INACTIVATION OF *CRYPTOSPORIDIUM PARVUM* OOCYSTS IN FIELD SOIL

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Abstract . *Cryptosporidium parvum* oocysts from dairy calves are believed to regularly contaminate watersheds. Identifying oocysts and measuring their viability in the natural environment are important elements in estimating the risk posed by this resistant organism. A 152 day field study was conducted to measure the viabilities of oocysts inoculated into 25 sampling points. Water potential, pH, and ammonium content were also measured at the same 25 sampling sites. A three-dimensional mapping program (Surfer®) was used to create 3-D maps of the viabilities of *C. parvum* oocysts and other factors measured during the experiment. The results indicate that 3-D graphical presentation may be a useful means to identify potential sites of greatest risk of oocyst survival and could indicate areas where natural conditions are causing the most rapid oocyst inactivation, and this method can be a means for the future measurement of microorganism inactivation in the natural environment.

INTRODUCTION

Cryptosporidium parvum oocysts shed in the feces of dairy calves can contaminate watersheds via leachate from bedding, the washing of calving houses, and the spreading of manure. Since the large outbreak of waterborne cryptosporidiosis in Milwaukee, WI (MacKenzie *et al*, 1994), watershed protection from non-point source contamination by these oocysts has received increased attention (Walker *et al*, 1998). The oocysts are resistant to environmental stresses and, in water, maintain their infectivity for long periods (Robertson *et al*, 1992). Thus, without understanding the capability of oocysts to survive in the environment, it will be impossible to establish legitimate management strategies to protect watersheds from oocyst contamination. The potential inactivation of pathogens in the environment was measured using the sentinel chamber system that was developed to follow pathogen viability under field conditions (Jenkins *et al*, 1999). In this work, the potential effects of various environmental conditions on oocyst inactivation in the field were examined to predict the potential risk of non point source watershed contamination with oocysts.

MATERIALS AND METHODS

Wild-type oocysts from naturally infected calves were inoculated into sentinel chambers that had been designed to safely contain *C. parvum* oocysts in a soil substrate that would equilibrate with the solutes of the external environment (Jenkins *et al*, 1999). Each chamber was buried 4 cm below the soil surface on a hillside of an old farm site at 25 points, 20 m apart, on a 5 × 5 grid. With each chamber, control oocysts in distilled water within a microfuge tubes were also buried. Chambers were sampled at 0, 2, 7, 19, 33, 66, and 152 days after placement, and oocyst viability was determined by a dye permeability assay using PI (Jenkins *et al*, 1997). Temperature probes were also buried. Soil samples from the site were taken to measure moisture content on Days 0, 7 19, 29, 40, and 152, ammonium content on Day 0, and pH was measured on Days 0, 2, and 66. Soil texture was determined by the Nutrient Analysis Laboratories at Cornell University (Ithaca, NY). The viability of *C. parvum* oocysts from each sampling site over the sampling period and each soil parameter were mapped using Surfer 7 software (Golden Software, Inc Golden, CO, USA).

RESULTS

The spatial relationship between oocyst viability in soil and water, and the other measured parameters, soil pH, moisture content, and ammonia content, are shown using 3-D topographical maps.

Oocyst viabilities

The oocysts in soils in the chambers were still viable at Day 152 (27% mean viability) in spite of the ground being frozen during the part of the study period (Figs 1-6). The mean viability of the oocysts in water at Day 152 was 30% (Figs 7-12). Statistical differences were observed between the viabilities of oocyst in the soil and water (Fig 21). Theoretical times required to achieve 99% inactivation of oocysts are 334 days in soil and 332 days in water.

Soil moisture content

Soil moisture content varied from 25% to 80% depending on field location and season of sampling (Figs 13-18).

