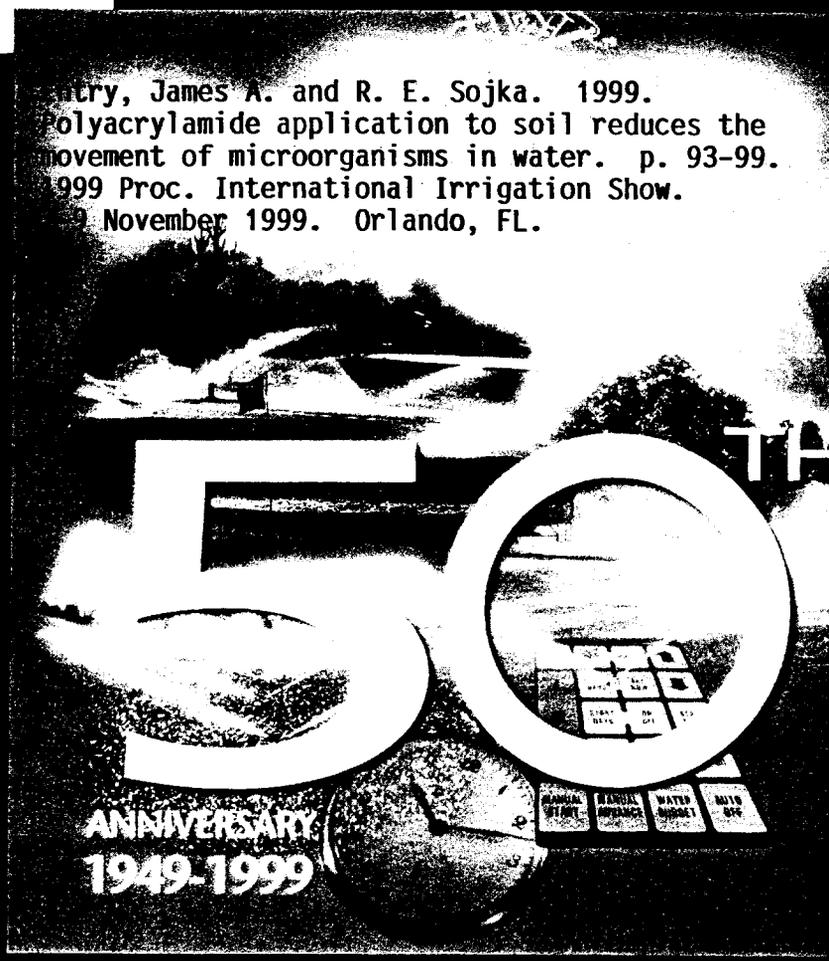


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Polyacrylamide Application to Soil Reduces the Movement of Microorganisms in Water

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Introduction

Early reports of polymer treatment of furrow irrigation inflows resulting in clarification of outflow water were made by Paganyas (1975) and Mitchell (1986). Paganyas did not specify the chemical polymer used, although its description suggests some type of polyacrylamide (PAM) formulation. Mitchell specified use of anionic PAM and noted stream advance time and infiltration increases. Both added PAM to furrow inflows during the advance (only) (before runoff, while water first traverses the dry furrow). Neither Paganyas nor Mitchell quantified sediment effects. Lentz *et al.* (1992) reported PAM-use in furrow irrigation for erosion control and increased infiltration, quantifying changes in sediment concentration and accumulation over time, sediment loss, infiltration and runoff. Lentz and Sojka (1994) reported that PAM-treatment reduced runoff water concentration of ortho-P, total-P, and nitrate, and lowered biological oxygen demand. Runoff sediment loss was reduced 94% and infiltration increased 15% in a 3 yr series of studies. Subsequently others further documented that PAM-treatment of furrow irrigation water improved runoff water quality parameters, including pesticide loads (Agassi *et al.*, 1995; Bahr and Steiber, 1996; Singh *et al.*, 1996). The most frequent approach in these studies involved PAM-treatment of the furrow advance stream (only). Good results were achieved with PAM application at about 1 kg/ha (1 lb/acre) applied at 10 ppm only in the advancing furrow. McCutchan *et al.* (1994) also reported runoff sediment load reductions, however, they applied PAM at 2.5 ppm continuously throughout the irrigation. On-farm PAM cost is about \$3 to \$5 per pound. Typical farmer seasonal PAM-use is 3 to 5 lbs per acre. The technology was adopted on about 1,000,000 acres in the United States as of 1998 and continues to grow rapidly in the US and overseas. The practice has received much attention in Western US states now seeking ways to meet mandated total maximum daily loads (TMDL) for various contaminants, including sediment, nitrogen, and phosphorus, in irrigation runoff and return flows.

