AN ABSTRACT OF THE THESIS OF

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Title: MOVEMENT OF LABELED ESCHERICHIA COLI THROUGH WESTERN OREGON HILLSLOPE SOILS UNDER CONDITIONS OF SATURATED FLOW

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Movement of microorganisms through two Oregon hillslope soil series was studied using strains of Escherichia coli labeled with antibiotic resistance. Field tests were undertaken to evaluate the potential use of these labeled organisms in monitoring bacterial translocation through the soil profile under conditions of saturated flow. Three horizontal injection lines at the depths of the A, B, and C horizons were installed in test sites located on the Dixonville and Hazelair soil series. Rows containing six sampling piezometers were installed downslope with the rows located at distances of 2.5, 5.0, 10.0, 15.0 and 20* meters from the injection lines. A row of six piezometers penetrated to a depth representative of the A, B, C_1,

* The 20 m row was absent at the Dixonville experimental site.

C_2, C_3 and C_4 horizons (the C_1, C_2, C_3, C_4 piezometers penetrated the "C" horizon to predetermined depths).

Field studies were undertaken to investigate the events which might occur when a septic-tank drainfield became submerged and fecal bacteria were subsequently released into the groundwater. The antibiotic-resistant bacteria were inoculated into the horizontal injection lines and their downslope movement monitored by withdrawing water samples from the piezometers at various time intervals. In comparative experiments, one antibiotic-resistant strain was injected in one horizon while a conventional fluorescein dye was injected simultaneously into a separate horizon. Organisms used were resistant to either 100 μg/ml naladixic acid, novobiocin or tetracycline. Pylam yellow fluorescein dye was used as a conventional tracer. Watertable data was collected prior to injection of the tracers and tensiometer observations were made throughout each sampling period.

Bacterial translocation was observed to be affected by soil moisture status, characteristics of the injection horizon and the type of C horizon contact in the soil profile. Distinct pulses of organisms were observed to move downslope, in the direction of subsurface flow, through the various soil horizons. Movement rates as rapid as fifteen meters in one hour were observed at the Dixonville site and all movement was observed under saturated flow conditions

created by irrigation. At the Dixonville site, tracer organisms were consistently recovered from an intermittent waterway located approximately twenty meters from the series of injection lines.

At recommended concentrations, fluorescein dye could be detected at the 10 meter line when tested with an Aminco-Bowman spectrophotoluorometer. No samples exhibited fluorescein concentrations that could be detected with the unaided eye. This investigation demonstrates the feasibility of employing antibiotic-resistant tracer bacteria under field conditions to monitor the movement of fecal contamination through soil and their value as one important criterion to evaluate the capacity of soil to adequately treat waste water being discharged from a septic tank-drainfield system.

Movement of Labeled *Escherichia coli* Through Western Oregon Hillslope Soils Under Conditions of Saturated Flow

by

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1. INTRODUCTION

The use of septic tank leach systems for on site treatment of domestic household waste is a widespread practice. Six million people in the United States depend on individual home sewage disposal systems (Palmer, 1974). In Oregon 37% of all housing units are served by septic tank leach systems according to the 1970 census. The interest in proper treatment and disposal of household waste stems from a demonstrated increase in the frequency of diseases transmitted by the fecal oral route (Craun and McCabe, 1973). It is suspected that this rise may be directly related to the increased use of septic tank leach systems coupled with individual and small community water systems (Palmer, 1974; Woodward et al., 1961). For these reasons it is very important that we develop an understanding of how and under what conditions pathogens move through the soil.

Movement of microorganisms through saturated profiles has been well documented. As early as 1937 movement of microorganisms was observed when experimenting with pit latrines. Movement was detected at a distance of 80 feet in 60 days (Caldwell, 1937). At that time movement under unsaturated conditions was also evaluated.

Movement of one foot laterally and vertically were documented. Recently more definitive research has indicated virtual lack of movement below certain moisture tensions. Investigations of the movement of Pseudomonas aeruginosa took place under various soil moisture tensions. It was concluded that bacteria rely on continuous water pathways for their movement. Even though certain pores may be filled in the soil they must be in excess of 1.5 μ or severe inhibition of movement will occur (Griffin and Quail, 1967). In summary, if soil moisture is far below field capacity movement will be in the range of a few centimeters.

Additional research has shown that in coarser textured soils the limits for movement may be at soil pressures which provide much greater pore size than 1.5 μ. Movements were observed to stop at pore sizes as great as 60 μ and therefore it was suggested that in these soils the limit for movement may be the availability of continuous water pathways.

Any study of microorganism movement in soil must utilize some techniques for determining the extent and direction of movement. In the past some studies of the effects of septic tanks on the environment dealt only with the chemical effects and left the pathogenic affects unexplored (Viraraghavan and Warnock, 1974; Polkowski and Boyle, 1970; Reneau and Pettry, 1975). Other researchers used increases in counts of indicator organisms to indicate the arrival of

contamination (Ziebell et al., 1974; Caldwell, 1937; Vaisman, 1964; Reneau et al., 1975). This technique has been challenged primarily on the basis of uncertainty about background counts and other possible sources of organisms. As a result, several researchers have used organisms with some special characteristic which allows that specific organism to be distinguished from all the background and natural interferring organisms in the soil. Ormerod (1964) used Serratia indica as a tracer to determine the mixing at an outfall along a Norwegian bay. That work followed the use of an antibiotic resistant Serratia indica for tracing sewage in sea water by Robson (1956). Rippon (1963) used an antibiotic resistant Serratia marcescens as a tracer in an estuary.

Recently researchers have began to use tracer organisms which have been tagged with antibiotic resistance. Wimpenny et al. (1972) used an antibiotic resistant strain of Serratia marcescens as a marker for river water in the heavily polluted Taft River. Recently antibiotic resistant strains of Escherichia coli and Streptococcus faecalis have been used to trace mevements of soil water in Willamette Valley terrace soils (Hagedorn et al., 1978). The investigators found that these organisms were most suitable as indicators of microbial movement and that the organisms survived over a sufficient length of time to be utilized satisfactorily as a tracer.

Current land use trends are toward saving the agricultural lands for food production. This trend has moved development pressures from the deep well-drained valley soils to the shallow soil rock mantles common to the marginal agricultural areas in the foothills of the coast and Cascade Mountain ranges. At present information regarding the movement of microorganisms in response to perched water tables commonly found in these shallow soils is very limited.

This thesis reports on the movement of antibiotic resistance labeled microorganisms through the soil rock mantle of two western Oregon hillslope soils. The study was intended to evaluate the movement of the organisms as it occurs under saturated flow conditions. Studies indicate that such conditions may exist for longer periods of time and more frequently than had been previously suspected in many northwest soils (Boersma et al., 1972). These studies simulate the events that would occur if a perched groundwater were to inundate the septic tank disposal trench and would duplicate the resultant discharge of organisms directly into the groundwater.

2. LITERATURE REVIEW

2.1 The Factors Affecting Movement of Microorganisms Through the Soil

2.1.1 Limits of Bacterial Velocities

Two possible sources of movement exist for microorganisms: the first is of microscopic scale such as flagellar movement while the second is the macroscopic translocation resulting from movement of the solution in which the organism is suspended.

When motile bacteria were observed in relation to varied soil moisture tensions, it was found that bacteria would move appreciably only when continuous water pathways existed. When sufficient pathways exist, movement on the order of 2 cm in 24 to 48 hours can be expected (Griffin and Quail, 1968; Thornton and Gangulee, 1926; Kellerman and Fawcett, 1907; Frazier and Fred, 1921). Therefore, when moisture tensions below field capacity are involved and continuous water pathways do not exist, no detectible movement will be observed. When continuous pores of adequate size are present, brownian motion and flagellar movement may account for distances of only a few centimeters.

Field work on soils containing adequate continuous water-filled pathways and sufficient hydraulic head provides some indication of

potential movement to be expected from microorganisms that travel with the solute. In a pit latrine located in a sandy aquifer, Escherichia coli was observed to move 40 ft. in less than 3.5 days. Eventually the organisms were detected as much as 80 feet from the pit latrine (Caldwell, 1937). The study indicated that at that distance the microorganisms were not simply present but rather represented gross pollution with recovery of 88 percent or more of the organisms injected. The author also stated that water samples had a foul stench associated with sewage. When Escherichia coli was injected into a pea gravel sand layer under saturated flow conditions, the organisms were observed to travel 13 feet in 15 minutes, 63 feet in 23 hours, and 100 feet in 24 hours (Kaufman and Orlob, 1956). From these reports it is evident that under saturated conditions the potential for movement is significantly greater than under conditions below field capacity.

2.1.2 Pore Size Limits to Movement

In recent years attempts have been made to relate certain physical aspects of the soil to the translocation of microorganisms. Griffin and Quail (1968) postulated the following regarding the movement of microorganisms in soil at low moisture tensions: bacteria will depend upon continuous water pathways for their movement to and upon solid substrates because they lack a hyphal system to bridge

air spaces; even though pores are water filled, bacteria will not be able to move appreciably if the relevant pore necks are too small. For rod shaped bacteria, a pore neck of radius 1-1.5μ is likely to restrict severely the rate of passage, whether by brownian or flagellar motion. Such a pore neck radius is equivalent to a suction of pF 3.0-3.2, and if the matrice suction exceeds this value, there will be no water-filled pores in the soil sufficiently large to permit easy passage of the microbial cells.

To permit appreciable movement of the bacteria there must be enough water-filled pores to provide a continuous pathway. It is not sufficient for there simply to be some water-filled pores of the requisite size. In turn, a continuous pathway will depend on the pore size distribution within the matrix.

Even though the pores of relevant size may be drained, movement of bacteria may be possible if lenses of water associated with the contact points of the soil particles are themselves in contact. Such movement is likely to be of limited extent as continuous water pathways of this type are likely to be short.

Griffin and Quail (1969) drew these conclusions after examining particulate systems which included one sandy clay loam soil and a series of aluminum oxide grits. A culture of Pseudomonas aeruginosa was placed on a 1 cm deep grit or in soil samples maintained at various pressures. After the desired time, samples were collected

at 0, .5, 1 and 2 cm from the innoculation point. This work caused Griffin and Quail to conclude that their postulates were correct.

Later investigators of pore neck size and soil suction as they affect microorganism movement reached slightly different conclusions. Hamdi (1971) evaluated the movement of Rhizobium trifolii in three soils, a Bungendore fine sand, a Spurwood coarse sand, and a silt loam soil. They found that a pF of 1.7 (50 cm H_2O) and a pore neck size of 60μ was limiting in coarse sand (1.25 percent moisture). In fine sand, movement was restricted at pF 2.4 (260 cm H_2O) which corresponds to a pore neck diameter of 11.5μ and a water content of 3.5 percent. In the silt loam, no movement was detected at pF 2.6 (pore neck diameter 6.8μ and moisture content 2 percent). Griffin and Quail (1968) found 1.5μ (pF 3.0-3.2) (18 percent water content) pore neck size to be limiting.

From this information Hamdi (1971) concluded that the limiting factor in microorganism movement is controlled by moisture content rather than reduced pore neck size. Research indicated that there was a loss of continuous water pathways long before the pore neck size became a restricting factor. It was pointed out that in finer textured soils with a predominance of fine pores the pore neck diameter may still be the limiting factor although the limiting size would more likely be related to the radius of gyration than to the diameter of the organism (Hamdi, 1971).

2.1.3 Darcy's Law and its Limits

Darcy (1856) determined experimentally that the volume flow of water per unit time (Q) through sands of constant cross-sectional area (A) was proportional to the hydraulic gradient. This relationship is called Darcy's Law and can be written as $\frac{Q}{A} = K \frac{\Delta H}{L}$ where Q/A is called the flux with the dimensions of velocity, $\Delta H/L$ is the hydraulic head drop over the distance along the axis of flow (hydraulic gradient) and K is a proportionality coefficient called Hydraulic conductivity with dimensions of velocity, depending on Fluid and medium properties.

It has been found that Darcy's law is applicable to most situations in porous media (Tolman, 1937; Meinzer, 1943; Muskrat, 1937). Hillel (1971) points out that as long as situations involve laminar and not turbulent flow, the flow will adhere to Darcy's law. According to the same author, the criteria to be used for detecting turbulent flow is the Reynolds number. (The Reynolds number is determined by the formula $R = \frac{d\pi p}{n}$ when π is the mean flow velocity (flux/porosity), d is the pore diameter, p and n the density and dynamic viscosity of the fluid, respectively.) Hillel (1971) states that flux in porous media is generally considered to be non-turbulent when $R < 1$. If turbulent flow occurs, the energy is partially lost in back currents and side flows, and the relationship between flux and

hydraulic gradient becomes non-linear. The author points out that this type of flow is possible in coarse sands and gravels with high hydraulic gradients.

Hillel (1971) states that flow of this type quite likely occurs in fissured bedrock. Sloping forest soils are also reported to have turbulent flow characteristics which are due to macroscopic root holes, soil cracks, and old root channels that conduct water even when the rest of the profile was less than saturated (Shipkey, 1969; Chamberlin, 1972; Cheng et al., 1975). Subsequent studies on one of Whipkeys' irrigated plots by Aubertin (1971) using fluorescein dye confirmed the presence of large biological structural voids. Finally, Jones (1971) reported that there was turbulent flow in water-worn pipes in Great Britain.

2.1.4 Effects of Macropore Theory

A considerable body of evidence has been put forth regarding the existance of large soil pores which may be responsible for the movement of massive quantities of water through the soil profile. It is appropriate to note that earlier work on these specific sites indicated that the macropores may play a major role in the movement of soil water and ions (Hammermeister, 1977). Hammermeister found that ions moved through a Dixonville soil series in western Oregon at a rate greater than 5.5 meters per hour. Evaluation of

several methods of mathematical analysis caused him to conclude that conventional predictive tools were not adequately explaining the observed results. His conclusion was that laminar flow through interconnected zones of high conductivity or saturated turbulent flow through macropores was responsible for the observed rates.

2.1.5 Permeability, Homogeneity and Isotropy

It is often found in field studies that soil permeability decreases with increasing depth from the surface (Coleman and Bodeman, 1944). In young residual soils this may be due to the decreasing degree of soil weathering with depth which results in little change in texture but an increase in bulk density. In more mature soils, this decrease in permeability may result from the formation of less permeable horizons. In addition to the mechanical stirring of the upper regions of the soil, cultivation may also cause compaction of the lower regions. Other properties of soil also change with depth, and this can result in some degree of layering or horizonation parallel to the soil surface. Unconsolidated, water-deposited sedimentary material sometimes found near the soil surface is layered, with the sequence of layers depending on the velocity of the water during the time of deposition. In laboratory studies and for the purposes of mathematical analysis, horizons are often idealized as homogenius isotropic layers. An

isotropic horizon is one which has identical transmission characteristics in all directions.

The effect on the movement of infiltrating water of a less permeable homogeneous isotropic layer beneath a more permeable layer was reported by Coleman and Bodeman (1944). They found that the infiltration of ponded water into initially dry soil columns resulted in the development of a positive hydrostatic pressure in the upper most permeable layer. They pointed out that if this sequence of layers occurred in a sloping field, lateral subsurface flow would take place within the upper layer when the rainfall rate exceeded the infiltration rate of the lower less permeable layer.

Maasland (1957) has proven that, for a layered porous media with the layers approximately parallel to the soil surface, the combined hydraulic conductivity normal to the layers is always less than the combined conductivity parallel to the layers. Thus, a layered profile as a whole can be considered to behave anisotropically.

Zaslavcki and Rodowski (1969) treated a layered profile as a homogeneous anisotropic profile and divided the flux into components normal and parallel to the layers. These are the two directions in an antisotropic media in which the driving force (hydraulic gradient) and the flux will coincide (Bear et al., 1968). Zasavski and Rodowski also showed the existance of a component of flux in the downslope direction of the resultant flux. Their analyses demonstrated that the greater the slope and degree of anisotropy, the closer the stream

lines approach a direction parallel to the surface. Further, plotting of stream lines on different topographies showed that stream lines will tend to diverge on convex portions of the landscape and converge on concave portions. When stream lines converge, saturated flow and seepage may result as is often found in the field. The authors emphasized that a lateral flow component will exist not only when the underlying layer is completely impermeable and saturated flow is occurring, but also when the underlying layer is only slightly less permeable, and even when light rainstorms do not cause saturated flow. In addition to concave situations, they point out that lateral flow may emerge on the surface when an impermeable barrier is in the way of lateral flow or when there is a transition to more restrictive soil along the rate of flow.

This indicates that saturated flow and therefore rapid transmission of microorganisms can occur in profiles with only slightly less permeable layers below it. Additionally the research would indicate that we are less likely to observe saturated flow in convex slopes and more likely to observe it in concave positions.

2.1.6 Saturated Flow in Soils

Weyman (1973) concluded from his work and that of others that vertical permeability breaks were necessary in order to have a substantial amount of saturated subsurface flow. He stated that since

vertical permeability breaks were widespread, then so was saturated flow. He also felt that the bulk of the water on the hillside moved away quickly under saturated flow conditions while a residual continued to move slowly away under unsaturated flow conditions.

Dunne and Black (1970a, 1970b) did not observe saturated flow even under very intense storm situations. They postulated that the thickness and the high permeability of the soil profile were responsible for the lack of observed saturated flow. They did observe perched water in concave slope positions where a less permeable layer was at 12-30 in (30-75 cm). There was also a report of overland flow under these conditions. Ragan (1968) indicated that perched water could be due to both rainfall and subsurface flow.

2.2 The Techniques for Evaluation of Microorganism Movement in the Soil

In the early 1900's research began tracing water as it moved through soil. Slichter (1905) worked with salt solutions in groundwater. He measured the resistivity changes in the groundwater and concluded that these represented the arrival of the ion front. In 1906 R. B. Dole published a paper entitled, "Use of Fluorescein in the Study of Underground Waters." From these papers came work in the 1950's which evaluated these techniques for use in soil water systems (Kaufman and Orlob, 1957). It is interesting to observe that the two

techniques which originated near the turn of the century are still the most common and widely used techniques today (Freedman, 1959; Viraraghavan and Warnock, 1974; Pruell and Schroepfer, 1968; Polkowski and Boyle, 1970; Reneau and Petty, 1976).

Very little work has been done on correlating the movement of tracers and microorganisms in the soil. One of the most extensive field studies was performed by Kaufman and Orlob (1957). The investigators compared the movement of coliform organisms, fluorescein, chloride, dextrose, and iodine-131 which had been injected through a 12-inch well into a confined aquifer 90 feet below the ground surface. They concluded that under the described circumstance none of the tracers were adequate to predict water movement.

A rather extensive paper by Kurtz and Melsted (1973) reviewed the uses of tracers and the most significant shortcomings of each. None were judged to be satisfactory for tracing the rate of soil water movement under field conditions. The techniques reviewed included the use of dyes, cations, deuterium, anions such as chloride, nitrate and sulfate. Of these techniques, the use of anions for tracing groundwater seemed to be the most reliable, although no comparisons were made to the rate of microorganism movement in relation to soil water conditions.

2.2.1 Ions as Tracers

It would seem that the most logical way to monitor soil water would be to mix some easily identifiable chemical with it. As Kurtz and Melsted (1973) pointed out, in a simple case the solvent would be moved at a uniform rate through a porous medium composed of uniform particles that are inert to both solvent and solute. The solvent and solute would move at the same rate through pores of uniform size that are completely filled. Under such conditions, the solvent and solute would be uniformly distributed, and the extent of the advance would equal the amount of pores filled which would be controlled by the volume of solvent added.

Such conditions rarely exist in the real soil situation. Soil particles are neither uniform in size nor completely inert to either water or dissolved salts. Furthermore, water, whether from rain or irrigation, is not added uniformly. As a result, a precisely predictable movement rate has not been defined. In some cases the rate of movement is ahead of that predicted while, at other times, it is behind (Kurtz and Melsted, 1973).

Cations are not considered to be good indicators of water movement because of cation exchange reactions that occur naturally in nearly all soils (Kurtz and Melsted, 1973; Miller and Reitemeier, 1963). Kurtz and Melsted point out that, when considering cations in

general, certain groups can be recognized as sharing characteristics. They stated that ions of the alkali metals--Group IA--and of the alkaline earth metals--Group IIA--undergo exchange reactions in soils and occupy much or all of the cation exchange capacity. Any cation added in a salt solution will equilibrate with those in the exchange system. For example, if KCl were added to the system, the K^+ would undergo exchange reactions with the other exchangeable reactions in the soil. As a result the leaching solution would soon be a mixture of chlorides of Ca^{++}, Mg^{++}, H^+, Na^+ and K^+. This mixture of ions would shift periodically as it moved laterally or vertically.

Kurtz and Melsted (1973) also point out that if the equivalents of salts in the leaching solution is assumed to be small in proportion to the exchange capacity of the soil, the K ions would be largely adsorbed in the surface horizon. Since calcium is the dominant exchangeable ion in agricultural soils, then the dominant salt in the leaching solution would soon be largely $CaCl_2$ rather than KCl. Thus the location of potassium ions in the soil would be a poor indicator of the path of water movement. Kaufman and Orlob (1957) concluded that cationic radiotracers were unsuitable largely due to the characteristics of the cations as discussed above.

Anions have received a great deal more attention as tracers in soil. Kaufman and Orlob evaluated the chloride ion along with several

other tracers and concluded that, even though the chloride ion did not reflect the true rate of water movement, it was relatively unaffected by the soil system and was the best tracer for water movement. Fairly good agreement was observed between the chloride ions and organisms in the group except that the heavy dosage of sodium chloride required to provide detectible concentrations of tracer at distant observation wells (500 feet) resulted in density separation of the tracer in the vicinity of the injection well and sporadic arrival of the tracer at points of observation.

Kurtz and Melsted (1973) stated that both chloride and nitrate ions are usually considered to move at the same rate as water and they reviewed a long list of publications that supported this contention. There are indications, however, that under irrigation, differing factors such as aggregation and rate of leaching do not affect the maximum depth of movement of the anions but do affect their distribution by depth. They further state that nitrates are sometimes subjected to denitrification as well as anion exchange. Anion exchange is thought to be most significant in southern U.S. and tropical soils and of less consequence in northern U.S. soils. The denitrification problem was also noted by Thomas (1970).

2.2.2 Dye Tracing Techniques

Fluorescent dyes have been used for many years to follow

surface and subterranean waters. Feuerstein and Selleck (1963) reviewed the use of these dyes in tracing surface waters where they evaluated three dyes: rhodamine B, Pontacyl Brilliant Pink B, and fluorescein. They concluded that even in surface waters it was essential that a fluorescent dye exhibit no adsorption or suspended sediments. However, both fluorescein and rhodamine B exhibited extensive adsorption and they concluded that these two dyes were unsuitable for work in any but the most pristine surface waters. Pontecyl Brilliant Pink B exhibited the most desirable characteristics and was judged suitable for use in surface waters.

It was also noted by these authors that Pontacyl Brilliant Pink B did exhibit optical quenching, i.e., further adsorption and scattering of fluorescence by suspended solids. It was therefore necessary to centrifuge samples before taking readings of the fluorescence. Furthermore, they reported that the dye exhibited a 50% chemical decay when moderately agitated in a light proof flask for 2 days. They suggested that in real systems this decay may be more significant than the well known photo decay common to fluorescent dyes.

The effects of chlorine on fluorescent dyes was explored by Deaner (1973). He stated that quenching of fluorescence is known to occur when the fluoresing material is mixed with chlorine. He concluded that at concentrations up to 9 mg/l quenching was not a serious consideration. Above that level, however, quenching was both a

predictable and significant occurrence. Chlorine in very high concentrations could be encountered in discharges from domestic washing facilities and could be a significant factor in the use of fluoresceint dyes.

Even though the many limitations of fluorescent dyes have been outlined, it is still common to see publications of their use in the soil-water system. Weyman (1973) reported a study that used a fluorescent dye known as "Pyranine Conc" to follow soil water for a distance of 6.5 m at a rate of 0.3 cm/min. Other reports of the use of fluorescent dyes are credited to Dole (1906), Kaufman and Orlob (1956), Reynolds (1966), and to Corey (1968). Fluorescent dyes are also commonly used in the public health profession for determining the functional status of soil absorption systems (Freedman, 1959).

Although fluorescent dyes have historically been the choice of most investigators using dye techniques (Kurtz and Melsted, 1973), there have been a group of non-fluorescent dyes which have received considerable attention. These dyes are commonly known in the dye industry as dispersed dye-stuffs which are formed as finely divided water insoluble "grains". The grains can be reconstituted by dispersion in water to yield particles predominantly 2 μm or less. These dyes are insoluble in water but can be readily extracted from the soil with hot acetone.

Corey (1968) described the testing of a series of dispersed dyes for use as soil-water tracers in studies for isolating nuclear waste in coarse grained materials. He concluded that the non-fluorescent dyes were not suitable for use in studies of movement through kaolinitic clay as they felt that the dye particles simply lodged on the surface of the soil particles.

Clarke and Vincent (1974) described the use of dispersed dyes in an investigation of rotational slips thought to be responsible for the formation of terracettes. They concluded that the dyes do move with the wetting front and suggested that their rate of movement is dependent upon moisture status in the soil.

No data could be found regarding the suitability of these dyes for use in predicting the movement of microorganisms through the soil. Comparison of their observed characteristics with those of microorganisms does yield some pertinent facts. The size of the dye particles and the size of microorganisms would seem to be approximately equal to 2.0 μ or less. Under saturated flow conditions, rates of movement limited by approximately the same pore neck size can be expected (Griffin and Quail, 1968). Under less than field capacity, the limits would quite likely be different. Hamdi (1971) suggests that at these lower moisture tensions, movement is limited by the radius of gyration of the microorganism. When using these dyes to simulate

movement of motile microbes, this would be significant since there would be no radius of gyration for an inanimate dye grain and there would also be differences in the frequence with which the dye particles lodged on the surface of soil micelles (Corey, 1968). Microorganisms which were motile would be expected to become lodged less frequently than dye particles due to their continuous motions, however random.

2.2.3 Radioactive Tracer Techniques

Several potential radioactive tracer ions exist (Kurtz and Melsted, 1963). One of the common ions is 131-I which was examined by Kaufman and Orlob (1956) as a potential tracer. When Iodine-131 was examined with other tracers such as dextrose, fluorescein, and coliform organisms, the researchers noted that in column studies Iodine-131 was observed to move more quickly than fluorescein, while in field trials it moved much slower. It was observed that increases in clay, silt and organic matter tended to reduce the peak Iodine-131 recovery and lowered the overall recovery. When tested along with fluorescein and chloride ions for movement rate through the soil columns, both I-131 and fluorescein dye exhibited chromotographic effects resulting in a separation in travel rates from the chlorine ions. In field tests the Iodine-131 tracer was observed to move much slower than coliform organisms, dextrose, or fluorescein.

Kurtz and Melsted (1973) discussed the use of deuterium hydroxide (DOH) as a tracer. They indicated that cation exchange effects on deuterium ions were neglegible so that this tracer would seemingly reflect water movement. No data is provided regarding the relationship between the movement of deuturium hydroxide and that of microorganisms.

Several investigators have noted the expense of utilizing radiotracers (Clarke and Vincent, 1974; Kaufman and Orlob, 1956). It is for this reason that radiotracer techniques are not more widely used. Any attempts to utilize this technique for monitoring microbiological movements in the soil would require considerable equipment and money. In addition to the expense of such investigations, there are the legal restraints placed upon the use of radioactive material by regulatory authorities. This technique, then, is limited to the well-financed and well-trained personnel found in the university or research laboratory setting.

2.3.4 Labeled Microorganism Studies

Antibiotic resistant Rhizobium were used for soil ecology studies as early as the 1950's. A review of their early use is provided by Schwinghamer (1967).

Organisms which are resistant to antibiotics have been used for tracing of surface waters in fresh and estuary environments.

Rippon (1963) used a strain of Serratia marcescens which was resistant to penicillin, aureomycin, actidione, and streptomycin. The culture was used to evaluate water movements in an estuary while the purpose of the antibiotic was to suppress the growth of indigenous marine organisms.

Wimpenny et al., (1972) used an antibiotic resistant Serratia marcescens to evaluate several different microorganism tracers for use in surface waters. This research indicated that a considerable drop in recovery rates on the antibiotic media occurred but the organism was judged as satisfying most criteria as a water tracer. The author suggested that the dye-off may have been due to the fact that antibiotic was not included in the media when the original cultures were grown. Consequently antibiotic sensitive organisms may have been dominant in the original culture.

Hagedorn et al., 1978, used antibiotic resistant Escherichia coli and Streptococcus faecalis as tracers in saturated soil. Both organisms were resistant to streptomycin. The investigators concluded that the organisms remained viable for at least 32 days and moved up to 1,500 cm. Speculation is offered that the flow of groundwater may have missed a 3,000 cm well. From studies by Caldwell (1937) that would seem very reasonable given the extreme directionality of organism movement observed by that investigator. Hagedorn

further concluded that this technique was suitable for use as a predictive tool in determining setbacks or buffer zones between drainfields and other features such as streams, wells, and property lines.

3. DESCRIPTION OF EXPERIMENTAL SITES AND EXPERIMENTAL DESIGN

3.1 General Description of Study Area

3.1.1 Geography and Geology of Area

The Willamette Valley is located in Western Oregon approximately 65 km inland from the Pacific Ocean. It is bordered by the Coast Range Mountains on the west and Cascade Mountains on the east. It extends from the Columbia River southward approximately 200 km to where the two mountain ranges converge near the town of Cottage Grove. The northward flowing Willamette River with its many tributaries is the major drainage system.

The study sites discussed are located in the foothills (120-150 m above sea level) on the western border of the southern portion of the Willamette Valley, west of the City of Corvallis. The soils in this area are underlain by a variety of sedimentary and volcanic rocks of Eocene age. These rocks are eastward extensions of Coast Range formations that presumably extend beneath the valley almost to the margin of the Cascade Range. The geology in this area is quite complex, the various geological formations were described by Baldwin (1976).

3.1.2 Soils and Vegetation of Area

Soils derived from igneous and sedimentary rocks situated along the edges of the valley on low hills are mainly Haplohumults with some Haploxerolls, Argixerolls, and Haploxeralfs. Soils developed in sandstone exhibit a wide range of characteristics ranging from shallow brown stoney loams, to deep reddish silty clay loams showing some clay accumulation. Soils derived from siltstone or shale parent materials are similar to those derived from sandstone, but are generally finer textured. Typically they have a silt loam surface horizon with a silty clay or clay textured B horizon.

Most of the soils developed in basalt in this area are deep, relatively stone free, and have well-developed profiles. Their surface textures are generally clay loam or silty clay loam with clay subsoil. In some locations the soil derived from basalt tends to be fairly shallow and stoney.

In the areas that are not cultivated, the vegetation on these upland soils consists of stands of Douglas-fir at higher elevations and Oregon white oak at lower elevations both interspersed with openings containing poison oak, wild rose, blackberry, brackenfern, and a variety of grasses.

3.1.3 Climate of this Area

Average yearly precipitation is about 100 cm (Knezevich, 1975). Approximately 70 percent of the annual precipitation falls from November through March, and less than 5 percent falls from June through August. Nearly all the precipitation falls as rain and the summers are moderately warm and dry and the winters cool and wet.

3.2 Location and Brief Description of Soil-Rock Mantle at Each Experimental Site

Table 3.1* gives a brief summary of the location, soils, underlying rock, vegetation, topography, and physiographic position of the experimental sites in this study. A detailed description of the soil profile and upper regions of weathered rock taken from a pit adjacent to each experimental site is given in Appendix 1. A summary description of the soil and rock in each of these pits is given below. In addition, the variation in depth to lithic or paralithic regions of rock across the actual area of each experimental site was determined by Hammermeister, 1977.

Hammermeister determined these measurements by carefully augering down to these materials at points next to porous cup sampling devices (C horizon, 110 cm, and 150 cm depths only) located in the 2.5 and 5.0 m lines. No augering took place adjacent to any devices

* All tables are found in Appendix 2.

used to collect samples for this study. The location of these devices at each site is given in Figure 3.1.* All holes were backfilled with bentonite clay.

3.3 Location and Brief Description of Field Equipment Used to Study the Movement of Antibiotic Resistant *Escherichia coli* at the Experimental Sites

The experimental layout of field equipment designed to study the movement of antibiotic-resistant bacteria was basically the same at each experimental site. Figure 3.1 shows the location of line sources, piezometers, and tensiometers. Each component is discussed more fully in section 4.1.3.1 to 4.1.3.3.

The three tracer organisms which were used in this study were all strains of Escherichia coli. Each organism was separately resistant to either Novobiocin, Tetracycline, or Naladixic Acid. No organism was resistant to more than one of the antibiotics. The bacteria were introduced into the soil horizons through injection lines. After injection of the various strains into a particular line source, ground water samples were collected periodically downslope from piezometers located at distances of 2.5, 5.0, 10, 15, and 20 m downslope. Piezometers were modified by the addition of a sample extraction device as outlined in section 4.1.3.2 and Figure 4.2.

Groundwater samples were collected from groundwater sampling devices in sample line 1 (Figure 3.1) located upslope from the

*All figures are found in Appendix 3.

injection lines. These samples provided background or control levels of antibiotic resistant microorganisms in the groundwater during the experiments.

Tensiometers and piezometers were used to measure water pressure potentials over the period of the experiment. Table 3.2 gives the depths of all instruments and injection lines in the upper horizons. The lower 110, 150 and 200 cm depths were the same for all sites (see Figure 3.1). The depth of the instruments and injection lines in the upper horizons varied with the morphology of the soil at each site.

4. MATERIALS AND METHODS

4.1 Materials

4.1.1 Introduction

Movement of microorganisms through the soil was measured by introducing antibiotic resistant organisms into the soil profiles through injection lines. These line sources were located in the A, B and C horizons of two soils in the foothills along the western edge of the Willamette Valley. The organisms were subsequently recovered from piezometer located at various distances and depths down hill from the injection lines.

4.1.2 Soil Descriptions

The following soil descriptions were made from soil pits prepared adjacent to the experimental sites described below.

The Hazelair experimental site is located on the lower footslope of a hillside immediately north of the OSU Turkey farm on the west edge of Corvallis, Oregon. The upper layer of soil at this site is dark brown silt loam and silty clay loam to a depth of 19 cm. The B and C horizons are grayish brown and dark yellowish brown clay extending to a depth of at least 200 cm from the surface. The structure of the B and C horizons is massive 32 cm from the surface and

downward. The depth of this massive substratum from the surface varies from 30 to 70 cm. The soil is classified as a very fine, mixed mesic, Aquultic Haploxeroll.

The Dixonville experimental site was located on the lower backslope of a hill located near the old OSU sheep barns just Northwest of Witham Hill in Northwest Corvallis. The upper layer of soil at this site is dark brown silty clay loam and is approximately 34 cm in thickness. The subsoil is dark brown silty clay and extends to a depth of 56 cm from the surface. This is underlain by reddish brown and yellow saprolite 10 cm thick which grades into sandstone. The thickness of the saprolite substratum varies from a few cm of granular saprolite to 50 cm of clayey saprolite over the area of the site. The depth to sandstone varies from 40 to 110 cm over the experimental site. This soil is classified as a fine, mixed mesic, pachic Ultic Argixeroll.

4.1.3 Experimental Design

4.1.3.1 Injection Lines

The injection lines were constructed of 1.25 (1/2 inch) 315 p.s.i. polyvinyl chloride (PVC) pipe and encased in a nylon sleeve sewn to nearly the same outside diameter as the PVC pipe. One-eighth inch diameter outlet holes were drilled every 5 cm through 3.05 and

6.10 meter lengths of the PVC pipe prior to encasement with the sleeve. In the field a 3.05 and a 6.10 meter section were cemented together to form a 9.15 meter perforated unit. Shorter unperforated pieces of PVC were then cemented into the joint at right angles to the above lengths. Six "T" joints were added to facilitate uniform distribution of the microorganisms along the line source. The ends of the units were stoppered with rubber bungs (Figure 4.1).