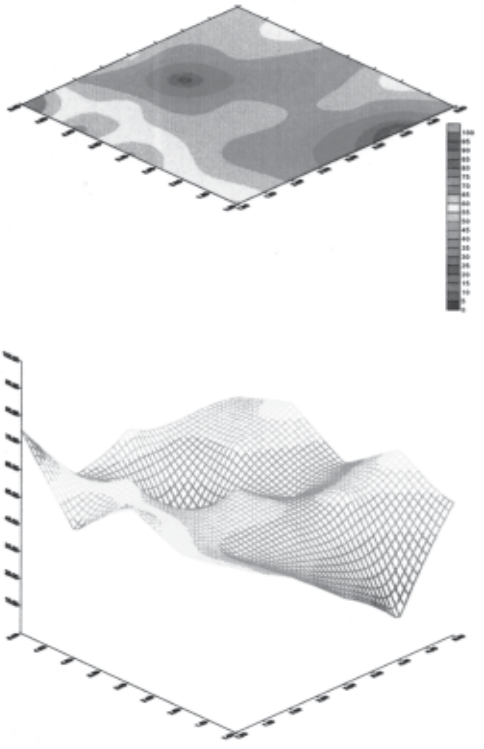

Fig 1- % Viability of oocysts in soil at Day 0.

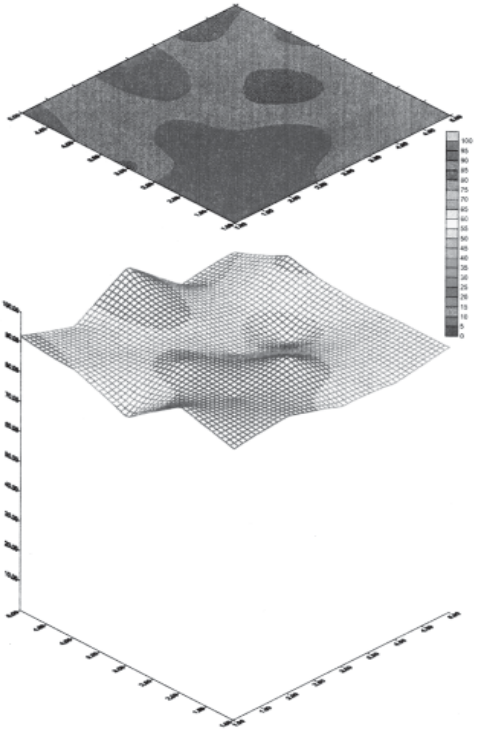

Fig 2- % Viability of oocysts in soil at Day 7.

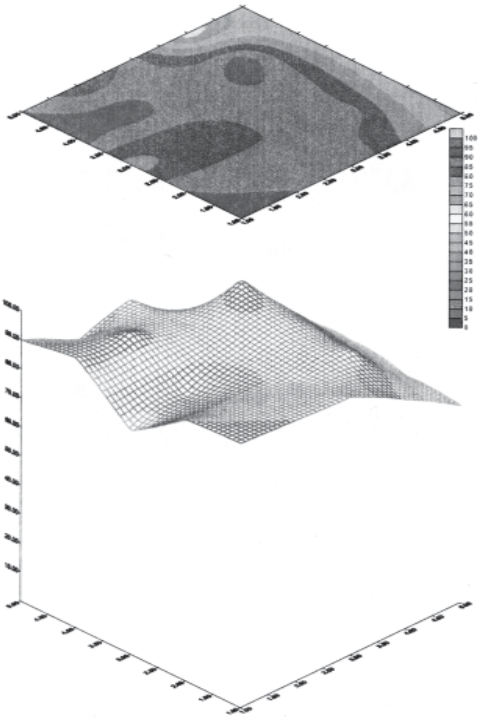

Fig 3- % Viability of oocysts in soil at Day 19.

