A Study of Human Waste Disposal in the Chebucto Bluff Trail, Nova Scotia.

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Summary and Recommendations:

Human stool specimens were deposited in the environment at mock wilderness toilet sites within Loop 2 of the Chebucto Bluff trail to monitor the disappearance of feces and the potential spread of fecal pathogens into the environment. Stool degradation was monitored by photographing the decrease in the volume of stool specimens over time. Spread of pathogens was monitored by performing coliform counts in drainage paths up to 15m from the toilet site. In addition 7 volunteers are identified who showed unique antibiotic resistance profiles of their *E.coli* and provided incontrovertible evidence when *E.coli* counts originated from the introduced human feces and not from visiting indigenous animals.

Results indicated that surface deposition was the fastest method of stool degradation. A "standard stool" of approximately 60 cm^3 disappeared after 85 to 100 days. Smearing feces thinly on rocks and leaving them exposed to the weather was the fastest method of stool degradation. When stools were buried either in conventional cat-holes at about 5cm depth, or deeper at 50 cm, disappearance time increased to about 240 to 260 days. Furthermore digging such holes in this terrain, with the intense mat of roots produced by the heath, was extremely difficult. Generally the soil depth is insufficient to permit holes of 50cm.

Coliform counts performed on water collected in crude lysimeters placed in the drainage path from the stool specimen, or performed on soil samples, showed that *E.coli* with antibiotic resistant profiles could be detected downstream from the deposition site - but surprisingly never more that 1m from the site. Spread of fecal bacteria from the introduced feces is therefore limited. Resistant *E.coli* were never detected in the receiving water of a pond at the experimental site – a distance of 36m to 60m. It is assumed that *E.coli* counts encountered at greater distances from the toilet sites must have come from visiting animals. Spread of fecal pathogens from deposited feces is not considered a large risk in this environment.

Recommendations:

- Surface deposition of feces, especially smearing, will provide a satisfactory means of human waste disposal for **low numbers** of wilderness campers. To be aesthetically pleasing toilet paper must be packed out. However with a disappearance time of approximately 100 days for such surface deposited feces, large numbers of campers would quickly cover the ground with stools or smeared rocks. The health threat of fecal pathogens contaminating the environment is minimal as marked *E.coli* were never detected more that 1m from the introduced stool specimen.
- Burial of feces, either in 5cm deep cat-holes or at greater depths, is impractical because of the dense mat of plant roots, the thin soil cover and the exposed bedrock. Furthermore the degradation of feces slows at least two-fold compared to surface deposition. Again spread of fecal pathogens from buried feces appears limited. If large numbers of campers are envisioned at some point in the future then mechanically excavated latrines or composting toilets will have to be considered.

Introduction:

Given the popularity of wilderness travel, there is a dearth of information on the most acceptable ways to dispose of human waste in such environments. The "Leave No Trace" movement is filling this void in the US with an extensive educational campaign which recommends waste disposal practices to minimize health risks and maintain wilderness in as pristine a state as possible. They are finding that these practices must be tailored to fit specific regions and biogeographic zones in the states (1). No such recommendations exist for the unique biogeographies of Canada and solid scientific data is needed on what happens to human waste when deposited in the natural environment.

The Chebucto Bluff region represents an exquisite pristine wilderness on the doorstep of Halifax. However, effective and safe disposal of human waste will be challenging in the "rock-land" areas where camping is envisioned on loops 3 and 4. This is because of the shallowness of the soil/duff and the extensive exposed granitic bedrock. This means that there will be little absorptive capacity in the ground where fecal degradation takes place.

In addition to fecal degradation and eventual disappearance of the feces, there is also a public health threat from fecal pathogens. The public health threat from human waste stems from a range of diseases spread via the fecal-oral route caused by viruses, bacteria, protozoa and helminths (2). Although the fate and survival of bacteria in the natural environment differs from the fate of viruses, protozoa and helminths, it is the coliform bacterial count which is used extensively as the principal indicator of fecal pollution. *E.coli* is routinely found in all mammalian bowels at concentrations of approximately 10^6 per gram of feces. This magnification factor serves as a convenient measure to track what is happening to feces as it degrades. The human population tends to be exposed to high concentrations of antibiotics, both through the consumption of pills and the use of antibiotics in animal feeds. *E.coli* are very good at absorbing antibiotic resistance genes to cope with this exposure, so many individuals show *E.coli* with characteristic resistance profiles. This enhances the capacity to say where the *E.coli* came from.

This project will address both aspects of human waste disposal in wilderness situations by examining the degradation of human fecal waste and also the spread of fecal bacteria from these stools.

Experimental Overview:

The fundamental approach taken in this project was to place known volumes of human feces into different habitats typical of Chebucto Bluff and follow 1) the disappearance of fecal material and 2) monitor the spread of coliform bacteria into the surrounding environment. This provided information on the most efficient way to dispose of human stools in this wilderness setting and also indicated the health threat of potential pathogens contaminating the environment.

Eight visits were made to the Chebucto Bluff throughout the summer and fall of 2004 to accomplish these objectives. The dates were:

May 18	Reconnaissance
June 21	Reconnaissance
June 25	Experiments started
July 22	Experiments continued
August 5	Experiments continued
August 12	Experiments continued
September 10	Experiments continued
October 15	Experiments terminated.

Fig 1: The Bluff Trail and the main experimental area at “The Pond”.

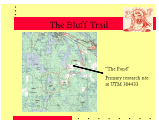


Fig 2: View of "The Pond" looking north.