The assembled injection lines were placed in trenches dug on contour of the hillside. The depth of these trenches are shown in Table 3.2. The trenches were then backfilled and covered with original soil material.

4.1.3.2 Piezometers

Piezometers which measure positive water pressures were modified from a design used by Yee (1975). The depths and location of piezometers at each site are shown in Figure 3.1 and Table 3.2. A 1.88 cm (3/4 inch) 200 p. s. i PVC pipe was cut into lengths at least 20 cm greater than the depths the piezometers were to reach into the soil rock mantle.

With exception of piezometers installed in A horizons, the last 10 cm of each length was perforated with six sets of 1/8 inch holes 90^{o} apart and spaced every 2 cm. Only the last 4 cm of the A horizon piezometers were perforated with three sets of holes 90^{o}

apart. Nylon window screen was taped in place over the holes in each tube to prevent the entry of surrounding filter sand. Each piezometer was sealed at the bottom with either a cap or a rubber bung. A removable vent cap was placed on top to prevent entry of rain (Figure 4.2).

The height of water within the piezometer was measured by observing a styrofoam float inside a .63 cm (1/4 inch) i.d. acrylic tube. As the water would rise it would carry the float upward where surface tension would then bind the float to the side of the tube marking the highest point (Figure 4.2).

A power auger (Haynes Manufacturing Co.) was used to drill holes for the piezometers. The auger bit used yielded holes approximately 4 cm in diameter. Each hole was then backfilled around the piezometer with approximately 500 ml of E.I. #8 sand to surround the bottom 20 cm of each piezometer. The "A" horizon piezometers had proportionately less sand. Fifty ml of dried soil was then added above the sand. This was followed by 50 ml of Bentonite clay. The Bentonite clay sealed the piezometer from the upper soil horizons. Succeeding layers of soil and Bentonite were added to backfill the hole to the surface.

Samples were extracted through the piezometers by adding a .63 cm (1/4 inch) o.d. glass nipple to the top of the acrylic tube and allowing it to extend 2 cm above the plastic cap. In addition each

piezometer had a tygon tube 25 cm long attached. By attaching a vacuum line to a sample bottle and then attaching the bottle to the piezometer tubing, a sample could be easily extracted (see Figure 4.2).

4.1.3.3 Tensiometers

Tensiometers after a design by Harr (1977) were used to monitor negative water pressures at each site (Figure 4.3). Tensiometers were located at the 2.5 m and 5.0 m distance only (Figure 3.1). All tensiometers were tested for air leaks by simulating their field operation in the lab prior to installation. All subsequent testing took place with the tensiometers installed in the field. Methylene bromide was used as a monometer fluid (Harr et al., 1976). Tensiometers were installed in each horizon at both 2.5 m and 5 m lines (Table 3.2 and Figure 3.1).

Installation of tensiometers included the boring of a 4 cm hole with a power auger, the mixing of soil from on site with water into a slurry in the bottom of the hole and then sealing them using the same procedure as described for piezometers.

4.2 Methods

4.2.1 Antibiotic Resistant Organisms

Antibiotic resistant Escherichia coli were selected from samples

of sewage treatment plant influent. Ten milliliter volumes of effluent were innoculated into 150 ml of Difco E.C. media with 100 μg/ml of the desired antibiotic present. Cultures were then incubated at 37° C on a rotary shaker until turbidity indicated growth. The cultures were then isolated on Difco Bacto Eosin Methylene Blue Agar (EMB) and a typical colony transferred to a slant of tryptic soy agar (TSA). These slants were grown at 37° C for 24 hours and transferred to 4° C for storage. Cultures were transferred to new slants on approximate 30 day intervals. Organisms that were resistant to Novobiosin, Tetracycline and Naladixic acid at a concentration of 100 μg/ml were developed. All organisms were tested to assure that there was no cross resistance and were then shocked to assure maintainance of antibiotic resistant characteristics.

Preparation of these organisms for introduction into the injection lines described above was accomplished by aseptic transfer of a loop full of the stock culture into 150 ml of Difco E.C. media with 100 μg/ml antibiotic present. The culture was then incubated for 18-24 hours and transferred to 1.5 l of E.C. media in a fernbach flask. The culture was again incubated at 37° C for 18 hours. Two such flasks were prepared for each organism to be injected. The two flasks were transferred to a 9 l carboy and brought to a 4 l volume with distilled water. The organisms were transferred to the site and injected within one hour.

4.2.2 Injection and Recovery of Tracer Organisms

Injection of the organisms into the experimental site was accomplished by elevating the 9 l carboy 1 m and siphoning the organisms through seven sections of .63 cm (1/4 inch) i.d. tygon tubing into the injection ports located along the length of the injection line. Once the organisms were injected into the groundwater table, periodic groundwater samples were extracted through the piezometers. Samples were collected in 130 ml (4 oz) sample bottles (Figure 4.2) by applying a vacuum. The vacuum hose was rinsed in 70% ethanol between samples to prevent possible cross contamination of samples. The water samples were then transported to the laboratory and tested immediately or stored for no more than 15 hours at 4° C.

Detection of the organisms was accomplished by serial dilution of a 1 ml portion of each water sample innoculated into 9.0 ml of Difco E. C. media with Bromothymol Blue and 100 μg/ml of the desired antibiotic added. The tube was mixed using a vortex mixer and 1.0 ml was then pipeted into the next tube. This pattern was followed until a series of 5 serialy diluted tubes were prepared. The number of organisms present was estimated by observing the number of serialy diluted tubes which demonstrated growth after incubation at 37° C for 24 hours.

The Difco media for the 9.0 ml tubes was prepared in 1.5 l volumes with Bromothymol Blue indicator added. The media was then autoclaved at 15 pounds pressure and 250° C for twenty minutes. It was then cooled to room temperature and antibiotic was then aseptically added to a concentration of 100 μg/ml. Media was then aseptically pipeted into pre-kap-uted and autoclaved 16 x 150 mm tubes using a Brewer Automatic pipeting machine model 40.

4.2.3 Fluorescein Dye Preparation

Pula-Tel Fluorescent yellow dye was mixed with distilled water at the rate of .959 g/4 l. This dilution rate was intended to simulate the introduction of 1 pound of dye into a septic system utilizing a 1,000 gallon septic tank. A second repetition was made using 1.918 g/4 l or the equivalent of two pounds of dye introduced into the same septic system. Dye was mixed in the laboratory and injected within 8 hours. All dyes and dye solutions were stored in dark containers to prevent photochemical decay. Dye solutions were transported to the experimental sites in glass carboys and injected in the same manner as the tracer bacteria. All results reported in this paper are those from the introduction of1.918 g/4l (2 lb/system) dye solutions.

5. RESULTS

The results of the experimental procedures outlined above are shown in Figures 5.10 to 5.58. These three dimensional graphs show the depth at which organisms were recovered on the X axis, the number of organisms that were recovered on the Y axis and the distance down the slope from the point of injection on the Z axis. The presence of a circle at the intersection of a downslope distance line and a depth in the soil profile line indicates that a sample was collected. The height from that point of intersection represents the number of organisms recovered. If the circle is directly on the intersecting line then no organisms were detected even though a water sample was collected. Intersecting lines without circles indicate that no sample was taken. Accordingly samples taken at time "0" (Figure 5.10) show all the circles directly on the points of intersection indicating the absence of the tracer organisms at all sample points.

Piezometric readings were taken only at the beginning of each twelve hour observation period to prevent cross contamination of sampling equipment. Statistical analysis of soil suctions as determined by tensiometric readings indicated that there was no significant change in soil moisture during the course of any of the observation periods at any given site (Table 5.1). This would indicate that the soil moisture remained constant throughout each of the experiments.

5.1 Soil Moisture at the Dixonville Site*

Analysis of piezometric and tensiometric data indicated the presence of a perched water table at this site. The average observed piezometric surface was 25 cm from the soil surface (Figure 5.10). Tensiometric readings indicate the presence of a restrictive layer below 110 cm and above 150 cm from the soil surface (Table 5.2). Piezometric and tensiometric measurements at the 12 cm depth indicated that the soil profile at this point was not saturated. A single exception to this statement is found in the 12 cm piezometer location at 15 m distance (Figure 5.12-5.18). The depth to the restrictive soil horizon responsible for the perching of water decreased at this point causing the piezometric surface of the perched water table to reach within 12 cm of the soils surface. Measurements made at the 45, 80, and 110 depths indicated saturated or near saturated soil conditions throughout the study. Higher soil water suctions at the 150 cm depth indicated that a restrictive layer existed at or slightly above that point in the soil profile (Table 5.2).

5.2 Inoculation of Tracer Bacteria at the Dixonville Site

Analysis of samples taken from the control line located uphill from the injection lines at this site did not give a positive test

* Due to similarities in results observed in the injection of organisms into A, B and C horizons, the figures cited are those representative of the observations in the "A" horizon.

(Figures 5.10-5.18). Also, analysis of soil solutions taken from the piezometers located downslope from the injection lines before injection of the tracer organisms indicated that no resistant organisms were present. Samples taken after the second and third repetitions of the experiment indicated a small carryover; however, amounts were less than ten organisms per milliliter. These amounts were used as background levels and increases above these levels were taken as positive movement.

Following injection of tracer organisms at the Dixonville site in the A horizon (12 cm depth), the organisms were found at the 15 m distance and at the 45 cm depth after one hour (Figure 5.11). They were found at a distance of 15 m and at depths of 80 and 110 cm after two hours (Figure 5.12). The depth to the restrictive layer decreased with distance down the slope. Consequently the 150 and 200 cm depths did not produce samples at the 15 m distance because they were below the restrictive layer. Examination of these same depths at the 10 m distance (Figures 5.10-5.16) indicate that water samples were collected but that they did not contain the tracer organisms.

The highest numbers of organisms were observed to be moving in the 45, 80 and 110 cm depths (Figures 5.12 and 5.13) with a distinct peak observed at the 200 cm depth at the 2.5 m distance (Figure 5.11-5.18). This peak is felt to be evidence of macropore movement and is evaluated in the discussion section. Over all evaluation indicated

that the numbers of organisms decreased significantly below 150 cm (Figures 5.12-5.16).

When tracer organisms were injected into the "B" horizon at the 45 cm depth, movement to a distance of 15 m was observed at the 12, 45, 80 and 110 cm depths within one hour (Figure 5.21). Movement characteristics of the organisms were similar to those observed when the organisms were injected into the "A" horizon at the 12 cm depth. The organisms were again observed to appear at the 12 cm depth at 15 m distance while the majority of movement was observed to occur at the 45, 80 and 110 cm depths (Figure 5.21-5.28). The observation of organisms at the 80 cm depth after only one hour is in contrast to "A" horizon injections. Where the organisms were not observed until two hours had passed. Counts of the tracer organisms were observed to reach a maximum (or plateau) within three hours and maintain that level throughout the remainder of the twelve hour sampling period (Figure 5.23).

Injection of microorganisms into the top of the "C" horizon at 80 cm resulted in somewhat similar movement patterns and rates as observed for injections in the "A" (12 cm) and "B" (45 cm) horizons (Figure 5.30-5.38). Organisms were observed to move 15 m in one hour and were recovered at the 45 and 110 cm depths (Figure 5.31). The bacteria were not recovered from the 12 cm depth except on the six hour sample (Figure 5.36) and somewhat lower peak numbers of

organisms were observed at the 2.5 and 5.0 m distances (Figures 5.31-5.38). Additionally, the numbers of organisms recovered began to decrease after the fourth hour following sampling (Figures 5.34-5.38).

Regardless of the point of injection, the tracer organisms moved in similar horizons and apparently at much the same rates. The effects of the rock layer which was at 70-150 cm in restricting bacterial movement at this site was observed in all results. Injection of organisms into the 80 cm "C" horizon depth resulted in slightly lower numbers of organisms being recovered at all distances and depths.

5.3 Soil Moisture at the Hazelair Site

Tensiometric and piezometric data indicate the existance of a restrictive layer located between 80 and 110 cm from the soil surface. The existance of low hydraulic conductivity rates further substantiates this observation (Table 5.3). This depth corresponds with the contact between the IIB2 and IIB3 horizon. The soil remained saturated above this level creating a perched water table with a piezometric surface at 9 cm. The 12 cm ("A" horizon) depth remained saturated throughout the study. The existance of a perched rather than a regional water table was further substantiated by the absence of water in piezometers at the 110, 150 and 200 cm depth (Figures

5.40-5.48) and by high moisture tensions at depths below 80 cm. In some cases the tensions at 150 cm exceeded the capability of the measuring device (455 cm H_2O).

5.4 Inoculation of Tracer Bacteria at the Hazelair Site

Samples collected from the control lines and down slope piezometers prior to addition of tracer organisms to the site indicated that antibiotic-resistant organisms were not detectable in the natural soil water solution. Subsequent to the initial injection of these organisms into the site they were observed in low but detectable numbers in the upslope control lines. For example, during the July 18 experimental run, the 12 cm ("A" horizon) piezometer in the control line was positive when sampled at twelve and twenty-four hours (Figures 5.41 and 5.42). The forty-eight hour sample was positive again and the piezometer continued to produce positive samples throughout the remainder of the ninety-six hour experimental period (Figures 5.46-5.48). Tracer organisms were also recovered from the 30 cm control line during the forty-eight, sixty and eighty-four hour sample periods (Figures 5.44, 5.45 and 5.47). This pattern was not observed during the 15 June experimental run. Residual organisms could be detected in down slope piezometers for up to four weeks from their date of injection. These background counts were taken into account when determining the number of

organisms recovered during the second repetition. The residual organisms were not present in numbers greater than 10 organisms per mil.

When tracer bacteria were introduced into the "A" horizon of the Hazelair site (12 cm), they moved to the farthest sampling distance of 20 m in less than 12 hours and were recovered at the 12 and 30 cm sampling depths. The organisms were observed to move only 5 m at the 80 cm depth in the same twelve hour period. At the 110 cm depth the organisms moved only 2.5 m in twelve hours (Figure 5.41). The number of organisms recovered at each distance and each depth in the Hazelair site was lower than at the Dixonville site.

Injection of tracer bacteria into the 30 cm B horizon at the Hazelair site produced results which were significantly different from those observed in the A horizon (12 cm) injections. The rate of movement was significantly reduced in that the organisms were observed to move only 5 m in 12 hours (Figure 5.51), 10 m in 24 hours (Figure 5.52) and were recovered at 20 m distance from the 30 cm depth after 72 hours (Figure 5.56). No organisms were recovered from the twelve cm depth after the seventy-two hour sample (Figures 5.56 to 5.58).

The highest number of organisms were observed to be moving at the twelve and thirty cm depths. Significantly fewer organisms

were observed to be moving at much slower speeds at the eighty cm depth. Organisms moved 2.5 m at a depth of 110 cm when injected into the A horizon (12 cm) (Figure 5.41), no movement was observed at the 110 cm depth when the organisms were injected into the B horizon (30 cm depth) (Figures 5.50-5.58).

In summary, the injection of tracer microorganisms into the Hazelair site resulted in the observation of organisms moving at much shallower levels in the soil than were observed at the Dixonville site. The total number of organisms recovered at each depth and distance was significantly lower in the Hazelair series and the rate of movement was less.

5.5 Experimental *in situ* Measurement of Fluorescein Tracer Dyes at the Dixonville Site

The addition of fluorescein dye into the A, B, and C horizons of the Dixonville site did not produce any water samples at any distance or depth which contained visible fluorescence when tested with a spectrofluorimeter. When the dye that was injected into the A horizon (12 cm) could be detected at a depth of 80 cm at the 2.5 m distance after two hours (6×10^{-5} μg/ml) (Appendix 3a). Injection of dye into the B horizon produced detectible increases at the 45 cm depth at 2.5 m distance after 3 hours, Appendix 3b. Evidence of an additional peak appeared to be present at the 10 m distance, 80 cm depth. When

dye was injected into the C horizon (80 cm depth) gradual increases occurred at the 45 and 110 cm depth in the 2.5 m line from the second hour on throughout the end of the sampling period. A slight increase was also observed in the 80 cm well in the 10 m line (Appendix 3c).

6. DISCUSSION

Water tables in all horizons remained nearly constant over time. Minor fluctuations of water tables were observed in the A horizon (12 cm) of the Dixonville site and were quite likely due to slight changes in the irrigation rate. The site was irrigated from a domestic well which also served a residence and utilization of water in the residence undoubtedly altered the amount of water available for irrigation. If irrigation rate were to change the most significant alteration in gravametric water would occur in the upper horizons.

High soil water tensions were observed in the lower horizons of both sites due to the presence of a restrictive soil layer. This soil layer reduced vertical infiltration of water and created a perched water table.

6.1 Soil Moisture Tensions at the Dixonville Site

The 12 cm depth continually showed an unsaturated condition with the exception of the sample point located 15 m from the injection line (Table 5.2). At this point the layer of consolidated parent material beneath the soil profile came to within 40 cm of the surface and caused the zone of saturation to rise at that point.

Observations of the soil water pressure at the 45 cm depth in

this profile shows a saturated condition throughout the study (Table 5.2). The pressures observed piezometrically agree closely with tensiometric measurements of soil water tensions made at the 12 and 45 cm depths while piezometric and tensiometric tensions below 45 cm indicated gradual increasing soil suctions. Evaluation of the soil profile as described in Appendix 1 indicated the presence of a large number of rodent holes some of which approached 10 cm in diameter above a clay or granular saprolite layer at a depth of 50 to 100 cm. This suggested that the progressive increase in soil suction with depth could be due to the soil being effectively underdrained by these large pores. In other words, at the same time the upper layers of soil allow infiltration of water into the profile the lower zones of higher conductivity are allowing the water to exit at an even greater rate. The result is an observed increase in soil suction with increasing depth. Increased soil water suction at the 150 cm depth could be caused by the presence of restrictive soil or rock layers above that point. Considerable variation was observed in the depth to the saprolite and rock layers across this site.

Data at these sites for the winter and spring of 1976 indicated that an average water table of 38.7 cm existed from natural rainfall (Hammermeister, 1977). The average depth for the irrigated water table was 25 cm for the spring and summer period covered by this

study. The water tables observed during storm cycles in 1976 research frequently exceeded the average observed under irrigation.

6.2 Injection of Tracer Organisms into the Dixonville Site

The continued absence of positive samples in the control lines at this site indicates the very low number of organisms present in this soil water system which could cause a false positive reading. Background counts of these organism in the soil water system was non-existant and we can therefore conclude that this technique is very sensitive in this soil water system.

During the latter repetitions of the experiment, residual organisms were observed in some of the piezometers. Detecting residual numbers of organisms after periods of twenty days would tend to substantiate previous findings regarding the stability of these organisms (Hagedorn et al., 1978). The residual organisms would also indicate that repeated use of these antibiotic resistant organisms as a tracer at a single site should be approached with caution.

When antibiotic resistance labeled E. coli were introduced into the 12 cm depth at the Dixonville site, the organisms moved down to 45 cm in the profile and then traveled downslope at rates as high as 15 m/hr (Figure 5.11). Hammermeister (1977) observed ion movement rates which were > 5.5 m in 2 hr. at the 45 cm depth and > 5.5

m in 1 hr. at the 110 cm depth. The reduction in soil profile depth with distance downslope at the Dixonville site created a situation where the water at the 15 m distance was raised to a shallower point in relation to the ground surface. This was due to reduction in depth to the rock-saprolite layer. Evaluation of the distance to the rock layer at the 15 m distance indicated that the depth to the restrictive rock layer varied from 40 to 90 cm from the surface. This would place the large pores in the 34-56 cm range and in the same general level as the 45 cm piezometer.

Hammermeister (1977) observed ions moving > 5.5 m in one hour at the 110 cm depth. The shortest time interval used by Hammermeister was one hour. The ions were observed to have moved past the furthest sampling point (5.5 m) within the first 1 hour sample period. It remains a possibility that the ions traced on this site moved at the same rate as microorganisms but that the movement was not detected due to the 1 hour time intervals and short sampling distance.

Very rapid movement of high numbers of organisms was observed at the 200 cm depth 2.5 m from the point of injection. This particular piezometer and the number of organisms recovered (Figures 5.11-5.18) indicated a direct link between it and the point of injection. The presence of macropores in the profile which could conduct large numbers of organisms at high velocities undoubtedly

account for this observation. An order of magnitude reduction in the number of organisms recovered was observed when tracer bacteria were injected into the 80 cm depth at the Dixonville site (Figures 5.31-5.38). In addition, a further reduction in the number of organisms appeared after the fourth hourly sampling period. It was possible that the installation of the 80 cm injection trench removed the saprolitic clay layer and exposed the fissures and broken parent material beneath. This could explain the relatively small number of organisms recovered after injection and the rapid drop in numbers with time. The injections in the A and B horizons would cause movement into all size pores with the most rapid in the large pores and the slowest in small pores. The result would be the maintenance of a relatively stable number of organisms over a longer period of time.

These results would seem to lend support to the concept of partial displacement in soils. Partial displacement was first recognized by Lowes, Gilbert and Warington (1882). They observed that a major part of the water moving through soil profiles at Rothamsted moved through macropores. The remainder of the water moved in smaller pores at much lower rates. The movement of water through macropores results in the partial displacement of a traceable material present in the soil water solution. Added irrigation water would displace only a part of the total soil water solution present in the soil profile. This situation would provide for rapid infiltration of tracer

material into the macropores with gradual movement into the finer pores. Conversely this would mean that the organisms would move through the macropores quickly with a much slower reduction in the number of organisms in the fine pores. This situation would create a condition in which the number of organisms would be gradually reduced rather than suddenly appear at a point and then be uniformly washed through.

Vertical movement of organisms at the Dixonville site appeared to be restricted to the upper 150 cm of the soil profile. The observed in situ hydraulic conductivity as determined by Hammermeister (1977) was .04 cm/hr. at 150 cm depth and this seemed to mark the lower limit of significant bacterial movement (Table 5.2). The upper limit of movement through this profile appeared to be the piezometric surface at approximately 24 cm. In both cases it was difficult to set a precise limit to movement due to the nature of the instrumentation. Samples and moisture tensions alike were taken at defined intervals and not on a continuum and more definitive work would be necessary to determine the precise extent of vertical movement.

Earlier work at these sites produced evidence of significant movement in macropores. Hammermeister (1977) observed that high ion concentrations were sometimes observed at the 5 m distance first while concentrations at the 2.5 m line increased more slowly. He concluded that this was evidence of rapid flow through zones of high

permeability or flow through macropores to the distant point with subsequent slower movement in a lateral direction through smaller pores. This same observation can be made at the Dixonville site with the injections into the A horizon and population recoveries of bacteria at 10 and 15 m (Figures 5.11 and 5.12).

Additional evidence of flow through zones of high permeability (or macropores) was observed in the numbers of organisms recovered. An average of 1.4×10^9 cells/ml were injected and it was not unusual to recover 10^5 cells/ml at distances of 2.4 m from the point of injection and to obtain numbers approaching 10^4/ml at 10 m distances. This recovery rate would indicate that once the organisms initially move into these zones of high permeability or macropores they experience little mixing or dilution but rather moved down the channel relatively unaffected by the media through which they were transported. This indicates mass flow. An additional line of investigation is to be found in the estimation of expected dilution over time. If the number of organisms known to be injected into the site were evenly distributed over the entire site in a static situation with no water being added to the system and no water leaving the system, the estimated number expected over the site would be 1.34×10^5/ml. However, the system in question is not static. Water is continually being added and continually leaving the site. The exact number of turnovers at the site is difficult to determine. However, if we

assume no water to be added to the system except the irrigation water and we estimate the irrigation water added per hour at .315 Cu meters, then we would observe .04 turnovers during the 8 hour run at the site. We know, however, that some of the water in the macropores turns at least once each hour. Therefore, we can conclude that some large pores in the system are emptied and filled again several times during the course of a run while other fine pores may transport tracer for only a few meters. An additional condition needs to be considered in the explanation of irregular flows. Subsurface topographic variation in the rock layer at the Dixonville site may be at least partially responsible for irregular flow patterns. Since high spots in the rock surface beneath the soil surface existed, the driving force toward these points would be less than the driving force toward ajoining lower areas. As a result, we would see less soil water and fewer organisms at these high points. The zones of rapid flow by laminar flow or turbulent flow through macropores may be located in the lower areas of the rock topography.

6.3 Soil Moisture Tensions at the Hazelair Site

The observation of the water table at higher levels at the Hazelair site was due to the presence of a restrictive layer with low hydraulic conductivity below 30 cm (Table 5.3). The average perched water table was within 9 cm of the surface. Soil suctions approaching

zero would be expected at the 12 cm depth and the tensiometer data indicated that as expected, the 12 cm depth was saturated (Table 5.3). Hydraulic conductivities and soil moisture tensions demonstrated considerable change at some point between the 30 and 80 cm depths. The C horizon which acted as a restrictive layer had a very low hydraulic conductivity (< 0.01 cm/hr). Both the 110 and 150 cm tensiometers indicated moisture tensions for those depths corresponding to dryer conditions than the soil above. This would be due to the inability of the soil water to move through the restrictive layer and wet the lower profile.

The average of the 1976 water tables observed by Hammermeister (1977) at the Hazelair site under natural rainfall was 15.3 cm while the average water table observed in this study under irrigation was 9.1 cm. Review of the data compiled by Hammermeister (1977) from winter rain cycles indicated a number of occasions occurred during storm cycles when water tables were at or above that maintained by irrigation.

6.4 Injection of Tracer Organisms at the Hazelair Experimental Site

The absence of positive samples in the control lines prior to injection of the tracer bacteria indicated that there were no organisms naturally present which were resistant to the antibiotics being used

as selective agents (Figure 5.40). Positive samples were observed in the upslope control lines when injections were made in the A horizon (Figures 5.41-5.48). Their presence can be explained in two ways. The first is the fact that leakage of tracer organisms may have occurred in an upward direction between the vertical PVC tubing and the soil. The organisms would have to move only 12 cm upward under the effect of the positive head placed on the injection line at the time of injection. Secondly the organisms may have traveled through a macropore and surfaced in a depression at some distance down slope from the injection line. The organisms could have easily been transported to the control line by foot traffic.

This observation points out the extreme sensitivity of this technique as well as demonstrating the amount of care necessary when employing antibiotic resistant microorganisms as tracers.

When labeled bacteria were introduced into the Hazelair experimental site, the movement rates were much slower and appeared to be due to two factors (Table 6.2). The first factor was that of reduced slope at the site (Dixonville 14%, Hazelair 10%). This factor decreased one of the major components of flux, that of hydraulic head. The second factor influencing the movement rates was the significantly lower hydraulic conductivities. A review of Table 5.3 will show that movement rates and hydraulic conductivities both decreased rapidly with depth at the Hazelair site.

Significantly lower numbers of organisms were recovered from down slope piezometers following injection of organisms into the A horizon (12 cm) at the Hazelair site compared to recoveries made from the same depth injection at the Dixonville site (Figures 5.11-5.18 and 5.41-5.48). The finer texture and lower hydraulic conductivities of the soil at the Hazelair site would tend to physically filter greater numbers of organisms than the relatively porous Dixonville soil. Macropores were not as evident in the profile at this site so the potential for very rapid movement through macropores under turbulent flow conditions would appear to be reduced. Any effects due to cell death would also be maximized under conditions which created longer time periods between injection and recovery of organisms.

Significant movement of microorganisms was observed to occur only to a depth of 30 cm in the soil profile (Figures 5.43-5.48 and 5.52-5.58). Hydraulic conductivities were measured at < .01 cm/hour at the next sampling depth of 80 cm (Table 5.3), and this seemed to be beyond the limit of significant downward movement. Upward movement was observed at the shallowest testing point of 12 cm. Observations made at the Dixonville site would indicate that organisms were quite likely evident upwards in the profile to the 9 cm piezometric surface.

6.5 Introduction of Fluorescein Dye into the Dixonville Site

Once it became evident that the microorganisms being utilized in this project were detectible for distances of up to 20 meters, it became of interest to determine how this compared to the detectibility of the more commonly used fluorescein dyes. Subsequently a fluorescein dye was introduced into the injection lines as described in Section 4.1.3.1. The failure of the dye to be visually detectible at 2.5 m and to be detectible only with a spectrofluorimeter at a distance of 10 m raises serious questions about the continued use of fluorescein dyes in soil water systems. Feverstein and Seeleck (1963) rated fluorescein dyes as suitable for only pristine surface waters due to sorbtion and quenching by suspended solids. Apparently these processes completely nulified the tracer within a distance of fifteen meters in this study.

Concentration of the dye in these studies were relatively high when compared to the quantities usually used by state health and environmental quality personnel. It is normal to introduce less than 1/8 pound of dye into each septic system when surveying for septic system failures. In this study we introduced the equivalent of one and two pound quantities of dye into the soil system. The review of one tracing dye is in no way intended to apply to all fluorescein dyes

or all tracing dyes. However, the results of this work do point out the need to carefully study the suitability of these dyes for tracing of sewage effluent through soil systems. Additionally, the comparison of detectible bacterial movements and the observed fluorescein movements points out the fact that a negative fluorescein dye test on a septic system can not be used to indicate that the septic system is functioning. A positive dye test is valid for detecting a failure but a negative dye test merely means that one does not know the functional status of the system. The bacteria may be moving freely over great distances while efforts to trace them with fluorescein dyes fail due to the reasons outlined above.

7. SUMMARY

The importance of macropores in the movement of large volumes of water at very rapid rates was discussed at length by Aubertin (1971). He cites, among other factors, structural pores resulting from the arrangement of primary soil particles, disturbed areas (such as crotoviena), open animal passageways ranging in size from small tunnels formed by insects to relatively large passageways formed by mammals, and structural cracks between soil units. All of these characteristics can be observed at the Dixonville site where the most rapid movement rates were observed. The hazelair site did not exhibit as many large pores or cracks as the Dixonville site but some were present. Old root channels from grasses, structural voids and some small tunnels from insects were observable and visual dye tests reported by Hammermeister (1977) indicated that these pores were effective in movement of tracer dyes.

Cores used for laboratory determinations of hydraulic conductivity do not properly reflect field conditions. Such factors as insect and rodent holes would not likely be included in a soil core since the rodent hole diameter might exceed the diameter of the intact core being tested and insect holes, if visually detectable, would be excluded as "unrepresentative".

The success or failure of an in situ hydraulic conductivity test would depend upon the sampling site location in relation to "representative numbers" of macropores. The partial displacement concept places a large portion of the movement in a small part of the total soil cross section and other tests are needed which include much larger sections that would more accurately reflect the influence of these conductive channels. These observations are subjective in nature. However, their importance is supported by the failure of previous quantitative measures to adequately predict the soil water velocities observed in this study (Hammermeister, 1977).

7.1 Implications Concerning Subsurface Disposal of Septic Tank Wastes

The data demonstrate the importance of adequate treatment of septic tank wastes before they are allowed to come in contact with a saturated soil horizon. There appears to be no doubt that microorganisms can move great distances in conditions of saturated flow. At the Dixonville site the tracer organisms were consistently recovered from a surface waterway located 20 m from the injection point within 2 hours.

Mottles provided a fairly accurate reflection of the persistent water table at the Hazelair site; however, they do not accurately reflect the transient high water table during storm cycles. Depth to

mottling did not correlate with the persistent or peak water table at the Dixonville site. The organic masking of oxidized and reduced soil colors seems a possible cause for this lack of morphological indicator. Evaluation of storm cycle data from the 1975-76 season indicated that the perched water tables under natural conditions may exist for only short periods of time. This period would undoubtedly be related to frequency and length of storm cycles and more work is needed before any definitive explanation of true water table characteristics can be made.

The very rapid rates of water and microorganism movement would indicate that the soil profile at the Dixonville site would tend to dry out much more rapidly after a storm cycle than the Hazelair site. This observation, coupled with the differences in microorganism movement in saturated and unsaturated conditions, would indicate that there would be important differences in the length of time that microorganisms would be discharged into the soil water at each site. A much more sustained discharge of organisms from a waste disposal facility would be observed in a Hazelair soil series whereas shorter intermittent discharges would be expected in a soil such as that found at the Dixonville site. Since most soils in Western Oregon would become saturated for some time during the winter season, the question arises as to what percent of time is acceptable for a failing situation.

The role of macropores in the movement of sewage effluent through soil profiles has been largely ignored by soil scientists and sanitarians alike. The concept of partial displacement and its role in movement of microorganisms under conditions of saturated flow must be considered if adequate protection of domestic drinking and surface water sources are to be provided.

The use of the antibiotic resistant microorganisms described here for determination of the functional status of existing septic tank disposal systems allows not only the identification of a failing septic system but also functioning septic systems. Failure to detect dye escaping from a septic system indicates that either the system is functioning or that the dye is inactivated by the soil media. On the other hand, the inability to detect tracer bacteria would indicate that the sewage treatment system is actually functioning in preventing the escape of fecal bacteria into the environment. This technique would allow detection of failing septic systems which previously have not been shown to be failures because of the inability of fluorescein dye to adequately model the movement of microorganisms through soil profiles.

This technique offers the opportunity for researchers to introduce tracer bacteria into additional soil profiles and observe the flow rate, structure, porosity, density and other parameters in order to construct a predictive model of microorganism movement through

soil profiles. Many problems remain to be worked out on the adequate measurement of soil characteristics such as distribution of macropores and true hydraulic conductivity rates. None the less, this technique offers a tool whereby this work may be undertaken.

7.2 Further Study Needed

Two very important aspects of microorganism movement remain to be investigated. The first involves the use of curtain drains and the second involves the effect of the biological mat on microbial movement. Adequate water table data already exists on these experimental sites to allow examination of the effects of the commonly used curtain or interceptor drain to lower the water table. Lowering of the water should significantly reduce the rate of microorganism movement in the Dixonville site and contrasts could be drawn to the Hazelair site. This information would be most valuable in planning for future waste disposal designs.

The existence of a biological mat at the interface of the sewage disposal trench and the soil has been studied to some extent by Bouma et al. (1972). No attempt was made to incorporate the biological mat variable into this study. There seems little doubt that under conditions of unsaturated flow the organisms move very short distances. The question of how microorganisms move when the soil near the trench approaches saturation or when the biological

mat is inundated will not be fully answered until the tracer organisms used in this study are subjected to a functioning sewage disposal system under the prescribed conditions.

Finally, it remains for the macropore system at these sites to be characterized morphologically. This description needs to be not only in a vertical direction but horizontal as well. The distribution and abundance of macropores or zones of high permeability in a vertical surface may be of less importance than their distribution and their interconnections in a horizontal direction.

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APPENDIX

APPENDIX 1

Appendix Tables

Appendix 1. Soil profile description by experimental site.