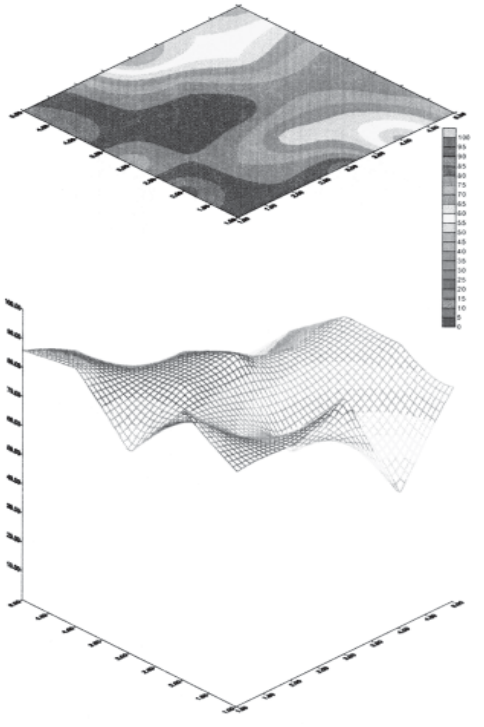

Fig 4- % Vability of oocysts in soil at Day 33.

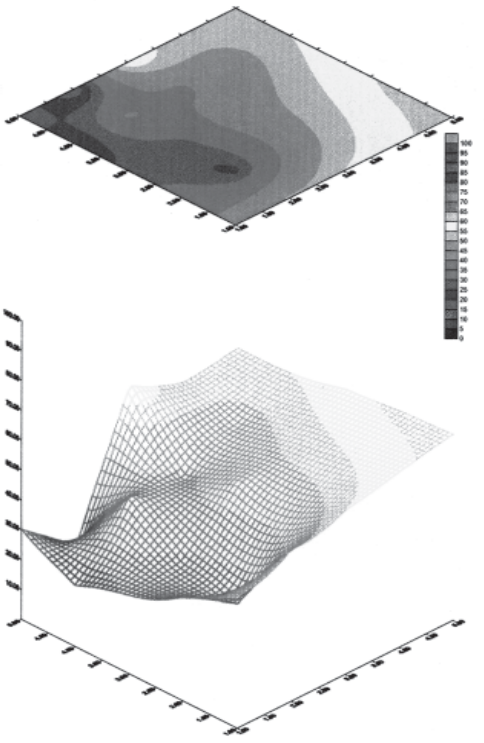

Fig 5- % Viability of oocysts in soil at Day 66.

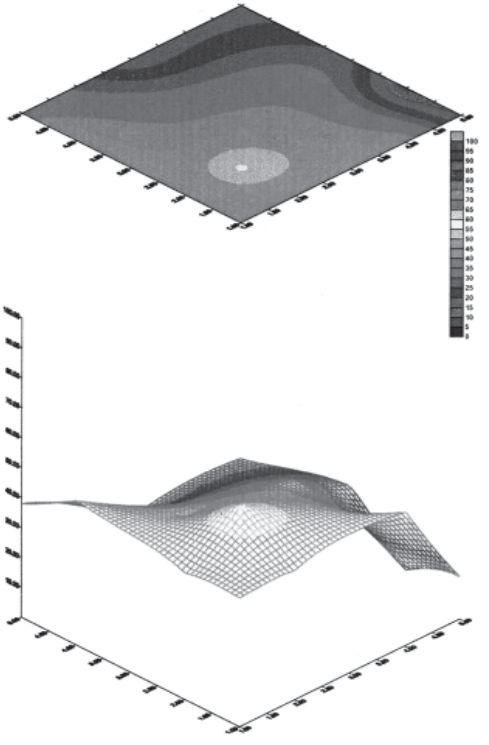

Fig 6- % Viability of oocysts in soil at Day 152.