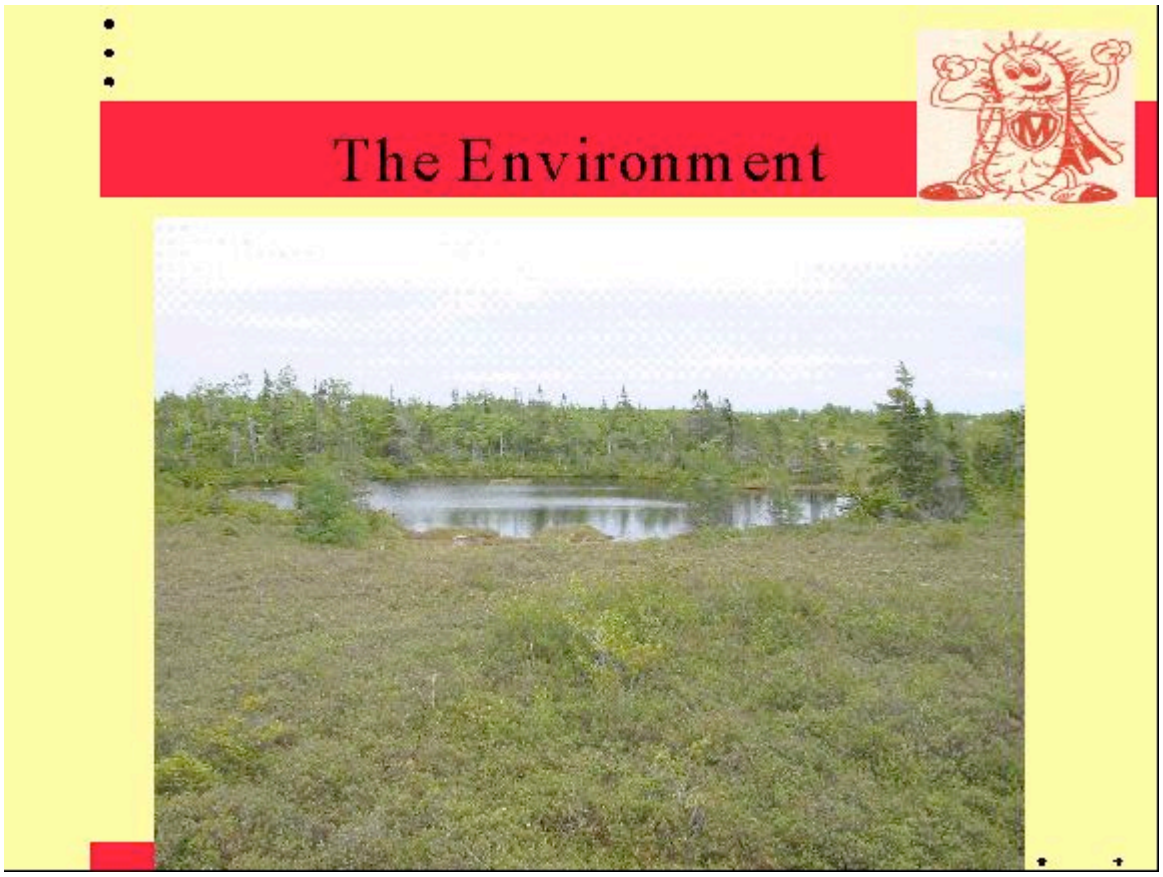
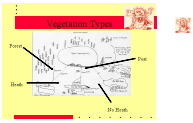


Fig 3: Vegetation types examined.



"No Heath" indicates areas of 3m square where all above ground vegetation was removed with shears.

Materials and Methods:

To accomplish the two objectives of this project some background microbiological preparations were necessary. These included:

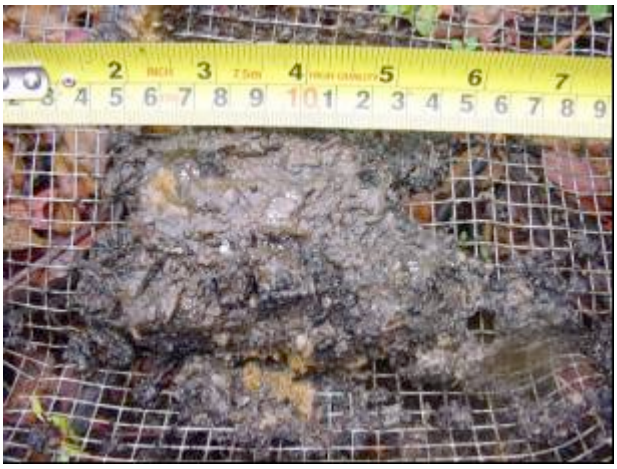
1. **Isolation Technique:** A comparison of techniques to extract bacteria from the soils and water of The Pond was performed (data not shown).

    a. **Soil Extraction -** Extraction protocols included treating the soil with a) homogenizers, b) vortex machines, c) sonicators, d) shakers and e) the Bead Beater ™. Two microbiological media were compared, Nutrient Agar and Tryptic Soya Agar (TSA) and as well as incubating the plates at 30^{o}C or at room temperature (approx 23 ^{o}C). Results indicated that higher numbers of bacteria, and more diverse types of bacteria, were obtained from soil sampled on May 18 using the Bead Beater, after dilution in Phosphate Buffered Saline (PBS), and incubating at room temperature on spread plates of TSA. This was adopted as the standard plating method for all subsequent soil studies. One gram of soil was placed in 9mL of PBS and processed in the Bead Beater for 150 seconds. The resulting suspension was diluted to 10^{-9} and 100 μL spread plated on TSA and incubated at room temperature for 4 days.

    b. **Water Extraction –** A similar comparison of media and incubation temperature revealed that spread plating on TSA at room temperature gave the best recoveries for water samples. This was adopted as the standard plating method for all subsequent water studies.

2. **Stool Degradation:** Human stool specimens were collected from volunteers and stored at 4 ^{o}C until required. An attempt was made to bury a uniform volume of feces in each experiment (a standard stool). In the field this was estimated as a piece the size of a forefinger which translated to approximately 60cm^{3}. The stools were placed in small wire cages constructed from ½ inch galvanized hardware cloth and placed at the site. Photos were taken at the beginning of the experiment and throughout the degradation at a constant magnification.

Fig 4: Photo of a stool specimen in a wire cage taken at constant magnification.



These photos were printed, at a constant magnification, on the same lot of printer paper. The stool images were cut out and weighed and the surface area of feces determined by reference to calibration graphs relating weight to surface area. These figures were converted to volume by estimating the average thickness of stools to be 2cm. Thick smeared stool was estimated to be 1cm thick, after a standard stool was smeared thickly on a rock. Thin smeared stool was estimated to be 0.5cm thick. At least three experiments were performed for each treatment and the averages and standard deviations (shown as error bars in all graphs) calculated.

Fig 5: A thinly smeared stool specimen.

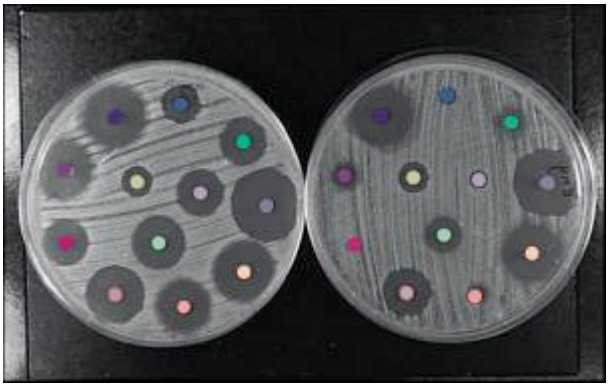
3. **Coliform Counts:** The standard membrane filtration technique of coliform counting was performed. Multiples of 100mL of water were filtered through a 0.45 μm filter, the filter transferred to mFC plates and incubated overnight at 44.5 ^{o}C. Presumptive *E.coli* colonies were picked at random and confirmation was performed with oxidase, indole and MUG tests. Water was collected from degrading stool samples by constructing crude lysimeters. These consisted of

sterile plastic 120mL urine specimen jars which had 4 holes (¼ inch diameter) drilled in their sides at the 100mL mark. The lysimeters were buried in the ground up to their tops at 1m intervals downhill from the stool deposition site up to 15m from the stool specimen.