The same water soluble PAMs, safely used for a variety of food, potable water treatment and other sensitive environmental uses, also provide erosion control with irrigation. These are very large anionic molecules (Barvenik, 1994). They should not be confused with, or evaluated with other PAM formulations, especially cationic PAMs, which have known environmental safety concerns related to their specific chemistries (Barvenik, 1994). They are very different from gel-forming non-water soluble cross-linked PAMs, which can cause severe application problems if used in flowing water. Environmental regulation, safety and toxicity of anti-erosion PAMs have recently been extensively reviewed (Seybold, 1994; Barvenik, 1994).

The amide functional groups in polyacrylamide are a ready microbial nitrogen source. Kay-Shoemaker *et al.* (1998a) reported that KCl extractable NO_3 and NH_4 was higher in irrigation water containing 10 mg L^{-1} PAM applied to potato (*Solanum tuberosum* L.) fields. However, there was no difference in KCl extractable NO_3 and NH_4 concentrations when the same amount and type of irrigation water was applied to beans fields. PAM degradation in soil is fairly rapid (Kay-Shoemaker *et al.*, 1998b, Shanker *et al.*, 1990, Lande *et al.*, 1979). Polyacrylamide was degraded completely within 5 days after applying $500 \text{ mg PAM kg}^{-1}$ garden soil (Shanker *et al.*, 1990). Lande *et al.* (1979) applied $25 \text{ mg PAM kg}^{-1}$ soil and reported that half life of acrylamide in soil was 18-45 hr. Bacteria were capable of utilizing PAM as a sole source of N in enrichment cultures (Kay-Shoemaker *et al.*,

1998a). The effect of PAM application to water or soils on soil microorganisms has been inconsistent (Nadler and Steinberger 1993, Steinberger *et al.*, 1993, Kay-Shoemaker *et al.*, 1998a). Changes in soil microbial populations likely depend on soil temperature, water, carbon, nitrogen and nutrient status as well as the amount and type of PAM. PAM effects on soil microorganisms may have been obscured by other factors (Kay-Shoemaker *et al.*, 1998a;1998b)

Because of the remarkable efficacy of non-toxic high molecular weight anionic PAMs for removing fine suspended solids from flowing water, we hypothesized that PAM-use would also remove and/or immobilize microorganisms in flowing water. We tested this hypothesis by monitoring the effect of PAM-treatment of furrow irrigation water used to irrigate an agricultural field, on several categories and measures of soil microorganisms in the inflow and runoff.

Materials and Methods

Study Site: The study was conducted at the USDA Agricultural Research Service's Northwest Irrigation and Soils Research Laboratory in Kimberly, Idaho. Soil in the test field was Portneuf silt loam (coarse-silty, mixed, superactive, mesic XericHaplocalcids), with 10-21% clay and 60-75% silt, and approximately 13 g/kg of organic matter. Saturated paste extract electrical conductivity (EC) of this soil's Ap horizon ranges from 0.7 to 1.3 dS/m. Exchangeable sodium percentage (ESP) is 1.4 to 1.7 with pH of 7.6-8.0 and with a CaCO₃ equivalent of 2-8%. Slope was 1.5%.

Experimental Design: Experimental design was a randomized complete block with three replications. Treatments were: 1) Control (no PAM applied) or PAM-treated, 2) three flow rates corresponding to three irrigation dates, 3) distance along the furrow (the inflow point, 1 m and 40 m down furrow) and 4) time during irrigation (1/2, 3 1/2 and 6 1/2 hr). Field plots were considered blocks. Three water samples were taken during each irrigation at each sampling point. Plots were 40 m long x 4 m wide.