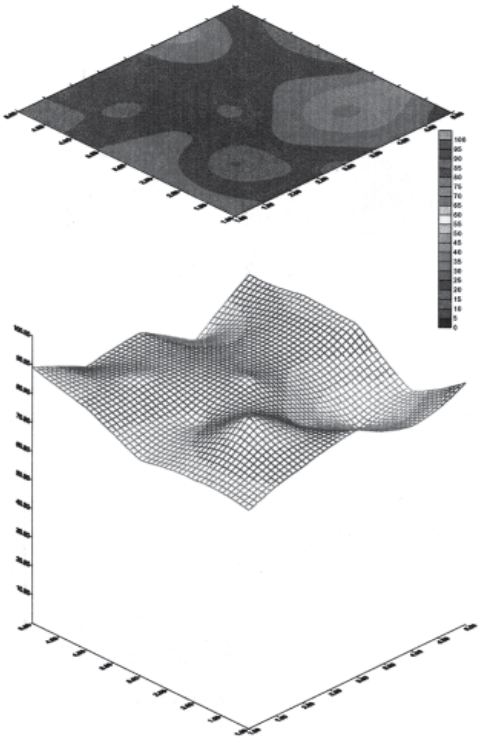

Fig 7- Viability of oocysts in water at Day 0.

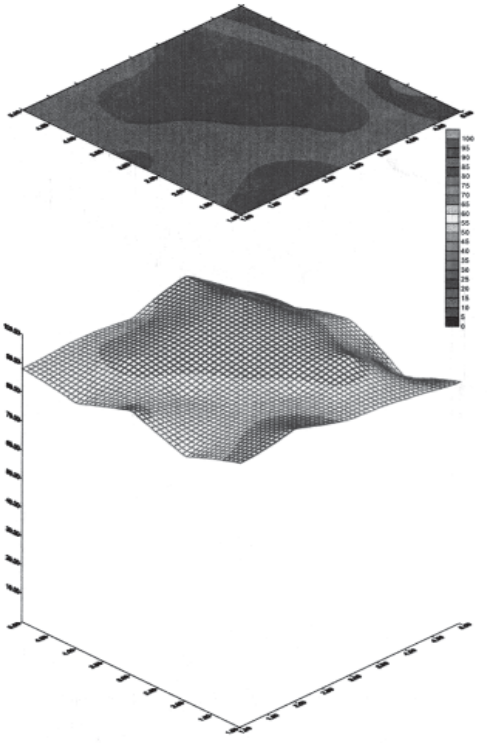

Fig 8- % Viability of oocysts in water at Day 7.

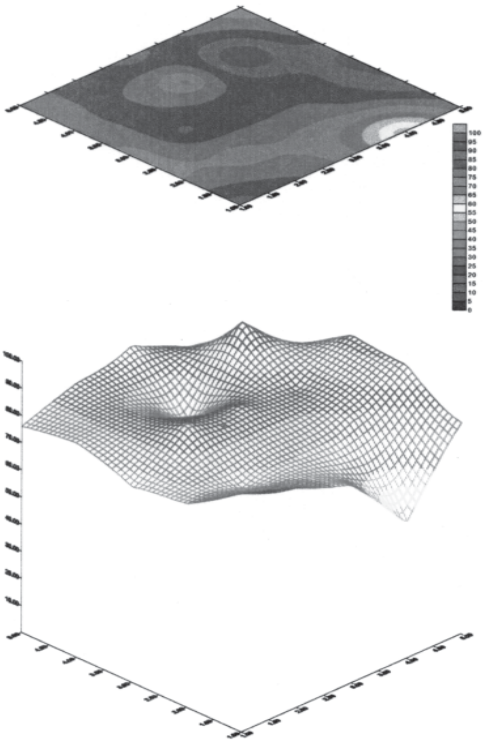

Fig 9- % Viability of oocysts in water at Day 19.