Fig 6: A lysimeter used to collect water from degrading stools.

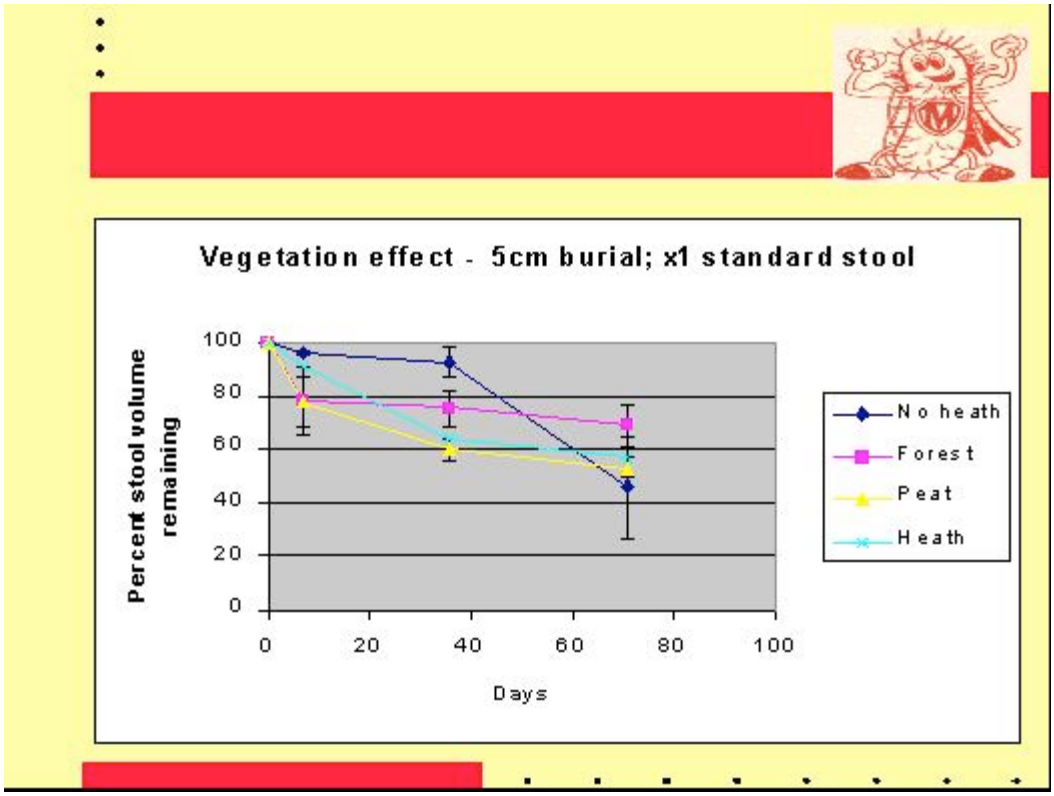
4. **Antibiotic Resistance Markers:** In order to distinguish fecal coliforms introduced into this environment from those deposited by the indigenous fauna, human stool specimens kindly provided by 19 volunteers was screened for resistance to 11 antibiotics. These antibiotics included: kanamycin, nalidixic acid, ampicillin, tetracycline, cefazolin, neomycin, gentamycin, carbenicillin, chloramphenicol, streptomycin and sulfamethoxazole. Once *E.coli* were confirmed from the membrane filter technique, 4 colonies from a MacConkey agar plate were placed into 100mL of Nutrient Broth, which was placed onto a shaker overnight at 35-37 ^{o}C. 1mL of each Nutrient Broth culture was diluted down to a MacFarland standard of 1 and was swabbed onto Mueller-Hinton Agar (MHA), and antibiotic disks were placed onto the plate. MHA plates were incubated overnight at 35-37 ^{o}C, and then zones of inhibition determined, indicating antibiotic resistance.

Fig 7: Zones of inhibition on MHA plates.



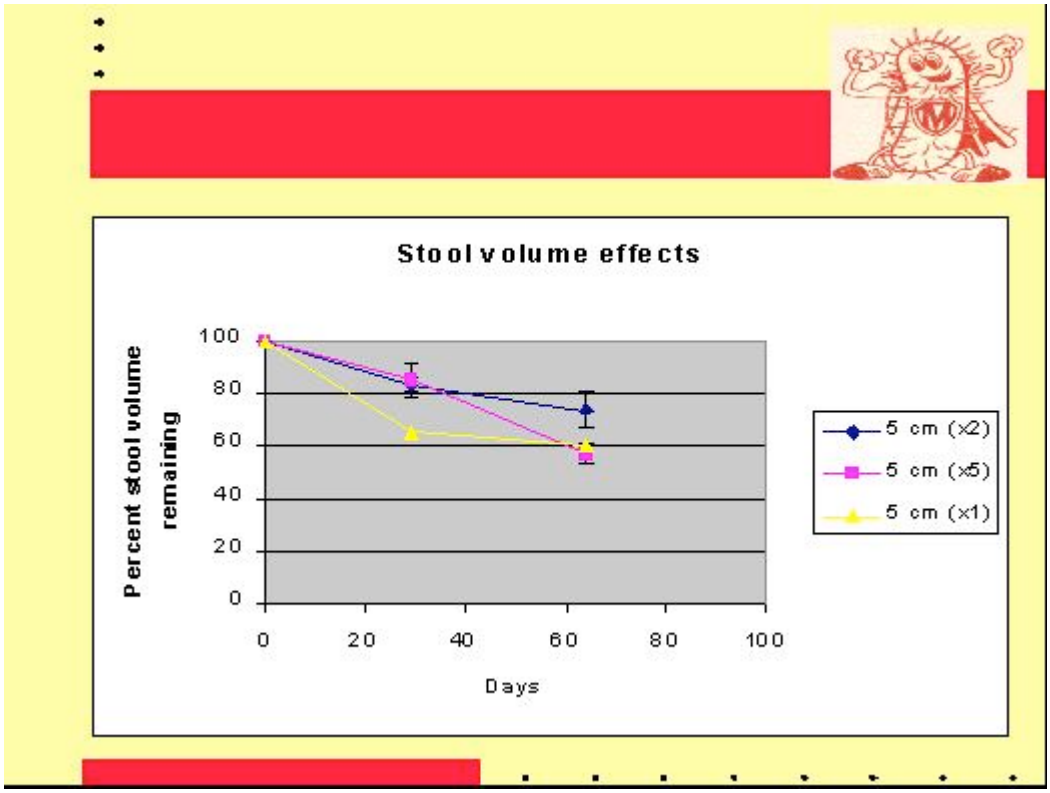
Results: Part 1 – Stool Degradation.