Horizon	Depth	Profile Description	Hydraulic Conductivities cm/hr (Horizontal)
Hazelair Soil Series; Classified as a Very Fine Mixed, Mesic, Aquultic Haploxeroll			
A1	0-5 in 0-12 cm	Dark brown (10YR 3/3) silt loam; few faint dark yellowish brown (10YR 4/4) mottles; moderate, medium granular structure; slightly hard, slightly sticky and slightly plastic; many fine and very fine roots; many fine and very fine interstitual pores; medium acid; abrupt smooth boundary.	---
A3	5-19 in 12-47 cm	Dark brown (10YR 3/3) silty clay loam; many distinct dark yellowish brown (10YR 4/4) mottles; moderate, medium subangular blocky structure; hard, slightly sticky and slightly plastic; many fine roots; many fine and very fine tubular pores; slightly acid; gradual, smooth boundary.	1.35
IIB2	19-32 in 47-80 cm	Grayish brown (10YR 5/2) clay; many distinct brownish yellow (10YR 6/6) mottles; moderate, medium prismatic to moderate, medium subangular blocky structure; very hard, very sticky and very plastic; very few fine roots; common fine and very fine tubular pores; medium acid; clear, smooth boundary.	.69
IIB3	32-44 in 80-110 cm	Dark yellowish brown (10YR 4/4) clay, massive structure, very firm, very sticky and very plastic; very few fine roots; common very fine tubular pores; medium acid; gradual, wavy boundary.	1.04
IIC	44 in to -- 110+ cm	Dark brown (10YR 4/3) clay, massive structure; very firm, very sticky and very plastic; very few fine roots; few very fine tubular pores; slightly acid.	0.01

Appendix 1. Continued

Horizon	Depth	Profile Description	Hydraulic Conductivities cm/hr (Horizontal)
Dixonville Soil Series, Classified as Fine Mixed Mesic Pachic Ultic Argixerolls			
A1	0-8 cm 0-3 in	Dark brown (7.5YR 3/2) silty clay loam; moderate, medium granular structure; loose, slightly sticky and slightly plastic; many very fine roots; many very fine and fine interstitual pores; medium acid; clear, and smooth boundary.	---
A3	8-34 cm 3-14 in	Dark brown (7.5YR 3/2) silty clay loam; moderate, medium subangular blocky structure; very hard, slightly sticky and slightly plastic; common fine roots; many very fine tubular pores; medium acid; clear, wavy boundary.	22.5
B2	34-56 cm 14-22 in	Dark brown (7.5YR 3/2) silty clay loam; moderate, medium and fine subangular blocky structure; hard, slightly sticky and slightly plastic; few fine roots; many very fine tubular pores; several rodent holes up to 10 cm in diameter present in 1 meter wide soil pit; medium acid; clear, wavy boundary.	26.2
C	56-67 cm 22-27 in	Reddish brown (2.5YR 5/4) and yellow (10YR 7/6) saprolite; granular to massive structure; very firm; sticky and plastic; few fine roots; clear to diffuse, broken boundary.	---
R	67 cm 27 in	Well indurated $CaCO_3$ cemented sandstone containing primarily mafic minerals and shell fragments; cannot be dug with a pick.	.04

Appendix 1b. Laboratory soil characterization analysis.

Site	Horizon	Percent Sand	Percent Silt	Percent Clay	pH	Percent Organic Matter	Cation Exchange Capacity	Total Exchangeable Base	Percent Base Saturation
Dixonville	A1	21.5	42.4	36.1	5.2	4.3	52.63	35.4	67.3
	A3	18.0	39.6	42.4	4.9	3.8	26.84	16.1	60.1
	B2	12.4	33.3	54.3	4.7	3.0	17.48	8.7	49.8
Hazelair	A1	17.8	52.8	29.4	6.1	4.8	20.85	17.6	84.4
	A3	17.6	44.8	37.6	5.9	3.3	16.91	13.4	79.3
	IIB2	9.1	26.6	64.3	5.7	1.2	14.36	10.5	73.1
	IIB3	6.8	40.4	52.9	5.6	0.9	12.40	8.5	68.5

Samples collected by C. Hagedorn

Analysis by Oregon State University Testing Laboratory.

Appendix 2a. Fluorescein recovered--Injection in A horizon (Relative intensity).*

Point	03	04	05	12	13	14	15	16	21	22	23	25	26	31	32	33	34	35	41	42	43	44	46	56
cm	120	150	80	80	150	200	110	45	200	111	45.5	80	150	80	150	110	45	200	80	45	100	80	100	Surface
Time																								
0	0	0	0																					
1	NT	NT	NT	.3	.4	.2	.4	.7																
2	--	--	--	26	--	.4	.6	.4	.6	.1	.8	.5	.6	.3	.6	.1	.3	.4	.6	.2	--	.2	--	.4
3	6	.3	.3	21	.2	.2	.9	.5	.3	.5	.2	.6	1.5	.2	.5	.2	.2	.5	.2	.2	.1	.2	.2	.2
4	1.9	.2	.2	18	.2	.2	.6	.2	.2	.4	.2	.2	.6	.4	.2	.1	.1	.4	.4	.2	.2	.2	.2	.5
5	.8	.2	.1	15	.2	.1	1.0	.6	.1	.6	.3	.3	.2	.4	.2	.2	.1	.2	.2	.1	.2	.2	.2	.7
6	2.5	.2	.2	14	--	.4	13	.6	.1	.2	.2	.1	.7	.4	0	.1	.2	.2	.1	.2	.2	.2	.1	1.1
7	1.4	.2	.2	12	0	.2	2.2	.6	.4	.3	.2	.2	.9	.5	0	.3	.1	.1	.1	.1	.1	.1	.2	.2
8	.6	.1	.2	11	--	.2	2.4	.7	0	.8	.2	.2	.5	.5	0	.2	.1	.1	.1	.1	0	.1	.1	.2

** First number indicates line, 0 = control
1 = 2.5 m
2 = 5.0 m
3 = 10.0 m
4 = 15.0 m

Second number shows position in line

Appendix 2b. Fluorescein recovered--Injection in B horizon (Relative intensity).

Point	03	05	12	13	14	15	16	21	22	23	25	26	31	32	33	34	35	41	42	43	44	46
cm	120	150	80	150	200	110	45	200	111	45.5	80	150	80	150	110	45	200	80	45	100	80	100
Time																						
0	2.5	.5	7.5	.5	.2	1.2	.2	.2	1.1	.2	.5	1.0	.5	.2	.2	.1	.1	.0	.5	.0	.1	.0
1	1.5	2.5	4.5	.5	.0	1.6	.3	.0	.5	.0	.6	.8	.6	.0	.1	.1	.0	.0	.2	.0	.1	.0
2	---	.4	4.5	.7	.2	2.7	.5	.3	1.0	.3	.6	1.3	1.1	.2	.4	.6	.3	.2	.5	.3	.4	.2
3	---	---	5.1	.6	.1	2.8	2.2	.2	.3	.2	.7	1.4	1.0	.2	.4	.4	.1	.4	.6	.3	.3	.2
4	---	.2	5.2	.5	.1	2.8	2.6	.3	2.7	.4	.7	1.5	1.7	.3	.5	.5	.1	.2	.3	.2	.3	.2
5	---	.0	6.0	.7	.2	3.0	3.5	.2	2.0	.4	.8	2.4	3.5	.2	.6	.2	.0	.4	.3	.3	5.2	.0
6	---	.2	6.6	.5	.2	2.9	4.0	.1	1.5	.2	.8	2.1	3.6	.0	.8	.3	.1	.1	.3	.4	.2	.3
7	---	.0	7.3	.8	.3	3.0	5.2	.2	2.7	.4	1.4	3.0	5.0	.2	1.1	1.0	.4	.5	.6	.3	.3	.1
8	---	.3	8.0	.1	.3	3.3	6.0	.2	2.0	.3	1.0	2.8	4.5	.1	1.3	.5	.3	.5	.7	.4	.5	.2

** First number indicates line, 0 = control
1 = 2.5 m
2 = 5.0 m
3 = 10.0 m
4 = 15.0 m
5 = stream sample point
Second number shows position in line

Appendix 2c. Fluorescein recovered--Injection in C horizon (Relative intensity).*

Point	05**	12	13	14	15	16	21	22	23	25	26	31	32	33	34	35	41	42	43	44	46
cm	80	80	150	200	110	45	200	111	45.5	80	150	80	150	110	45	200	80	45	100	80	100
Time																					
0	1.0	6.7	1.2	.2	2.5	1.5	.3	1.0	.5	1.2	3.0	4.5	.5	1.4	1.0	.4	.6	.4	.4	1.0	.2
1	1.0	6.3	1.5	.2	6.4	1.1	.3	1.5	.4	1.5	3.0	3.5	.3	.9	.6	.1	.6	.1	.2	.5	.1
2	.5	6.4	1.5	.5	8.3	2.5	.5	.7	3.0	2.0	3.0	5.3	.5	1.0	.5	.3	1.0	.7	.7	1.0	.5
3	.5	6.0	1.5	.5	8.7	4.5	.4	2.0	.7	1.8	3.0	7.0	.3	1.2	.7	.5	1.1	.8	.8	1.0	.4
4	.3	6.2	1.4	.5	9.0	5.5	.3	3.0	.5	1.5	3.3	7.2	.1	1.0	.5	.3	1.0	.6	.8	1.2	.2
5	.2	6.0	1.5	.4	9.0	7.2	.2	.5	.5	1.6	4.5	7.6	.2	.9	1.4	.1	1.2	.6	.6	1.0	.2
6	.2	6.0	1.6	.4	12.0	11.0	.2	2.0	.5	2.0	3.9	8.2	.3	.9	.5	.3	1.4	.8	1.0	1.3	.3
7	.6	6.2	1.8	.6	14.0	12.0	.3	2.7	.5	2.0	5.0	8.5	.2	.7	.5	.2	1.4	.7	1.0	1.4	.4
8	.4	5.5	1.5	.4	11.0	10.0	.2	.5	.4	2.2	4.9	7.7	.2	.4	.5	.2	1.4	.8	1.0	1.2	.2

* Relative intensity as recorded directly from the spectroflorimeter. Absolute concentrations (Appendix Tables are determined from a standardized curve constructed at the time of testing.

Appendix 3a. Fluorescein recovered--Injection in the A Horizon (μg/ml).

Time	03	04	05	12	13	14	15	16	21	22	23	25
0	---	---	---	---	---	---	---	---	---	---	---	---
1	---	---	---	4×10^{-7}	6×10^{-7}	2.5×10^{-7}	6×10^{-7}	1.2×10^{-6}	---	---	---	---
2	---	---	---	6×10^{-5}	---	6×10^{-7}	1×10^{-6}	6×10^{-7}	1×10^{-6}	1×10^{-7}	1.5×10^{-6}	8×10^{-7}
3	1.5×10^{-5}	4×10^{-7}	4×10^{-7}	5×10^{-5}	2.5×10^{-7}	2.5×10^{-7}	1.7×10^{-6}	8×10^{-7}	4×10^{-7}	8×10^{-7}	2.5×10^{-7}	1×10^{-6}
4	4.5×10^{-6}	2.5×10^{-7}	2.5×10^{-7}	4.5×10^{-5}	2.5×10^{-7}	2.5×10^{-7}	1×10^{-6}	2.5×10^{-6}	2.5×10^{-7}	6×10^{-6}	2.5×10^{-7}	2.5×10^{-7}
5	1.2×10^{-6}	2.5×10^{-7}	1×10^{-7}	3.5×10^{-5}	2.5×10^{-7}	1×10^{-7}	2×10^{-6}	1×10^{-6}	1×10^{-7}	1×10^{-7}	4×10^{-7}	4×10^{-7}
6	5.5×10^{-6}	2.5×10^{-7}	2.5×10^{-7}	3.5×10^{-5}	---	6×10^{-7}	2.7×10^{-6}	1×10^{-6}	1×10^{-7}	2.5×10^{-7}	2.5×10^{-7}	1×10^{-7}
7	2.7×10^{-6}	2.5×10^{-7}	2.5×10^{-7}	3×10^{-5}	---	2.5×10^{-7}	5×10^{-6}	1×10^{-6}	6×10^{-7}	4×10^{-7}	2.5×10^{-7}	2.5×10^{-7}
8	1×10^{-6}	1×10^{-7}	2.5×10^{-7}	2.5×10^{-5}	---	2.5×10^{-7}	5.5×10^{-6}	1.2×10^{-6}	---	1.5×10^{-6}	2.5×10^{-7}	2.5×10^{-7}

Time	26	31	32	33	34	35	41	42	43	44	46
0	---	---	---	---	---	---	---	---	---	---	---
1	---	---	---	---	---	---	---	---	---	---	---
2	1×10^{-6}	4×10^{-7}	1×10^{-6}	1×10^{-7}	4×10^{-7}	6×10^{-7}	1×10^{-6}	2.5×10^{-7}	---	2.5×10^{-7}	---
3	3×10^{-6}	2.5×10^{-7}	8×10^{-7}	2.5×10^{-7}	2.5×10^{-7}	8×10^{-7}	2.5×10^{-7}	2.5×10^{-7}	1.0×10^{-7}	2.5×10^{-7}	2.5×10^{-7}
4	1×10^{-6}	6×10^{-7}	2.5×10^{-7}	1×10^{-7}	1×10^{-7}	6×10^{-7}	6×10^{-7}	2.5×10^{-7}	2.5×10^{-7}	2.5×10^{-7}	2.5×10^{-7}
5	2.5×10^{-7}	6×10^{-7}	2.5×10^{-7}	2.5×10^{-7}	1×10^{-7}	2.5×10^{-7}	2.5×10^{-7}	1×10^{-7}	2.5×10^{-7}	2.5×10^{-7}	2.5×10^{-7}
6	1.2×10^{-6}	6×10^{-7}	---	1×10^{-7}	2.5×10^{-7}	2.5×10^{-7}	1×10^{-7}	2.5×10^{-7}	2.5×10^{-7}	2.5×10^{-7}	1×10^{-7}
7	1.7×10^{-6}	8×10^{-7}	---	4×10^{-7}	1×10^{-7}	1×10^{-7}	1×10^{-7}	1×10^{-7}	1×10^{-7}	1×10^{-7}	2.5×10^{-7}
8	8×10^{-6}	8×10^{-7}	---	2.5×10^{-7}	1×10^{-7}	1×10^{-7}	1×10^{-7}	1×10^{-7}	---	1×10^{-7}	1×10^{-7}

Appendix 3b. Fluorescein recovered--Injection in the B horizon (μg/ml).

Time	03	04	05	12	13	14	15	16	21	22	23	25
0	5.5×10^{-6}	---	8×10^{-7}	1.75×10^{-5}	8×10^{-7}	2.5×10^{-7}	2.3×10^{-6}	2.5×10^{-7}	2.5×10^{-7}	2.1×10^{-6}	2.5×10^{-7}	8×10^{-7}
1	3×10^{-6}	---	5.5×10^{-6}	1.2×10^{-5}	8×10^{-7}	---	3.3×10^{-6}	4×10^{-7}	---	8×10^{-7}	---	1×10^{-6}
2	---	---	6×10^{-7}	1.2×10^{-5}	1.2×10^{-6}	2.5×10^{-7}	6.5×10^{-6}	8×10^{-7}	4×10^{-7}	1.8×10^{-6}	4×10^{-7}	1×10^{-6}
3	---	---	---	1.3×10^{-5}	1×10^{-6}	1×10^{-7}	6.5×10^{-6}	4.7×10^{-6}	2.5×10^{-7}	4×10^{-7}	2.5×10^{-7}	1.2×10^{-6}
4	---	---	2.5×10^{-7}	1.4×10^{-5}	8×10^{-7}	1×10^{-7}	6.5×10^{-6}	8.5×10^{-6}	4×10^{-7}	6.2×10^{-6}	6×10^{-7}	1.2×10^{-6}
5	---	---	---	1.5×10^{-5}	1.2×10^{-6}	2.5×10^{-7}	7×10^{-6}	8.5×10^{-5}	2.5×10^{-7}	1.3×10^{-6}	6×10^{-7}	1.5×10^{-6}
6	---	---	2.5×10^{-7}	1.7×10^{-5}	8×10^{-7}	2.5×10^{-7}	6.7×10^{-6}	1.0×10^{-5}	1×10^{-7}	3.1×10^{-6}	2.5×10^{-7}	1.5×10^{-6}
7	---	---	---	1.8×10^{-5}	1.5×10^{-6}	4×10^{-7}	7×10^{-6}	1.3×10^{-5}	2.5×10^{-7}	6.2×10^{-6}	6×10^{-7}	2.8×10^{-6}
8	---	---	4×10^{-7}	1.9×10^{-5}	1×10^{-7}	4×10^{-7}	8.9×10^{-6}	1.4×10^{-5}	2.5×10^{-7}	4.3×10^{-6}	4×10^{-7}	1.8×10^{-6}

Time	26	31	32	33	34	35	41	42	43	44	46
0	1.8×10^{-6}	8×10^{-7}	2.5×10^{-7}	2.5×10^{-7}	1×10^{-7}	1 x10	---	8×10^{-7}	---	1×10^{-7}	---
1	1.5×10^{-6}	1×10^{-6}	---	1×10^{-7}	1×10^{-7}	---	---	2.5×10^{-7}	---	1×10^{-7}	---
2	2.6×10^{-6}	2.1×10^{-6}	2.5×10^{-7}	6×10^{-7}	1×10^{-6}	4×10^{-7}	2.5×10^{-7}	8×10^{-7}	4×10^{-7}	6×10^{-7}	2.5×10^{-7}
3	2.8×10^{-6}	1.8×10^{-6}	2.5×10^{-7}	6×10^{-7}	6×10^{-7}	1×10^{-7}	6×10^{-7}	1×10^{-6}	4×10^{-7}	4×10^{-7}	2.5×10^{-7}
4	3.1×10^{-6}	3.6×10^{-6}	4×10^{-7}	8×10^{-7}	8×10^{-7}	1×10^{-7}	2.5×10^{-7}	4×10^{-7}	2.5×10^{-7}	4×10^{-7}	2.5×10^{-7}
5	4.9×10^{-6}	8.6×10^{-6}	2.5×10^{-7}	1×10^{-6}	2.5×10^{-7}	---	6×10^{-7}	4×10^{-7}	4×10^{-7}	1.3×10^{-5}	---
6	4.4×10^{-6}	8.9×10^{-6}	---	1.5×10^{-6}	4×10^{-7}	1×10^{-7}	1×10^{-7}	4×10^{-7}	6×10^{-7}	2.5×10^{-7}	4×10^{-7}
7	2.7×10^{-6}	1.3×10^{-5}	2.5×10^{-7}	2.1×10^{-6}	1.8×10^{-6}	6×10^{-7}	8×10^{-7}	1×10^{-6}	4×10^{-7}	4×10^{-7}	1×10^{-7}
8	6.6×10^{-6}	1.1×10^{-5}	1×10^{-7}	2.6×10^{-6}	8×10^{-7}	4×10^{-7}	8×10^{-7}	1.2×10^{-6}	6×10^{-7}	8×10^{-7}	2.5×10^{-7}

Appendix 3c. Fluorescein recovered--Injection in the C Horizon (μg/ml).

Time	03	04	05	12	13	14	15	16	21	22	23	25
0	---	---	1.8×10^{-6}	1.4×10^{-5}	2.3×10^{-6}	2.5×10^{-7}	5.6×10^{-6}	3.1×10^{-6}	4×10^{-7}	1.8×10^{-6}	8×10^{-7}	2.3×10^{-6}
1	---	---	1.8×10^{-6}	1.5×10^{-5}	3.1×10^{-6}	2.5×10^{-7}	1.6×10^{-5}	2.1×10^{-6}	4×10^{-7}	3.1×10^{-6}	6×10^{-7}	3.1×10^{-6}
2	---	---	8×10^{-7}	1.6×10^{-5}	3.1×10^{-6}	8×10^{-7}	2.1×10^{-5}	5.6×10^{-6}	8×10^{-7}	1.2×10^{-6}	7×10^{-6}	4.3×10^{-6}
3	---	---	8×10^{-7}	1.4×10^{-5}	3.1×10^{-6}	8×10^{-7}	2.2×10^{-5}	1.1×10^{-6}	6×10^{-7}	4.3×10^{-6}	1.2×10^{-6}	3.9×10^{-6}
4	---	---	4×10^{-7}	1.6×10^{-5}	2.8×10^{-6}	8×10^{-7}	2.1×10^{-5}	1.3×10^{-5}	4×10^{-7}	7×10^{-7}	8×10^{-7}	3.1×10^{-6}
5	---	---	2.5×10^{-7}	1.4×10^{-5}	3.1×10^{-6}	6×10^{-7}	2.1×10^{-5}	1.8×10^{-5}	2.5×10^{-7}	8×10^{-7}	8×10^{-7}	3.3×10^{-6}
6	---	---	2.5×10^{-7}	1.4×10^{-5}	3.3×10^{-6}	6×10^{-7}	2.7×10^{-5}	2.5×10^{-5}	2.5×10^{-7}	4.3×10^{-6}	8×10^{-7}	4.3×10^{-6}
7	---	---	1×10^{-6}	1.6×10^{-5}	3.9×10^{-6}	1×10^{-6}	3.4×10^{-5}	2.6×10^{-5}	4×10^{-7}	6.2×10^{-6}	8×10^{-7}	4.3×10^{-6}
8	---	---	6×10^{-7}	1.3×10^{-5}	3.1×10^{-6}	6×10^{-7}	2.6×10^{-6}	2.4×10^{-5}	2.5×10^{-7}	8×10^{-7}	6×10^{-7}	4.6×10^{-6}

Time	26	31	32	33	34	35	41	42	43	44	46
0	7×10^{-6}	1.1×10^{-5}	8×10^{-7}	2.8×10^{-6}	1.8×10^{-6}	6×10^{-7}	1×10^{-6}	6×10^{-7}	6×10^{-7}	1.8×10^{-6}	2.5×10^{-7}
1	7×10^{-6}	8.5×10^{-6}	4×10^{-7}	1.7×10^{-6}	1×10^{-6}	1×10^{-7}	1×10^{-6}	1×10^{-7}	2.5×10^{-7}	8×10^{-7}	1×10^{-7}
2	7×10^{-6}	1.3×10^{-5}	8×10^{-7}	1.8×10^{-6}	8×10^{-7}	4×10^{-7}	1.8×10^{-6}	1.2×10^{-6}	1.2×10^{-6}	1.8×10^{-6}	8×10^{-7}
3	7×10^{-6}	1.7×10^{-5}	4×10^{-7}	2.3×10^{-6}	1.2×10^{-6}	8×10^{-7}	2.1×10^{-6}	1.5×10^{-6}	1.5×10^{-6}	1.8×10^{-6}	6×10^{-7}
4	8.9×10^{-6}	1.7×10^{-5}	1×10^{-7}	1.8×10^{-6}	8×10^{-7}	4×10^{-7}	1.8×10^{-6}	1×10^{-6}	1.5×10^{-6}	2.3×10^{-6}	2.5×10^{-7}
5	1.1×10^{-5}	7.8×10^{-5}	2.5×10^{-7}	1.75×10^{-6}	2.8×10^{-7}	1×10^{-7}	2.3×10^{-6}	1×10^{-6}	1×10^{-6}	1.8×10^{-6}	2.2×10^{-7}
6	9.8×10^{-6}	2×10^{-5}	4×10^{-7}	1.75×10^{-6}	8×10^{-7}	4×10^{-7}	2.8×10^{-6}	1.5×10^{-6}	1.8×10^{-6}	2.6×10^{-6}	4×10^{-7}
7	1.3×10^{-5}	2.1×10^{-5}	2.5×10^{-7}	1.2×10^{-6}	8×10^{-7}	2.5×10^{-7}	2.8×10^{-6}	1.2×10^{-6}	1.8×10^{-6}	2.8×10^{-6}	6×10^{-7}
8	1.2×10^{-5}	1.8×10^{-5}	2.5×10^{-7}	6×10^{-7}	8×10^{-7}	2.5×10^{-7}	2.8×10^{-6}	1.5×10^{-6}	1.8×10^{-6}	2.3×10^{-6}	2.5×10^{-7}

APPENDIX 2

Tables

Table 3.1. Site, location and description.

Soil Series	Location	Soil Family	Underlying Rock	Vegetation	Relief	Aspect	Hillslope Position	Shape of Landform
Hazelair	NW 1/4, NE 1/4, NW 1/4, Sec. 33, T. 11S., R.5W., Willamette Principle Meridian	Very fine, mixed, mesic, Aquultic Haploxerolls	Massive clay layer	Native grasses	10%	West	Lower Footslope	Concave, convex
Dixonville	SW 1/4, SE 1/4, NW 1/4 Sec. 28, T. 11S., R.5W., Willamette Principle Meridian	Fine, mixed, mesic Ultic Haploxeroll	Clayey saprolite and somewhat poorly sorted, $CaCO_3$ cemented sandstone containing primarily mafic minerals and shell fragments	Native grasses, poison oak	14%	North East	Lower Backslope	Convex, convex

Table 3.2. Depth from soil surface for piezometers, tensiometers and injection lines. Horizons or levels in soil rock mantle: A Horizon = 1, B Horizon = 2, C Horizon = 3, C_2 Horizon = 4, C_3 Horizon = 5, C_4 Horizon = 6.

Site	Horizon or Level in Soil-Rock Mantle	Depth of Instrument cm
Dixonville	1	12
	2	45
	3	80
	4	110
	5	150
	6	200
Hazelair	1	12
	2	30
	3	80
	4	110
	5	150
	6	200

Table 5.1. F ratios and probability of water table fluctuations at both sample sites.

Site/Horizon	F Ratio	F Probability
Dixonville, "A" horizon:		
2.5 m line		
12 cm	.135	.996
45 cm	.112	.998
80 cm	.054	1.0
110 cm	.058	1.0
150 cm	.062	1.0
Dixonville, "A" horizon:		
5.0 m line		
12 cm	.210	.971
45 cm	.016	1.0
80 cm	.049	1.0
110 cm	.701	.686
150 cm	.025	1.0
Dixonville, "B" horizon:		
2.5 m line		
12 cm	.967	.490
45 cm	.013	1.0
80 cm	.060	1.0
110 cm	.075	1.0
150 cm	.020	1.0
Dixonville, "B" horizon:		
5.0 m line		
12 cm	.015	1.0
45 cm	.195	.988
80 cm	.070	1.0
110 cm	.084	.999
150 cm	.000	1.0
Dixonville, "C" horizon:		
2.5 m line		
12 cm	.051	1.0
45 cm	.130	.997
80 cm	.049	1.0
110 cm	.046	1.0
150 cm	.045	1.0

Table 5.1 (Continued)

Site/Horizon	F Ratio	F Probability
Dixonville, "C" horizon:		
5.0 m line		
12 cm	.336	.932
45 cm	.024	1.0
80 cm	.129	.997
110 cm	.152	.995
150 cm	.229	.956
Hazelair, "A" horizon:		
2.5 m line		
12 cm	1.235	.386
30 cm	.823	.605
80 cm	1.203	.400
110 cm	.177	.978
150 cm	.316	.938
Hazelair, "A" horizon:		
5.0 m line		
12 cm	.032	1.0
30 cm	.875	.572
80 cm	.729	.667
110 cm	.253	.918
150 cm	*	*
Hazelair, "B" horizon:		
2.5 m line		
12 cm	1.236	.377
30 cm	.456	.859
80 cm	.611	.750
110 cm	.191	.985
150 cm	.163	.991
Hazelair, "B" horizon:		
5.0 m line		
12 cm	.044	1.0
30 cm	.523	.813
80 cm	.548	.795
110 cm	.396	.892
150 cm	*	*

* All readings exceed the capacity of the tensiometer.

Table 5.2. Dixonville site, hydraulic conductivities and soil moisture tensions.

Depth (cm)	Directions+ of K	Hydraulic (K) Conductivities cm/hr ++	Hydraulic Conductivities in situ ++	Average Soil* Moisture Tension
12	H	28.13		-4.4 cm
	V	49.51		
45	H	26.21	15.15	-8.8 cm
	V	11.25		
80			8.55	-22.6 cm
110			17.97	-19.8 cm
150			.04	-105 cm

+ H = Horizontal
V = Vertical

Table 5.3. Hazelair site, hydraulic conductivities and soil moisture tensions.

Depth (cm)	Direction of K ++	Hydraulic (K) Conductivities cm/hr ++	Hydraulic Conductivities in situ ++	Average Soil* Moisture Tension
12	H	1.35		+1.4
	V	1.446		
30	H	.76		-0.2
	V	14.07		
80	H	< .01	1.60	-2.3
	V	< .01		
110	H	< .01	.01	-343.6
	V	< .01		
150	H	.02	.03	-387.6
	V	.15		

* Plus or minus 1.25 cm H_2O.

++ Hammermeister, 1977.

Table 6.1. Comparative movement rates.

Site	Depth cm	Ions*	Bacteria
Dixonville	12	> 35.7 cm/hr	> 1500 cm/hr
	45	> 275 cm/hr	> 1500 cm/hr
	80	> 300 cm/hr	> 1500 cm/hr
	110	> 550 cm/hr	< 1500 > 1000 cm/hr
	150	> 275 cm/hr	< 1500 > 1000 cm/hr
Hazelair	12	> 9.6 cm/hr	> 166.6 cm/hr
	30	> 9.6 cm/hr	> 166.6 cm/hr
	80	> 9.6 cm/hr	> 41.6 < 83.3 cm/hr
	110	> .52 cm/hr	> 20.8 < 41.6 cm/hr

* Hammermeister, 1977.

Table 6.2. Comparative movement rates by hours to first recovery.

Site	Depth of Injection	Depth of Recovery	Distance of Recovery				
			2.5 m	5.0 m	10.0 m	15.0 m	20.0 m
Dixonville	12	45	1 hr	1 hr	1 hr	1 hr	1 hr
		80	1 hr	1 hr	2 hr	2 hr	2 hr
	45	45	1 hr	1 hr	1 hr	1 hr	1 hr
		80	1 hr	1 hr	1 hr	1 hr	1 hr
	80	45	1 hr	1 hr	1 hr	1 hr	1 hr
		80	1 hr	1 hr	1 hr	2 hr	2 hr
Hazelair	12	30	12 hr	12 hr	12 hr	12 hr	12 hr
		80	12 hr	12 hr	>96 hr	>96 hr	>96 hr
	30	30	12 hr	12 hr	12 hr	24 hr	72 hr
		80	24 hr	48 hr	>96 hr	>96 hr	>96 hr

APPENDIX 3

Figures

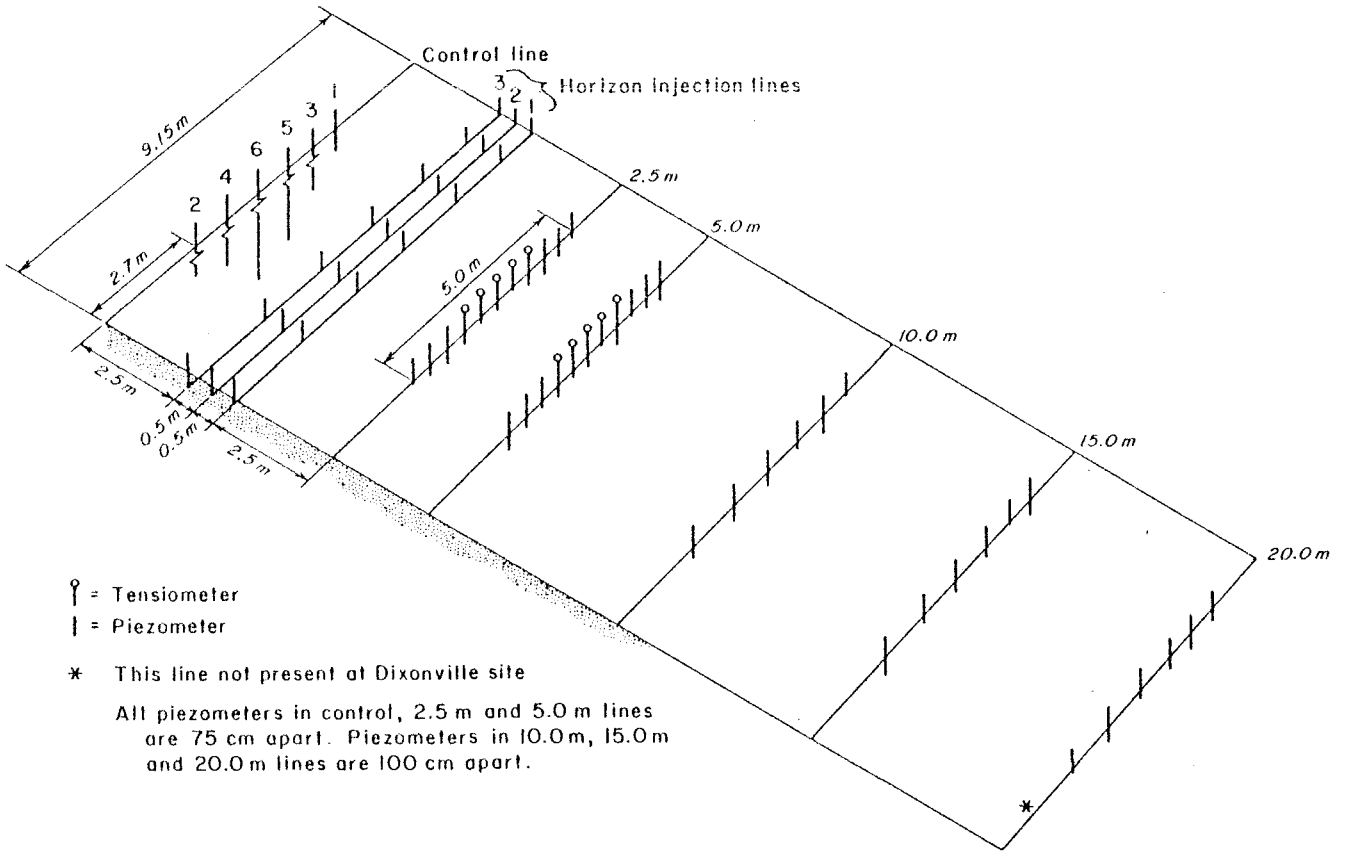

Figure 3.1. Site lay out.