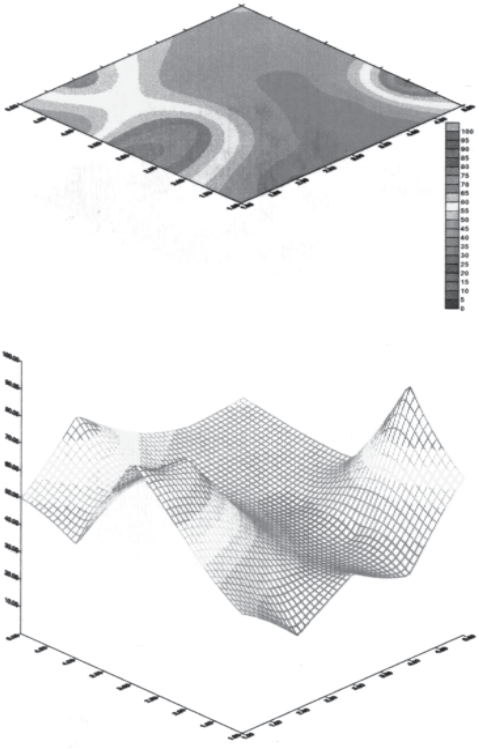

Fig 11- % Viability of oocysts in water at Day 66.

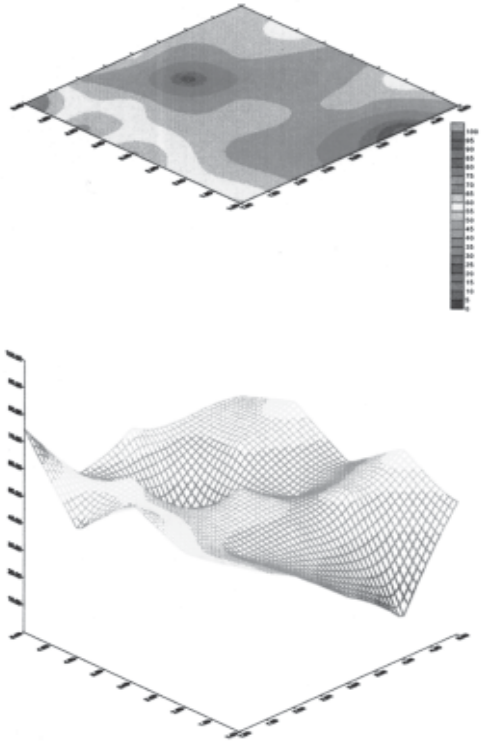

Fig 10- % Viability of oocysts in water at Day 33.

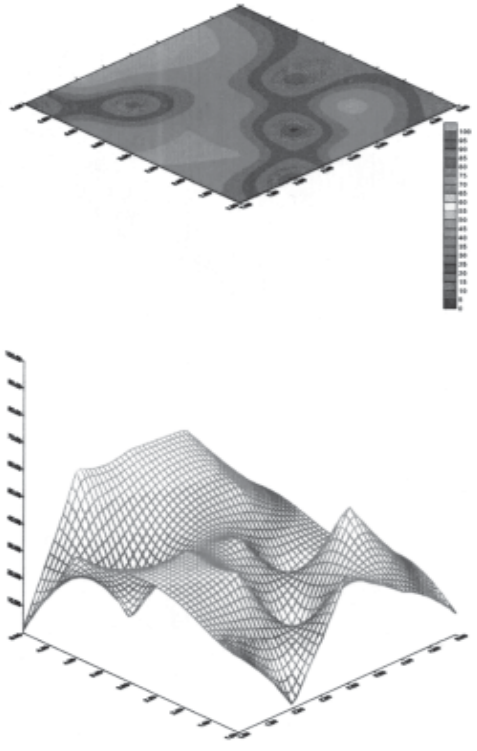

Fig 12- % Vability of oocysts in water at Day 152.