Fig 8: The effects of different vegetation on degradation with 5cm burial.



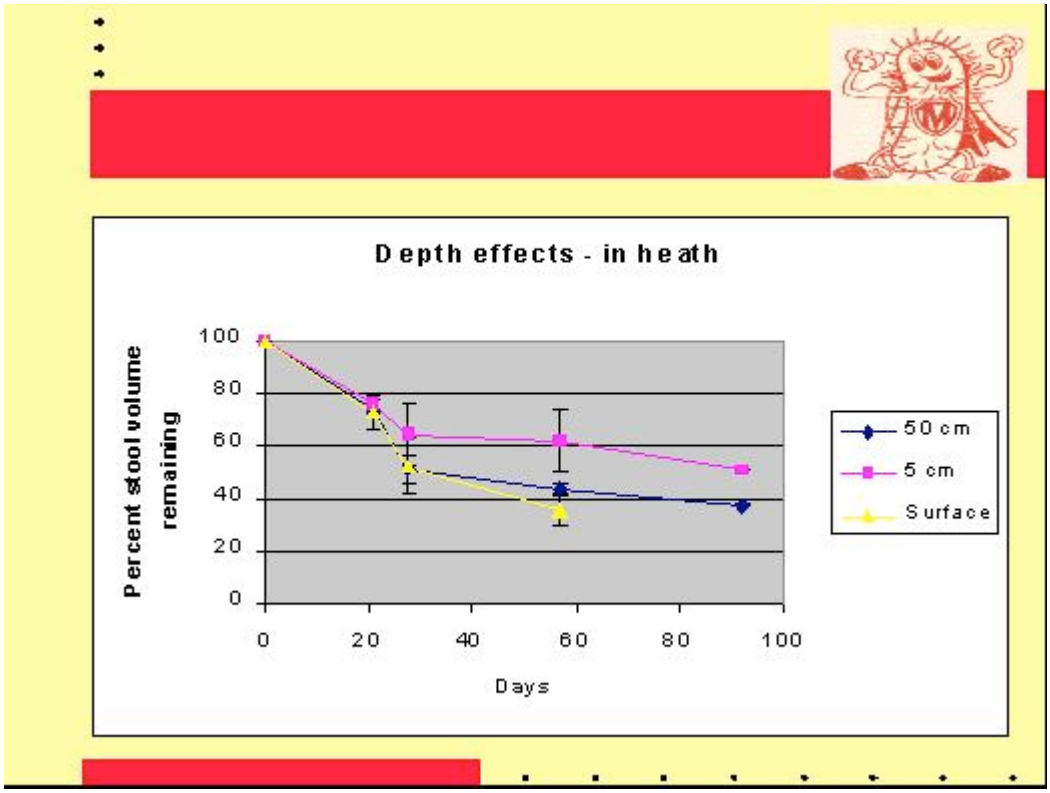
Cat-hole sites in the forest to the west of the pond consistently produced the slowest degradation. Removal of the heath in the "no heath" treatment appeared to have little effect on the final degradation which was similar to that observed in the heath and in the peat next to The Pond.

Fig 9: The effect of increasing feces volumes on degradation with 5cm burial in heath.



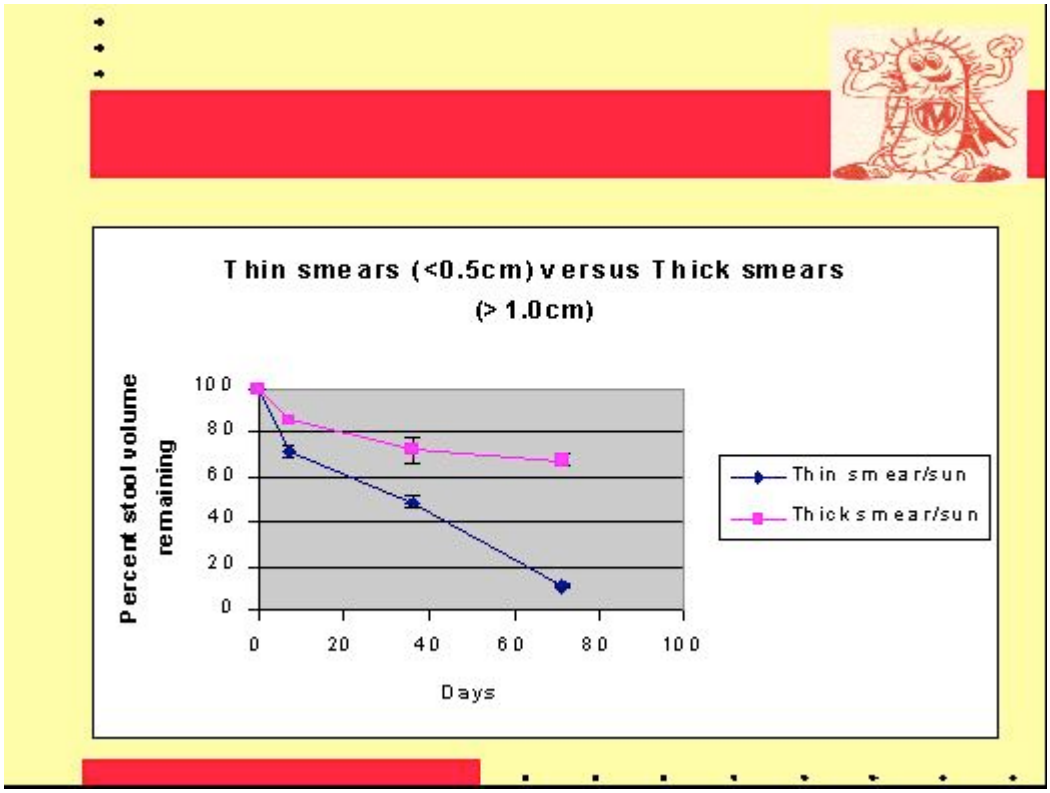
Increasing the volume of feces buried in 5cm cat-holes had inconsistent effects. Doubling the volume (x2) significantly slowed up the degradation, but increasing the volume five times did not seem to slow down degradation compared to the standard stool (x1).

