Use of Two Adsorption Models to Describe the Fate of Entomopathogenic Nematodes in Soils

Sam O. Dennis[1]*, Teferi Tsegaye[2], Robert E. Harrison[1] and Sunnie A. Aburime[3]
[1]Tennessee State University, Nashville TN [2]Alabama A&M University, Normal AL [3]Clark Atlanta University, Atlanta GA

Abstract

Entomopathogenic Nematodes (ENs) are potential bio-control agents for soil-borne insects like the white grubs of the scarab beetles. Knowledge of their fate in soils is important for their release into the soil environment. The study seeks to examine the fate of *Steinernema carpocapsae* TN 18 strain, an entomopathogenic nematode, in nursery soil at the column scale. Two field soils were used to pack the columns. The soils had history of nursery crop production for at least ten years. A Darcian flux (0.8 cm hr^{-1}) was used to facilitate movement (leaching) of the nematodes in disturbed soil columns. Bromide concentration of 0.8 M, non-toxic to the nematodes was added to the soil surface as a conservative tracer. Leachate samples were assayed for nematodes and bromide concentrations. Bromide and nematode breakthrough curves (BTCs), as well as two adsorption models, were used to provide evidence of entomopathogenic nematodes' fate and transport processes in the soils. The amount of ENs sorbed to soils was tested with two adsorption isotherms (Freundlich and Langmuir). A good fit of the data was obtained with the Freundlich isotherm while the Langmuir isotherm exhibited a non-linear fit. Relatively, the bromide and nematodes' BTCs indicate that the ENs movement was retarded in both field soils, suggesting that sorption might be a key factor in determining nematodes transport through soil.

Introduction

Nematodes are worm-like animals, usually microscopic in size. Some are free-living, plant parasites and animal parasites. Entomopathogenic nematodes (ENs) are exclusively soil organisms. They attack insects by releasing bacteria while inside the insects (Figure 1). The bacteria will eventually decimate the insect(s). As a result they used as potential bio-control agents for soil-borne insects pests. In field nursery production systems, white grubs, e.g. Japanese beetle grubs, present an increasing threat to growers because the pests destroy crops. Additionally, presence of grubs in nursery root balls may result in quarantine actions and/or revocation of nursery certification. The primary means of controlling these soil-inhabiting insects is chemical insecticides. With the use of chemicals, the potential for surface and groundwater contamination abounds; and the active ingredients of these chemicals are usually highly toxic and persistent. Entomopathogenic nematodes offer many advantages over chemical products. Therefore, knowledge of their fate in Soils and implications on water resources is important. The objective of the study is to examine the fate of *Steinernema carpocapsae* TN 18 strain, an entomopathogenic nematode, in nursery soil at the column scale. Freundlich and Langmuir isotherms were used to describe the sorption of ENs to nursery soils.

Figure 1. Nematode infested Grub.

Methodology

I. Soil Characterization

The soil samples that were used for the study were collected from the Alabama A&M University's Winfred Thomas Research Station in Normal, Alabama and from the Tennessee State University Nursery Crop Research Station, in McMinnville, Tennessee. The soils were used to pack the soil columns. The soils collected from the Alabama A&M University's location were classified as Decatur silt loam (Rhodic Paleudult soil). Those soils were identified in the study as Alabama soils (AS). The soils collected from the Tennessee State University's location were classified as the Waynesboro sandy clay loam (Clayey, kaolinitic, thermic Typic Paleudults). Those soils were identified in the study as the Tennessee soils (TS). Various nursery crops have been grown on both sites for at least ten years. The soil samples were collected from 0-35 cm depth. The collected soil samples were sterilized with an Amsco model 3021 autoclave with temperature set at 254^{0F} for 60 minutes to kill existing nematodes in the soils. The sterilized soil samples were then used to pack the soil columns. The soils in the columns were packed to a bulk density of 1.20 gm cm^{-3}. A total of eight (8) soil columns were used for the study.