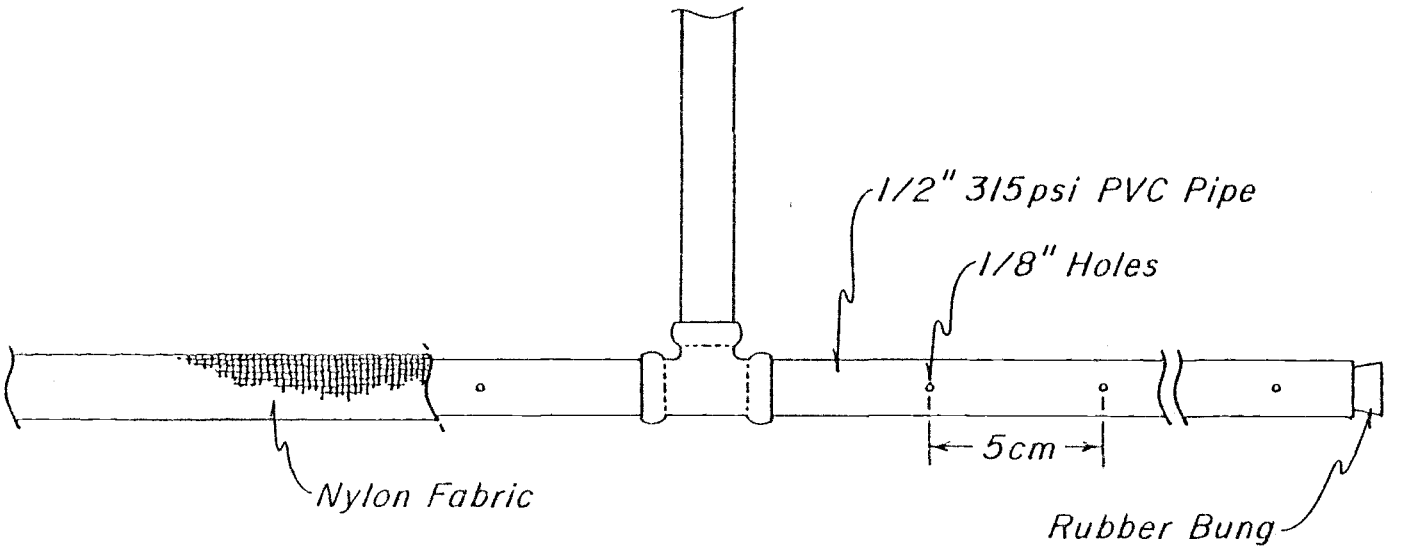

Figure 4.1. Injection Line

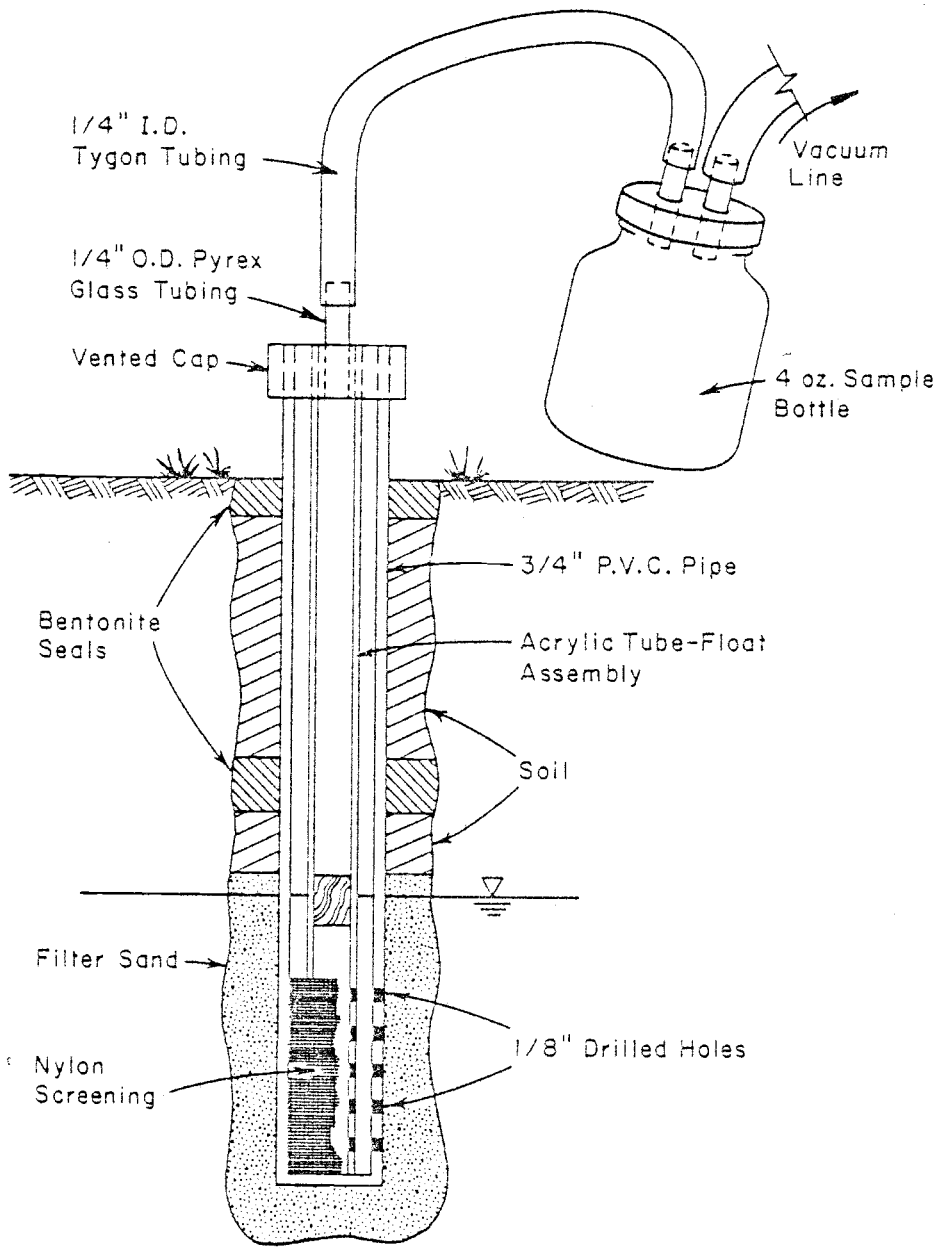

Figure 4.2. Piezometer design.

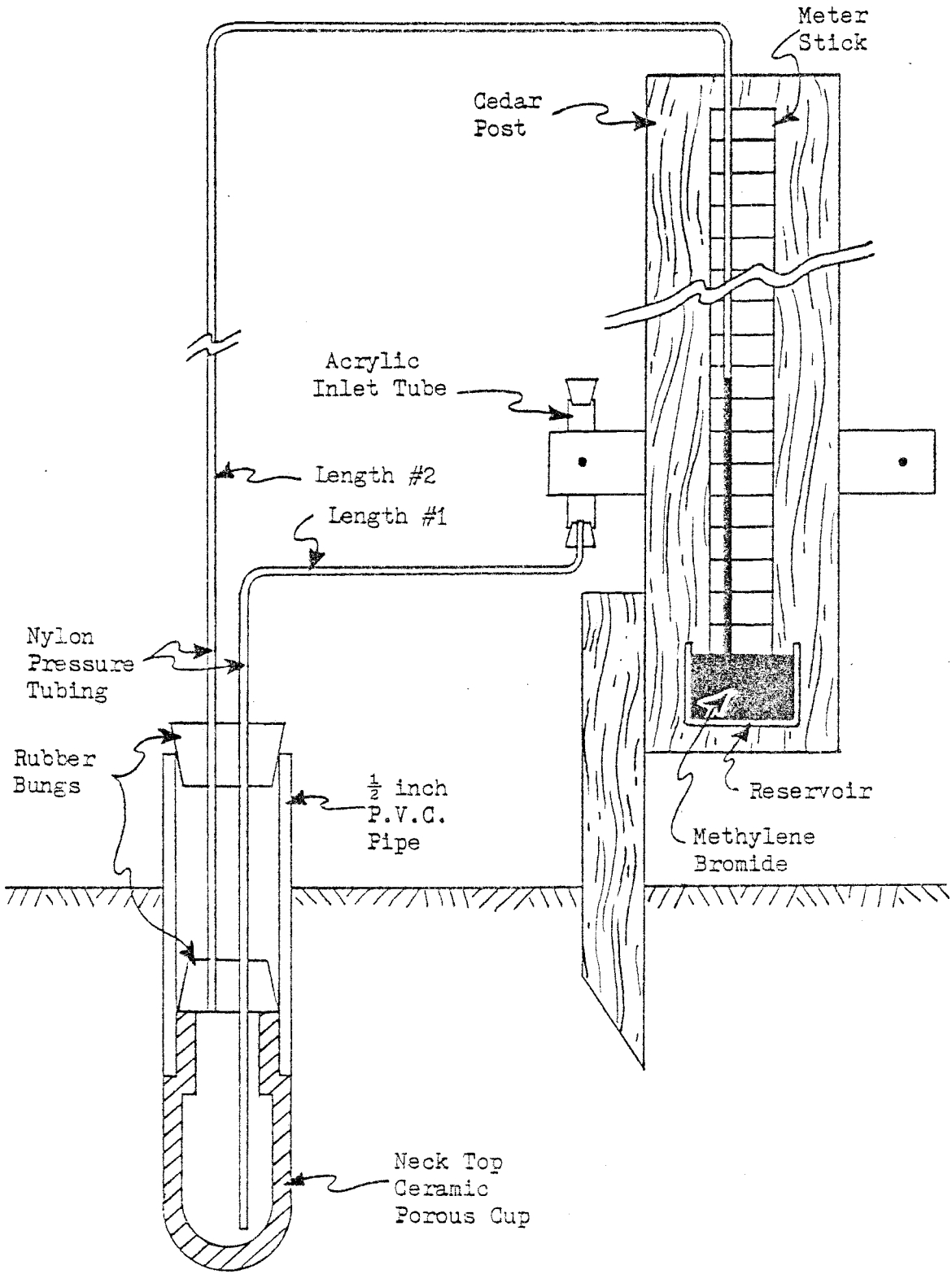

Figure 4.3. Diagram of a Tensiometer-Manometer System. (Hammermeister 1977)

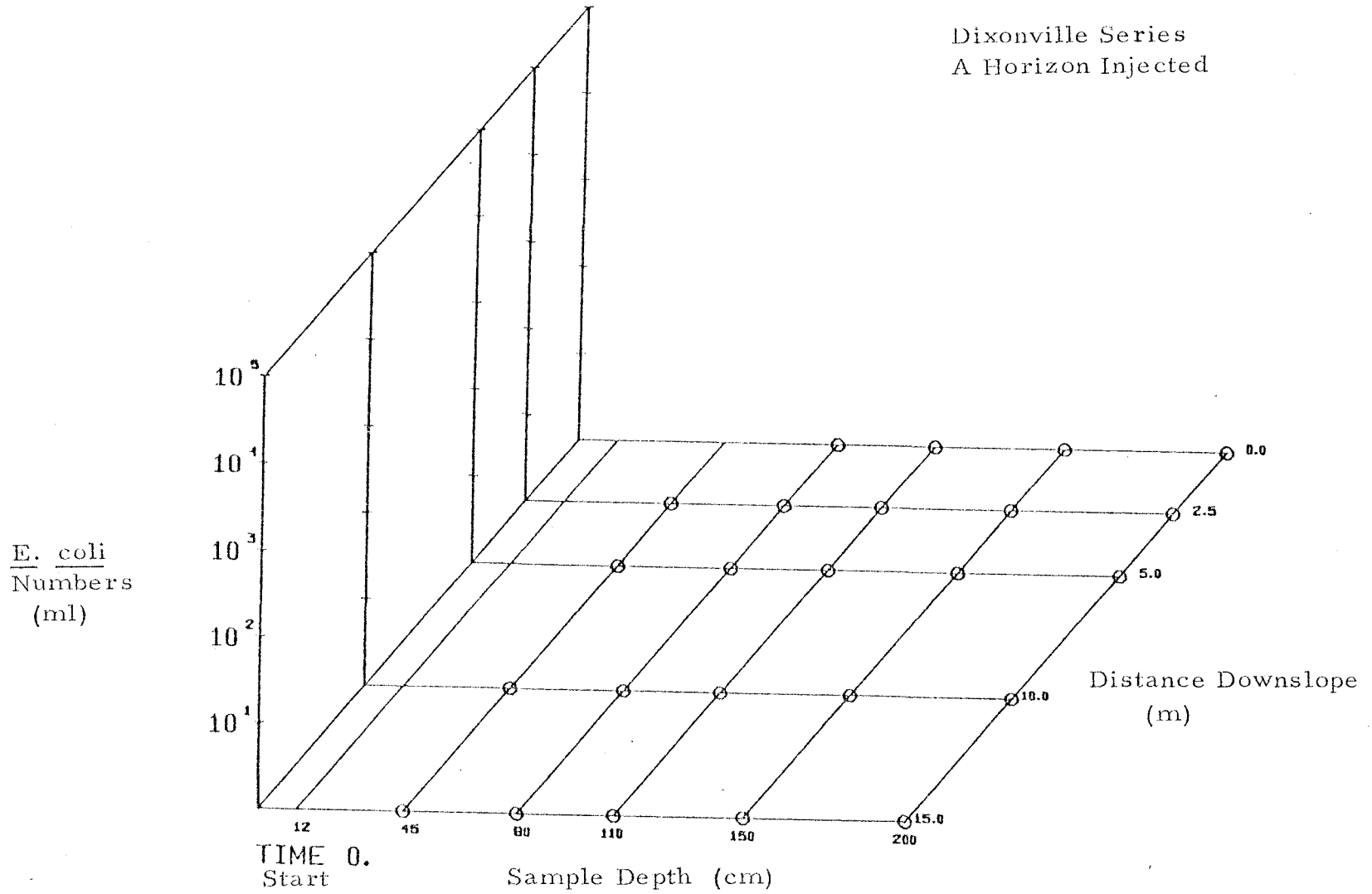

Figure 5.10. Organism movement at Dixonville Site, A horizon, pre-injection.

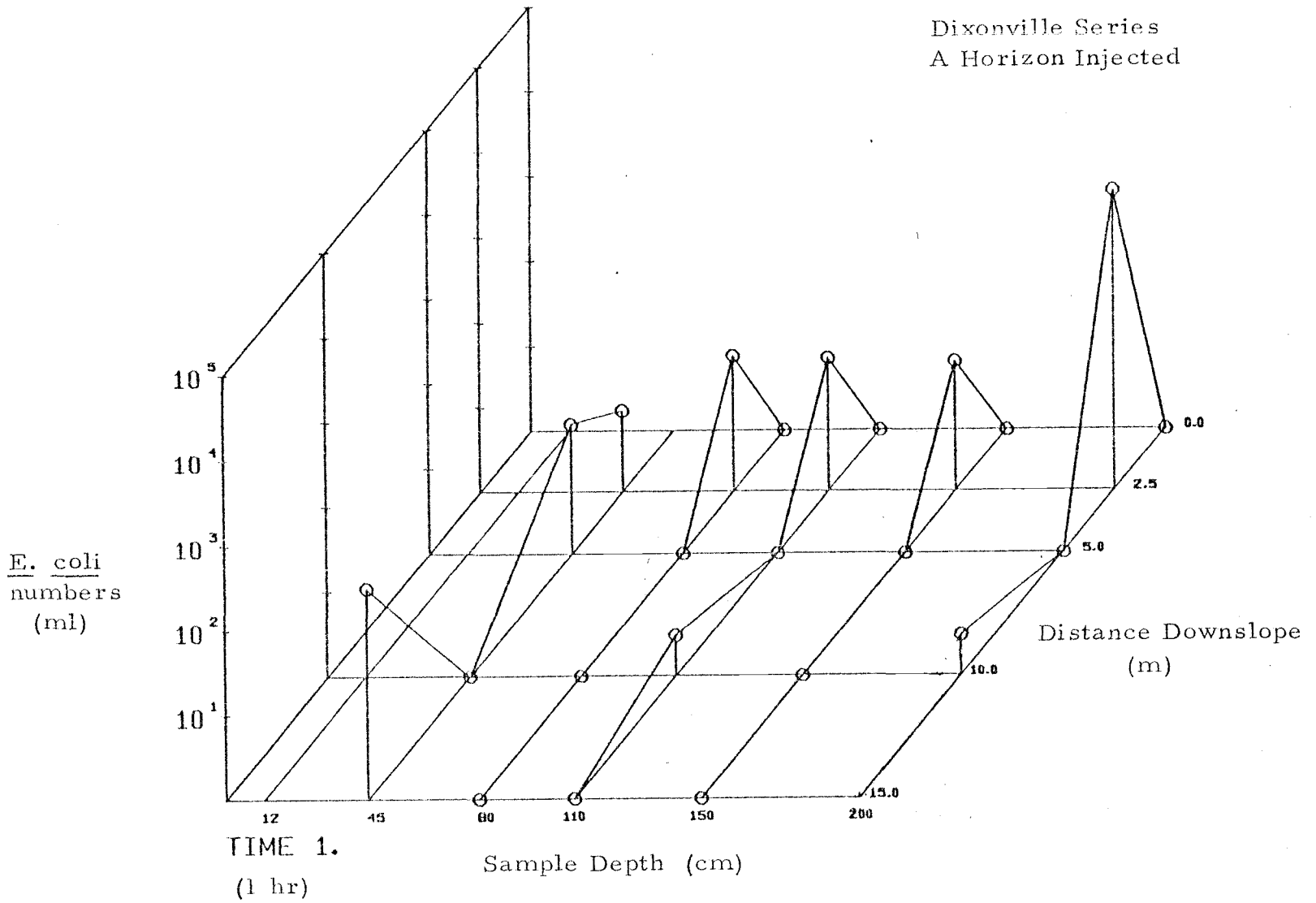

Figure 5.11. Organism movement at Dixonville site, A horizon, 1 hour.

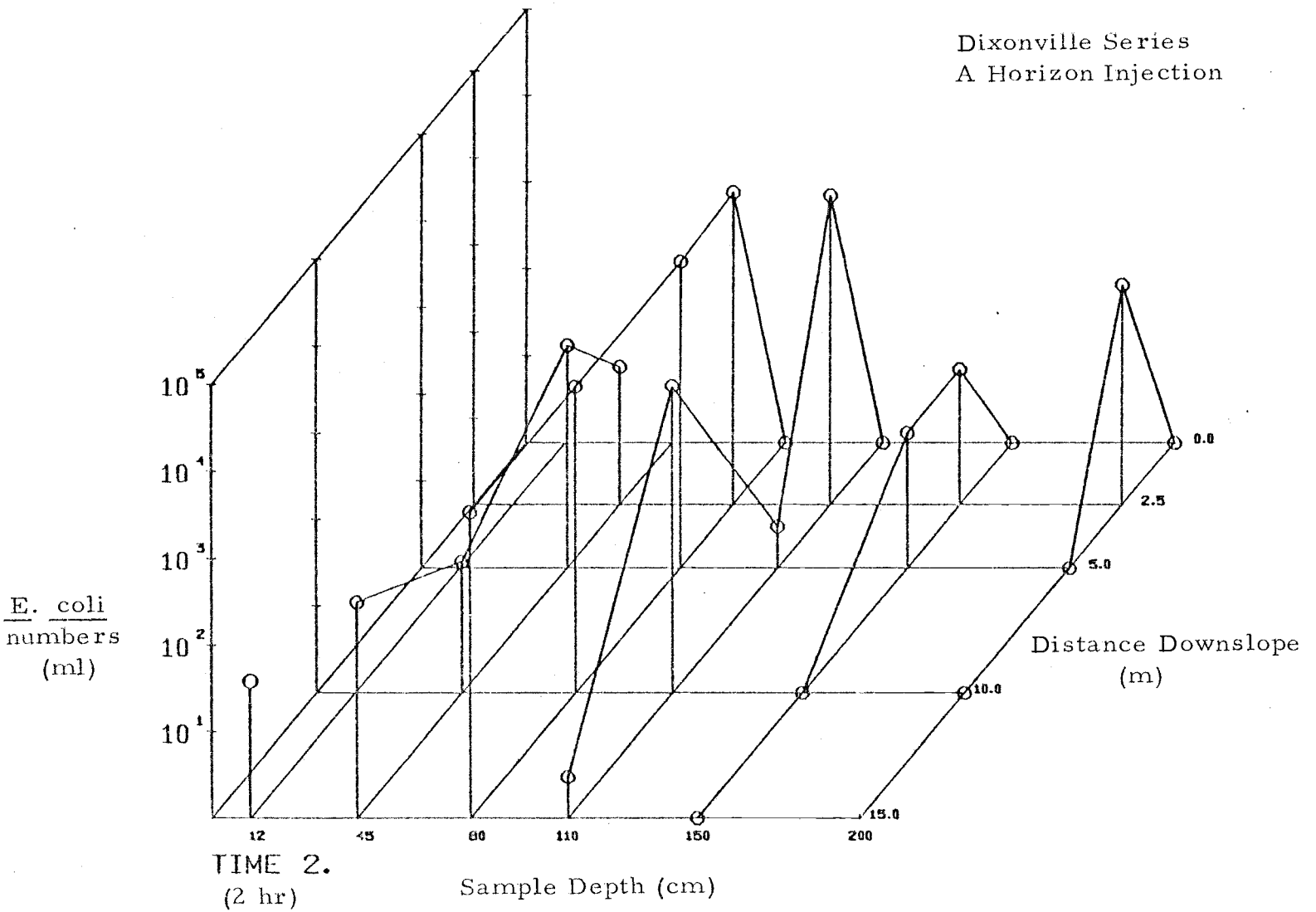

Figure 5.12. Organism movement at Dixonville site, A horizon, 2 hour.

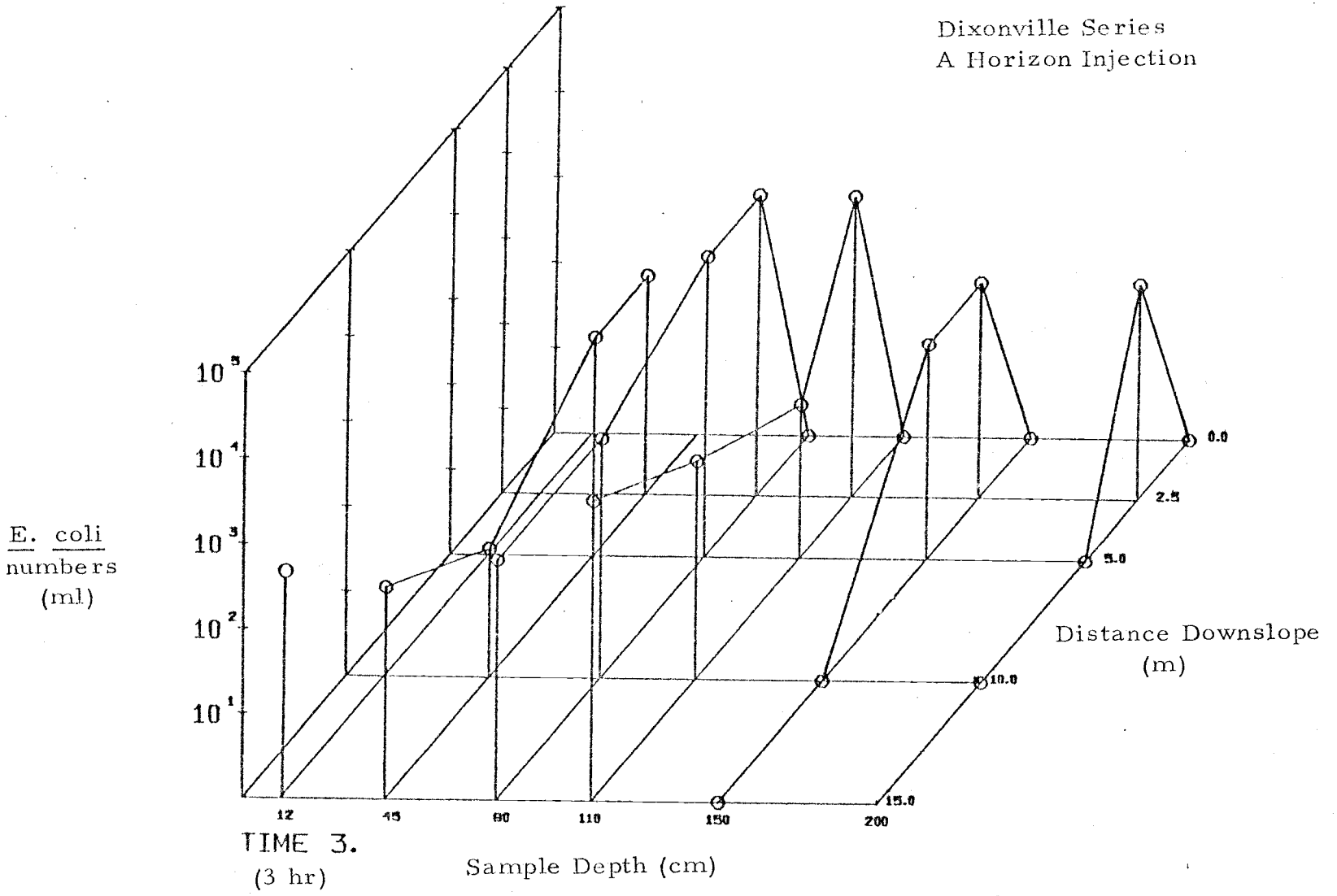

Figure 5.13. Organism movement at Dixonville site, A horizon, 3 hour.

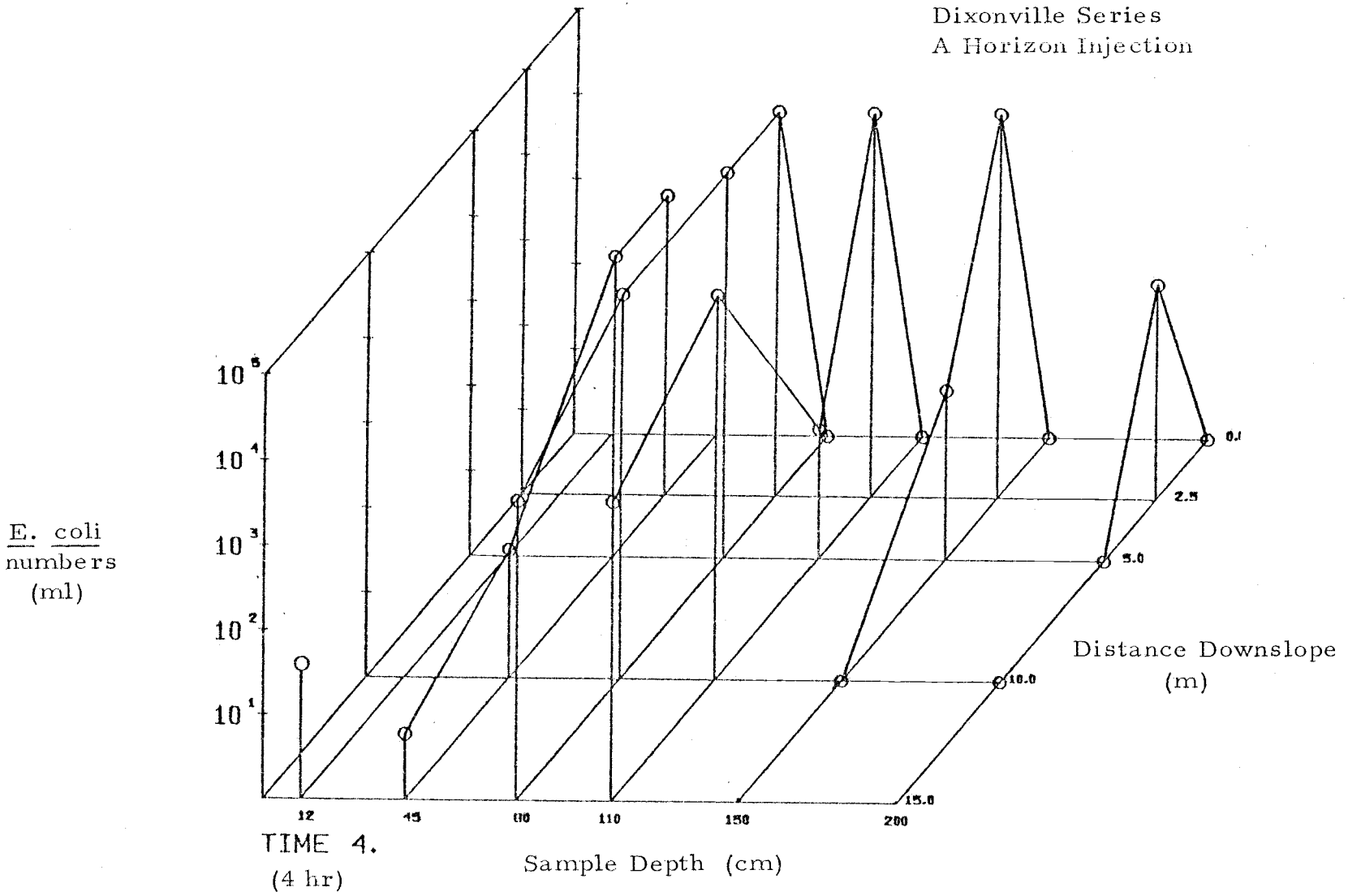

Figure 5.14. Organism movement at Dixonville site, A horizon, 4 hour.

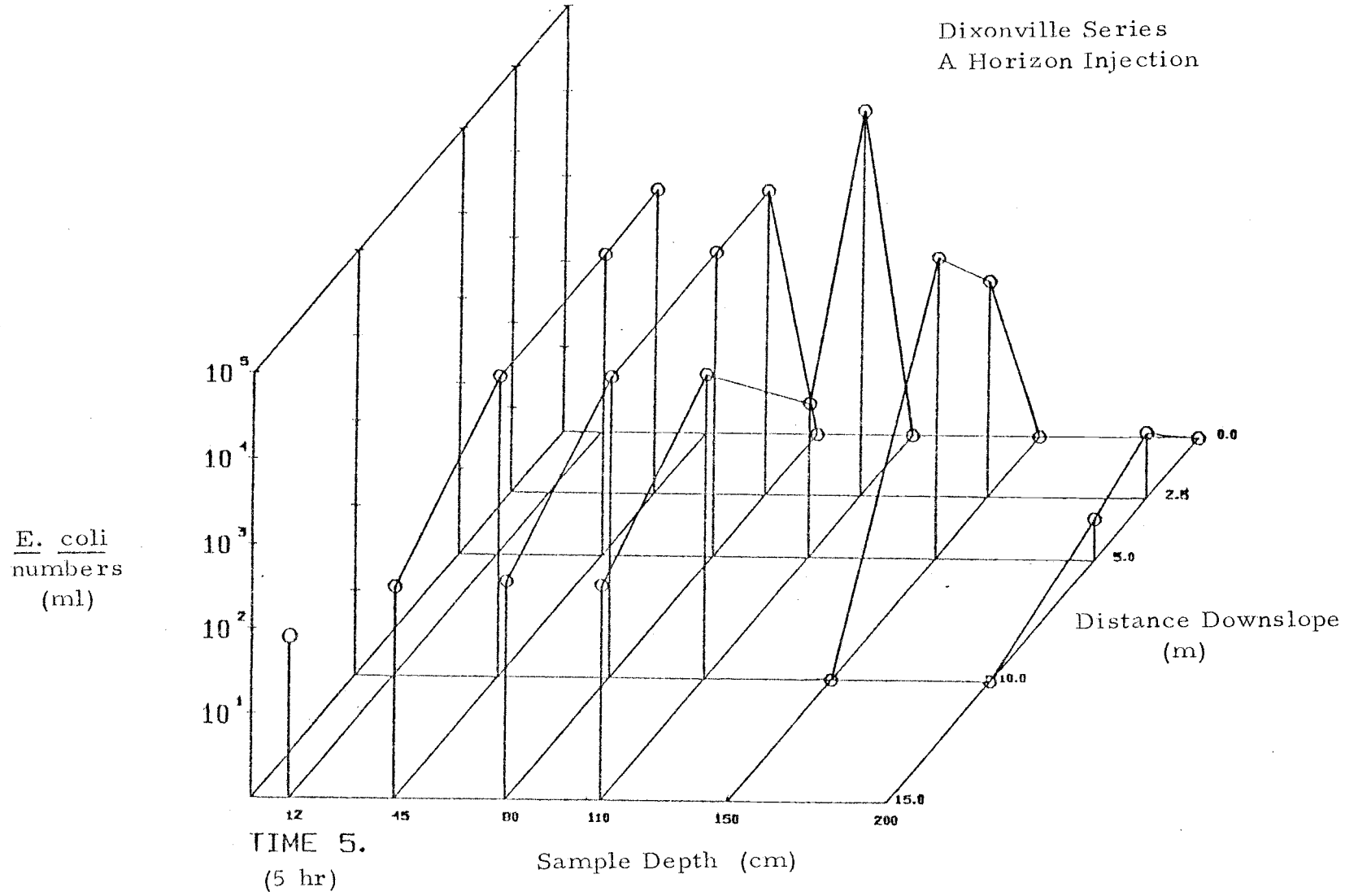

Figure 5.15. Organism movement at Dixonville site, A horizon, 5 hour.

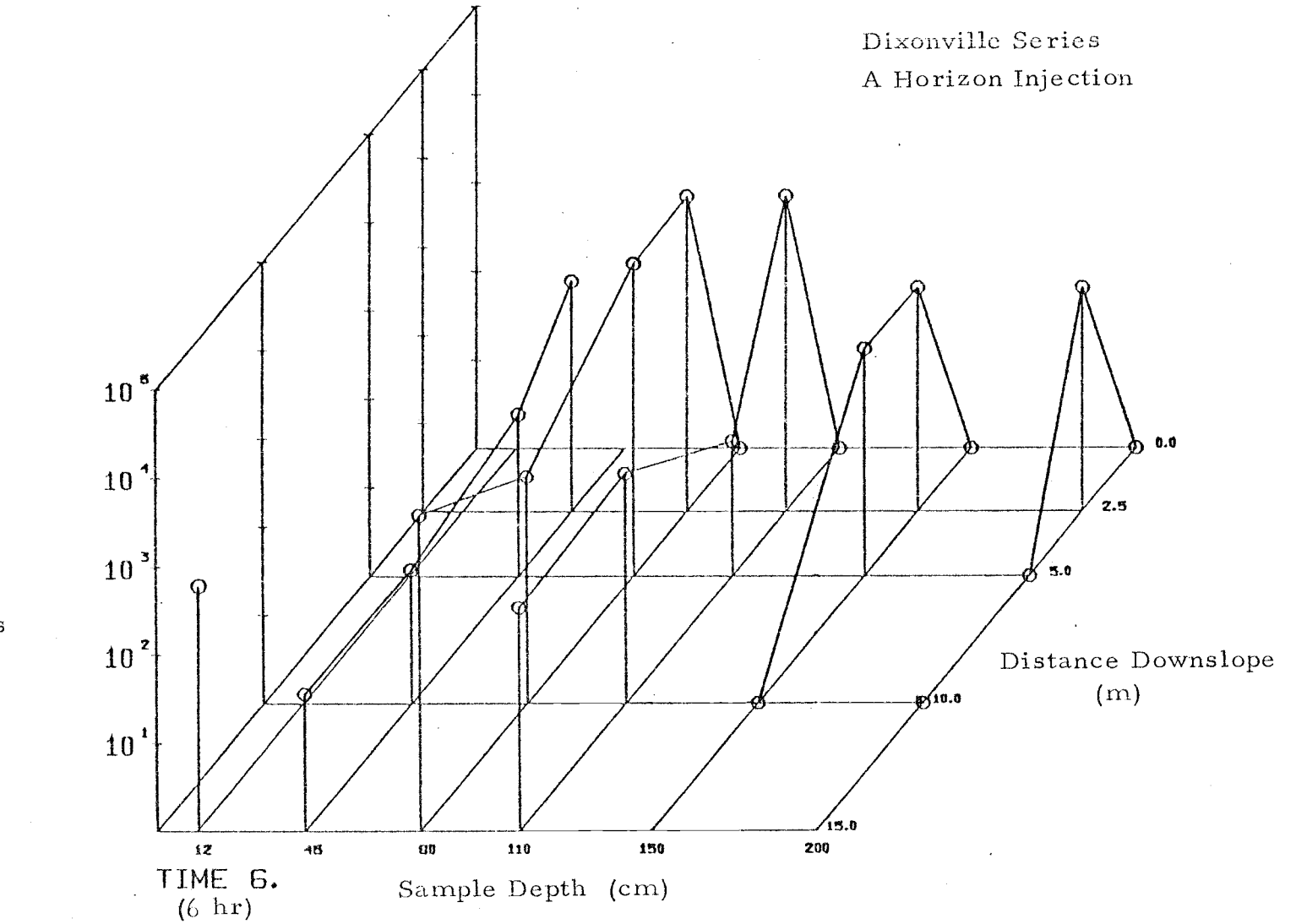

Figure 5.16. Organism movement at Dixonville site, A horizon, 6 hour.