Fig 10: The effects of burial at different depths in heath.



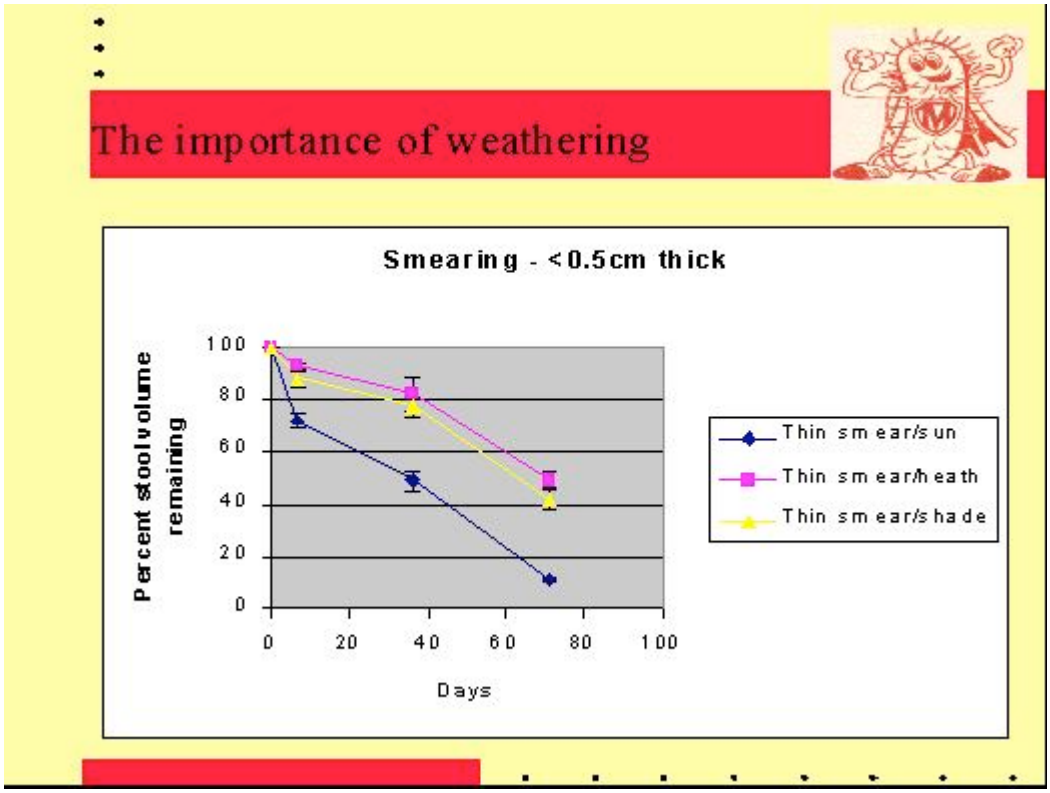
Leaving the feces to degrade on the surface was definitely the fastest disposal method. When these curves are extrapolated to zero it shows that stools left on the surface will completely disappear after 110 days, those buried at 50cm in 240 days and those buried in 5cm cat-holes in 260 days.

Fig 11: The effect of thin versus thick rock smears on degradation – exposed to weathering.



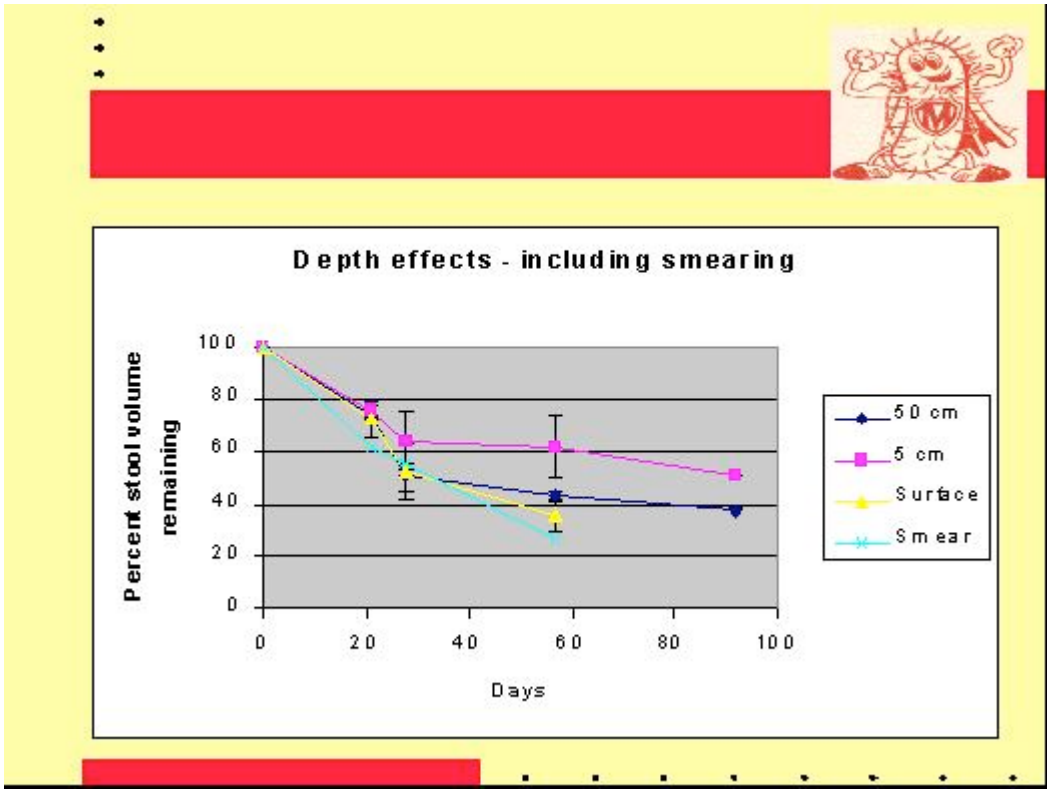
Smears consistently produced the fastest degradation. The thinner the smear the faster the degradation. Extrapolation of these curves indicates that the stools would reduce to nothing after 85 days when smeared thinly. In fact at the end of this experimental period it was difficult to see any feces left on the rocks.

Fig 12: The importance of weathering on degradation of smears.



The rapid degradation of stools smeared thinly only occurred when the rock was left exposed to the sun, and presumably rain. When the rock was placed back into the thick vegetation cover of the heath or left under the shade of large boulders, degradation slowed to the rate observed for non-smeared surface deposited stools.