II. Leaching Experiment

Each soil column (8 columns total: 4 for Tennessee soil and 4 for Alabama soil) received approximately 11,000 nematodes, an amount equivalent to a field application rate of about 1 billion nematodes per acre. The nematodes were left to equilibrate with the soil for 24 hours. Bromide solution, 0.8M (0.095kgL^{-1}) non-toxic to the nematodes, was uniformly applied to the surface of each column as a solute tracer. The rainfall simulators with water dripping from them were then placed on top of the soil columns to leach the soil for bromide and nematodes. The leaching experiment was performed in duplicate and was run for 48 hours. Leachate samples were collected in 500-ml polyethylene bottles at specific time interval (pore volumes). The leachate samples were assayed for the presence of nematodes and bromide that may have been transported through the soil columns by water. The breakthrough curve for bromide concentration was plotted using the relative concentration C/C_0 (concentration recovered divided by initial concentration) versus pore volumes of the leachate. At the conclusion of the leaching experiment, the soil columns were cut into six-depth increments as described by Dennis et al. (1999). The nematodes in each soil depth increment were extracted and quantified under a stereomicroscope connected to an image analyzer programmed for nematode recognition.

III. Nematode sorption experiment

In the kinetics experiment we found that equilibrium adsorption of the nematodes was reached after 4 hours, and that 24 hour was an appropriate equilibrium time for the sorption experiment. Thus, the sorption experiment was conducted the same way the kinetics experiment was done except for the following deviations: (a) The concentrations of nematodes inoculated were varied; six (6) concentrations including blank (0, 50, 100 200, 500 and 1000) were used. Due to difficulty in counting the nematodes and in order to minimize error for miscount nematodes, concentration over 1000 was not used. (b) The flasks were shaken for 24 hours. After shaking, the soils samples were allowed to settle for about 40 seconds and the supernatant was poured into a watch glass. The nematodes in the suspension were counted and recorded as the concentration of nematodes in solution. This value was then subtracted from the initial concentrations (50, 100 200, 500 and 1000 nematodes) to obtain the amount of nematodes sorbed to the soil at each concentration. Data analysis (isotherms) was developed using the linear form of both the Freundlich and Langmuir equations. (c) The remaining soil samples in the flask were not discarded as in the kinetics experiment; instead they were diluted with 100 ml of deionized water and shaken vigorously for 10 minutes with a Vortex-Genieä shaker. This was done in order to desorb the nematodes that were sorbed to the soil. Nematodes in suspension, if any, were counted and recorded as the concentration of nematodes desorbed.