Polyacrylamide Application: The polyacrylamide copolymer used was a dry granular material having molecular weight of 12-15 Mg/mole, with an 18% negative charge density (provided by CYTEC Industries of Wayne, NJ; marketed as Superfloc 836A). PAM application involved spreading granular PAM on the surface of a 0.1 m² area of soil in the furrow, corresponding to the first meter of furrow below inflow spigots. Application amounts were 35, 35, and 15 g of material (approximately 28, 28, and 12 g of active ingredient) on three sequential irrigation dates respectively.

Water Application: Using spigoted plastic pipe, irrigation water was applied from a storage pond to a field that was disked in autumn and spring, then roller harrowed after incorporation of fertilizer and herbicides prior to planting edible dry beans (*Phaseolus vulgaris*). Furrows of 40 m length and 10 cm depth were prepared with weighted 75° V-shaped forming tools. Furrow spacing was 76 cm. Irrigation was on every other furrow only, in wheel-track furrows on the first and third irrigations and on non-wheel furrows in the second irrigation. Three flow rates of water were used (7.5 liter min⁻¹, 15.0 liter min⁻¹, 22.5 liter min⁻¹), which corresponded to the three different irrigation dates: June 19, July 2, and July 17, 1997.

Sample Collection: Water samples were collected from the surface to a 3 cm water depth at the furrow inlet, 1 meter below the inlet and 40 meters below the inlet. Water samples were taken 1/2 hr following the initiation of each irrigation (advance), and at 3 1/2 and 6 1/2 hr after beginning irrigation. Three separate water samples were collected at each sampling point at each sampling

time. Samples were collected and analyzed for active fungal biomass, active bacterial biomass, total fungal biomass, total bacterial biomass, and algae. Water was collected and stored in air-tight and water-tight plastic 125 ml bottles and prepared for microbial testing within 24 hr of collection to minimize storage effects on microbial activity.

Microbial Analysis: Active and total bacteria and fungi in surface flow were determined for each treatment using methods described by Ingham and Klein (1984). Active fungi were estimated by taking a 1.0 ml water sample which was diluted in 9 ml of a phosphate buffer (pH 6.0) and shaken at approximately 120 rpm for 5 min. A 1 ml aliquot was removed and stained with 1 ml of a 20 $\mu\text{g ml}^{-1}$ fluorescein diacetate (FDA) solution in a 0.2 M phosphate buffer for 3 min. One ml of 1.5% agar in a 0.1 M phosphate buffer (pH 9.5) was added to the FDA suspension. The sample was mixed and an aliquot placed on a microscope slide containing a cavity of known volume (Ingham and Klein, 1984). Immediately after preparation, slides were examined for FDA-stained hyphal length by epifluorescent microscopy. Total fungal biomass was estimated by measuring the length and diameter of hyphae in 3 to 60 fields with phase - contrast microscopy. Three slides were evaluated from each sample and ten fields per slide were evaluated with phase contrast microscopy for total hyphal length, and three transects were evaluated for FDA-stained (active) hyphal length at X160 total magnification. Iodonitrotetrazolium (INT) stain was used for counting active bacteria (Stamatiadis *et al.*, 1990). A 1 ml sample of initial soil suspension was diluted to a final dilution in a 0.2 mg soil in 4 ml buffer. The suspension was incubated with 4 ml of filtered INT buffer for 60 min in the dark at 20 °C. Total bacteria per ml of water were estimated from the mean number of bacteria (fluorescent and non-fluorescent bacteria), their average diameter and length per field. Three slides were evaluated for each sample and ten fields per slide were evaluated using epifluorescent oil-immersion microscopy to determine numbers and size of fluorescent and total bacteria (Lodge and Ingham, 1991). Bacterial volume was computed from the number of soil bacteria per gram of soil considering that bacterial spheres were 1 μm in diameter (Jenkinson and Ladd, 1981). A carbon to volume conversion factor of 120 $\mu\text{g C mm}^{-3}$ was used for both bacteria and fungi, assuming 1.1 g cm^{-3} wet density, 20% dry matter content, and a 0.41 carbon content in the bacterium or fungus (Jenkinson and Ladd, 1981).