Fig 13: A comparison of all treatments on degradation.



Burial of stools to any depth slowed degradation appreciably. Smearing was the most effective way of disposing of feces provided the material was left exposed to weathering. The rate of surface deposition was very comparable to smearing.

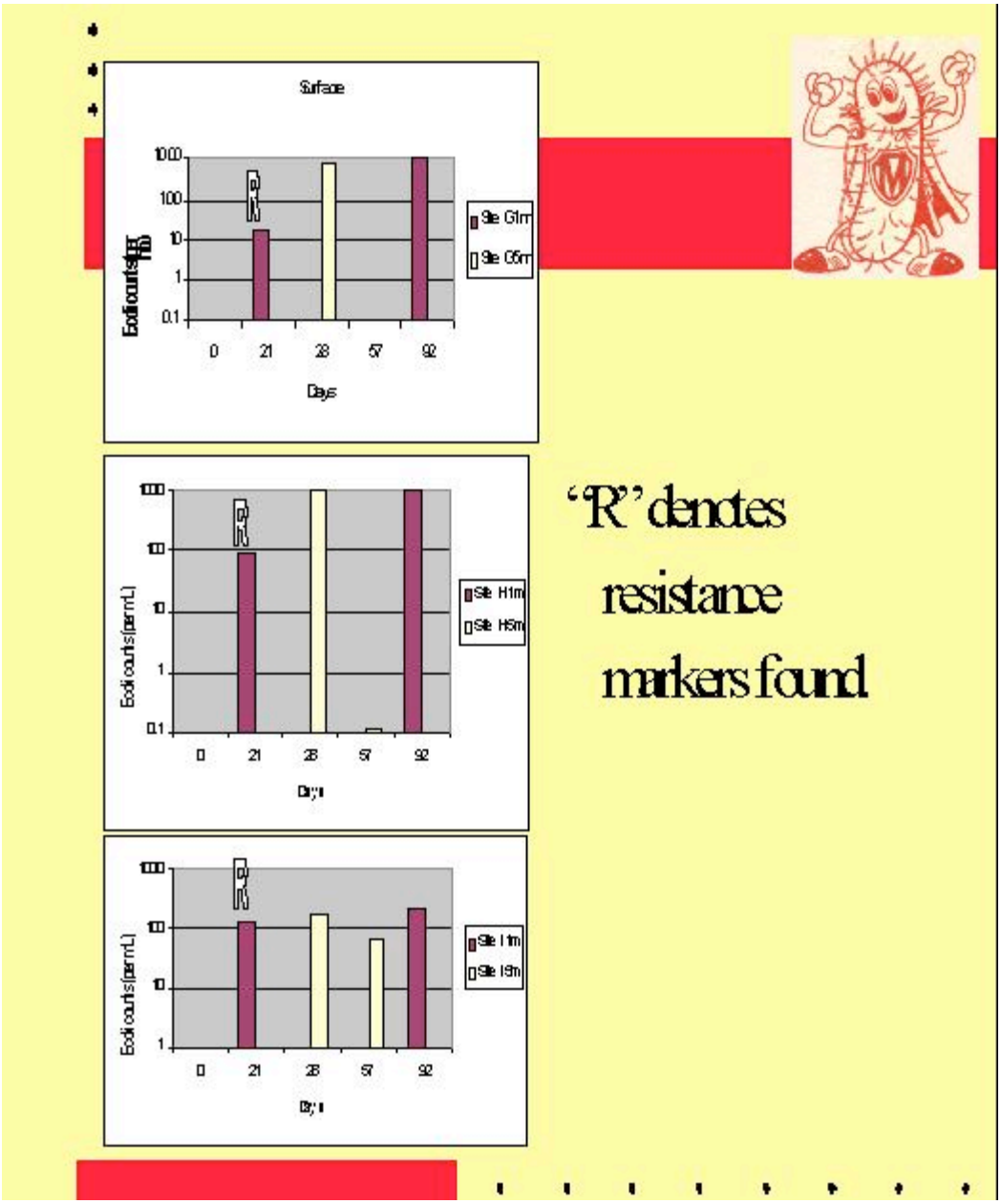
Results: Part 2 – Potential spread of fecal pathogens.

Table 1: Seven of 19 volunteers who showed antibiotic profiles.

Subject Number	Resistances
3	Tetracycline, streptomycin, ampicillin, trimoxazole, carbenicillin, chloramphenicol
4	Streptomycin
6	Ampicillin, cefazolin
9	Carbenicillin, ampicillin
14	Carbenicillin, ampicillin
15	Tetracycline, oxytetracycline
17	Gentamycin, streptomycin, nalidixic acid, ampicillin, carbenicillin

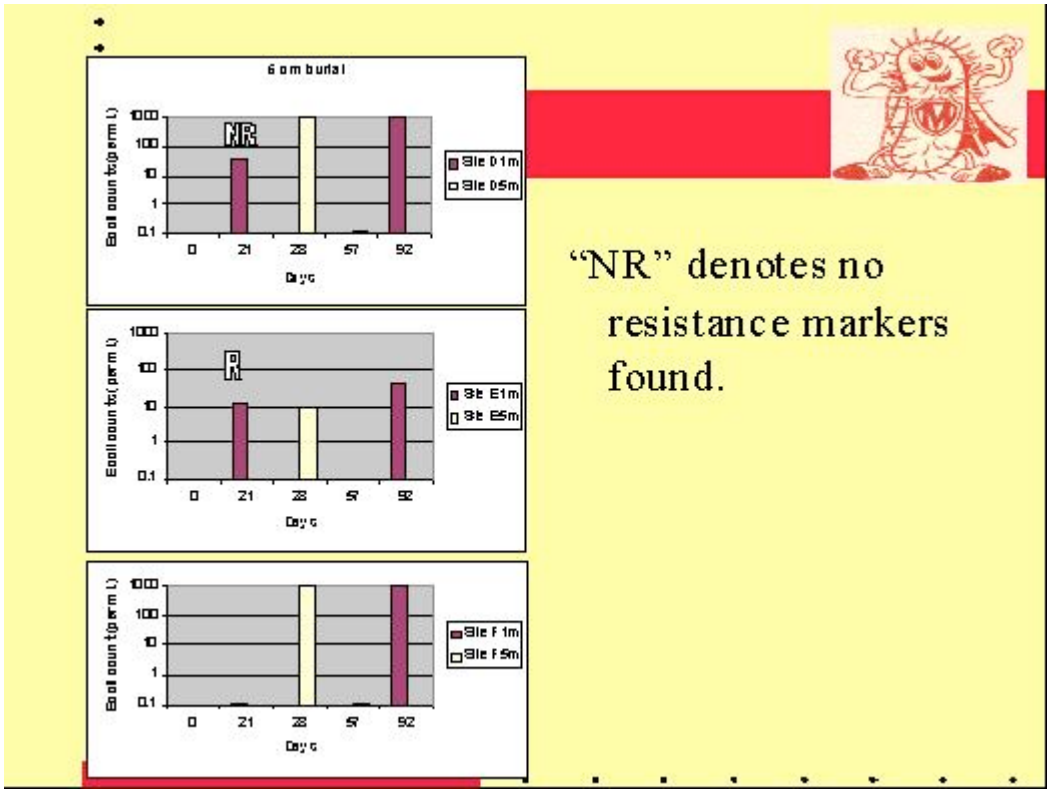
These seven volunteers were asked to donate as many stool specimens as possible. Although most of them complied as much as possible, there was not enough material for all treatment sites. In some experiments, fecal material without fecal markers had to be used. Fortunately base-line studies conducted on The Pond at the beginning of the project indicated exceedingly low fecal coliform counts from the indigenous fauna. Indigenous *E.coli* colonies averaged 5 per 100mL (0.05/mL) with no indication of any of the antibiotic resistances shown above.

