on forage crop irrigation with sewage effluent. If this type of disposal of municipal waste and its utilization by agriculture is to gain public acceptance, then the absence of health hazards for man and animals must be demonstrated. Furthermore, it is important with regard to groundwater quality and soil fertility to assess microbiological changes in the irrigated soil.

The objectives of the microbiological studies were:

1. To monitor pathogens and principal bacterial indicators in the lagoon effluent;

2. To assess the quantity, distribution and survival rate of intestinal bacteria in irrigated soils; and

3. To determine the effects of effluent irrigation on the indigenous soil microflora.

Although the test site contained four different soil types, only some results from the Orthic soil are presented in this paper.

METHODS

I. Sanitary Bacteriological Analyses

1. Analyses for bacterial pathogens were conducted by the provincial Public Health Laboratory in Regina.

2. Liquid and soil samples were analysed in Swift Current for principal bacterial indicators of fecal pollution by means of multiple-tube fermentation techniques according to the standard methods of the American Public Health Association (1971). The following groups of intestinal bacteria were enumerated by presumptive and confirmed MPN tests:

   (i) Total coliform bacteria
   (ii) Fecal coliform bacteria
   (iii) Fecal streptococci.

II. General Microbiological Analyses

1. The total count of viable bacteria and actinomycetes

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in liquid and soil samples was determined by dilution plating onto:

(i) Plate count agar, and
(ii) Soil extract agar.

2. Viable fungi in liquid and soil samples were counted by dilution plating onto Rose bengal streptomycin agar.

In assessing the effects of effluent irrigation on indigenous soil microorganisms it was desirable to differentiate between the effect of moisture input and the effect of nutrient inputs. This was facilitated by taking replicate samples before and after each irrigation from each of the three treatments: effluent irrigated, creek water irrigated and dryland soil.

RESULTS AND DISCUSSION

Repeated analyses of the effluent for presence of intestinal pathogens, such as Salmonella spp., Shigella spp., and Staphylococcus spp., were consistently negative. This was not surprising because in comparison to waste waters from many other Prairie towns and cities the chlorinated effluent from the large and shallow aerobic secondary lagoon at Swift Current is relatively "clean". The effluent was therefore judged to be safe for handling of irrigation equipment by field staff.

When the bacteriological quality of sewage effluent is determined in order to assess its suitability for crop irrigation the sampling procedure is very important. As shown in Table 1, the microbial density in dip samples

Table 1. Effect of irrigation pump pressure on microorganisms in sewage effluent (October 1, 1973)

Type of organisms and culture conditions	Mean number*/100 ml liquid		Loss of viability during pumping %
	Dip samples from lagoon	Line samples from main pipe+	
Total bacteria on PCA, 48 hr at 20°C	5.3×10^6	3.8×10^6	28
Total bacteria on PCA, 24 hr at 35°C	5.2×10^6	2.8×10^6	46
Fungi on RBSA, 6 days at 20°C	10.5×10^3	5.6×10^3	47
Total bacteria on SEA, 14 days at 20°C	9.6×10^5	9.2×10^5	4
Total coliforms, MPN	10,600	4,000	62
Fecal coliforms, MPN	863	297	66
Fecal streptococci, MPN	350	32	91

*Average of three replicates

+At a pressure of 60 psi

drawn directly from the lagoon was considerably higher than in line samples drawn simultaneously at a port behind the pump. It seems that some microbial cells were physically damaged (possibly ruptured) during the instantaneous pressure change from atmospheric to 60 psi in the impeller of the centrifugal pump or during the sudden pressure release at the sprinkler. It is noteworthy that the intestinal bacteria of human and animal origin such as streptococci, coliforms and others (plate count at 35°C) are much more susceptible to the detrimental effects of pumping than are the bacteria indigenous to water and soil (plate counts at 20°C). According to U.S. recommendations (Finstein, 1973) the dip-sampled effluent would have been assessed as unsuitable for crop irrigation, because it contained more than the recommended limit of 5,000 total coliforms per 100 ml, while the effluent drawn behind the pump was perfectly safe (Table 1). These microbiocidal effects of pumping could be of considerable sanitary significance and this phenomenon warrants further investigation.