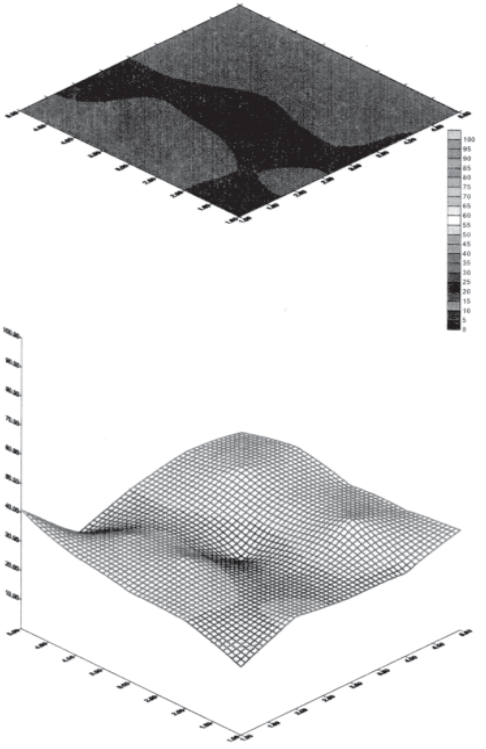

Fig 13- % Soil moisture content at Day 0.

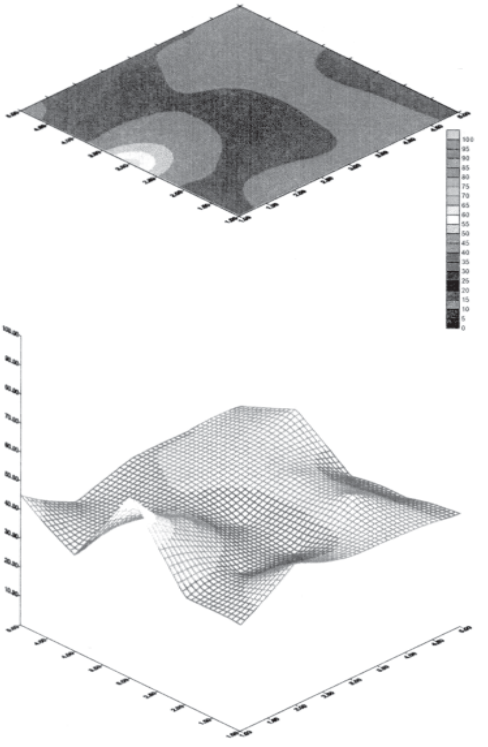

Fig 15- % Soil moisture content at Day 19.

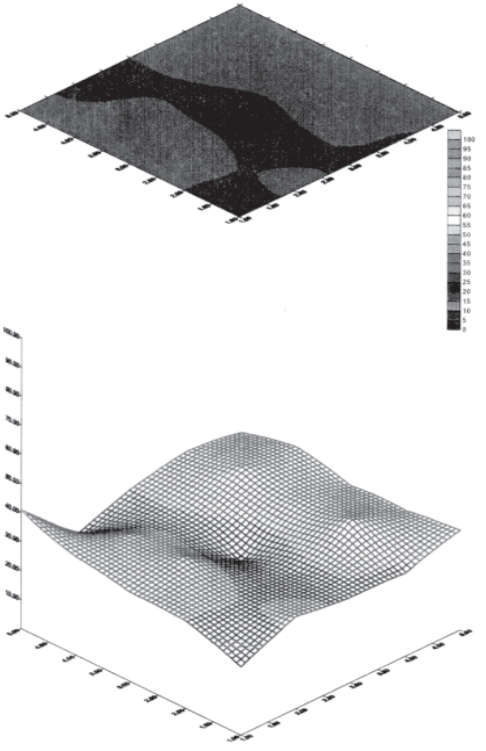

Fig 14- % Soil moisture content at Day 7.