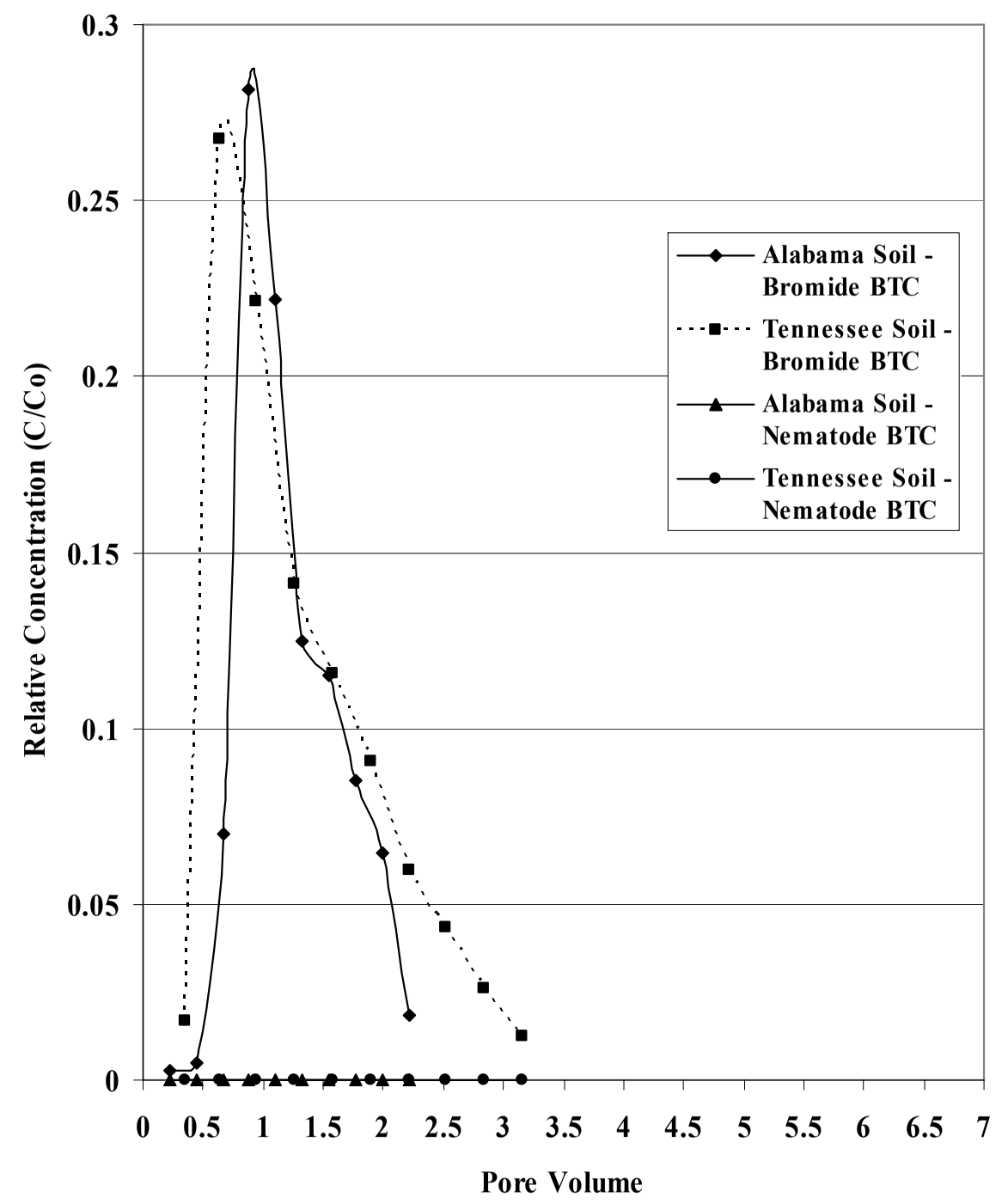

Figure 3. Observed breakthrough curves (BTCs): bromide