Public health authorities will only sanction the use of sewage for irrigation if the foliage of the forage crop is not contaminated with pathogens at the time of ingestion. Among the potentially pathogenic bacteria in municipal sewage, the salmonellae are most likely to cause serious health hazards to livestock. At Swift Current the irrigated alfalfa foliage was not monitored for intestinal bacteria, because a field study had already been undertaken at Taber, Alberta to determine how long fecal coliforms, indicators of the salmonella group of enteropathogenic bacteria, survive on alfalfa irrigated with sewage effluent. The results of this field study were recently published by Bell (1976) and show that fecal coliforms on effluent-irrigated alfalfa plants are completely destroyed by exposure to 10 hours of bright sunlight. To completely eliminate the risk of salmonellosis to livestock consuming that forage, Bell recommends that at least two sunny days elapse between the cessation of effluent irrigation and harvest or ingestion of the forage.

The quantity, distribution and survival of the principal intestinal bacteria in soil irrigated with sewage effluent was investigated, because these bacteria, although they are nonpathogenic, can serve as indicators of some enteropathogenic bacteria with similar survival characteristics (McFeters et al. 1974) and can simulate the potential fate of pathogens in soil. Although indicator bacteria were enumerated in all soil samplings throughout the 1973 and the 1975 irrigation seasons, the results from a three-week period in 1975 will suffice to illustrate their behaviour in soil. Examination of surface soil sampled two days before, one day after and 19 days after the second effluent irrigation during midsummer of 1975 reveals that the number of total coliforms in the irrigated soil was always more than 30 times greater in the top 2.5 cm than in the 2.5-15 cm depth and that there were small but persistent numbers of coliforms in the dryland soil (Table 2).

Effluent irrigation effected a sharp increase in coliforms at both depths, but during the subsequent 19 days of slow drying there occurred a killing of roughly 90% and numbers approached pre-irrigation levels. The presence of total coliforms in dryland soil and their increase in the 2.5-15 cm depth of the irrigated soil should be attributed to the reportedly

Table 2. Effect of effluent irrigation* on total coliforms in Birsay Orthic soil

Site	Depth cm	Mean number/g soil			Decrease between irrigations %
		June 22	June 25	July 13	
Effluent irrigated	0-2.5	121,500	906,300	123,900	86
	2.5-15	51	26,800	1,500	94
Dryland	0-2.5	118	85	71	-
	2.5-15	4	11	4	-

*5.84 cm of effluent containing 13,000 total coliforms/100 ml was applied on June 24, 1975

natural occurrence of Aerobacter aerogenes, a coli-type bacterium, in soils (Geldreich et al. 1962), and its growth stimulation under high soil moisture, rather than to pollution of the soil.

Numbers of fecal coliforms in soil underwent similar changes but were much smaller than numbers of total coliforms (Table 3), attesting to the greater specificity and sanitary significance of fecal coliforms as pollution indicators.

Table 3. Effect of effluent irrigation* on fecal coliforms in Birsay Orthic soil

Site	Depth cm	Mean number/g soil			Decrease between irrigations %
		June 22	June 25	July 13	
Effluent irrigated	0-2.5	128	144,700	26,700	82
	2.5-15	0	322	95	71
Dryland	0-2.5	0	4	8	-
	2.5-15	0	0	1	-

*5.84 cm of effluent containing 6,100 fecal coliforms/100 ml was applied on June 24, 1975

In half of the dryland samplings and one sampling of irrigated soil, no fecal coliforms could be detected. It is interesting that the fecal coliforms

population in soil decreased very slowly after irrigation (Table 3) when compared to the very rapid die-off observed on alfalfa, where Bell (1976) repeatedly found a kill of more than 99.9% within 48 hours after irrigation. The longer survival in soil can be attributed to the much higher moisture levels and the protection from bright sunlight under the plant canopy.