Fig 14: Coliforms detected downstream from the three surface sites.



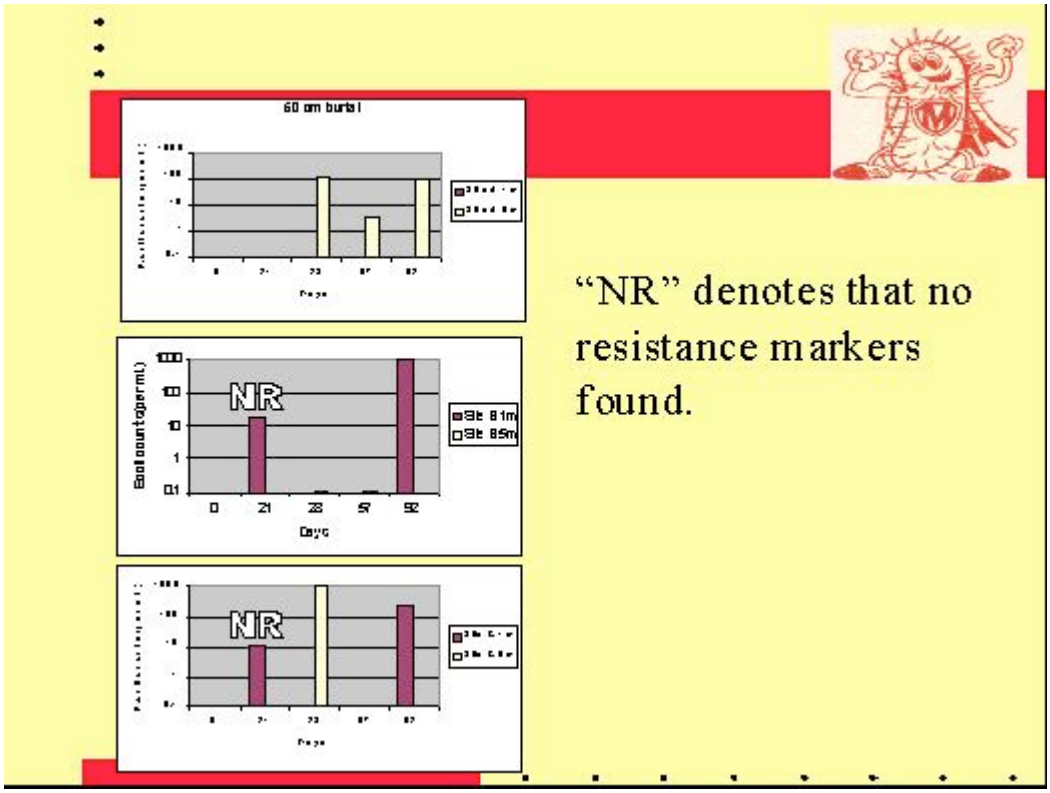
The large numbers of resistant coliforms detected 1m from the stool specimen could only mean that they originated from this stool specimen. Although such high levels of coliforms were detected 5m from the specimen, no coliforms were detected at any greater distance than 5m. Lysimeters were placed up to 15m from the specimen but never gave any positive coliforms.

Fig 15: Coliforms detected downstream from the three 5cm burial sites.



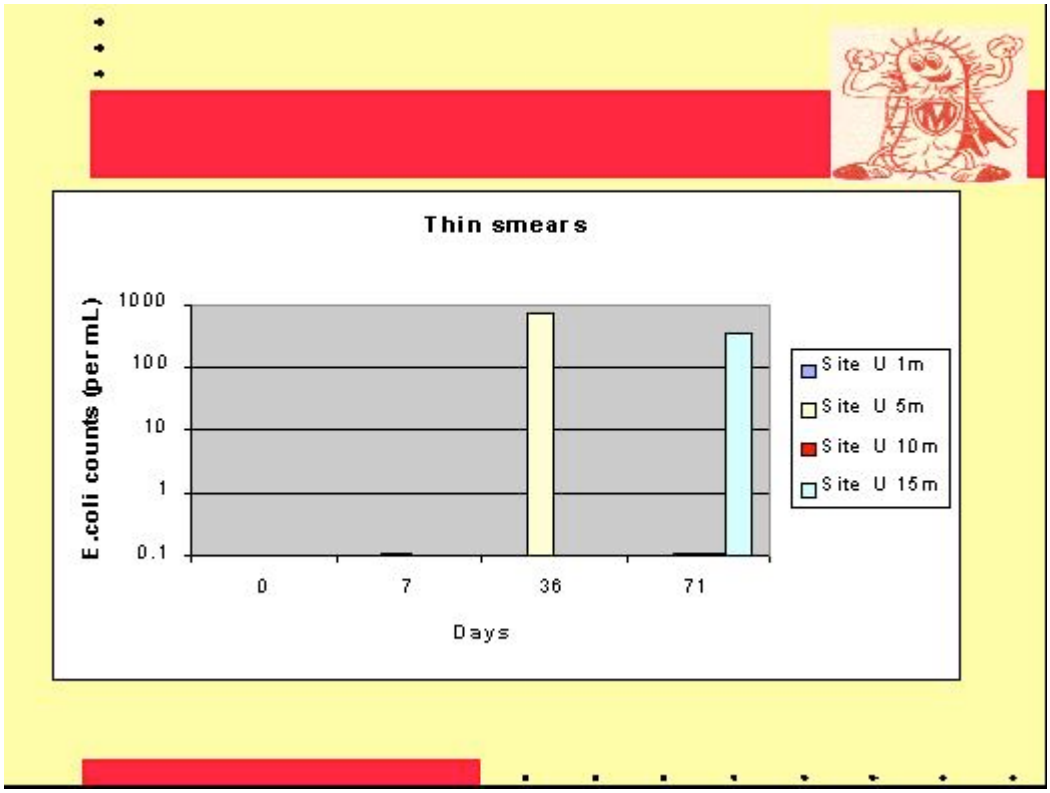
One site (site E) had resistant profiles showing that the coliforms must have originated from the human stool specimen, however site D, which had fecal markers, did not show any resistant profiles. This suggest that some of these coliform counts presumably came from indigenous animals. As the experiment progressed, scats became more and more evident suggesting that animals were visiting the site. Again, resistant profiles were never detected at large distances from the human stool specimen.