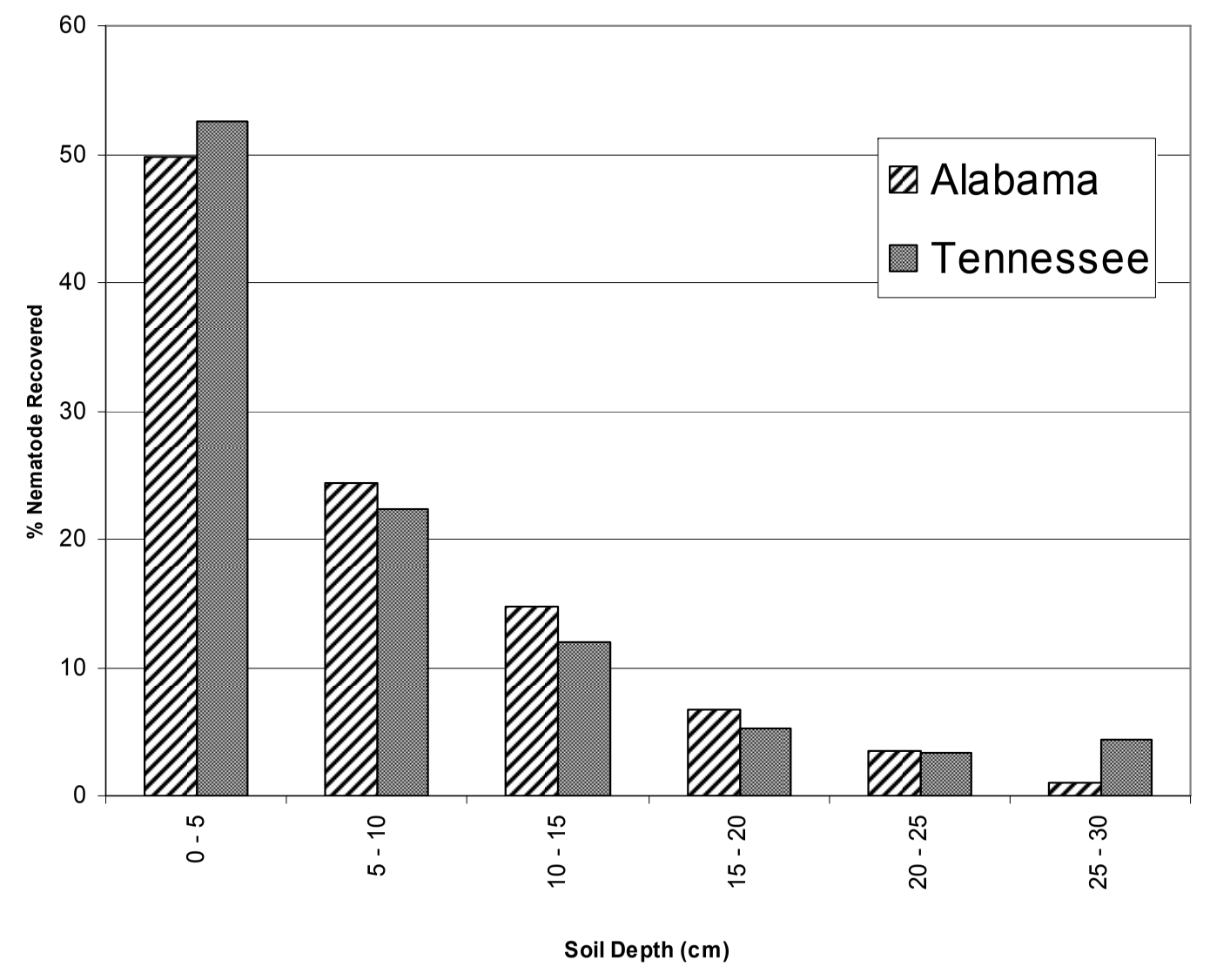

Figure 4. Distribution of nematodes in the soil columns.