The streptococci in the fecal streptococcal group are reported to be good indicators of public health hazards from sewage in soils and on vegetables (Mallmann and Litsky 1951). In the present study, the number of fecal streptococci at the surface of the irrigated soil did not increase in response to the second irrigation (Table 4), primarily because at this time the effluent contained very few fecal streptococci and also because a high number of these organisms had survived since the first irrigation 12 days earlier. However, the fecal streptococci population at both soil depths

Table 4. Effect of effluent irrigation* on fecal streptococci in Birsay Orthic soil

Site	Depth cm	Mean number/g soil			Decrease between irrigations %
		June 22	June 25	July 13	
Effluent irrigated	0-2.5	80,200	21,400	7,500	65
	2.5-15	74	460	211	54
Dryland	0-2.5	2,200	606	117	-
	2.5-15	2	2	14	-

*5.84 cm of effluent containing 223 fecal streptococci/100 ml was applied on June 24, 1975

did decrease, although more slowly than the coliforms, between irrigations. Again, as with the coliforms, there was little if any occurrence of downward movement of these intestinal bacteria through the soil profile under effluent irrigation. The absence of a marked downward movement of fecal bacteria was confirmed in the fall of 1975 when deep cores were subjected to sanitary analyses and no fecal coliforms or fecal streptococci could be detected below a depth of 30 cm. There are, however, published reports of marked downward dispersal of fecal coliforms in lighter textured soil and under much higher irrigation rates (Dazzo et al. 1972; Reneau and Pettry 1975).

Soil dilution plate counts were made throughout the 1975 irrigation season (Fig. 1) in all three treatments. The results show very clearly that the microbial population in the top 2.5 cm increased very sharply in response to each irrigation, similar to the response to rainfall in

dryland soil (Campbell et al. 1973), and that the increases in effluent irrigated soil were considerably greater than in creek water irrigated soil. As the surface soil dried between irrigations the bacterial population decreased markedly but generally not to the pre-irrigation level. Thus it appears that irrigation, particularly with effluent, exerted a cumulative effect on the soil microbial population boosting the number of viable bacteria to levels of over 4 billion cells per gram of soil. The