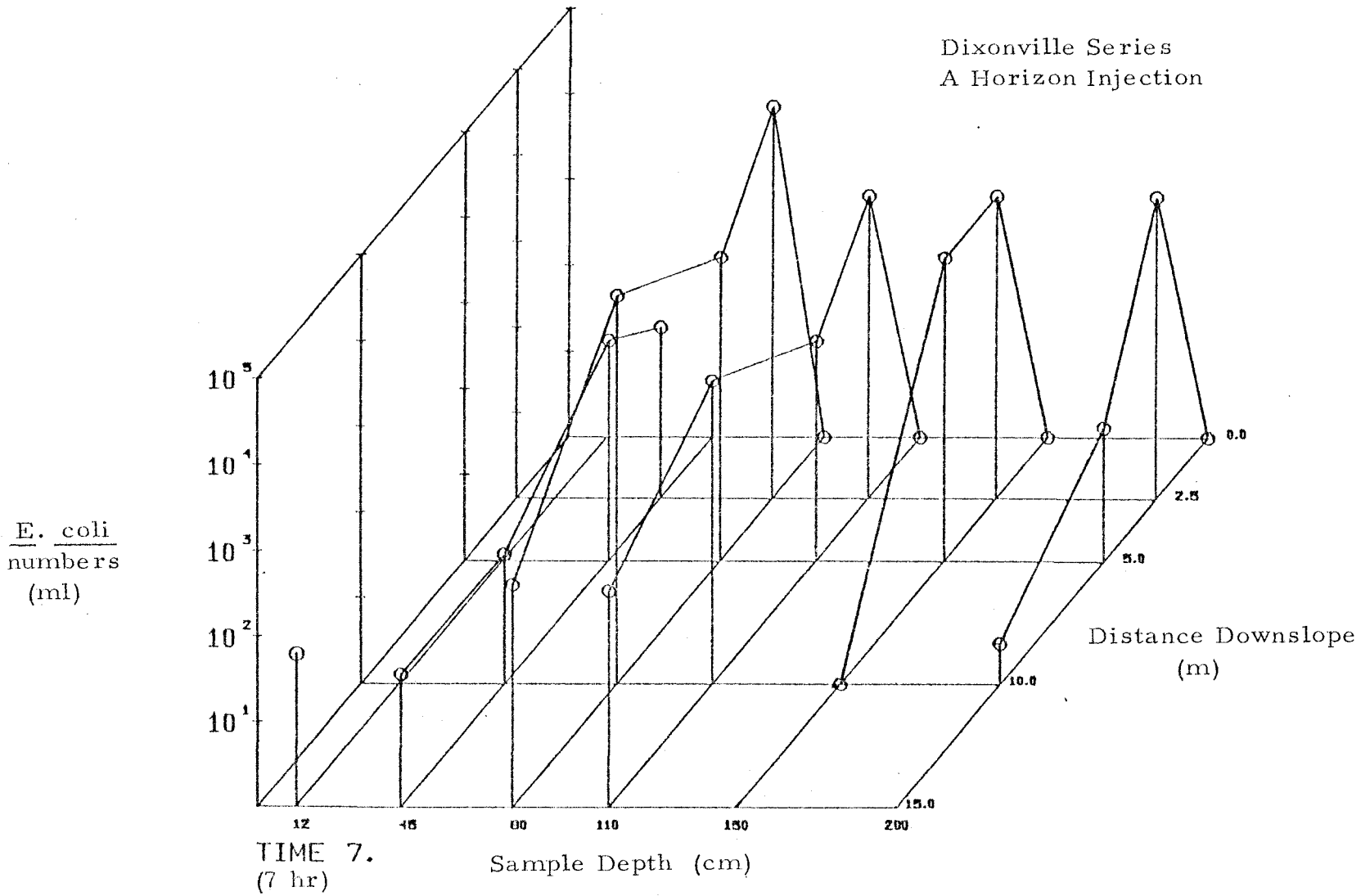

Figure 5.17. Organism movement at Dixonville site, A horizon, 7 hour.

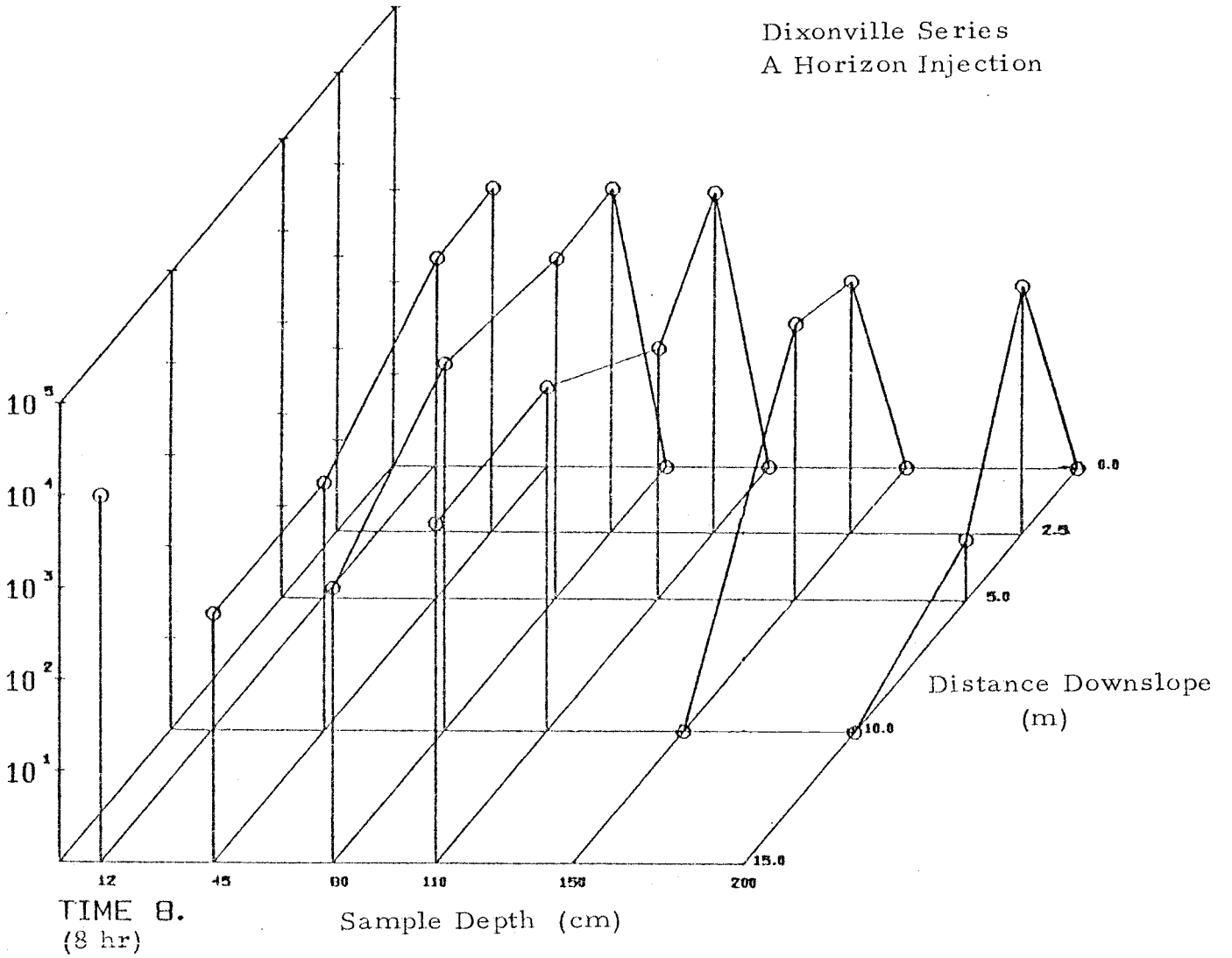

Figure 5.18. Organism movement at Dixonville site, A horizon, 8 hour.

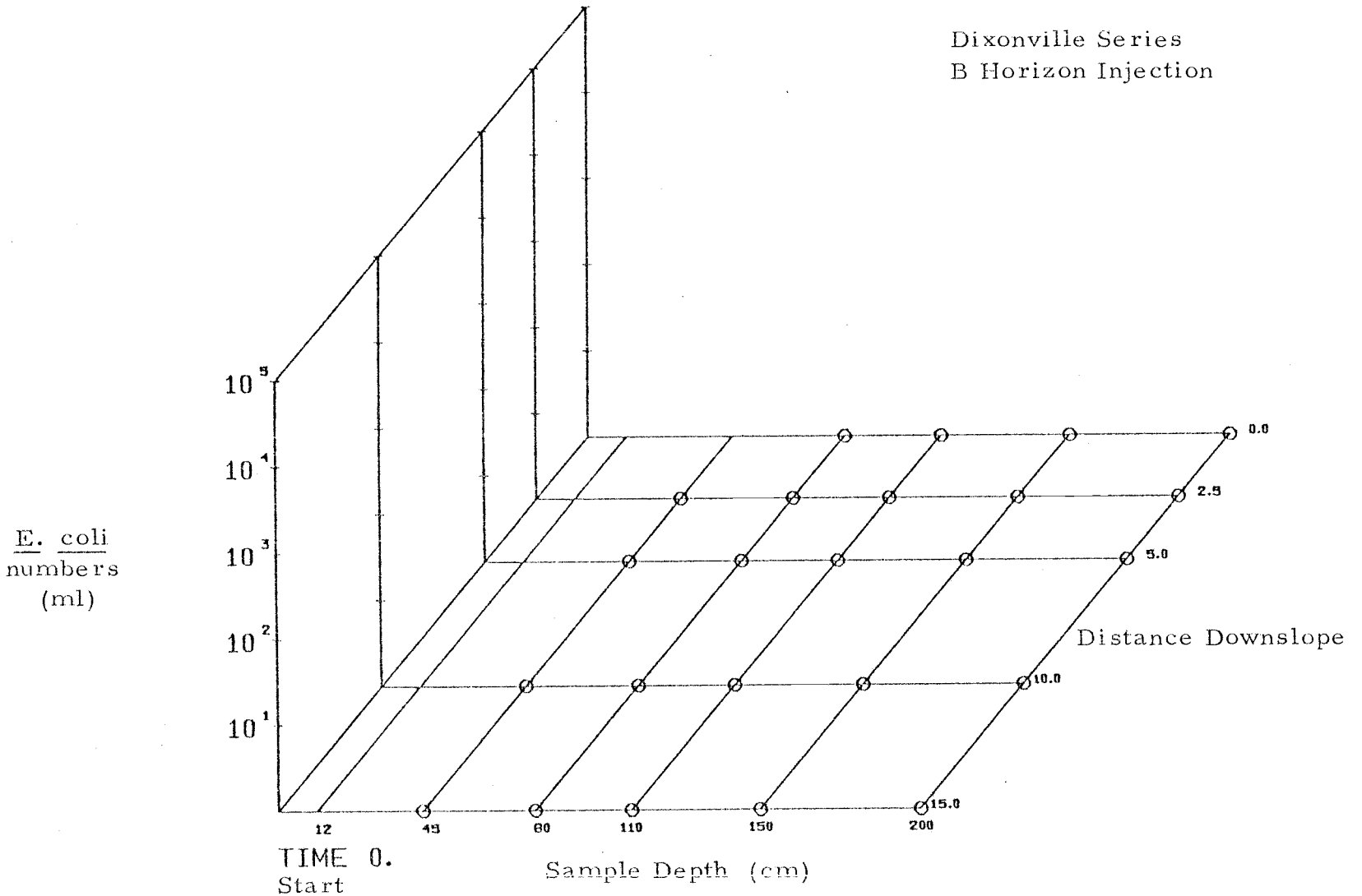

Figure 5.20. Organism movement at Dixonville site, B horizon, pre-injection.

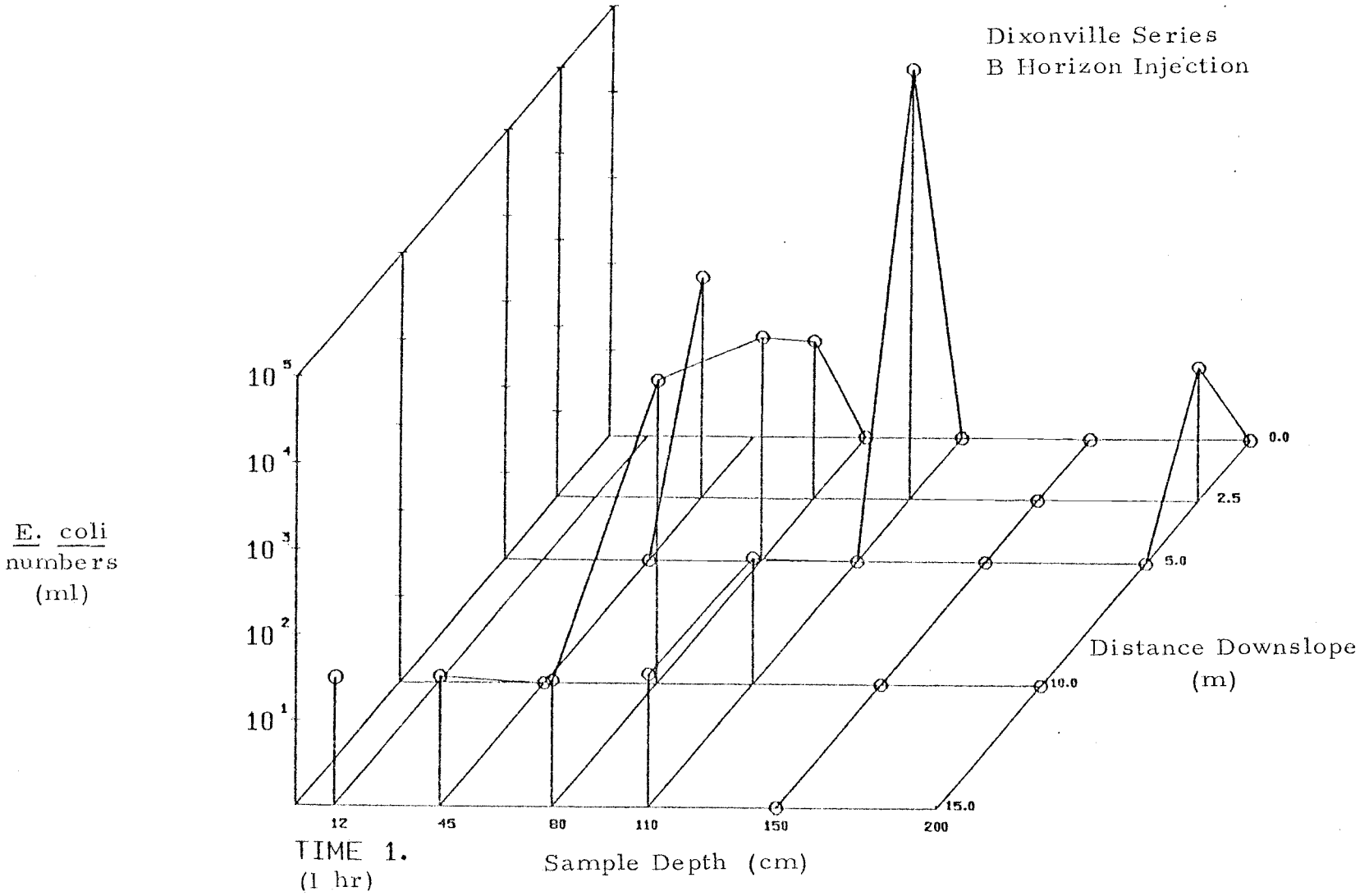

Figure 5.21. Organism movement at Dixonville site, B horizon, 1 hour.

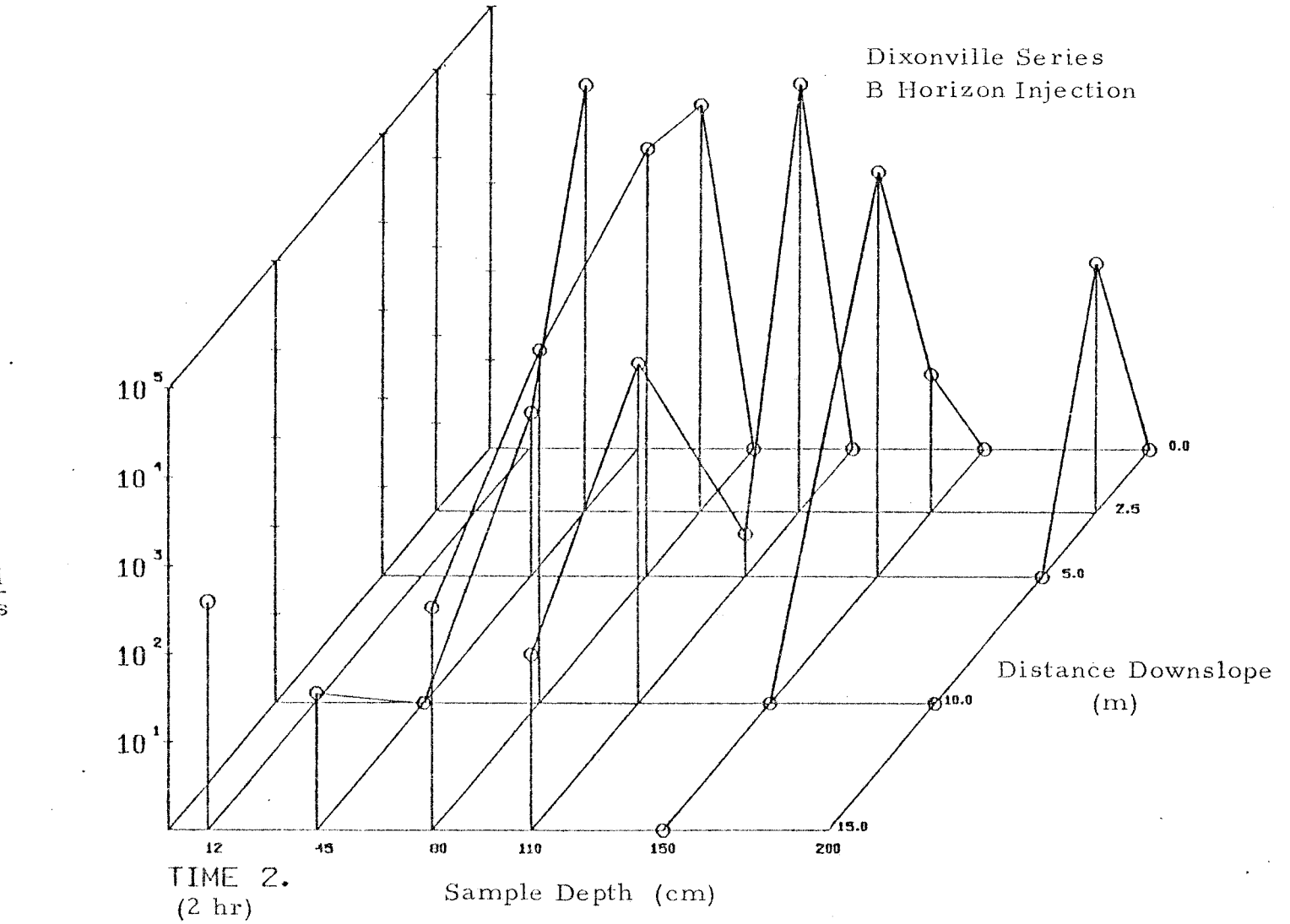

Figure 5.22. Organism movement at Dixonville site, B horizon, 2 hour.

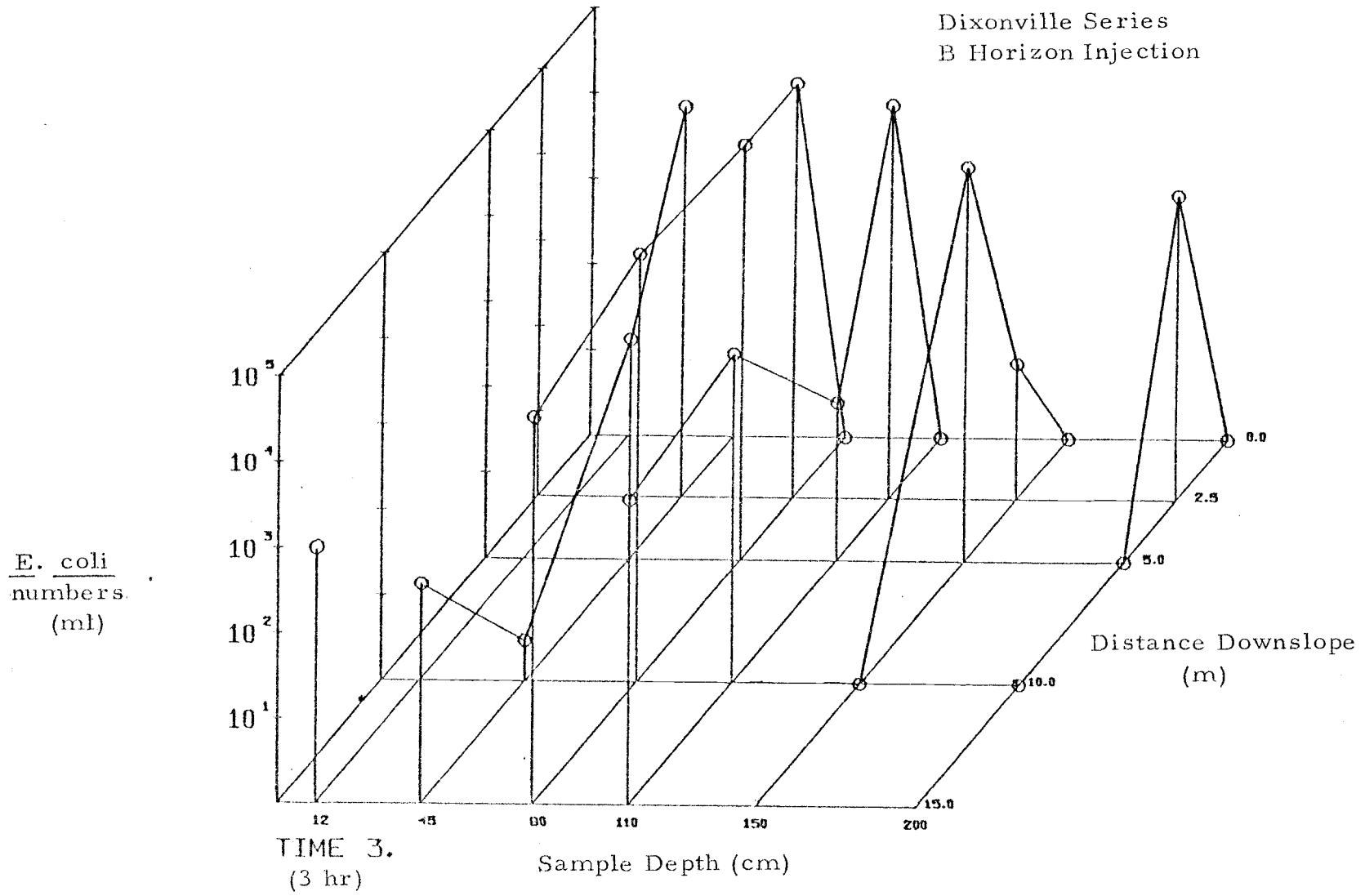

Figure 5.23. Organism movement at Dixonville site, B horizon, 3 hour.

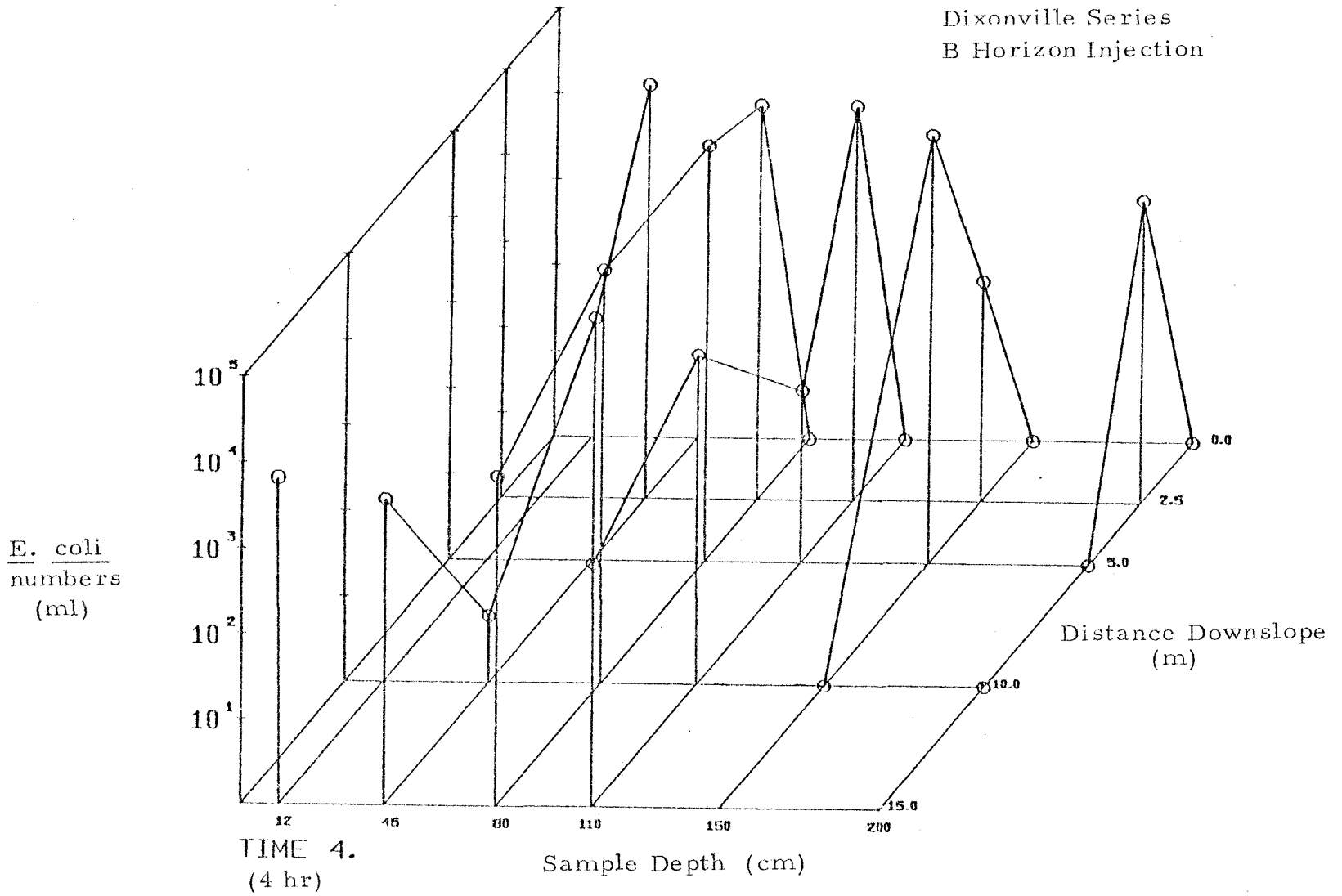

Figure 5.24. Organism movement at Dixonville site, B horizon, 4 hour.

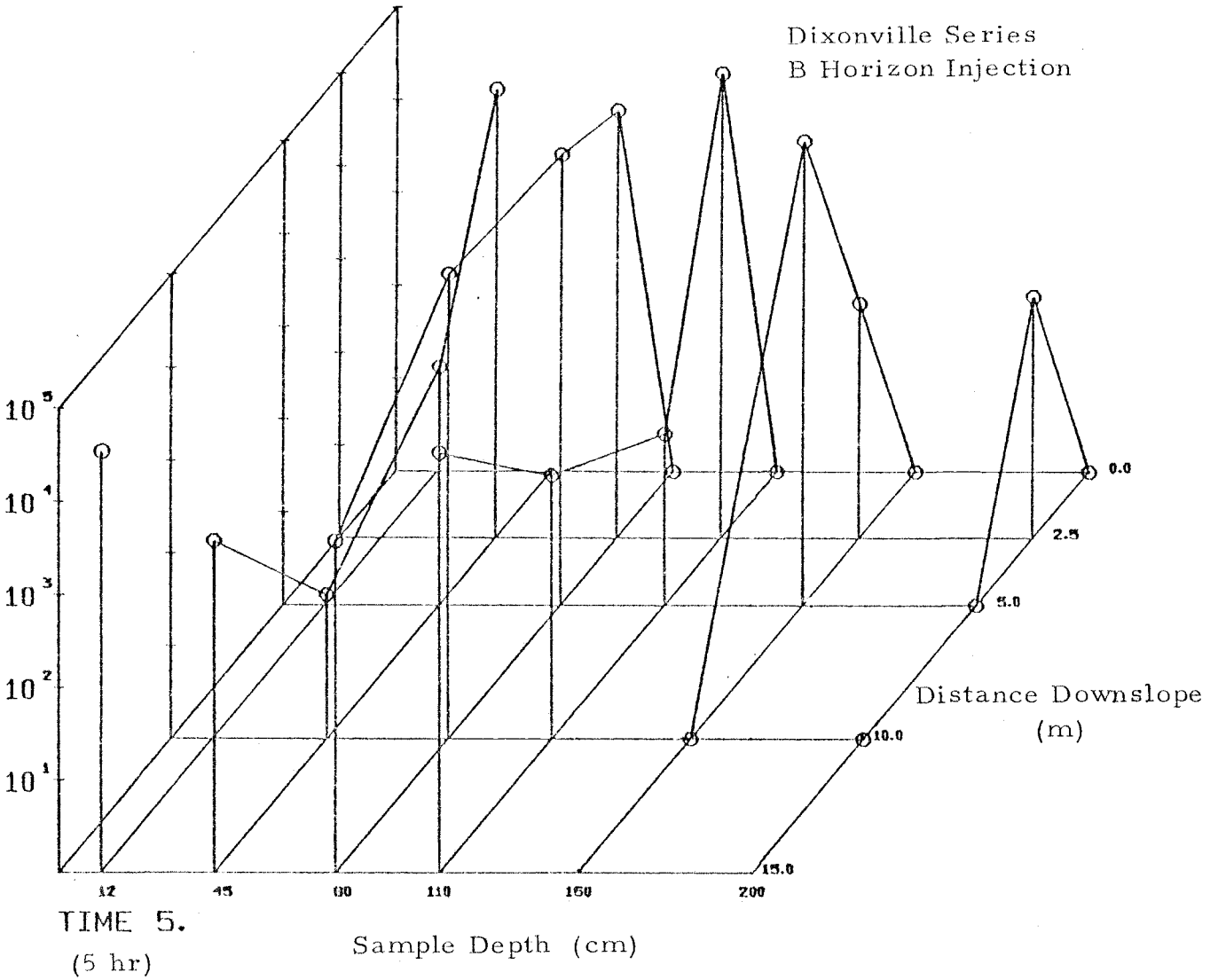

Figure 5.25. Organism movement at Dixonville site, B horizon, 5 hour.

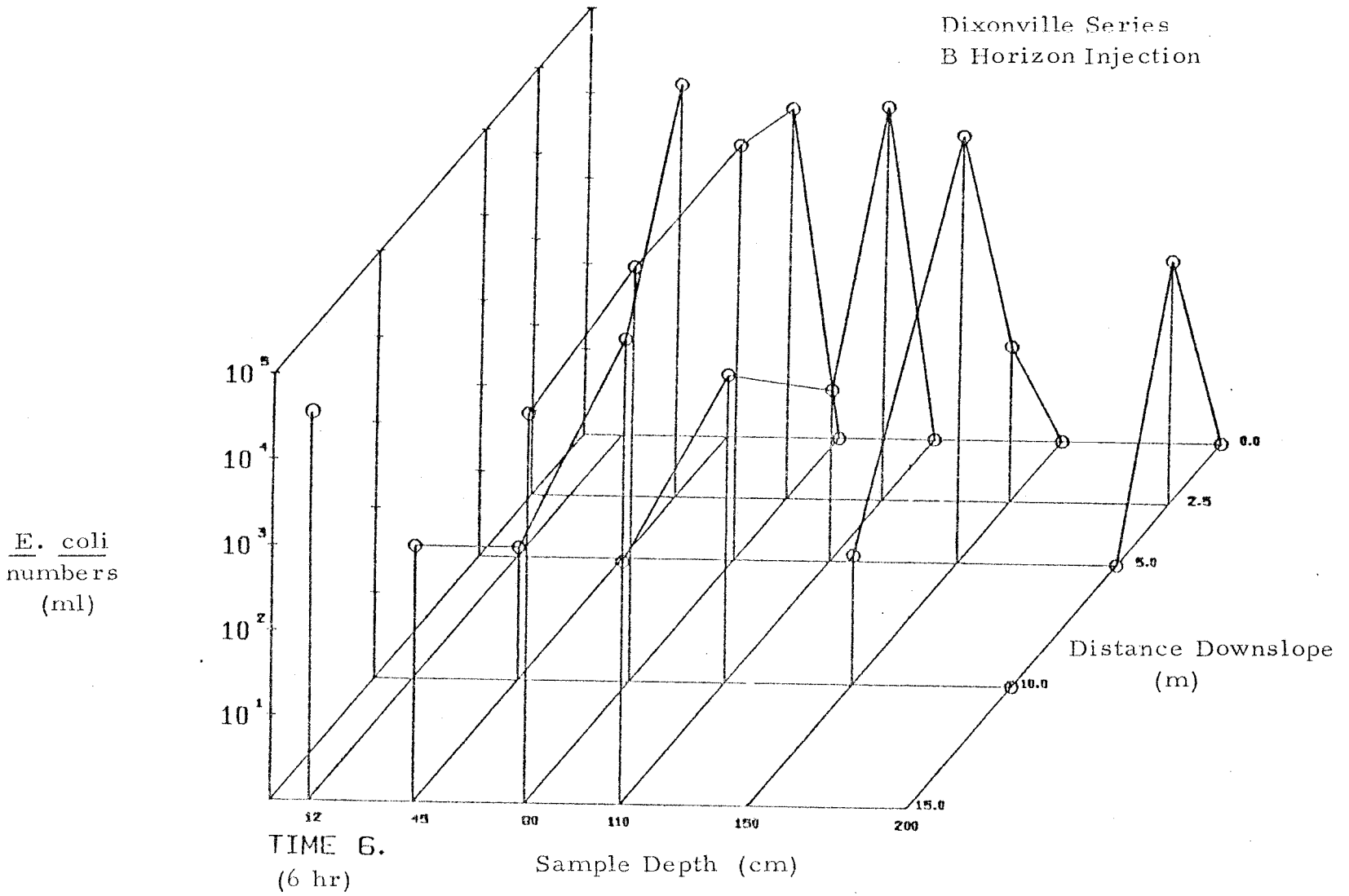

Figure 5.26. Organism movement at Dixonville site, B horizon, 6 hour.

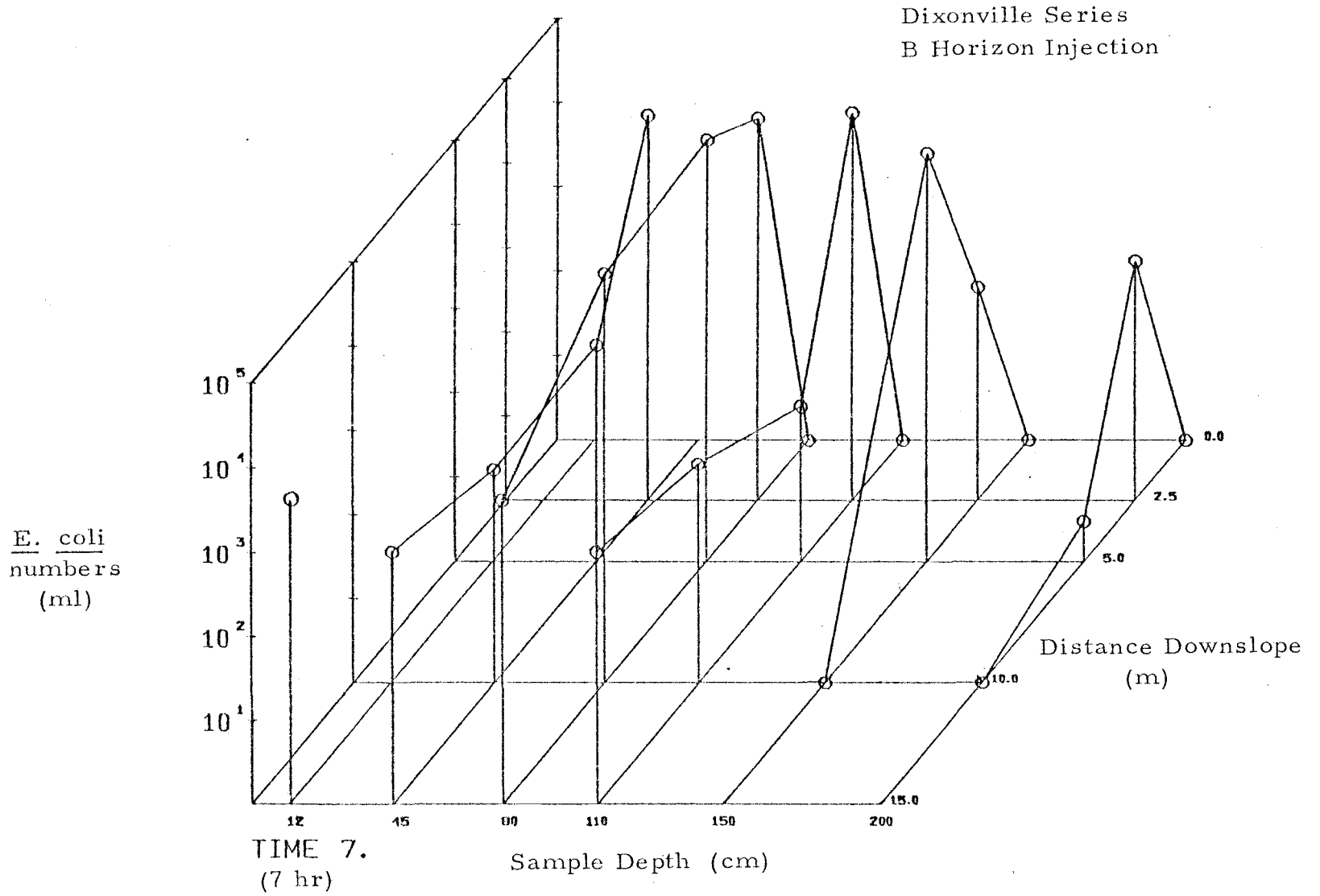

Figure 5.27. Organism movement at Dixonville site, B horizon, 7 hour.