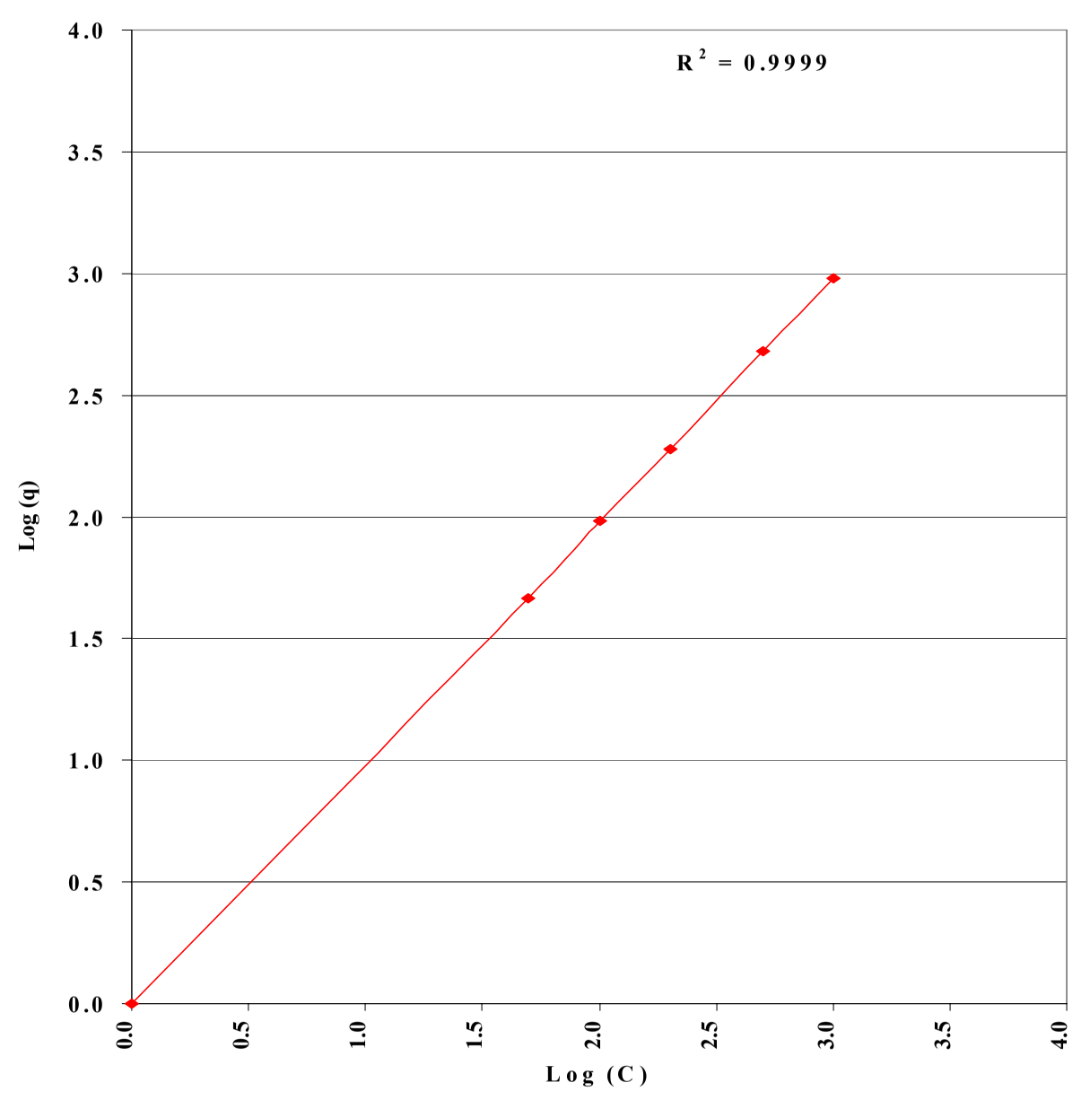

Figure 5. Freundlich adsorption isotherm: Alabama soils

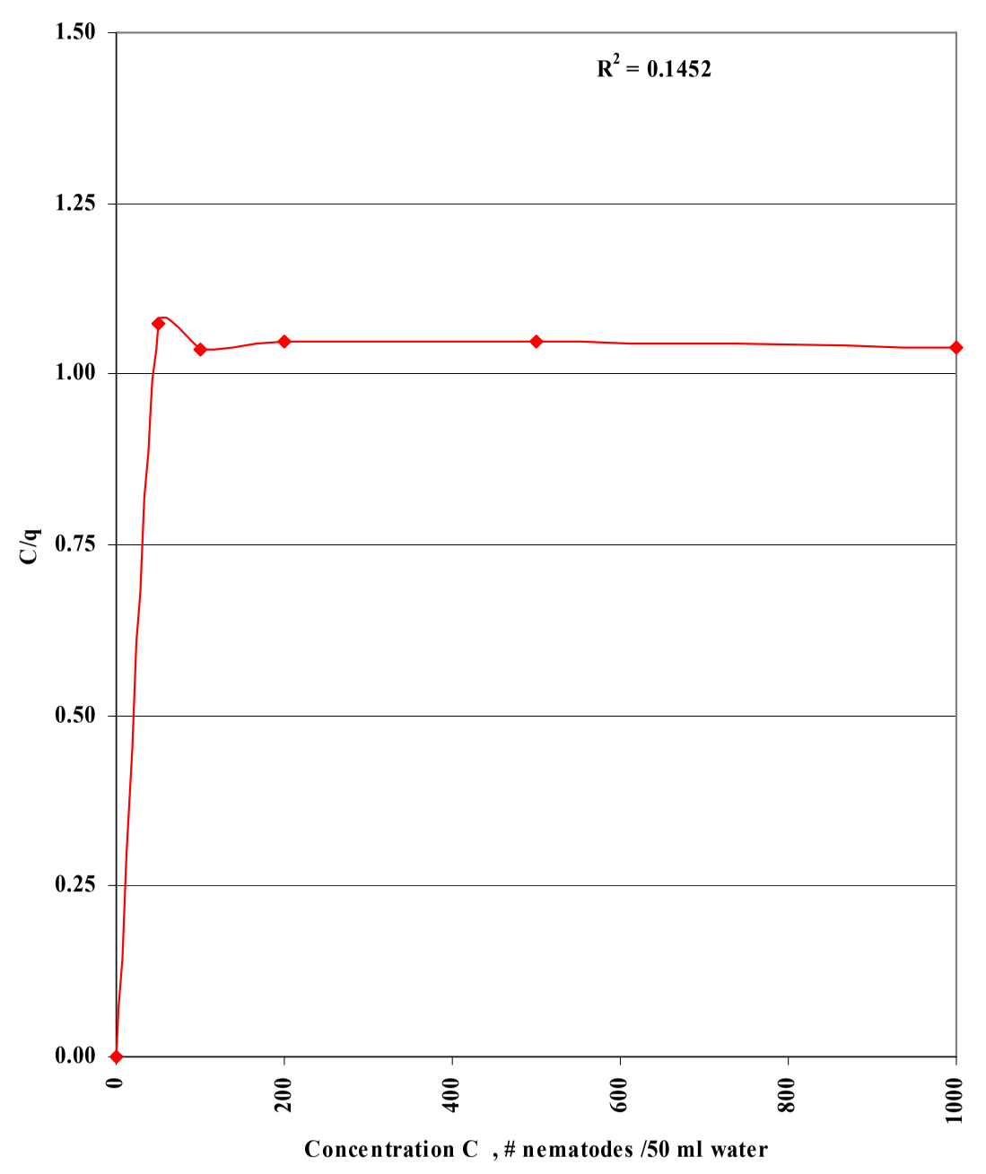

Figure 6. Langmuir adsorption isotherm: Alabama soils

Conclusion

The primary goal of this study was to understand the fate of micro-organisms like ENs in media that are predominately used by nursery crop growers to grow ornamentals. These growers are one of the stakeholders for research of this nature. Nursery crop production needs to be sustainable and environmentally friendly. Since ENs might be released into nursery soil as part of growers' pest management strategy, it was postulated that some of these nematodes could migrate below the root zone and contaminate groundwater. However, this study suggests that will not be the case. These nematodes tend to have an affinity for soil; our data (Figure 4) shows that the majority of the infective juveniles of the ENs were recovered in the 0-15 cm depths. This had practical implication on the efficacy of these nematodes in controlling soil-borne pests. It is evident that the goal of most nursery crop growers is to maximize the effectiveness of these nematodes around the root zones because this is where recalcitrant pests do most damage. Thus, if ENs are applied to nursery soils it may offer an alternative to current pesticides used in the control of white grubs, the root feeding larvae of scarab beetle (Coleoptera: Scarabaeidae).

Literature Cited

Corapcioglu, M. Y. and A Haridas. 1984. Transport and fate of microorganisms in porous media: A theoretical investigation. J Hydrol. (Amsterdam) 72:149-169.

Dennis, S. O., R. E. Harrison, S. A. Aburime and T. Tsegaye. 1999. Distribution of water facilitated entomopathogenic nematodes in soil columns. Proc. Southern Nursery Association. 44:258-261.

Powelson, D. K., and C. P. Gerba. 1995. Fate and transport of microorganisms in the vadose zone. In Vadose Zone Characterization & Monitoring. Wilson et al. (Eds.). Pp. 123-135.

Wagenet, R. J., and W. Chen.1998. Coupling sorption rate heterogeneity and physical non- equilibrium in soils. In: Selim, H. M. and L. Ma (eds). Physical Non- equilibrium in Soils *Modeling and application*. Ann Arbor Press Inc. pg 1-36.

Results