Statistical Analysis: Microbial data were transformed using natural logarithms to achieve a normal probability distribution; data were then subjected to a general linear models analysis of variance (Kirk, 1982). Residuals were normally distributed with equal variance. Differences among treatment means were computed using the Least Square Means test ($p \leq 0.05$, $n=9$).

Results

Since analysis of variance indicated that treatment x flow rate x distance x time, treatment x flow rate x time, treatment x distance x time, and flow rate x distance x time interactions for active bacterial and fungal biomass, total bacterial and fungal biomass, algae, active fungal biomass/active bacterial biomass, total fungal biomass/total bacterial biomass and total microbial biomass were not significant, results are discussed below with regard to treatment x flow rate x distance (Kirk, 1982). After water flowed 1 meter at 7.5 liter min^{-1} , PAM-treatment reduced the number of active bacteria, total fungi, and total microbial biomass compared to control treatment (Figures 1, 4, and 7). After water flowed 1 meter at 15.5 liter min^{-1} , PAM-treatment reduced active and total bacterial numbers, total fungal lengths and active and total microbial biomass compared to control treatment (Figures 1, 3, 4, 6, and 7). After water flowed 1 meter at 22.5 liter min^{-1} , PAM-treatment reduced numbers of

active bacteria, length of active and total fungi and total microbial biomass compared to control treatment (Figures 1, 2, 4, and 7). After water flowed 40 meters at 7.5, 15.5, and 22.5 liter min⁻¹, PAM-treatment reduced all microorganisms examined compared to control treatment (Figures 1 to 7). Although several measures of microbial constituents of irrigation water were reduced by PAM treatment even after only one meter of flow, more consistent results were obtained (all microbial measurements were reduced by PAM) after 40 meters, indicating that some mixing and time (flow distance) in the furrow is needed to maximize the organism sequestering effect compared to controls.

Discussion

Furrow irrigation water is an in-field vector of numerous soil-borne and water borne plant pathogens. Irrigation return flows which are collected and reused in many district-wide irrigation schemes can also carry algae to distribution laterals and canals, and pathogens to neighboring fields. Irrigation runoff also often carries micro-organisms that threaten human hygiene to surface waters used for drinking or for recreation. This initial study demonstrated that PAM-treatment, now routinely used to control erosion and enhance infiltration, can reduce the spread of micro-organisms in runoff and return flows. Further development of this technology may reduce vectoring of plant pathogens and human hygiene-threatening microorganisms in runoff and return flows. This has potential for reduced algal inoculation of surface waters, reduced spread of plant disease in irrigated fields and reduced water-borne human health problems affected by return flow water quality. Further development of this aspect of PAM technology could conceivably also reduce the need for and use of pesticides used to control these organisms.

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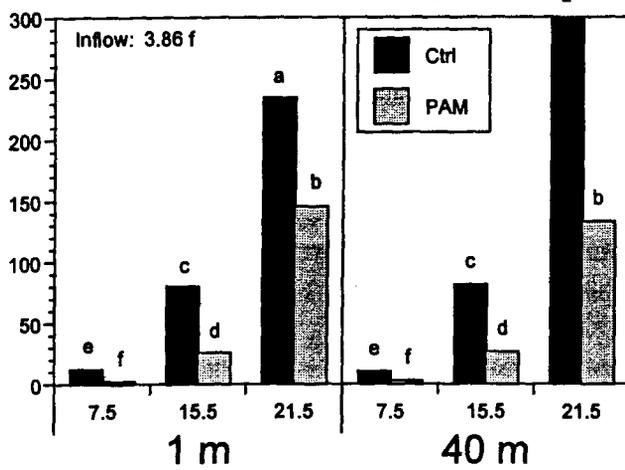
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Active Bacteria

(bacteria $\times 10^5 \text{ ml}^{-1} \text{ H}_2\text{O}$)