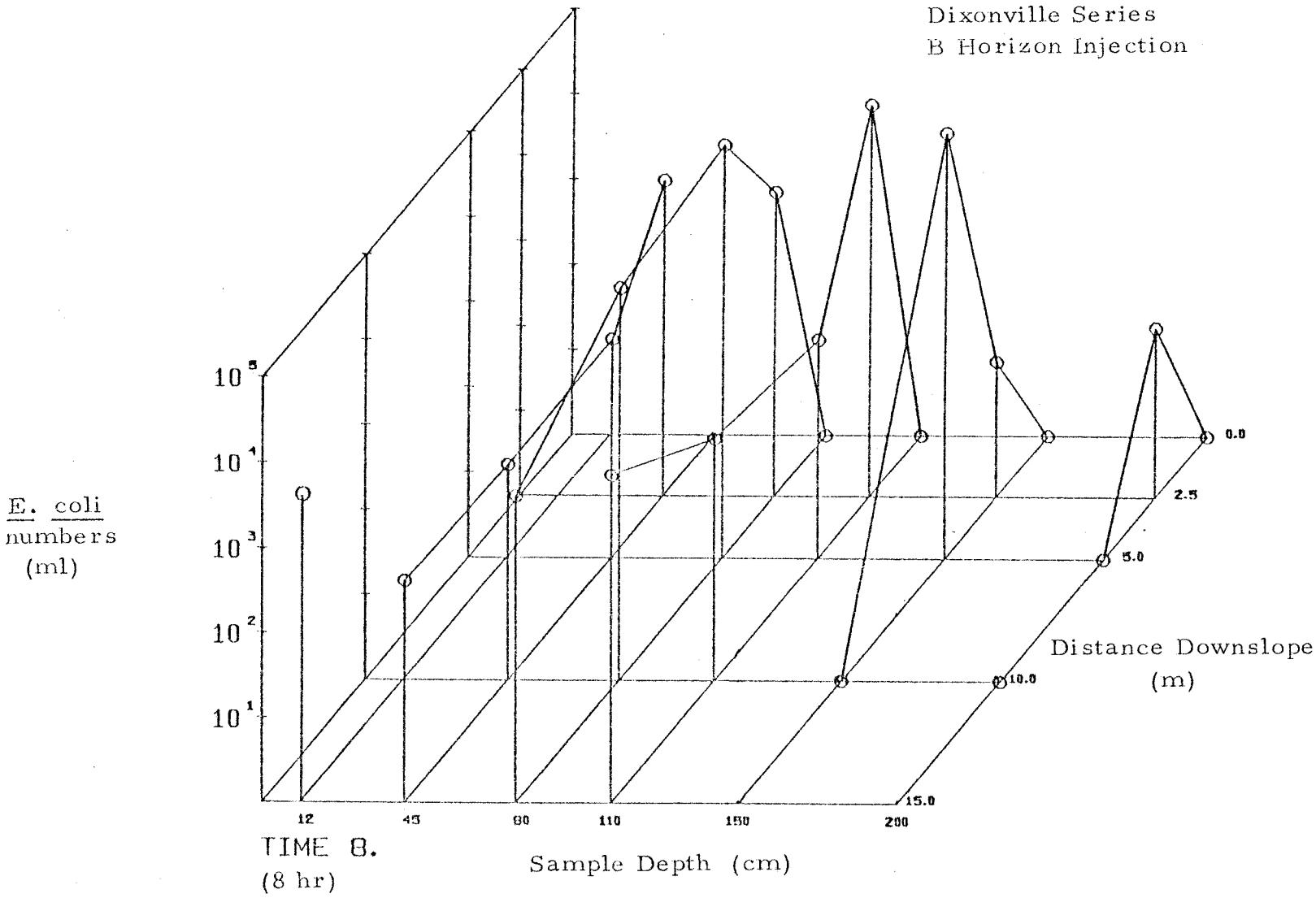

Figure 5.28. Organism movement at Dixonville site, B horizon, 8 hour.

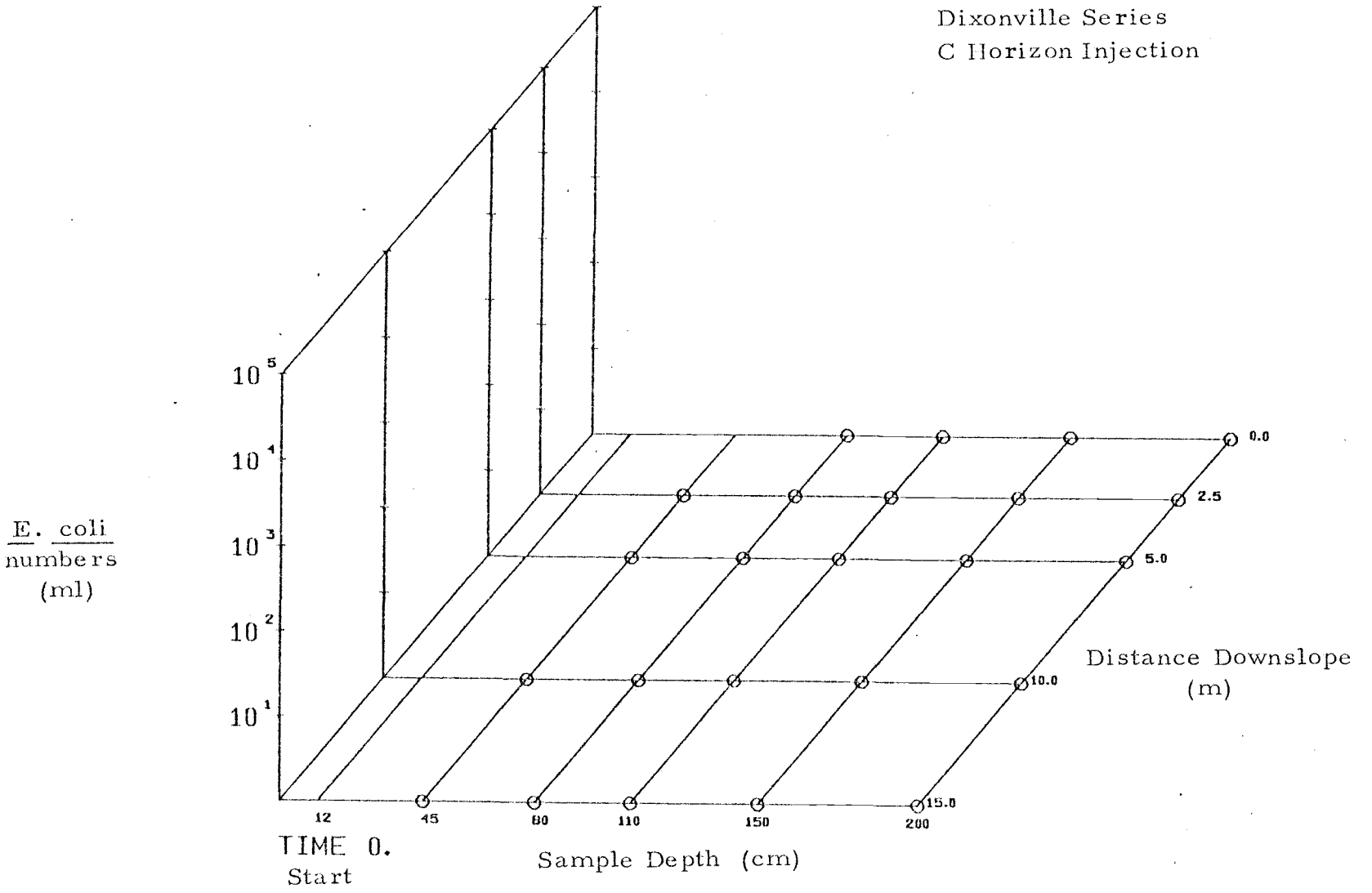

Figure 5.30. Organism movement at Dixonville site, C horizon injection, pre-injection.

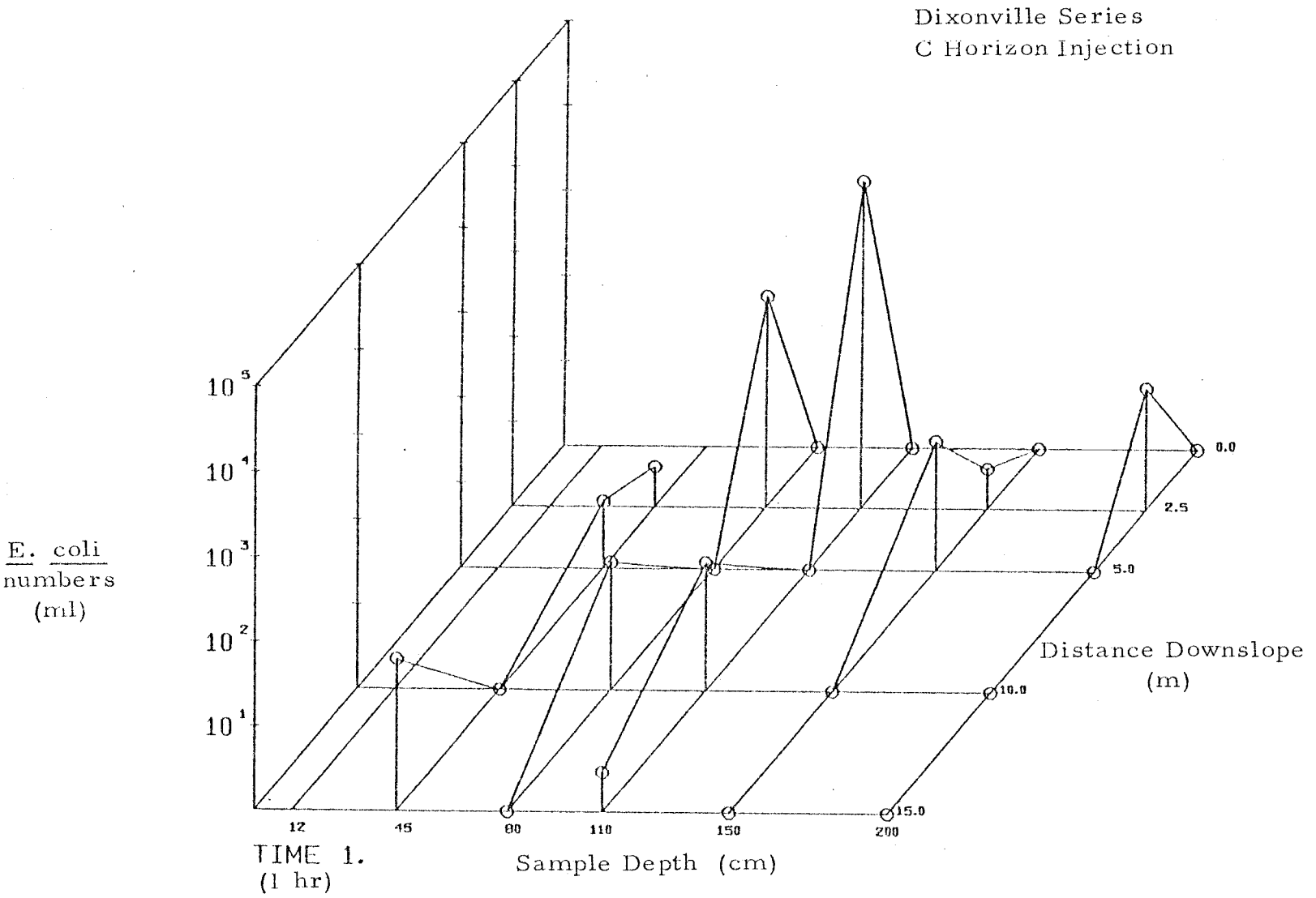

Figure 5.31. Organism movement at Dixonville Site, C horizon injection, 1 hour.

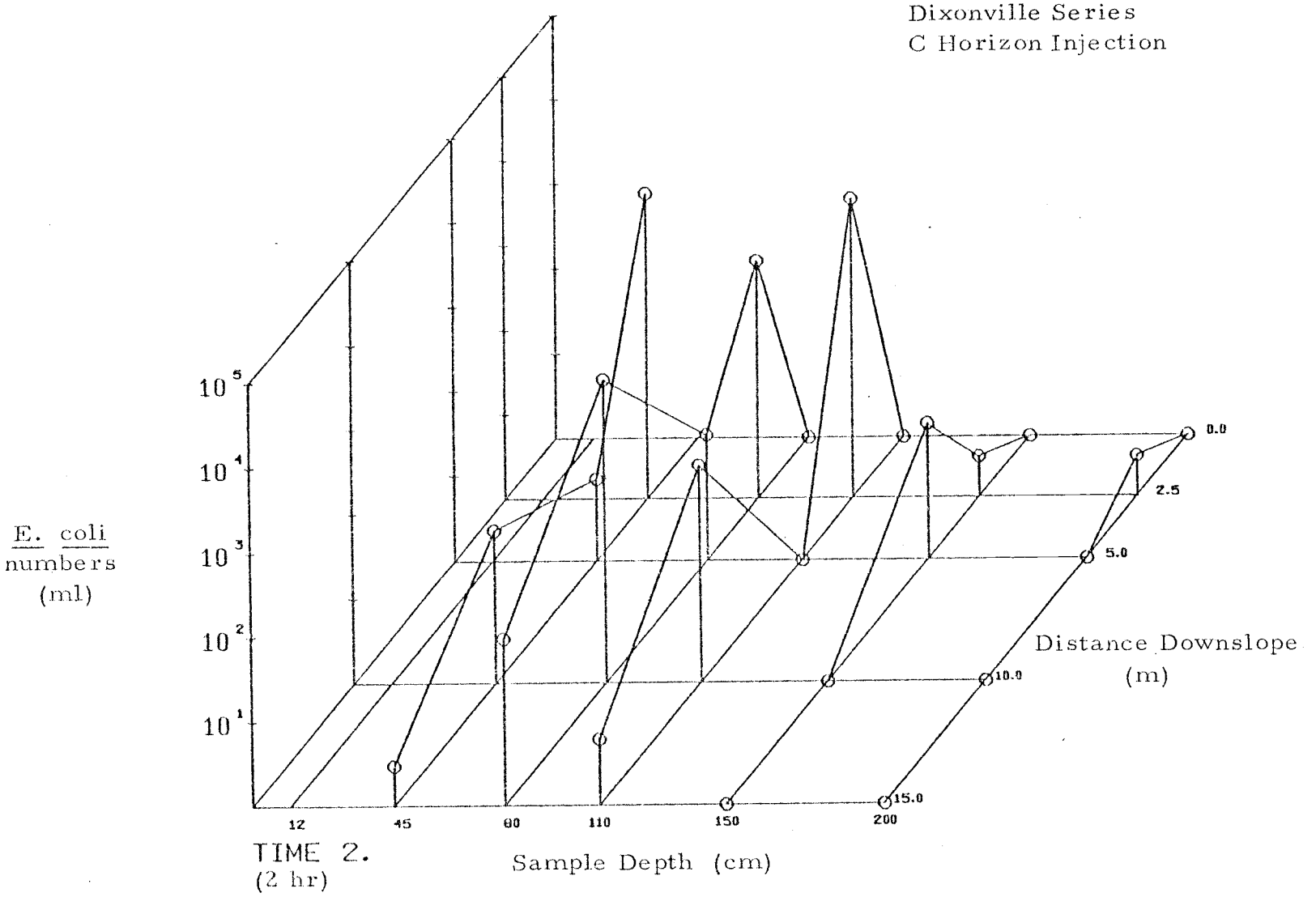

Figure 5.32. Organism movement at Dixonville site, C horizon injection, 2 hour.

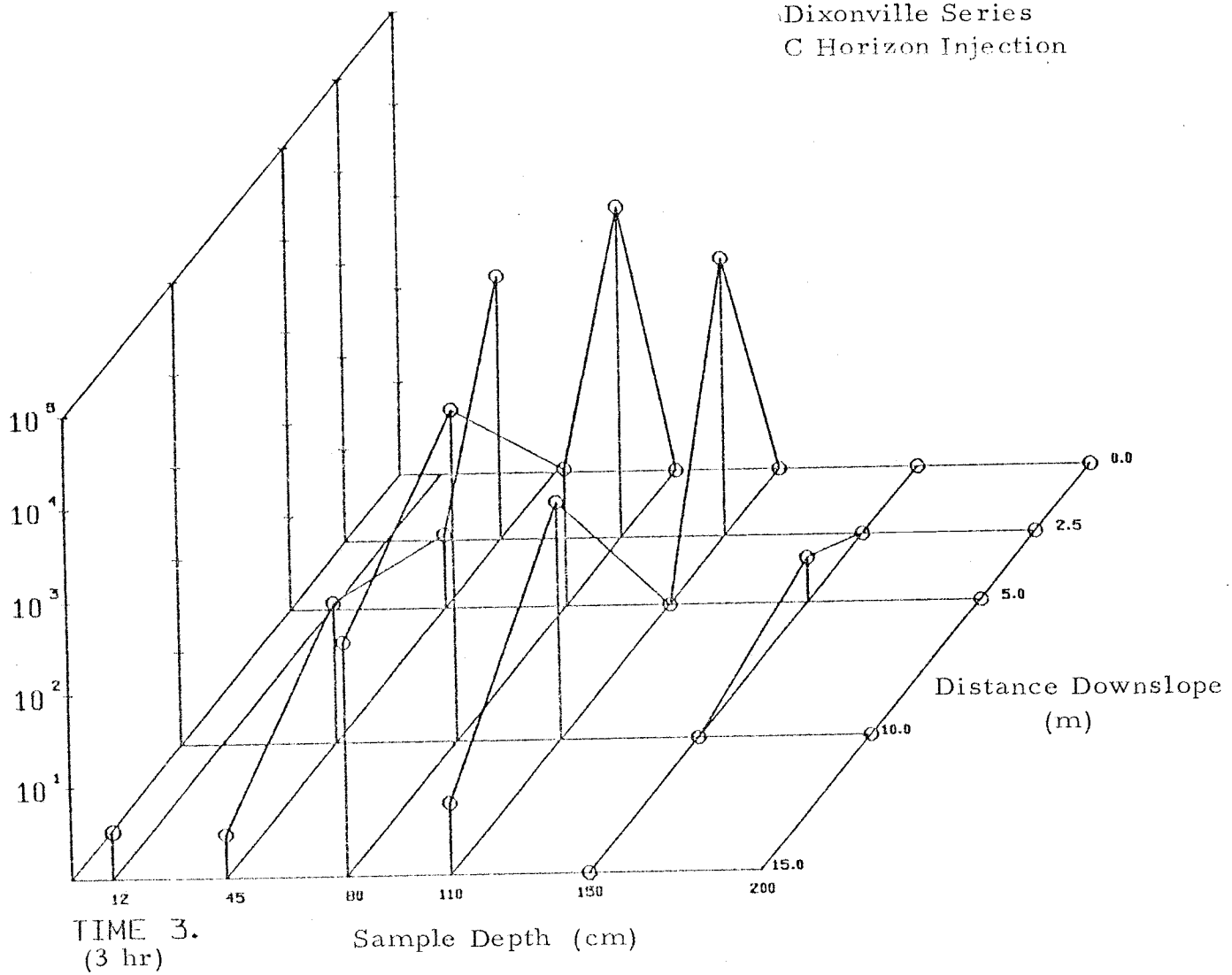

Figure 5.33. Organism movement at Dixonville site, C horizon injection, 3 hour.

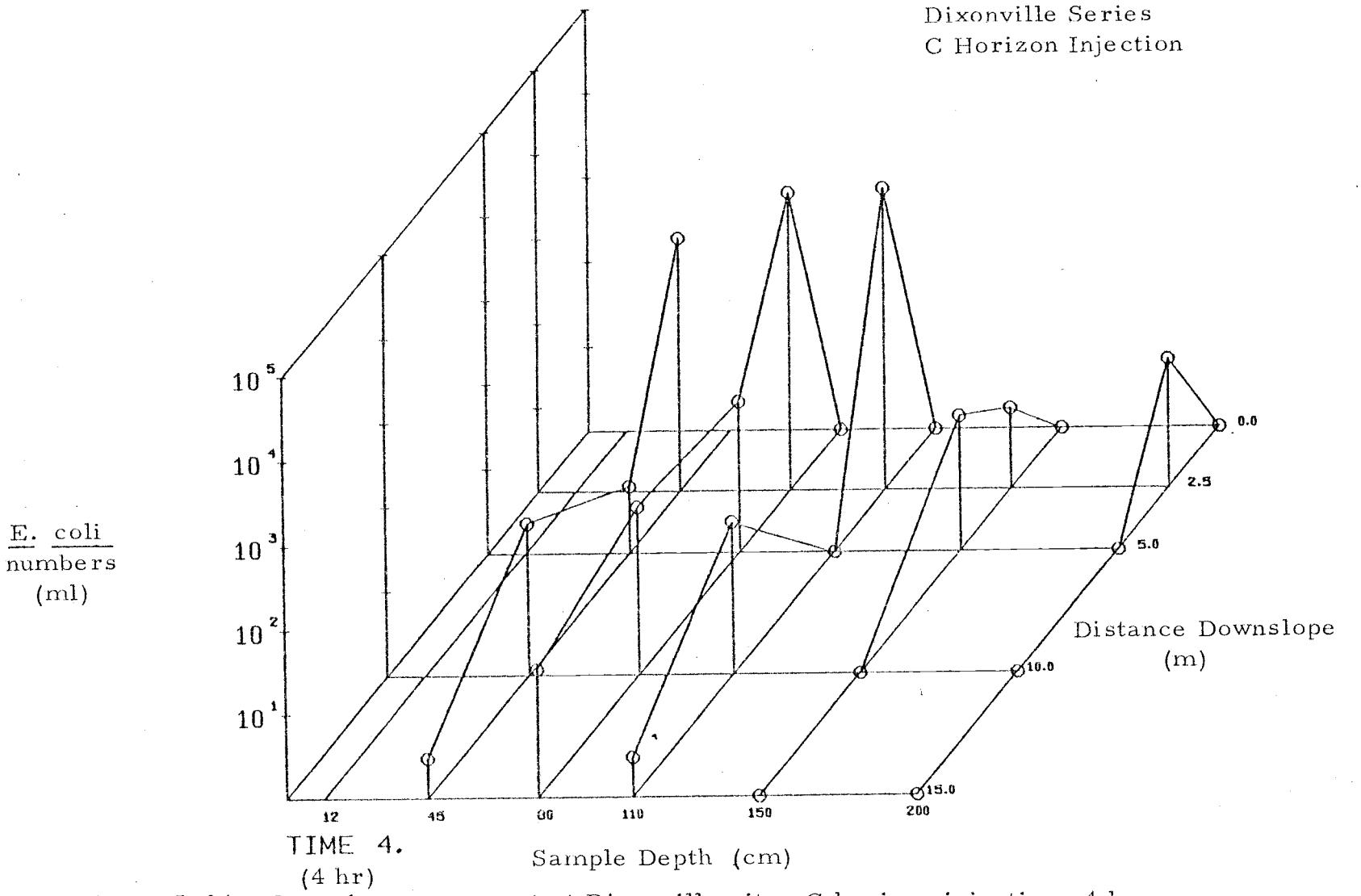

Figure 5.34. Organism movement at Dixonville site, C horizon injection, 4 hour.

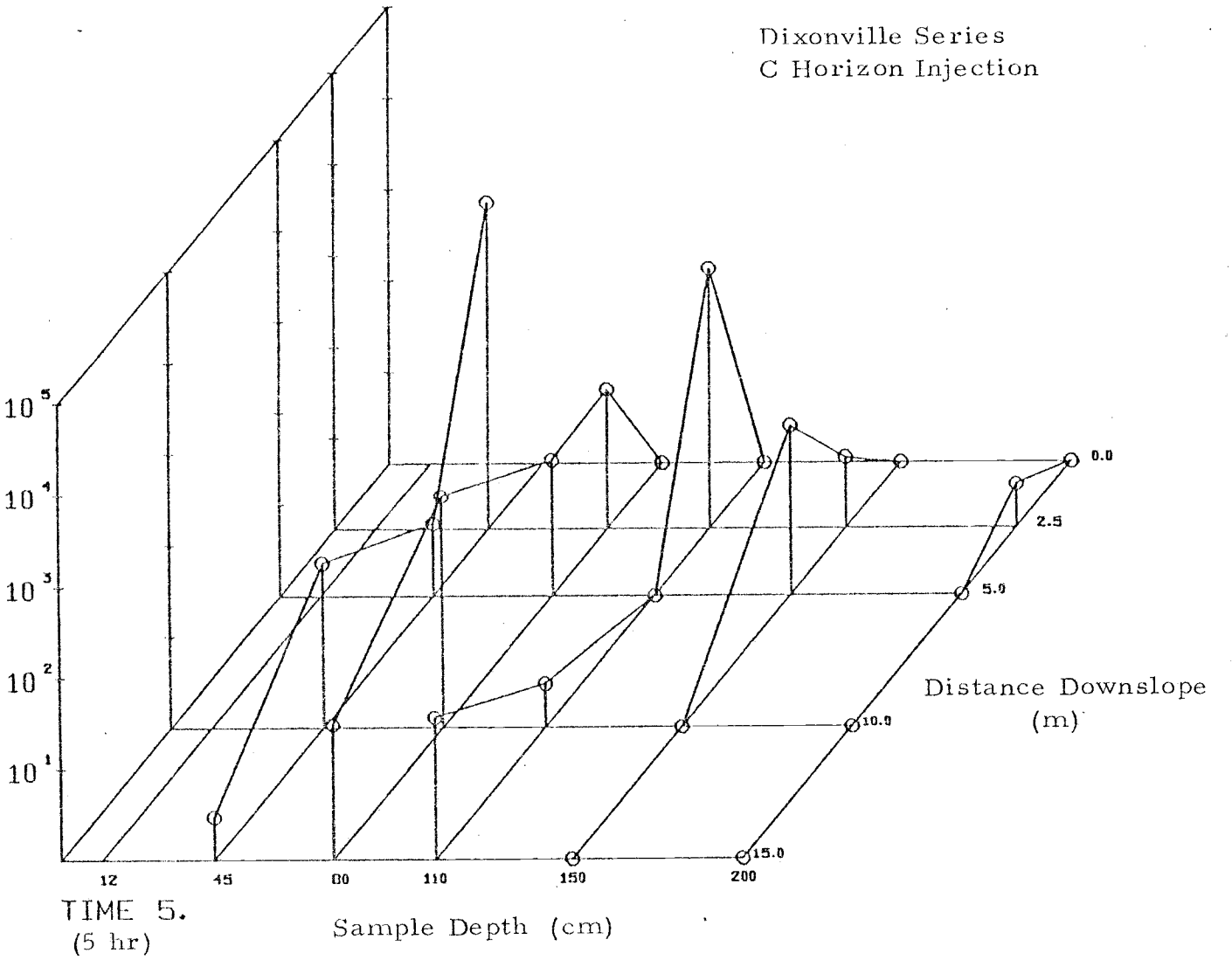

Figure 5.35. Organism movement at Dixonville site, C horizon injection, 5 hour.

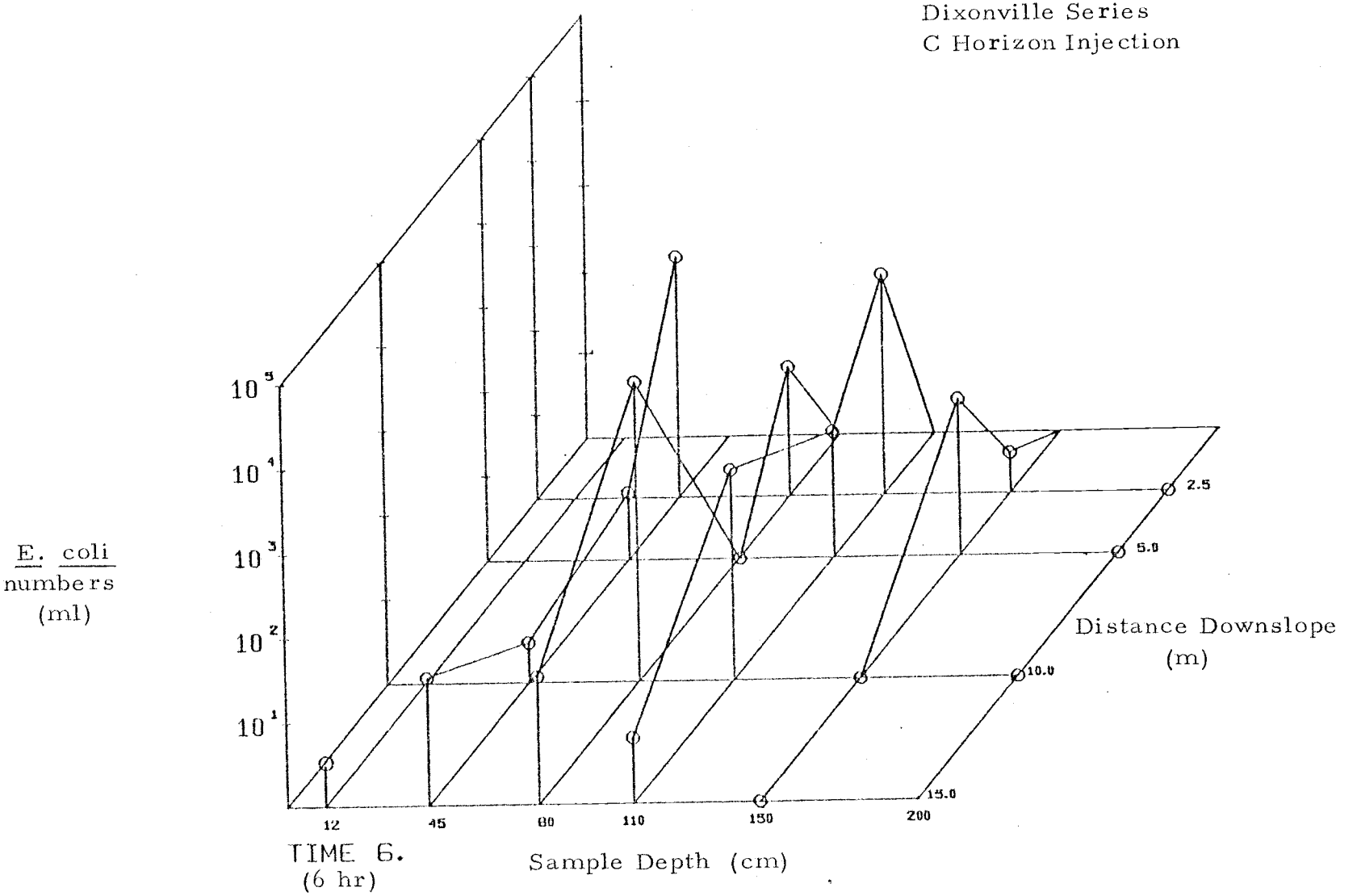

Figure 5.36. Organism movement at Dixonville site, C horizon injection, 6 hour.

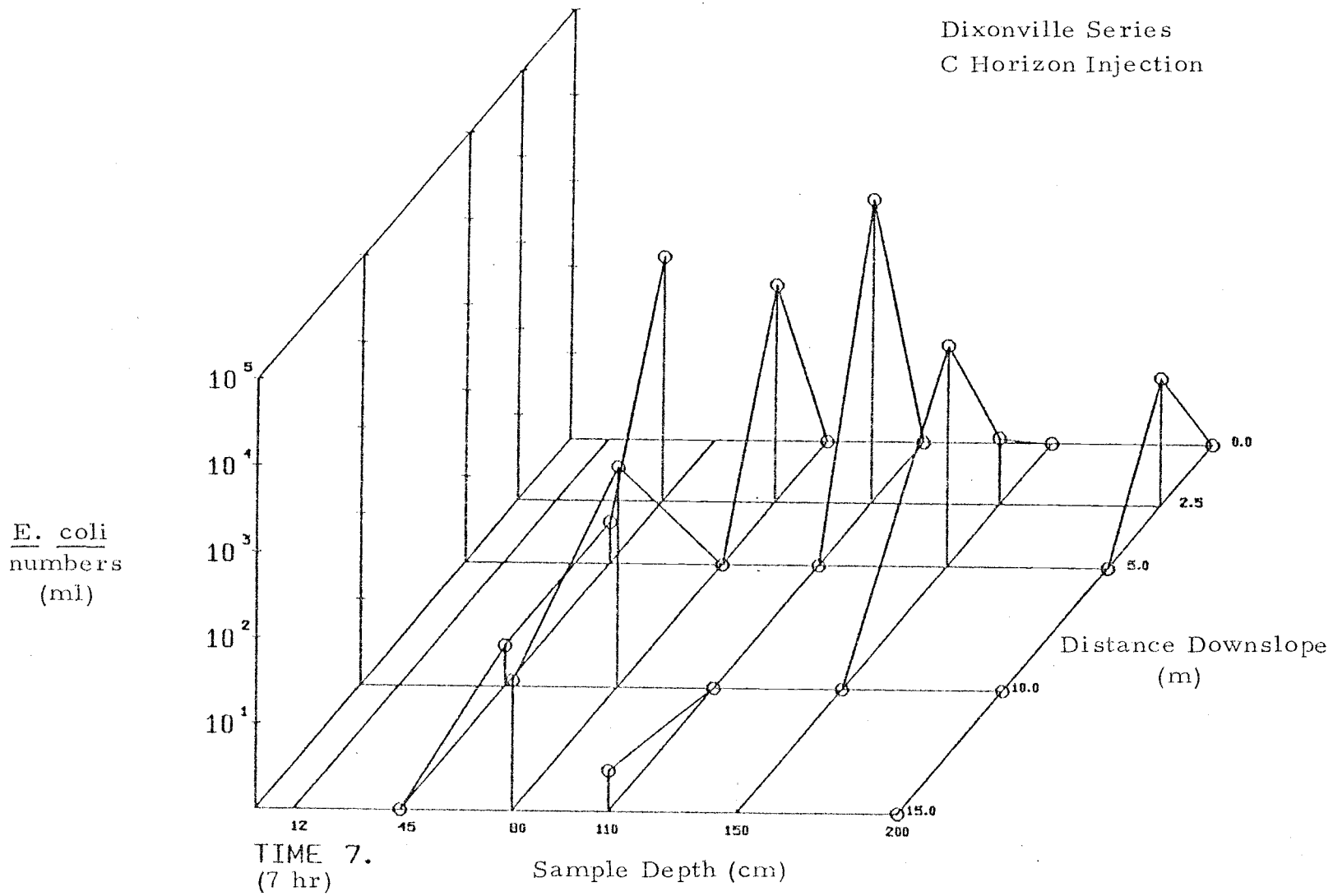

Figure 5.37. Organism movement at Dixonville site, C horizon injection, 7 hour.