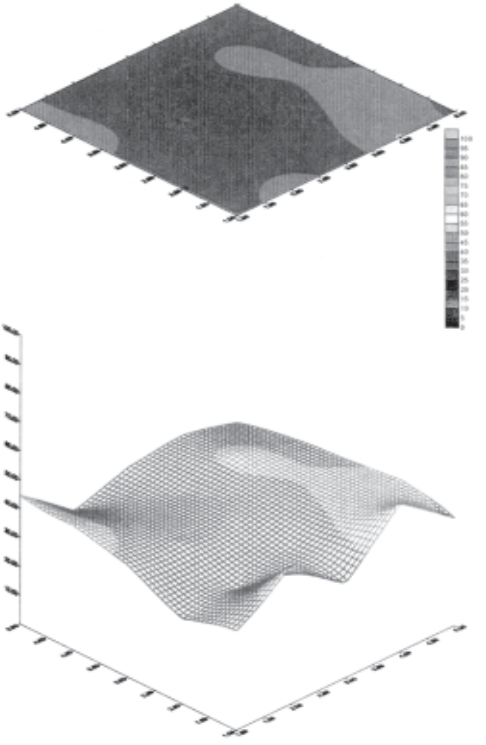

Fig 16- % Soil moisture content at Day 29.

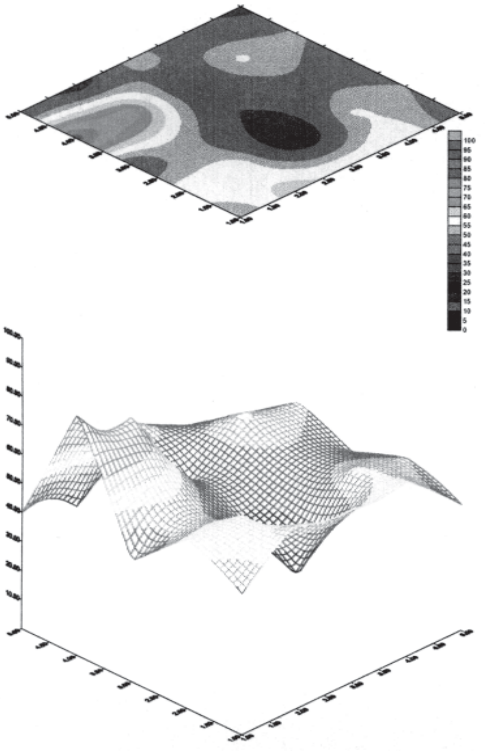

Fig 17- % Soil moisture content at Day 43.

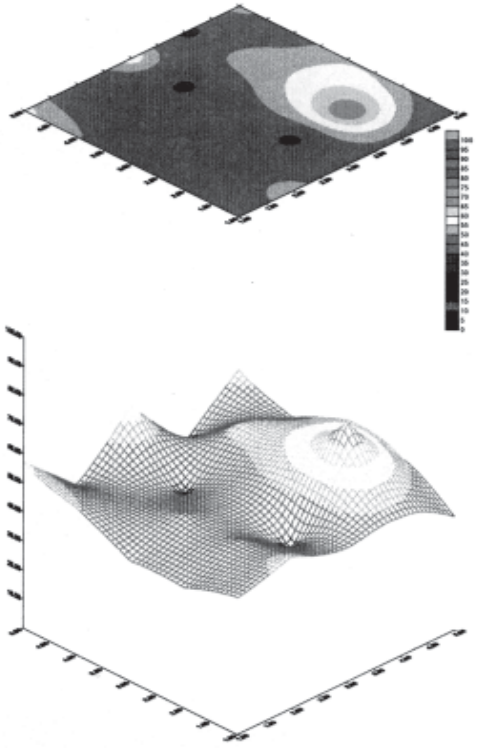

Fig 18- % Soil moisture content at Day 52.