Active Fungi

(μm hyphae $\times 10^4 \text{ ml}^{-1} \text{ H}_2\text{O}$)

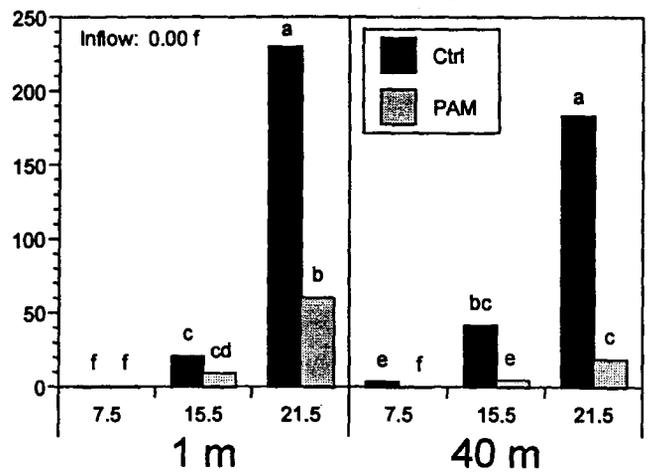
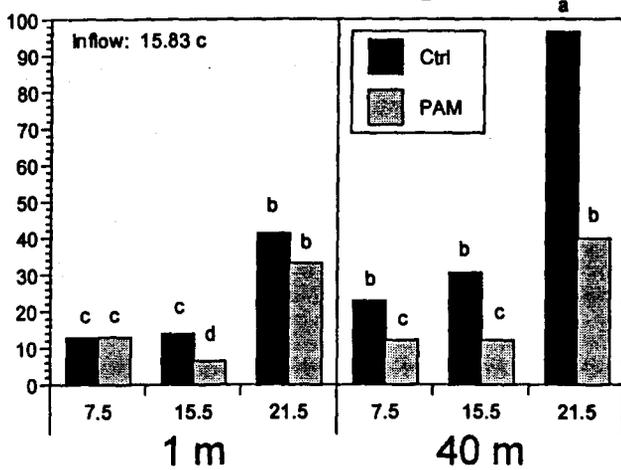


Figure 1. Numbers of active (actively metabolizing) bacteria in irrigation water at 1 and 40 meters from the inflow point at flow rates of 7.5, 15.5, and 21.5 liters per minute; mean inflow value for all 3 irrigations is noted.

Figure 2. Lengths of active (actively metabolizing) fungi in irrigation water at 1 and 40 meters from the inflow point at flow rates of 7.5, 15.5, and 21.5 liters per minute; mean inflow value for all 3 irrigations is noted.

Total Bacteria

(bacteria $\times 10^5 \text{ ml}^{-1} \text{ H}_2\text{O}$)



Total Fungi

(μm hyphae $\times 10^4 \text{ ml}^{-1} \text{ H}_2\text{O}$)

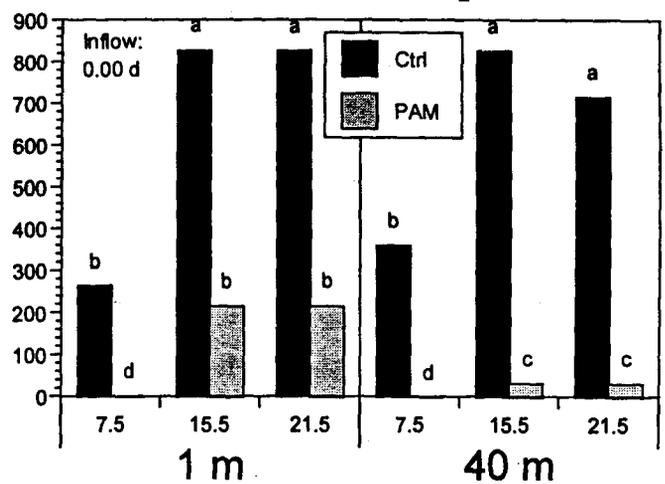


Figure 3. Numbers of total bacteria in irrigation water at 1 and 40 meters from the inflow point at flow rates of 7.5, 15.5, and 21.5 liters per minute; mean inflow value for all 3 irrigations is noted.