Fig 16: Coliforms detected downstream from the three 50 cm burial sites.



All the 50cm sites contained feces with marker profiles, yet no resistances were ever detected. The *E.coli* shown here must have originated from visiting animals.

Fig 17: Coliforms detected downstream from the thin smear sites.



Unfortunately there was insufficient marked stool specimens to use in the smearing trials. It is impossible to know whether these coliforms came from the smeared material or from visiting animals. Given the ease with which coliforms could be washed off smeared rocks sitting outside in an exposed situation, it is surprising that no coliform counts were ever detected close to the smeared rocks.

Fig 18: Coliforms in The Pond.

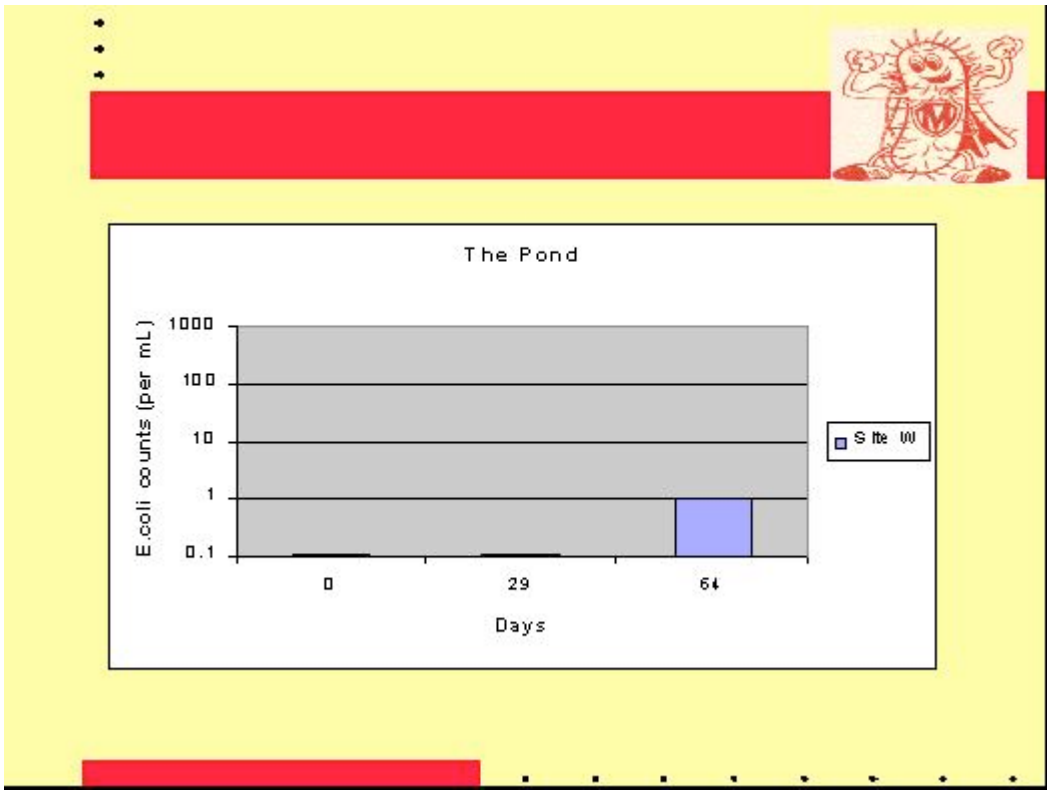


Fig. 3 indicates that all experimental sites were within 36m to 60m of the pond. Furthermore they were all above The Pond on drainage paths. No coliforms from the marked stool specimens were ever detected in The Pond and the 1 *E.coli* shown in the above figure, which was found on day 64, must have come from a visiting animal.

Conclusions:

Stool Degradation – Surface deposition, either as smeared material or as non-smeared stools left on the surface, was the fastest method of stool degradation compared to any depth of burial. Thin smears were the most effective way of disposing of human waste with almost complete disappearance of the feces after 70 days. Not only was burial ineffective when compared to surface deposition but because of the dense nature of the heath root structure, plus the exposed granitic bedrock, digging holes would be impractical for the recreational camper. The scientific team, even when equipped with shovel, pick-axes and shears, had great difficulty digging down to 5cm; the 50cm holes took the better part of an afternoon to locate a site with soil this deep. Surface deposition would be aesthetically acceptable for small numbers of campers provided that toilet paper was packed out. Taking toilet paper home is rapidly becoming the accepted wilderness standard.

Potential Spread of Fecal Pathogens – The availability of antibiotic markers on the *E.coli* in seven volunteers provided incontrovertible evidence that *E.coli* from human waste could wash-out into the surrounding environment. The surprising outcome from these experiments, given the huge numbers of marked *E.coli* present in a standard stool (in the order of 10^9), was the relatively low numbers of resistant *E.coli* sometimes detected close to the stool (i.e. within the first meter) and the fact that resistant *E.coli* were never detected at greater distances from the stool. This implies that fecal bacteria are not spreading away from the site of stool deposition and thus the public health threat is minimal. The fact that no resistant *E.coli* were ever detected in The Pond confirms the absence of long range transport. Recommendations from the literature to defecate at least 200 ft away from water bodies are erring on the safe side.

Bibliography:

1. Christensen, N.A. and Cole, D.N. 2000. Leave No Trace practices: Behaviours and preferences of wilderness visitors regarding use of cookstoves and camping away from lakes. USDA Forest Service Proceedings RMRS-P-15-VOL-4, 77-85.
2. Cilimburg, A., Monz, C., Kehoe, S. 2000. Wildland recreation and human waste: A review of problems, practices and concerns. Environmental Management Vol 25, No. 6, 587-598.