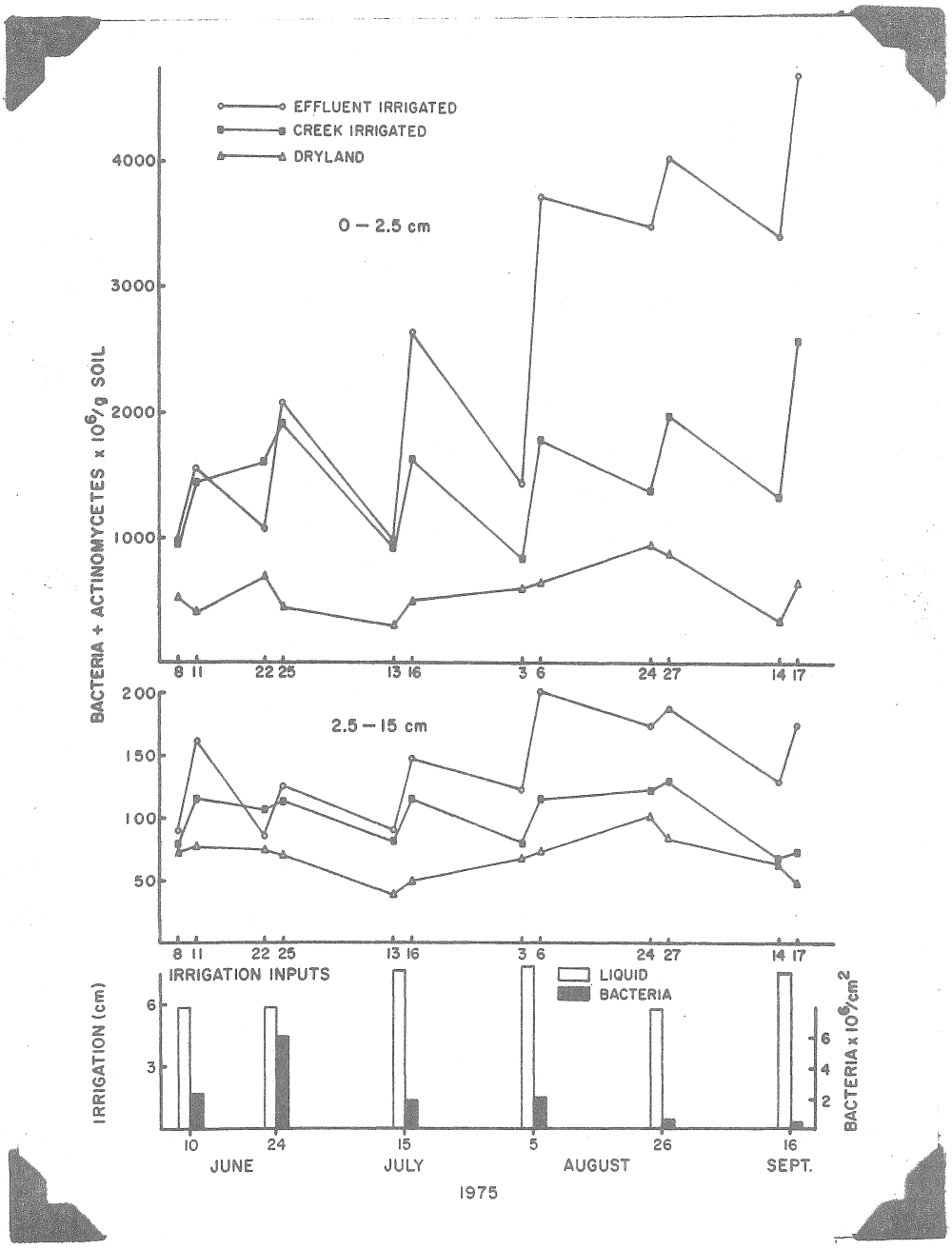

Fig. 1. Effect of irrigation with effluent and creek water on microbial populations near the surface of Birsay Orthic soil

magnitude of these population increases can only be fully appreciated when one realizes that even direct microscopic counts of bacteria in nearby dryland soils yielded total counts of less than 2 billion cells per gram of surface soil (Campbell and Biederbeck, unpublished). There is no doubt that the observed population increases were due to multiplication or growth of indigenous soil microorganisms rather than the addition of alien organisms to soil via irrigation, because the bacterial inputs accounted always for less than one percent of the soil bacteria at the surface (Fig. 1). In the 2.5-15 cm depth, bacterial population changes were considerably less extensive but followed a pattern similar to that observed at the soil surface (Fig. 1).

The long-term effects of effluent irrigation on the indigenous soil microflora were assessed by microbiological analyses of deep cores taken each spring one month before the start and each fall one month after cessation of irrigation. The results obtained during the first three years demonstrate that the fungal population in soil under effluent irrigation was markedly higher than in dryland soil at each sampling, but only to a depth of 30 cm (Fig. 2). Below 30 cm, there were generally no significant differences between fungal numbers in effluent irrigated and in dryland soil. The greatest stimulation of fungal growth was observed in the surface layer (0-2.5 cm) where the fungal population, even one month after the last irrigation season, was still boosted to the unusually high level of 4 million propagules per gram of soil. This is an increase of more than 600% over the respective population in dryland soil.

The differences in numbers of viable bacteria plus actinomycetes between dryland and effluent irrigated soil were generally smaller than those observed for fungi, but they were still very noticeable to a depth of 30 cm (Fig. 3). At greater depth, there were again very few significant differences.

Effluent irrigation effected not only quantitative but also qualitative differences in the indigenous soil microflora, because the ratio of bacteria to actinomycetes was always much higher in effluent irrigated than in dryland soil, indicating a preferential stimulation of soil bacteria over actinomycetes.

CONCLUSIONS

The results of sanitary and soil microbiological studies conducted to date at Swift Current can be summarized by the following conclusions:

1. From the standpoint of sanitary bacteriology the effluent from the secondary sewage lagoon at Swift Current is safe and suitable for crop irrigation as it was found to be free of enteropathogenic bacteria and not excessively loaded with fecal indicator bacteria.