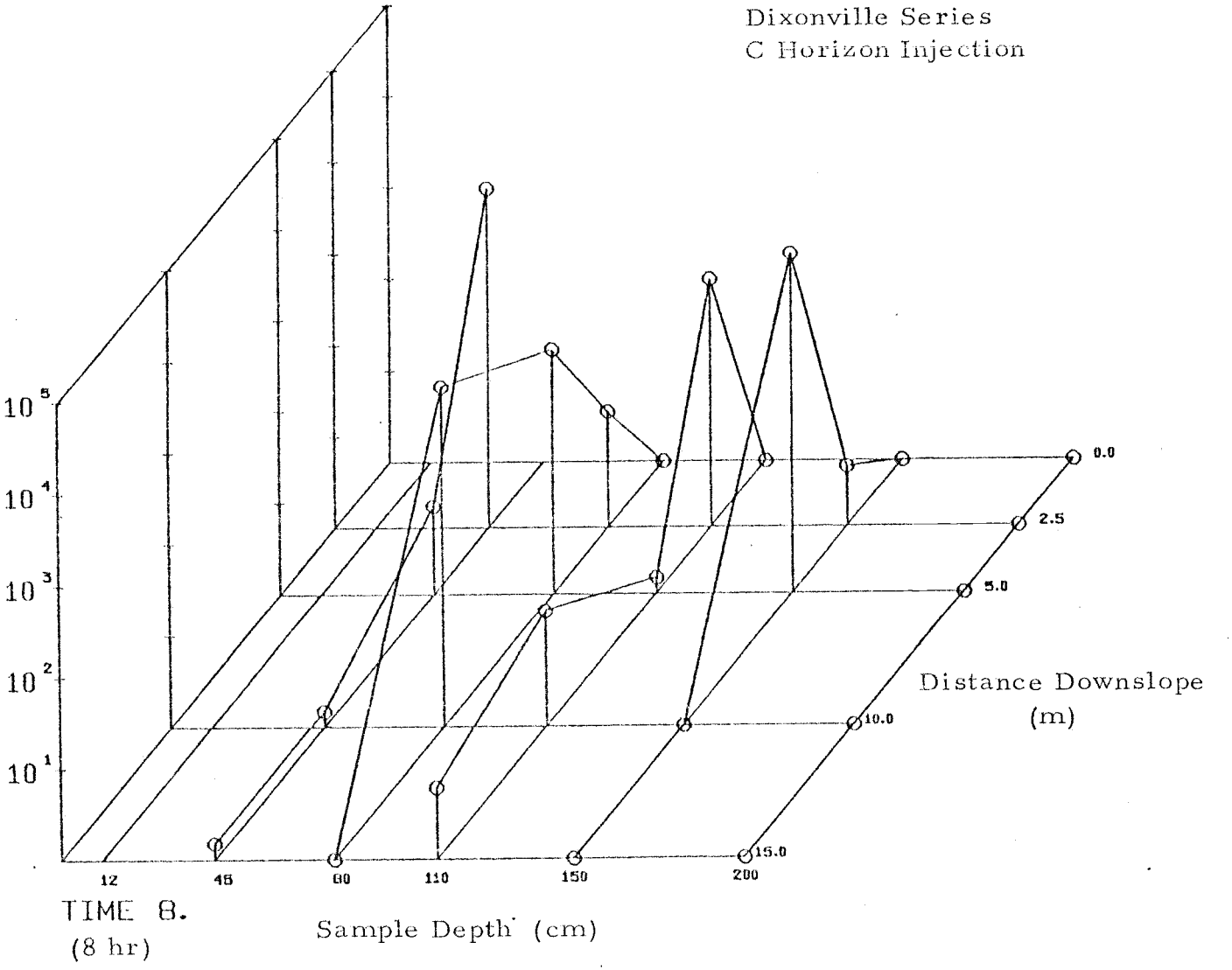

Figure 5.38. Organism movement at Dixonville site, C horizon injection, 8 hour.

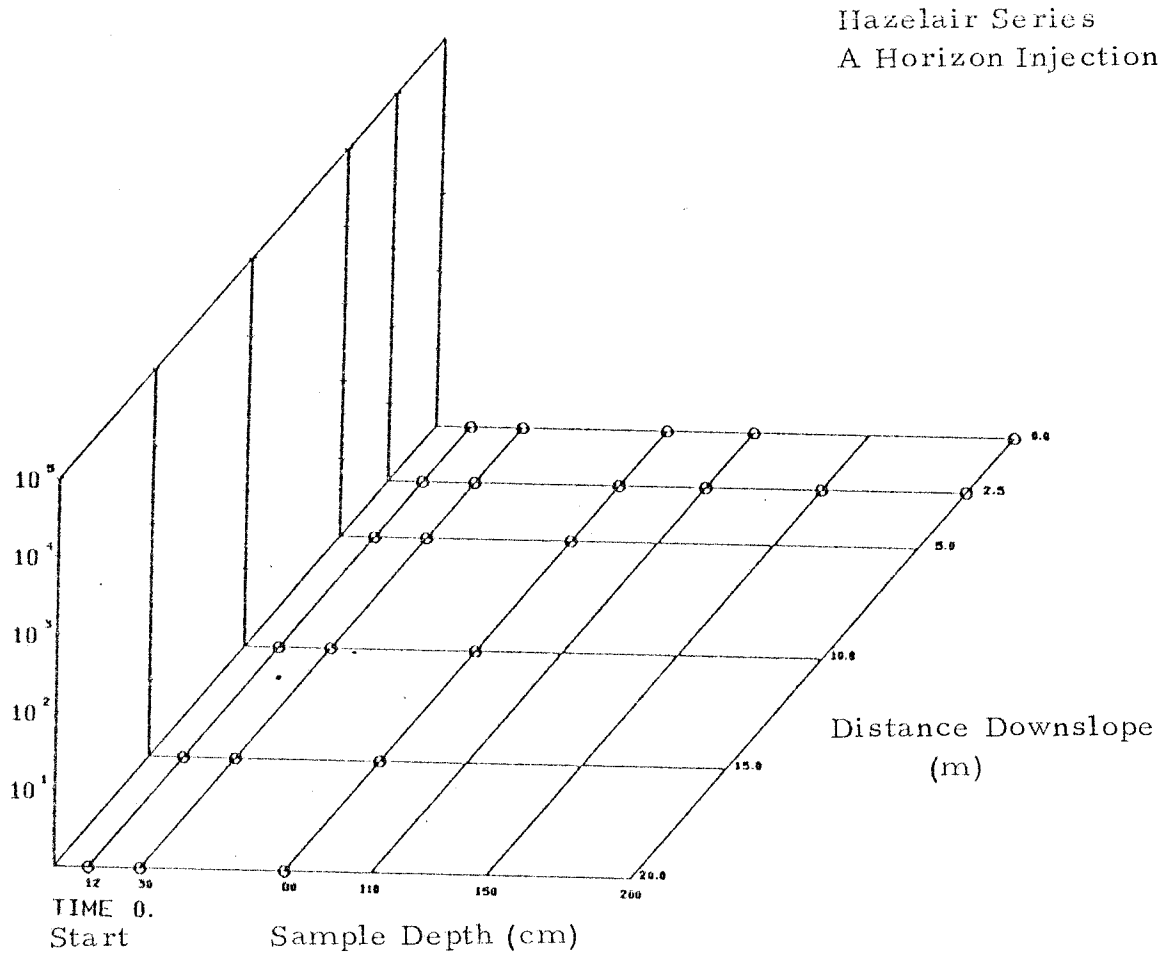

Figure 5.40. Organism movement at Hazelair site, A horizon injection, pre-injection.

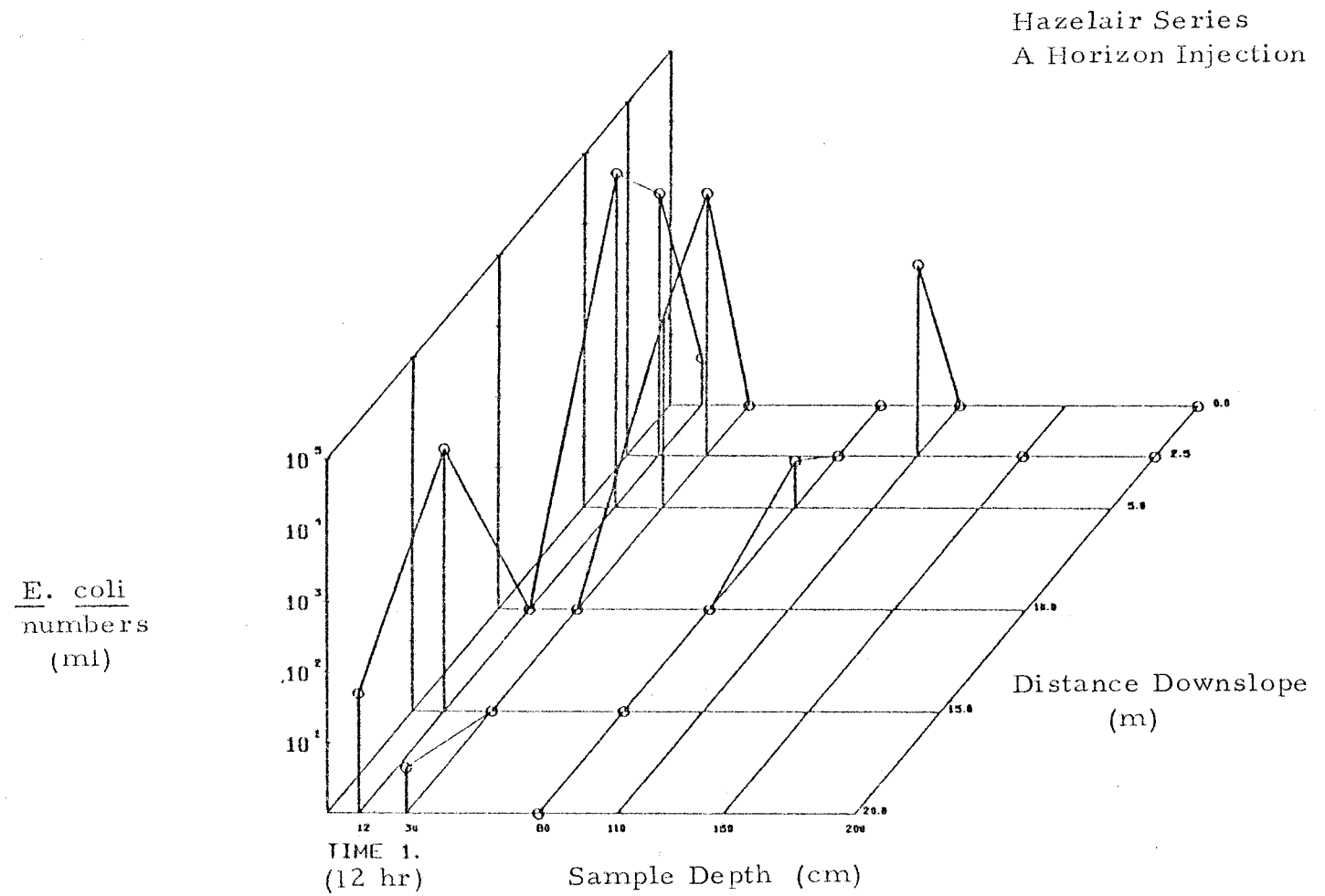

Figure 5.41. Organism movement at Hazelair site, A horizon injection, 12 hour.

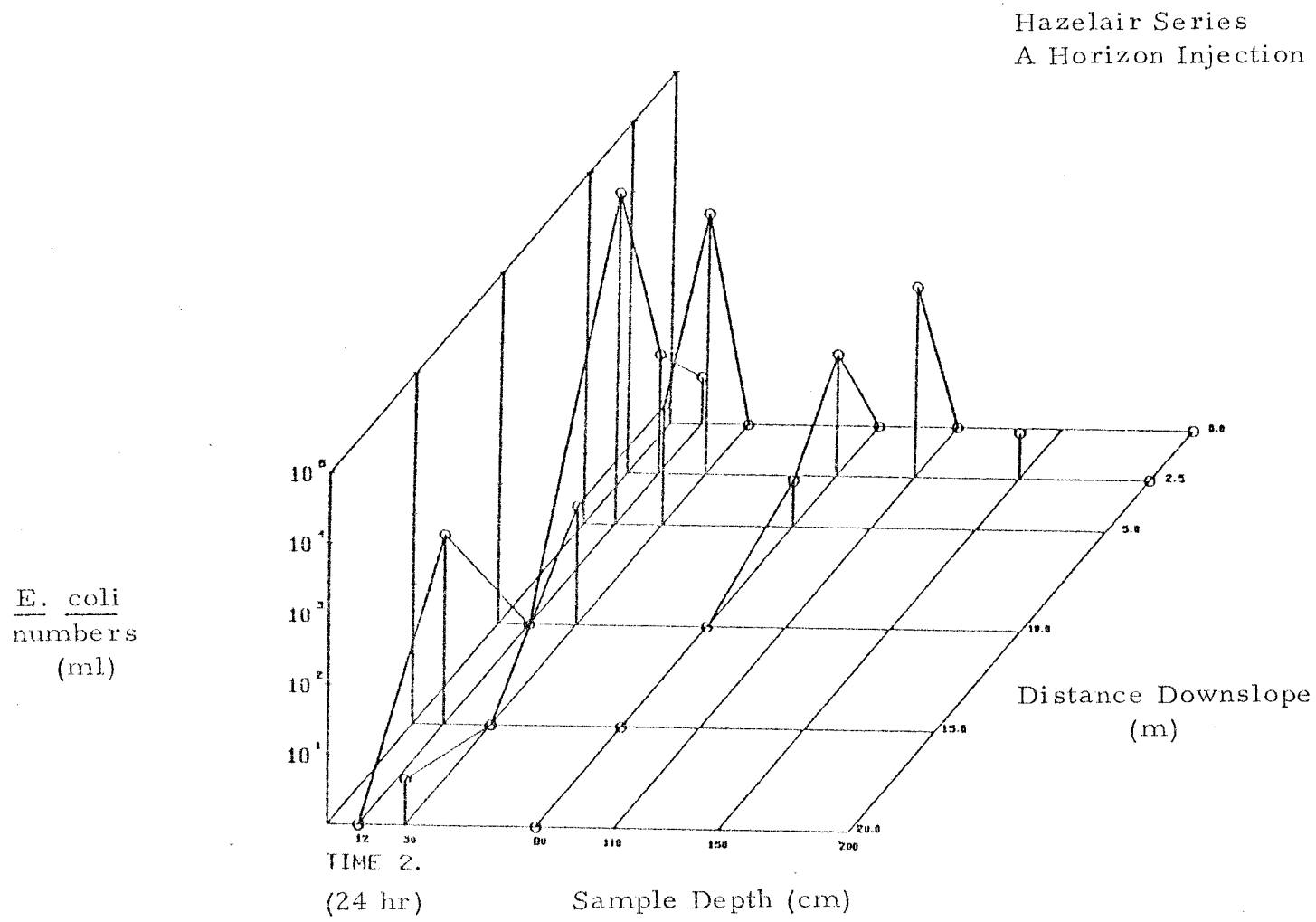

Figure 5.42. Organism movement at Hazelair site, A horizon injection, 24 hour.

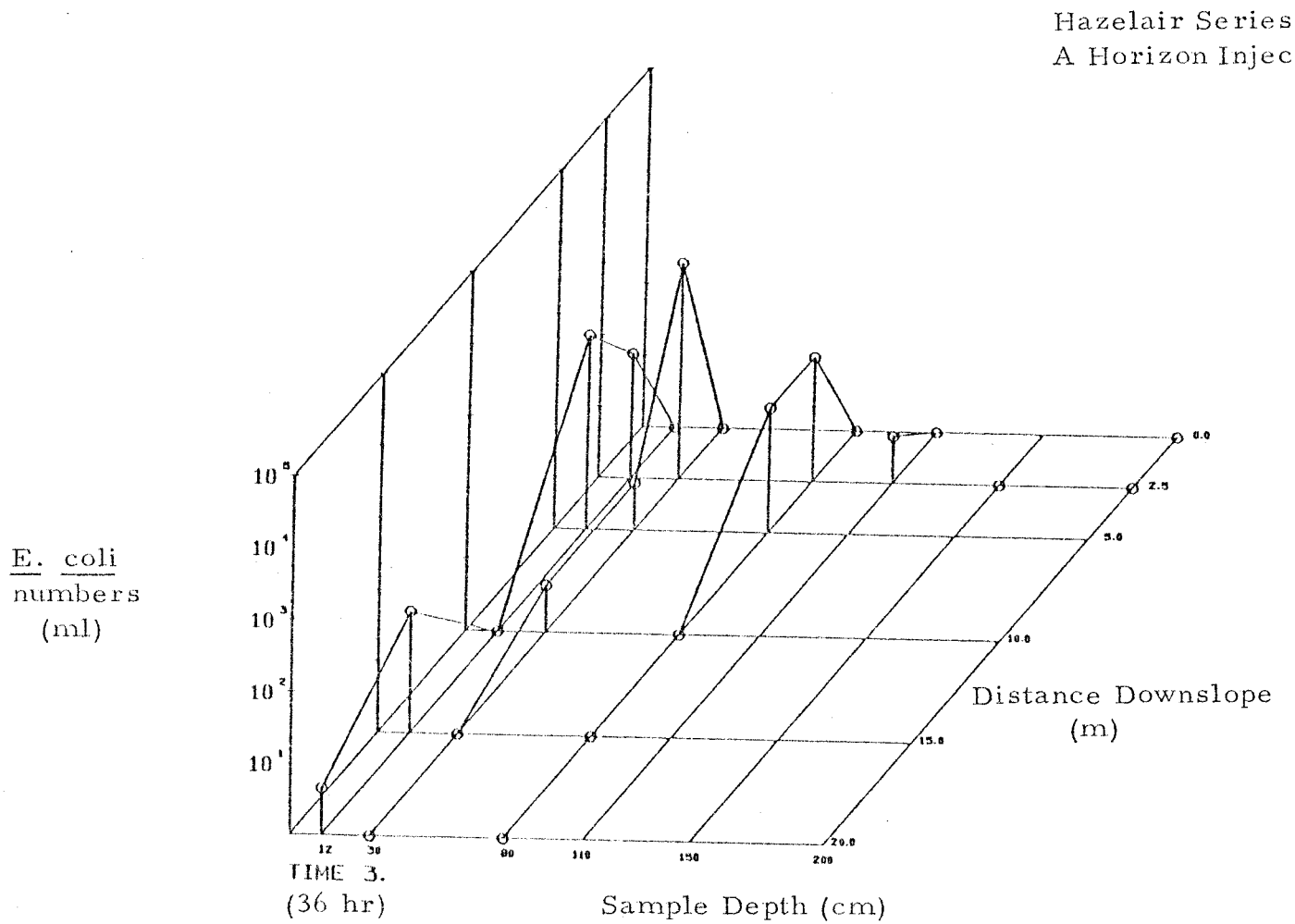

Figure 5.43. Organism movement at Hazelair site, A horizon injection, 36 hour.

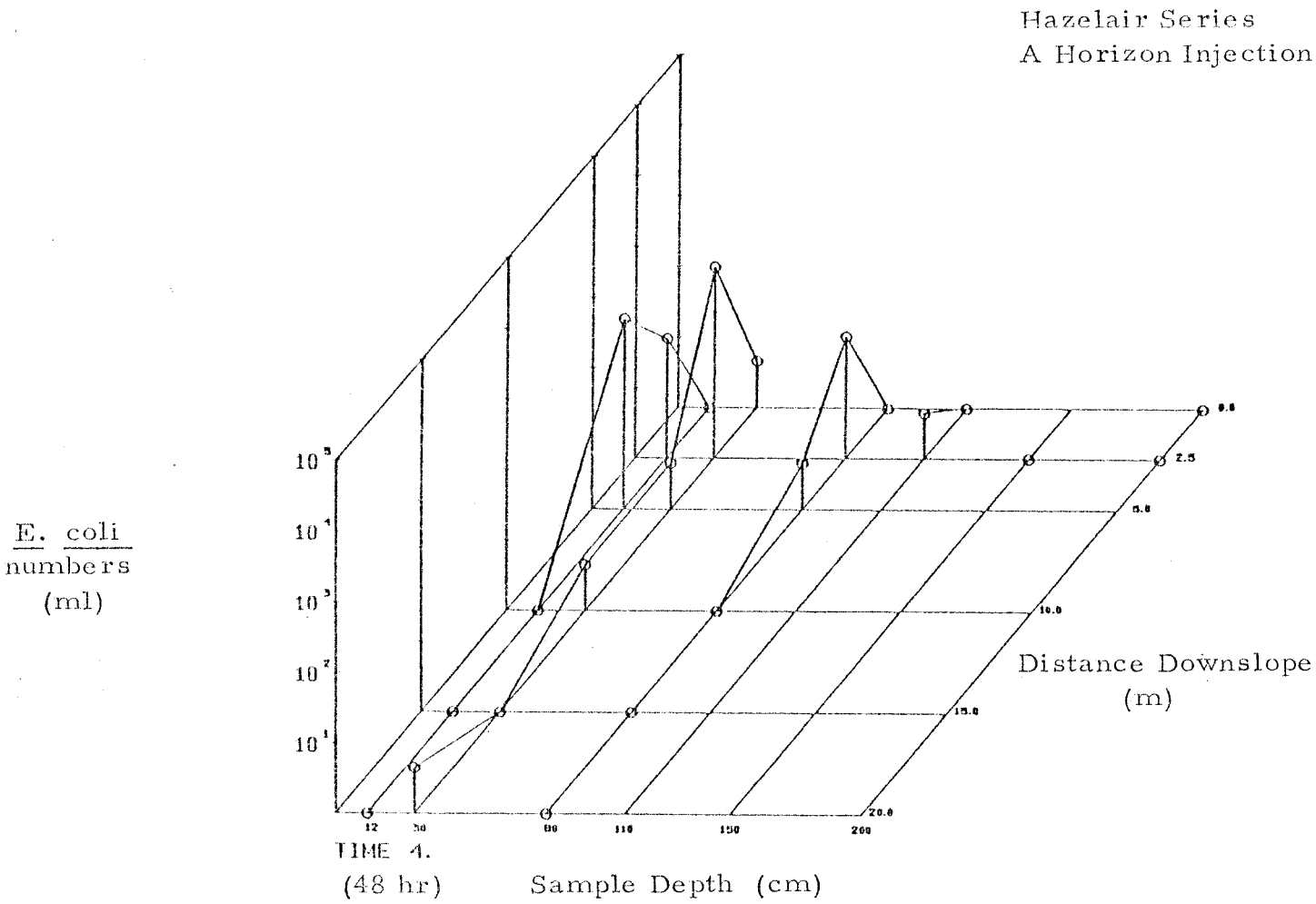

Figure 5.44. Organism movement at Hazelair site, A horizon injection, 48 hour.

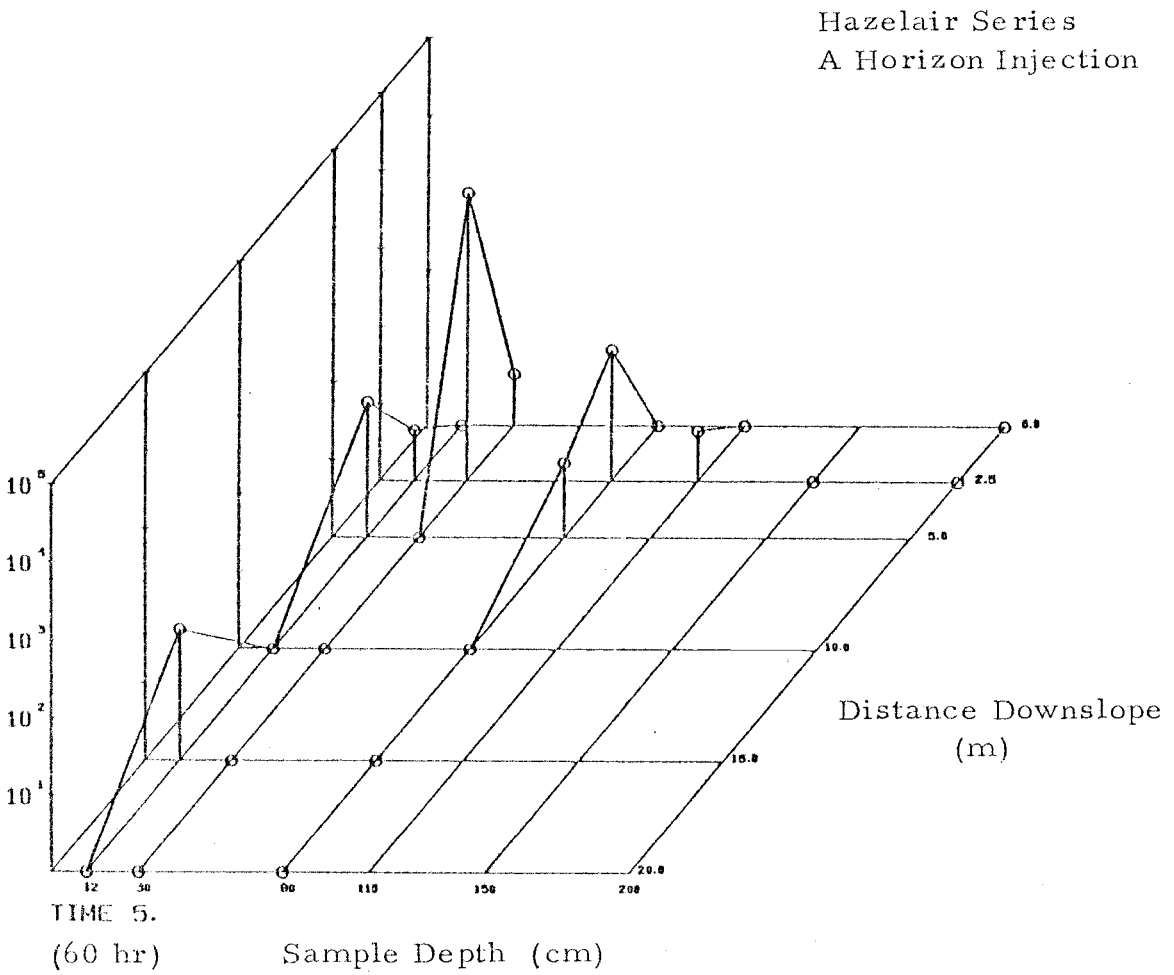

Figure 5.45. Organism movement at Hazelair site, A horizon injection, 60 hour.

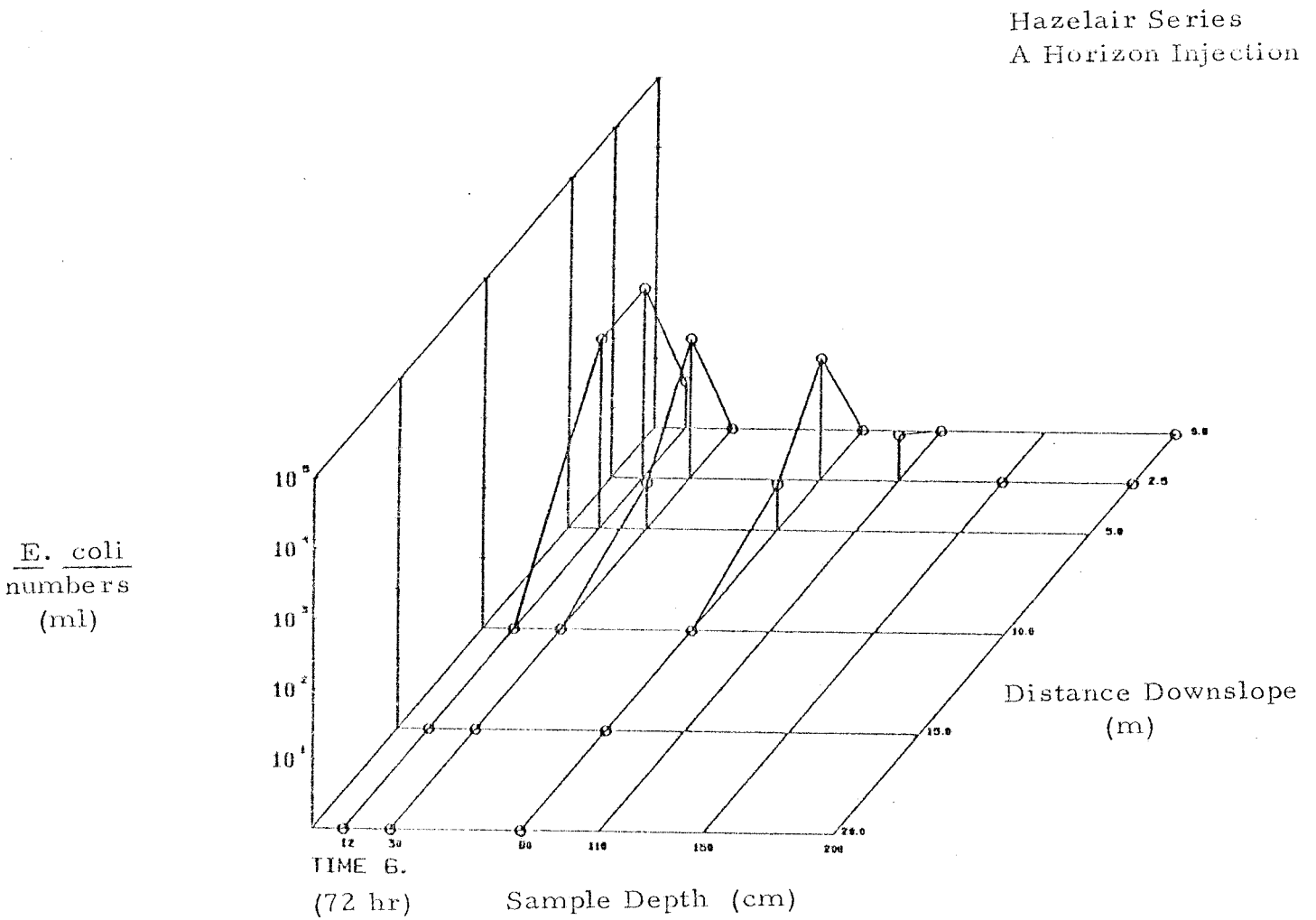

Figure 5.46. Organism movement at Hazelair site, A horizon injection, 72 hour.

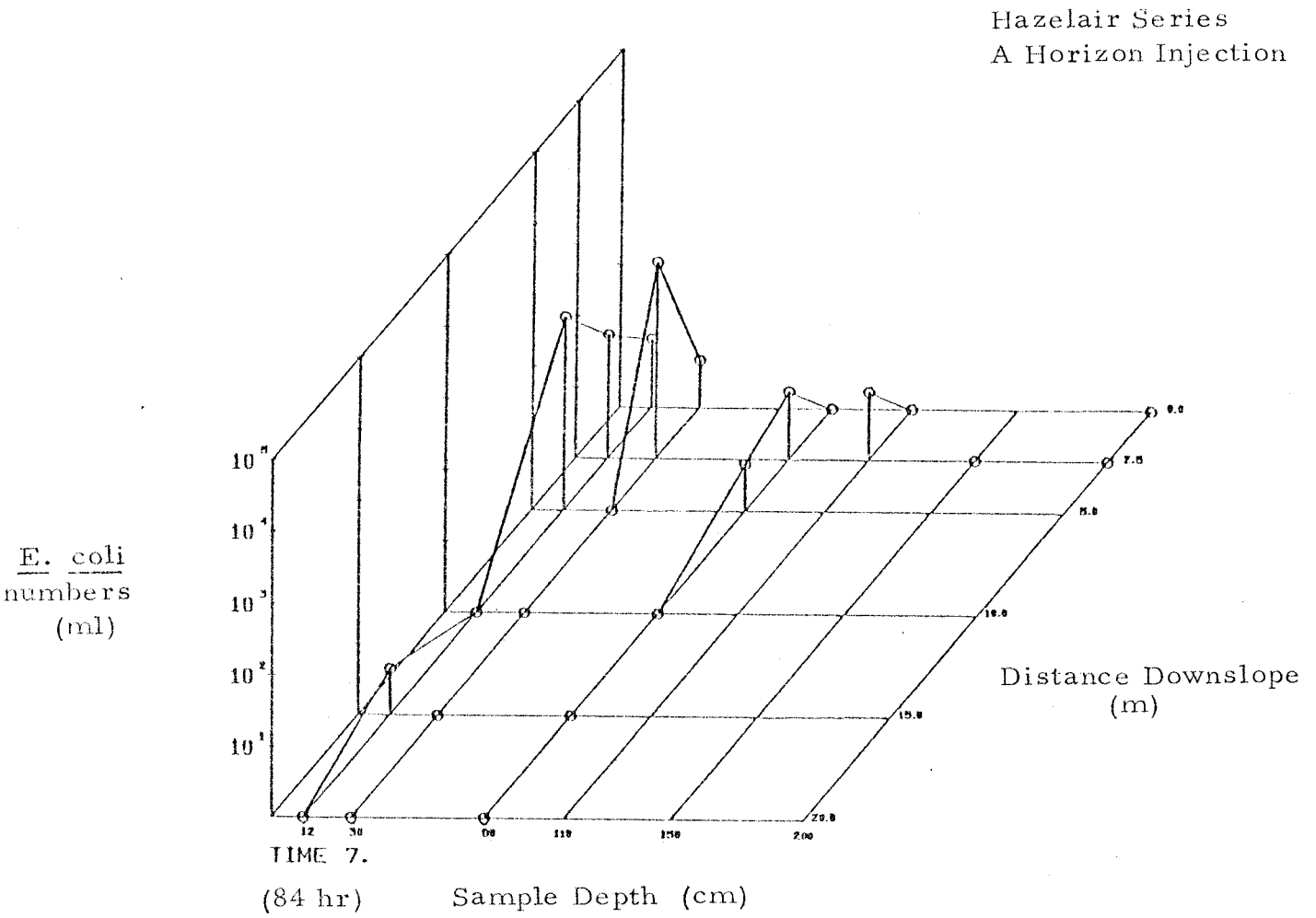

Figure 5.47. Organism movement at Hazelair site, A horizon injection, 84 hour.

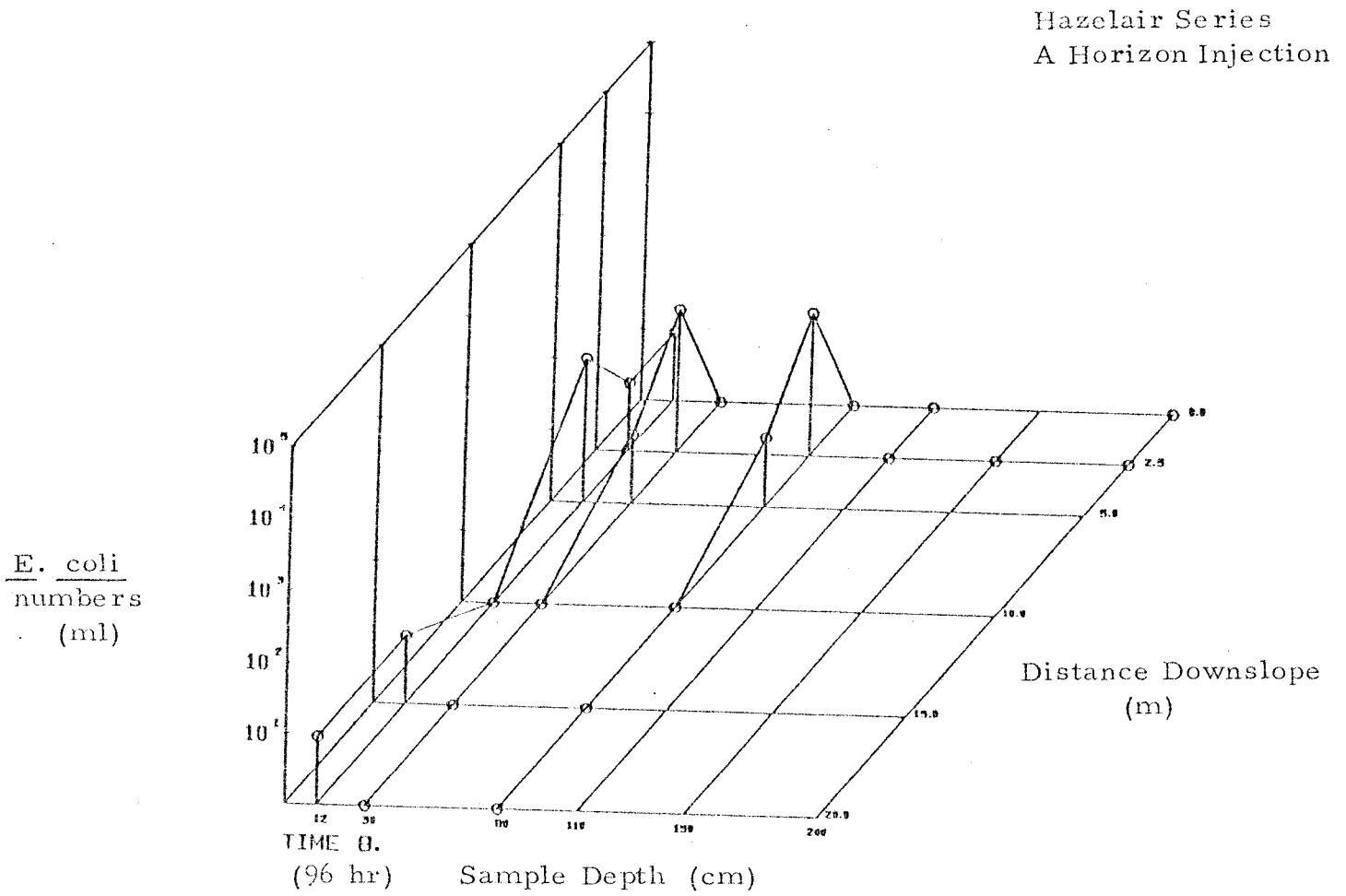

Figure 5.48. Organism movement at Hazelair site, A horizon injection, 96 hour.