Figure 4. Lengths of total fungi in irrigation water at 1 and 40 meters from the inflow point at flow rates of 7.5, 15.5, and 21.5 liters per minute; mean inflow value for all 3 irrigations is noted.

Algae

($\mu\text{g C ml}^{-1} \text{H}_2\text{O}$)

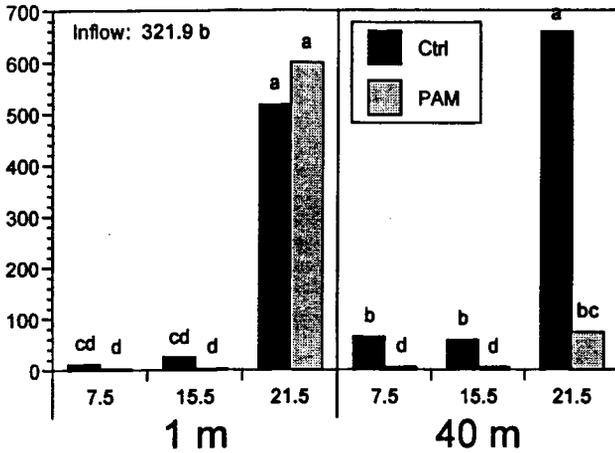


Figure 5. Lengths of algae in irrigation water at 1 and 40 meters from the inflow point at flow rates of 7.5, 15.5, and 21.5 liters per minute; mean inflow value for all 3 irrigations is noted.

Active Microbes

($\mu\text{g C ml}^{-1} \text{H}_2\text{O}$)

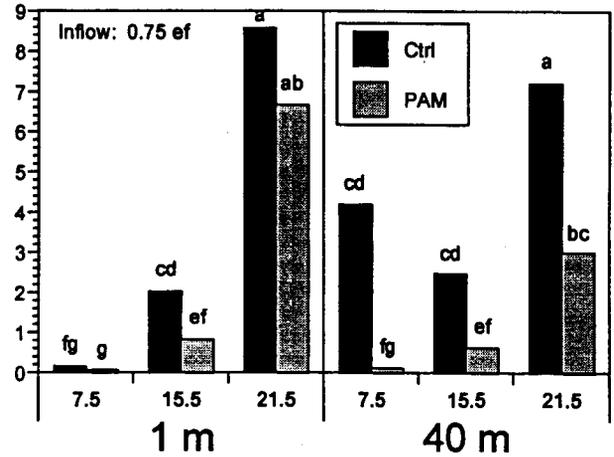


Figure 6. Active (actively metabolizing) microbial biomass in irrigation water at 1 and 40 meters from the inflow point at flow rates of 7.5, 15.5, and 21.5 liters per minute; mean inflow value for all 3 irrigations is noted.

Total Microbes

($\mu\text{g C ml}^{-1} \text{H}_2\text{O}$)

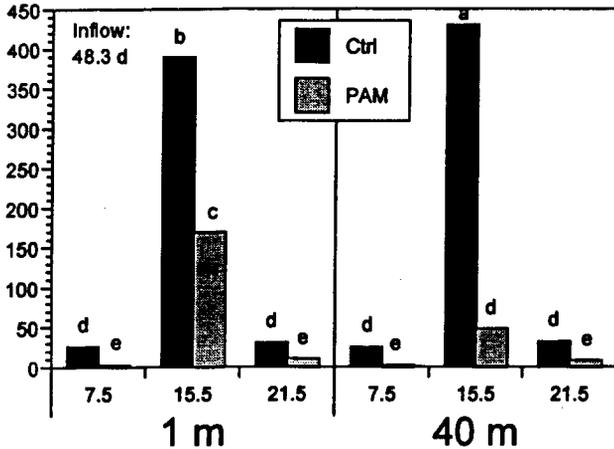


Figure 7 Total microbial biomass carbon in irrigation water at 1 and 40 meters from the inflow point at flow rates of 7.5, 15.5, and 21.5 liters per minute; mean inflow value for all 3 irrigations is noted.