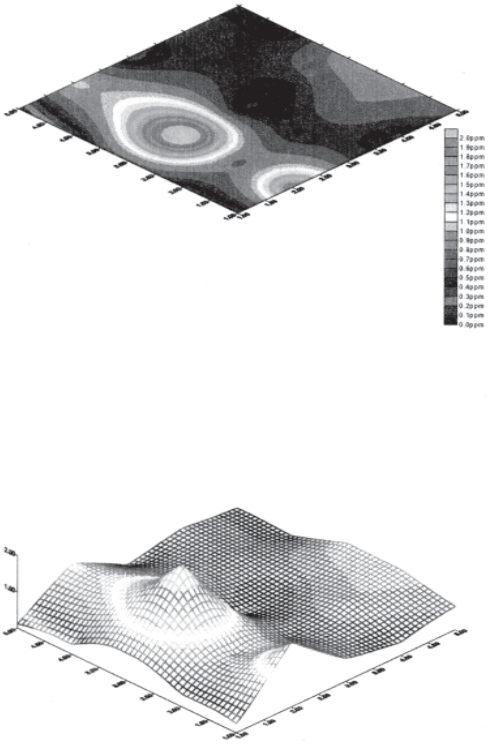

Fig 19- Soil ammonia content at Day 0.

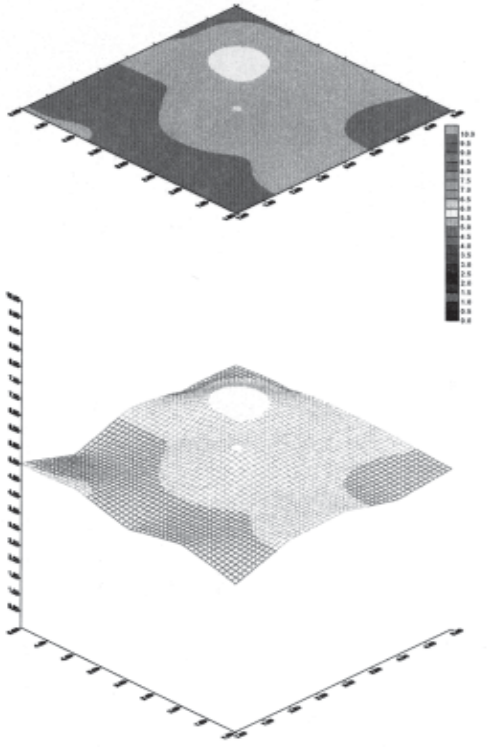

Fig 20- Soil pH at Day 0.

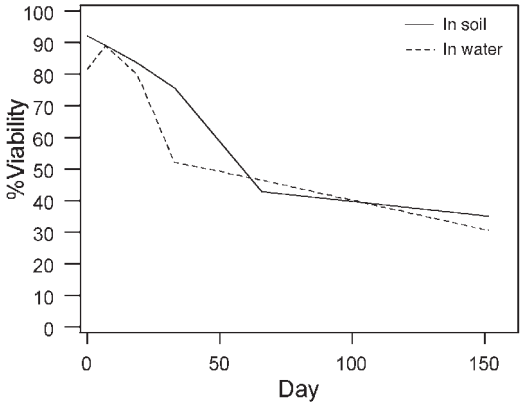

Fig 21- Mean oocyst viability % in water and soil.

Soil ammonia content

Ammonia levels were 0 to 2 ppm (Fig 19).

Soil pH

Difference of soil pH varied from 4.5 to 6.0 (Fig 20).

CONCLUSION

The 3-D graphical representation of the data provided a means for examining the effects of different environmental factors on oocyst viability over time. As these studies on the inactivation of oocysts in soils continue to progress, it is expected that more reliable methods will develop for assessing the inactivation of pathogens in soils that might occur following contamination via domestic or wild animals, wastewater treatment discharges, or the land application of biosolids. A large scale field study will be conducted to produce more precise 3-D graphical presentations of oocyst viabilities and environmental factors using USGS and GIS. The information will be used to establish management strategies for protecting watershed from oocyst contamination and for providing safe drinking water.

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