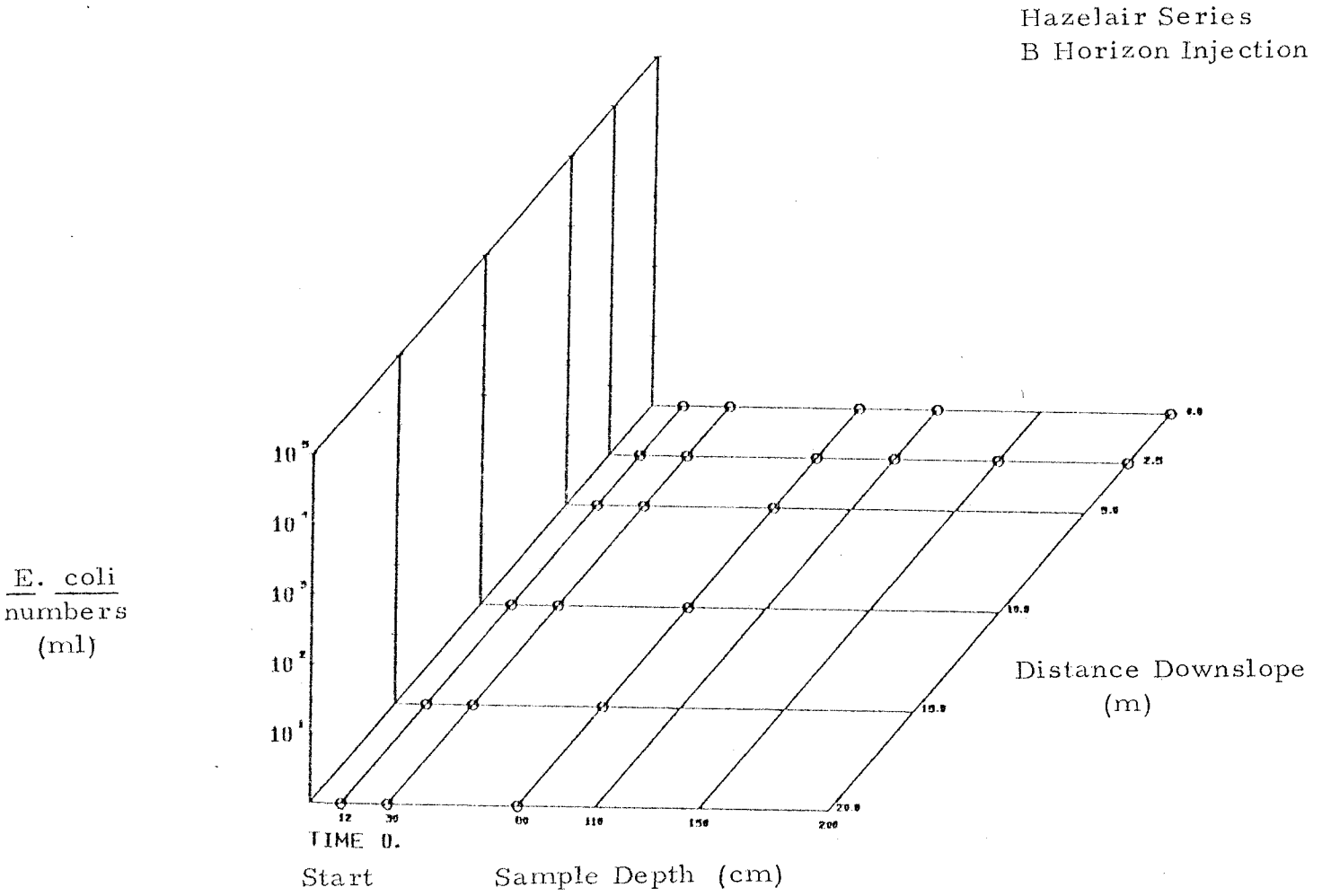

Figure 5.50. Organism movement at Hazelair site, B horizon injection, pre-injection.

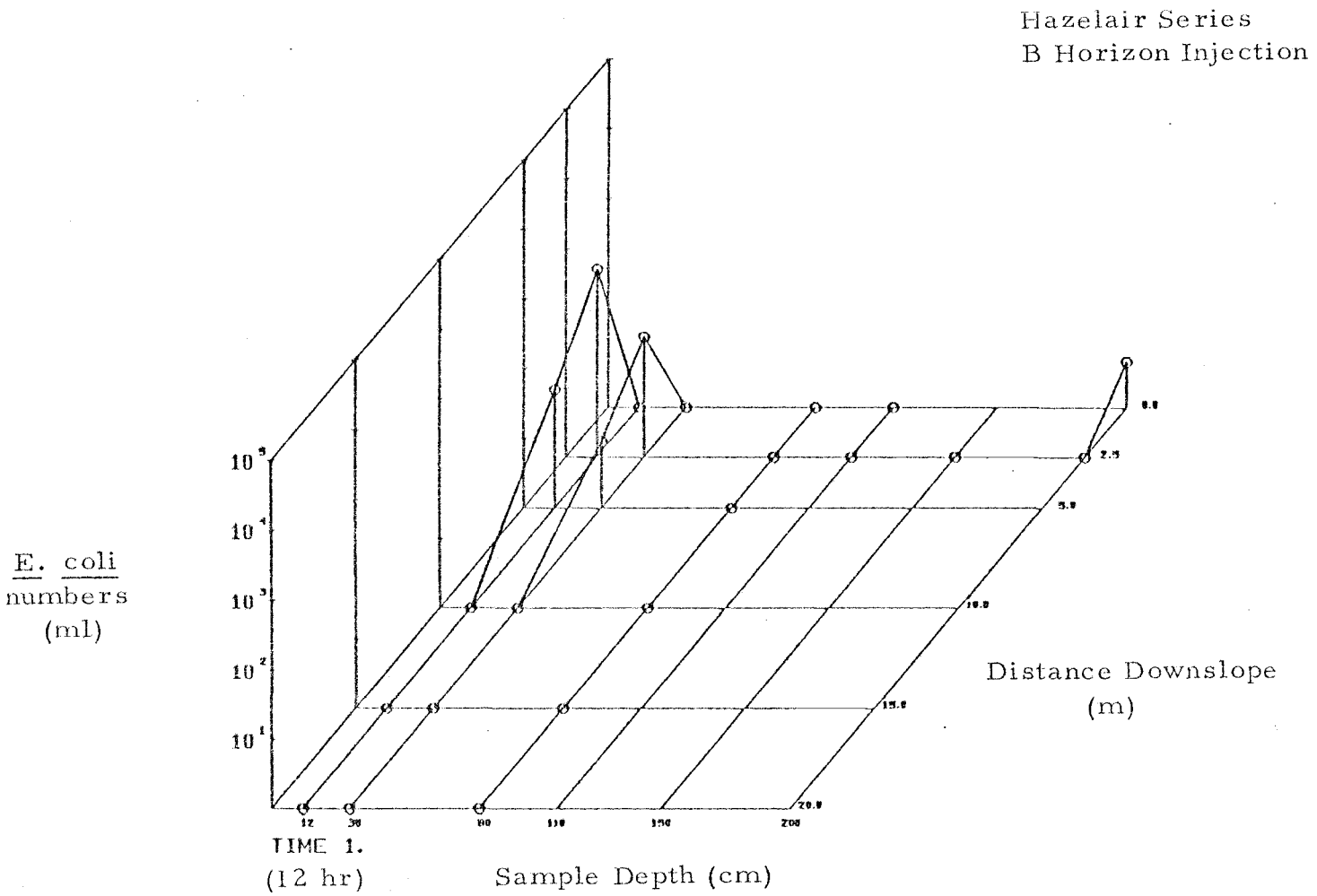

Figure 5.51. Organism movement at Hazelair site, B horizon injection, 12 hour.

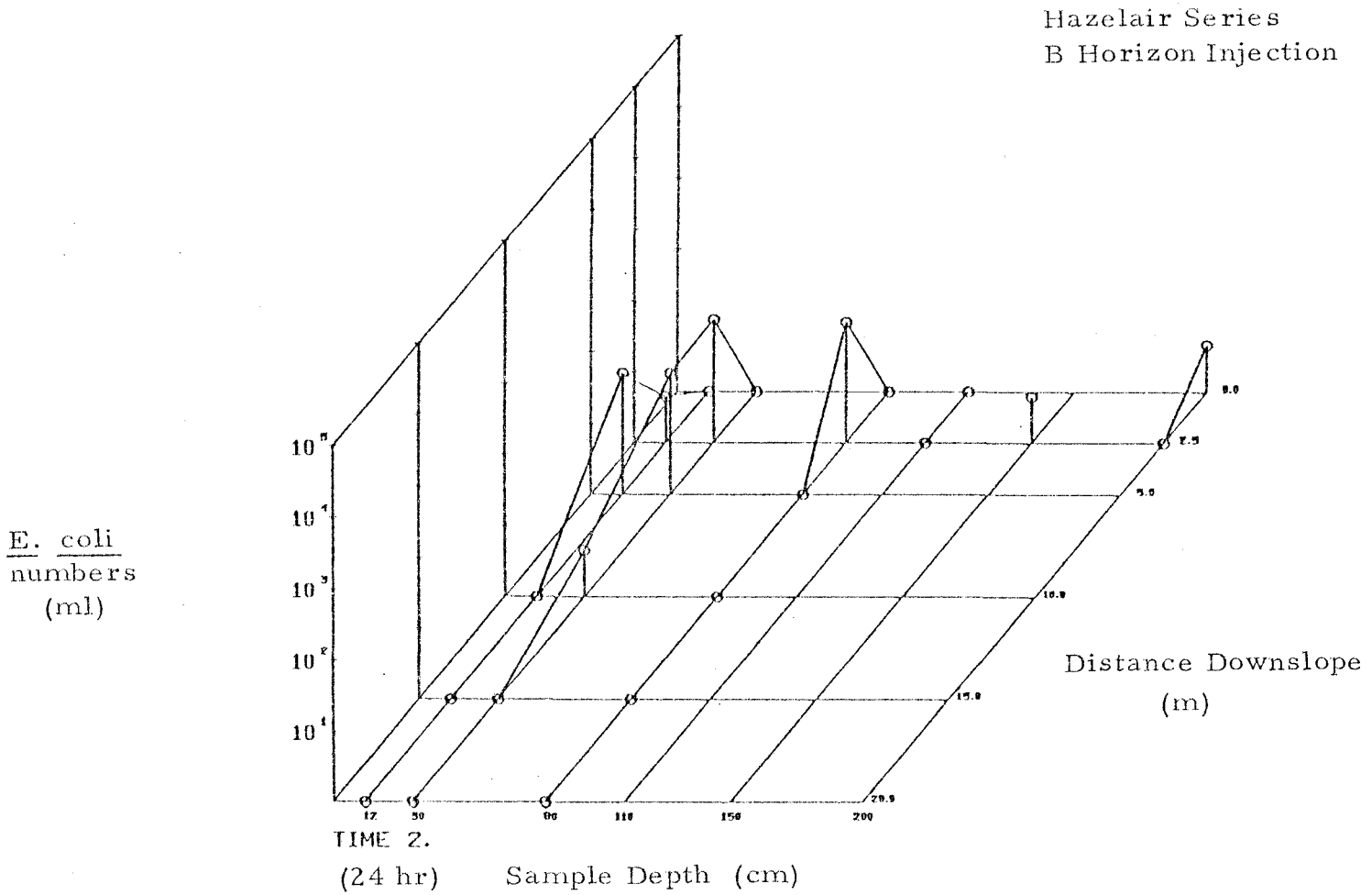

Figure 5.52. Organism movement at Hazelair site, B horizon injection, 24 hour.

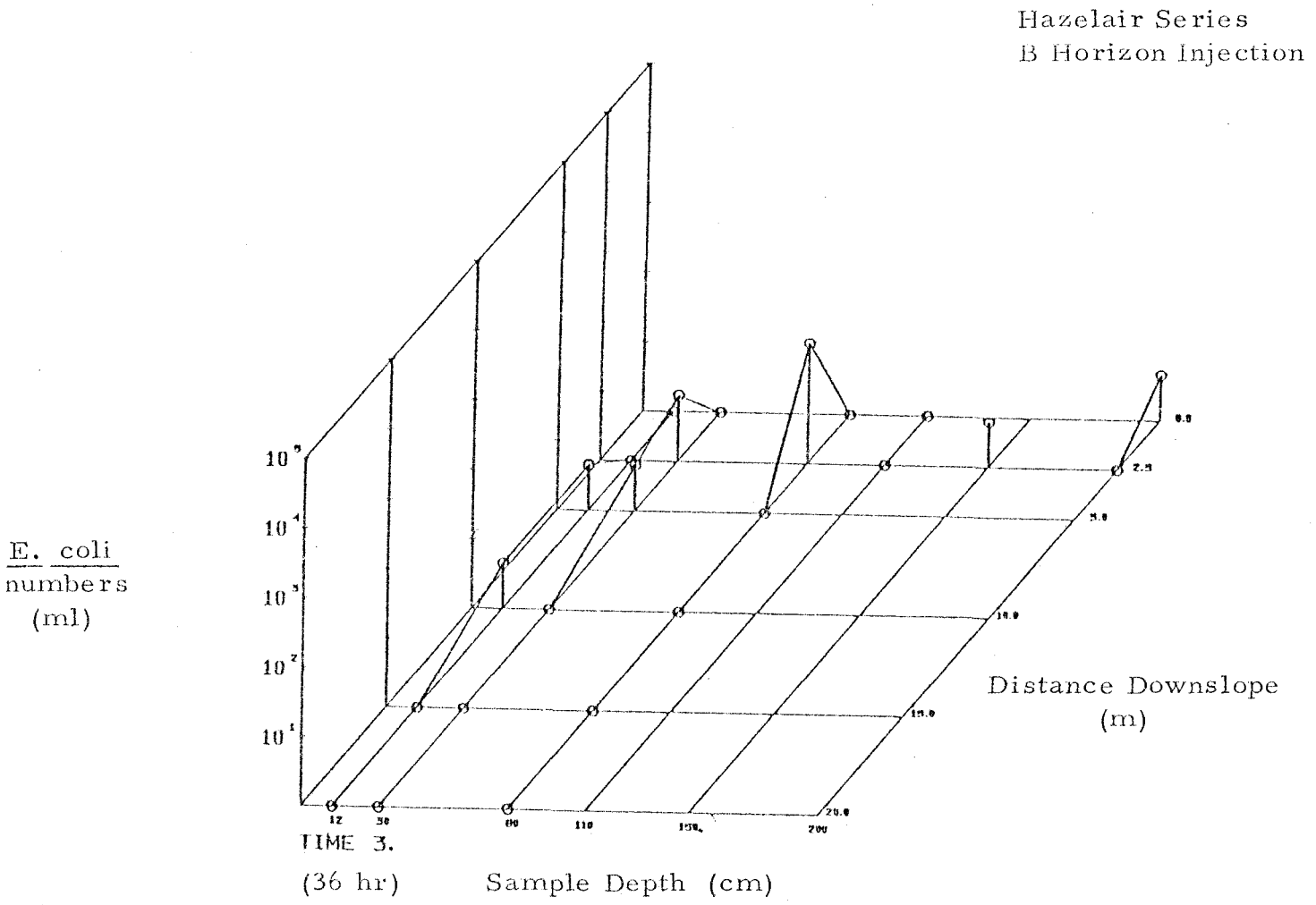

Figure 5.53. Organism movement at Hazelair site, B horizon injection, 36 hour.

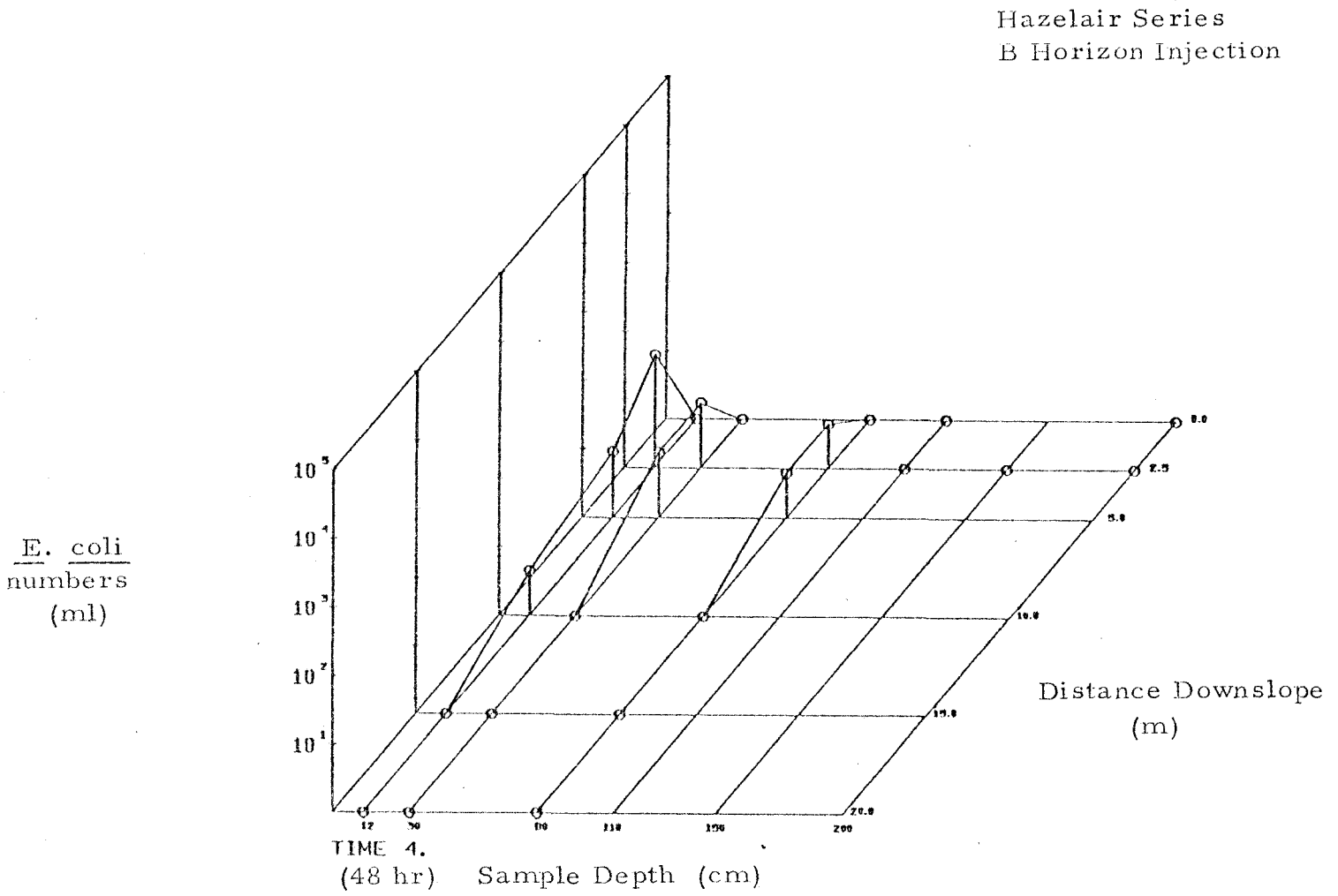

Figure 5.54. Organism movement at Hazelair site, B horizon injection, 48 hour.

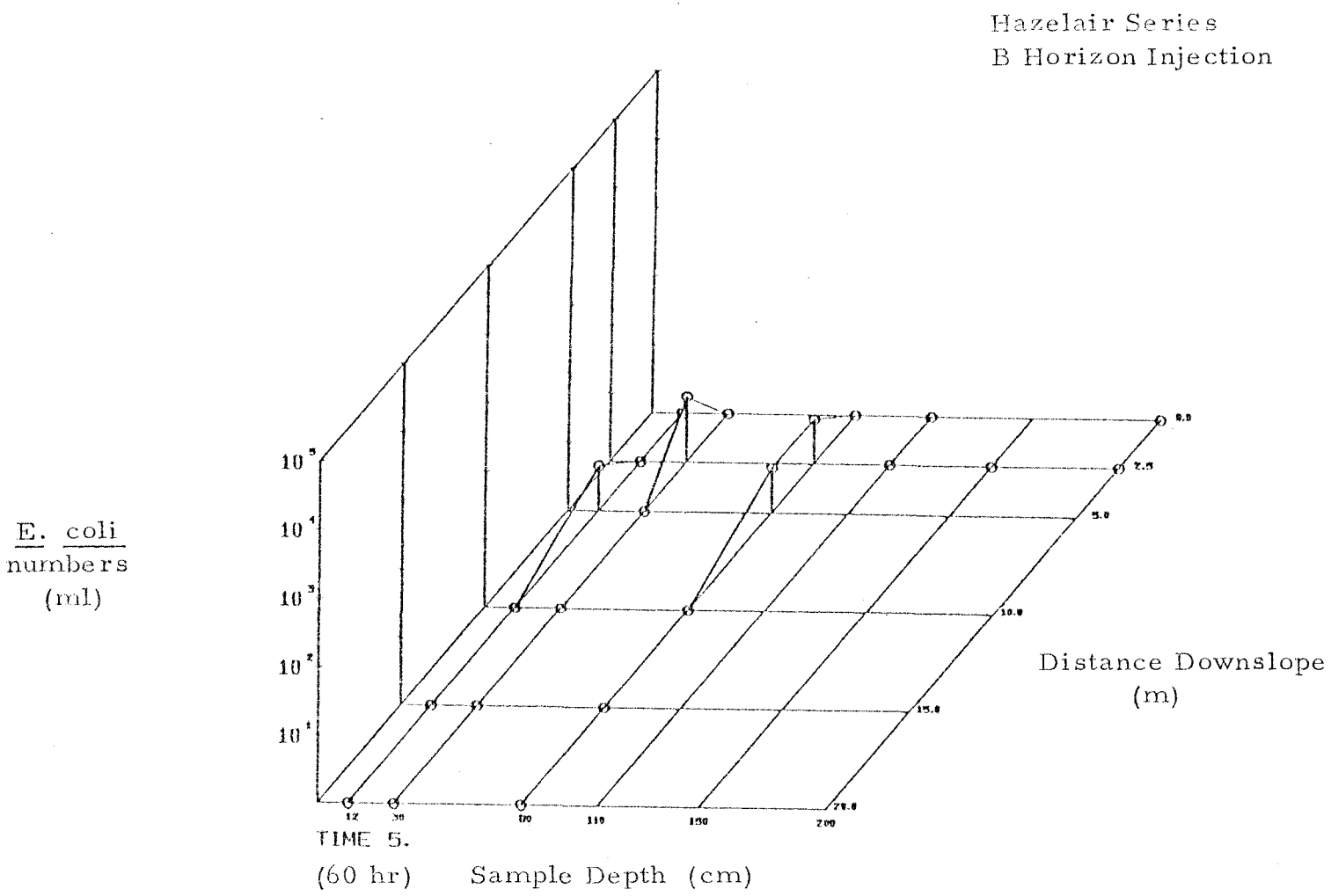

Figure 5.55. Organism movement at Hazelair site, B horizon injection, 60 hour.

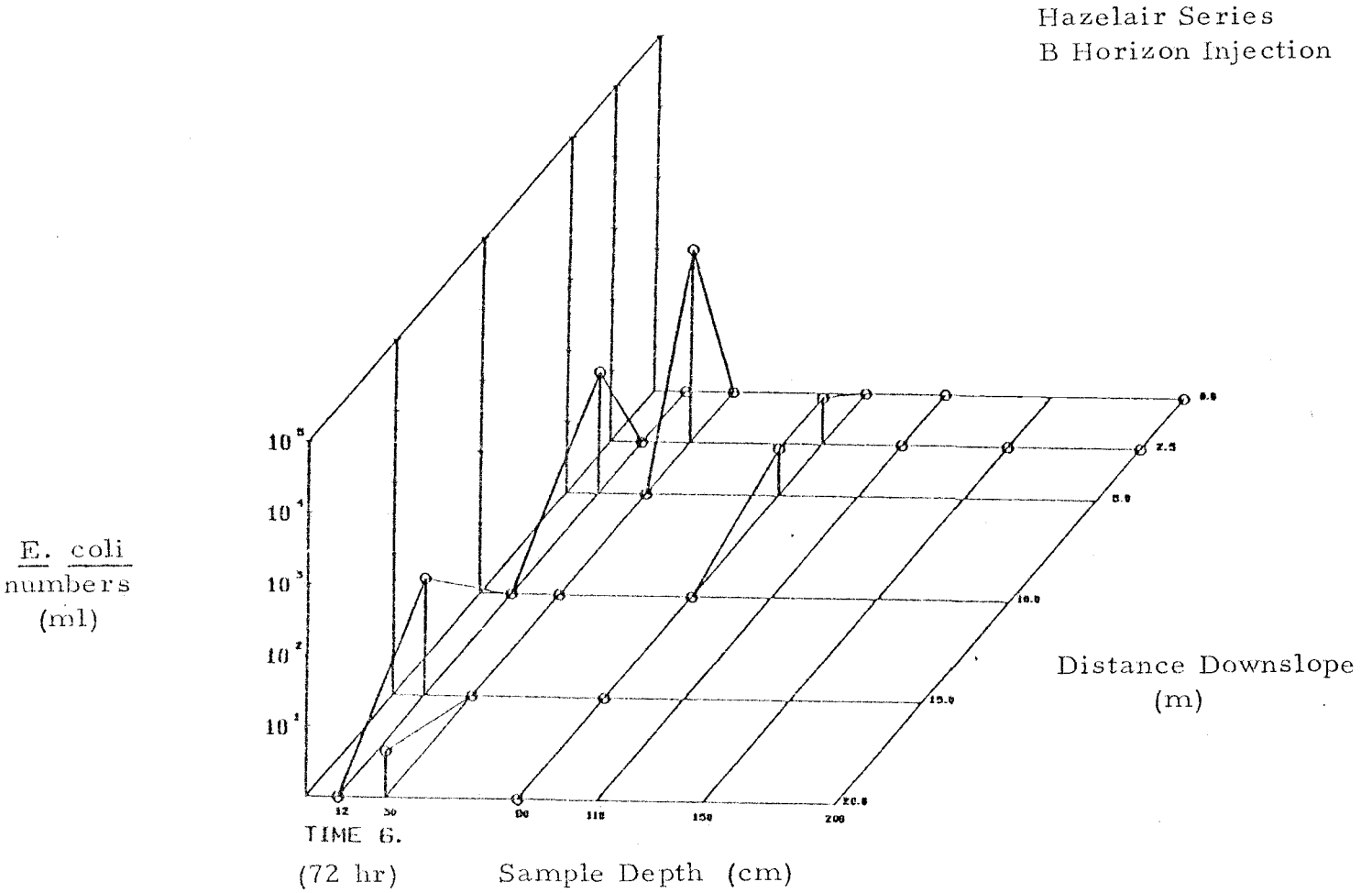

Figure 5.56. Organism movement at Hazelair site, B horizon injection, 72 hour.

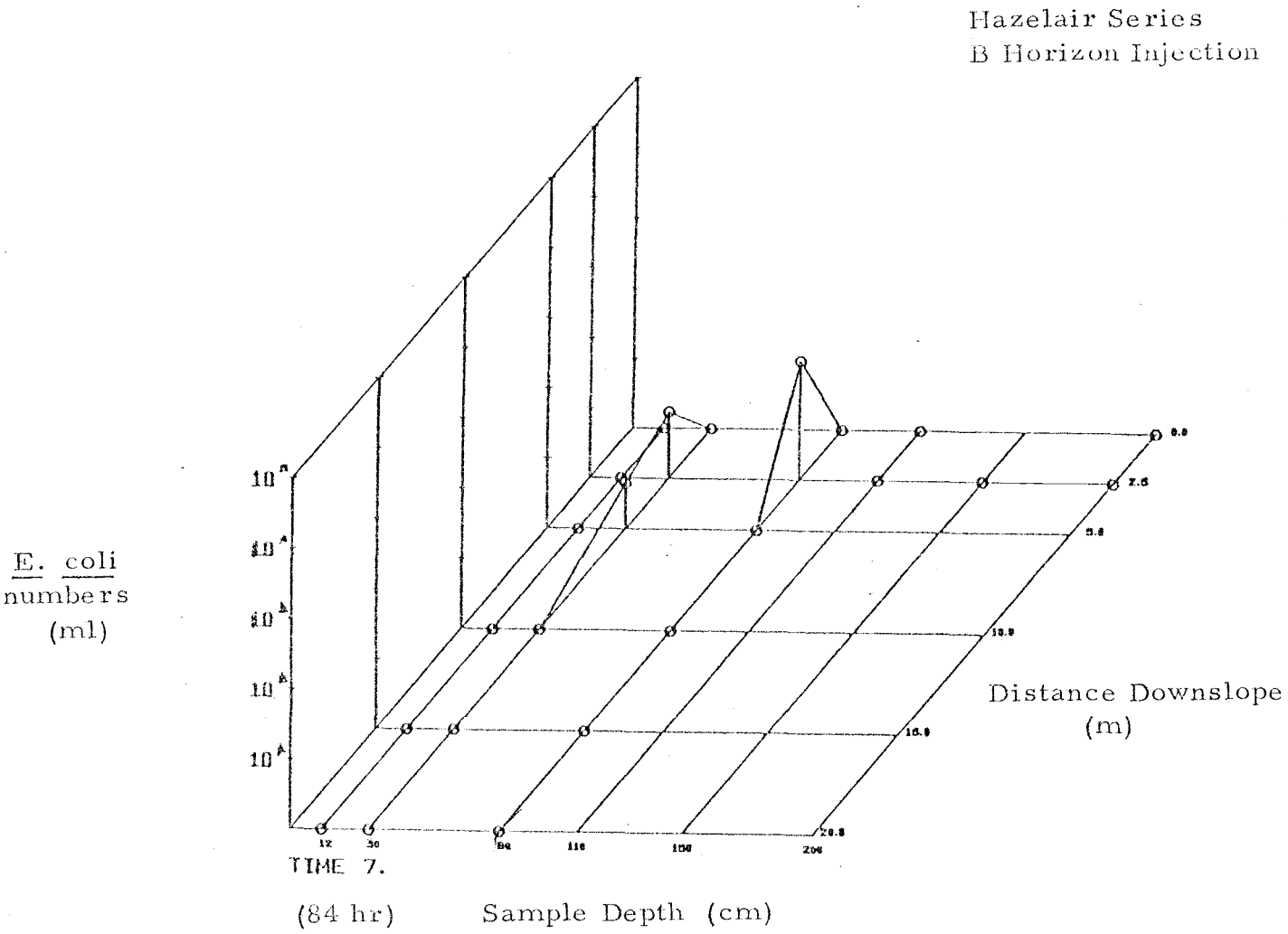

Figure 5.57. Organism movement at Hazelair site, B horizon injection, 84 hour.

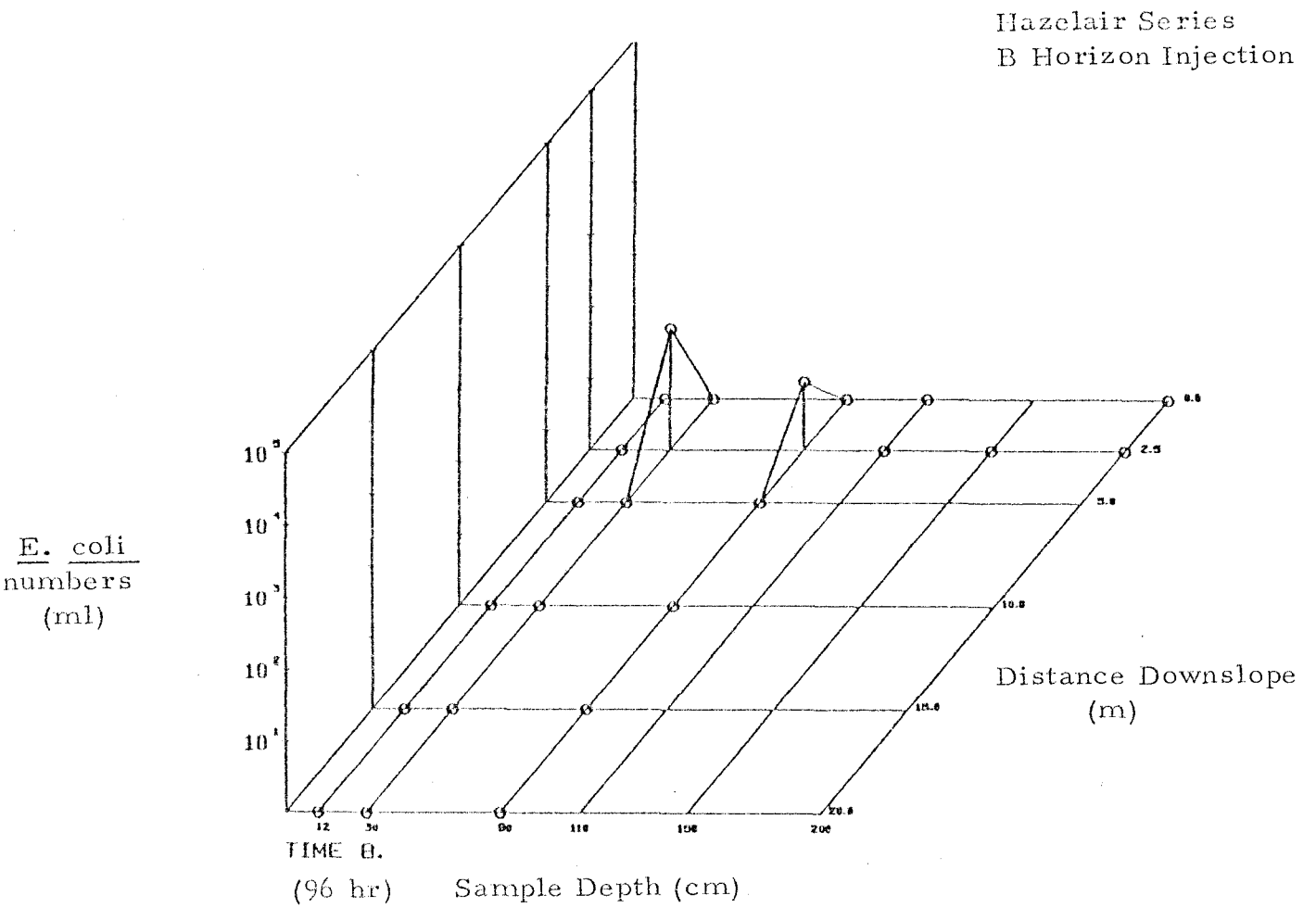

Figure 5.58. Organism movement at Hazelair site, B horizon injection, 96 hour.