2. A large proportion of the undesirable fecal bacteria can be killed by pressure changes normally occurring within the

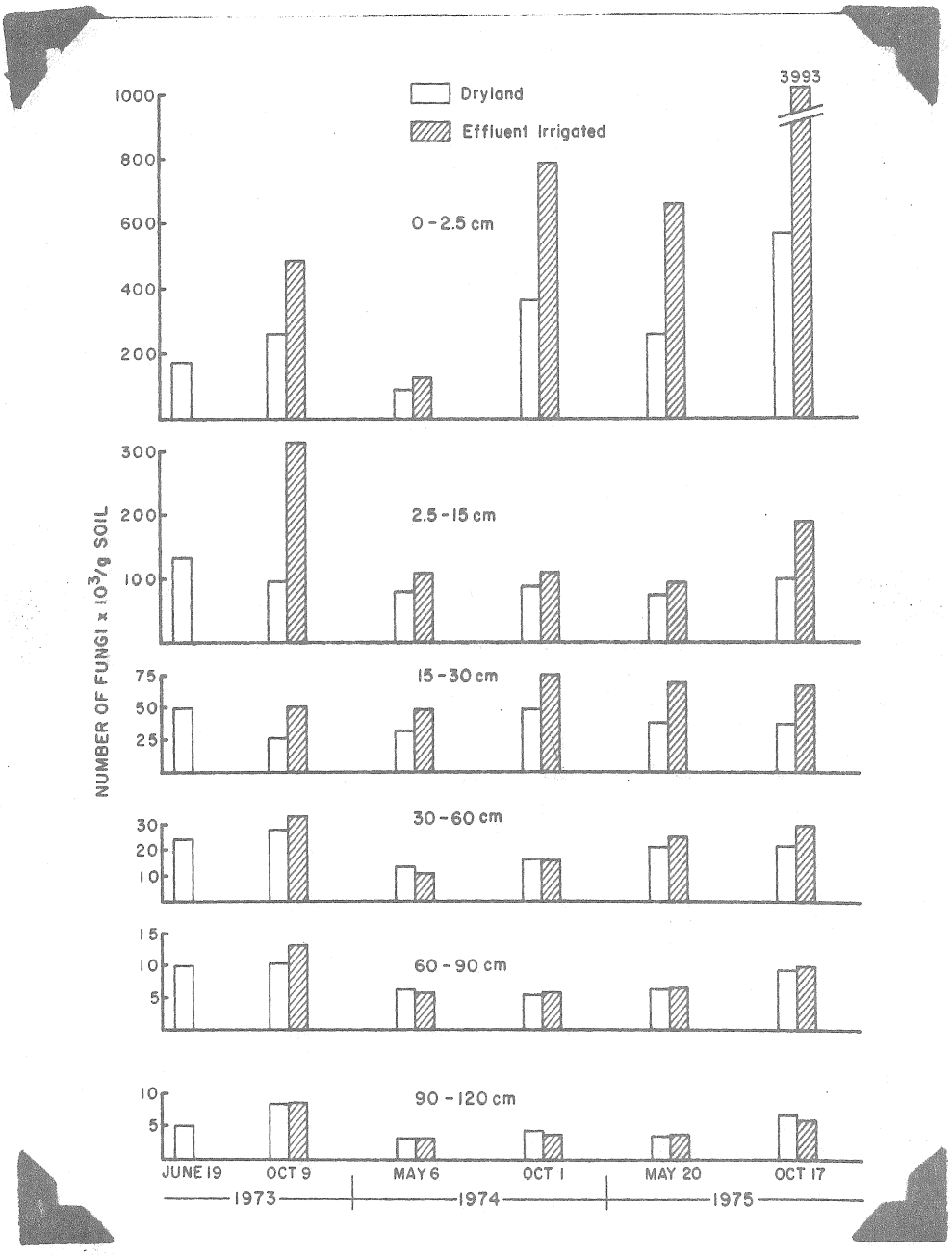

Fig. 2. Fungi at different depths in dryland and effluent irrigated Birsay Orthic soil

irrigation pumping system.

3. Under properly managed crop irrigation with effluent, well over 90% of the pollution indicator bacteria were retained at the soil surface and there was negligible downward dispersal in this clay loam, thus alleviating any possibility of bacteriological groundwater contamination.

4. The majority of intestinal bacteria added to the soil die-off between irrigations.

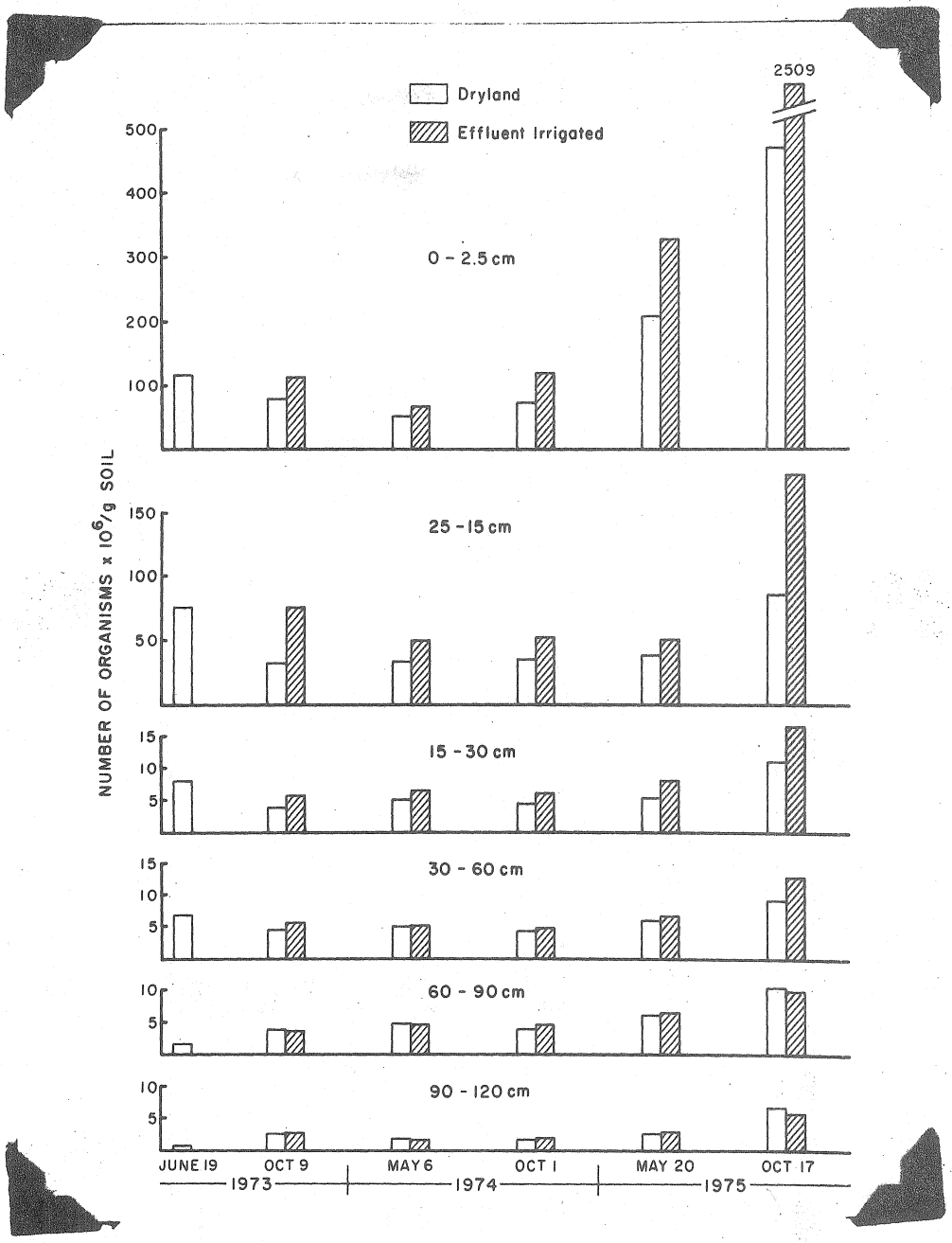

Fig. 3. Viable bacteria plus actinomycetes at different depths in dryland and effluent irrigated Birsay Orthic soil

5. Populations of indigenous soil microorganisms increased considerably more in response to irrigation with effluent than to irrigation with "fresh" water, demonstrating that the organics and nutrients in the effluent were primarily responsible for the strong stimulation of microbial growth.

6. The effluent-associated soil population increases

extended to a depth of 30 cm and persisted for long periods of time (e.g., from fall to spring).

7. The maintenance of high levels of plant-available phosphorus and nitrogen under the creek water as well as under the effluent irrigated alfalfa (Nicholaichuk and Biederbeck 1976) should be attributed to the very high soil microbial activity observed and the corresponding acceleration in mineralization of soil organic-P and N.

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