Selected soil physical and chemical properties of both the Alabama soils (AS) and the Tennessee soils (TS) are shown in Table 1. The percentage of clay and silt in the AS soils amounts to 78 percent indicating that these soils have relatively smaller pore space than the Tennessee soils. The saturated hydraulic conductivity (Ksat) for both soils was low; the data shows that the Tennessee soils will conduct more water than the Alabama soils. Not withstanding both soils have diverse soil characteristics that are unique to nursery crop production.

Table 1. Soil Characteristics: The Alabama and Tennessee soils

Soil Properties	Alabama Soil	Tennessee Soil
pH_w (1:2)	5.3	5.9
Organic Matter (%)	1.20	1.76
Bulk Density (gm cm^{-3})	1.51	1.50
K_{Sat}. (cm hr^{-1})	0.76	3.16
Particle size analysis		
Sand (%)	22	57
Silt (%)	41	29
Clay (%)	37	14
Porosity	0.43	0.44

The average size of the ENs used in the study range from 500 to 700 microns in length and 18-30 microns in width. These nematodes were the infective juveniles (IJs) of the TN18 strain and have been tested for insect pathogenicity. They were maintained in the laboratory in the refrigerator and were brought to room temperature before use. The IJs are morphological and physiological adapted to remain in soil for a prolong period of time without taking food while in search for an insect host.

The observed breakthrough curves (BTCs) for nematodes and bromide for both the Alabama and Tennessee soil columns are shown in (Figure 3). Bromide breakthrough was observed after 2 and 3 pore volumes in Alabama and Tennessee soils respectively. The Alabama soils have more clay and silt content therefore bromide was slow breaking through. However, a mass balance of the tracer was almost achieved (97-99%) in both soils (Table 2). The movement of the ENs through soil columns did not follow the same pattern as the tracer, thus the ENs did not breakthrough the soils. The water application rate did not have any significant effect on the distribution of the nematodes in the soil columns. Instead, the water rate was used to mimic the ideal situation of irrigation intensity after ENs are applied to field soils. The fact that some nematodes (< 1%) were recovered in the leachate of the TS soils suggests some incidence of transport of ENs in porous media. It also shows that when ENs are applied to soils they tend to remain in the media to which they were applied. Most of the ENs (~ 85%) recovered from the soil columns in both the Alabama and Tennessee soils were in the 0-15cm surface depth (Figure 4).

In order to describe the fate of the nematodes in the nursery field soils, nematodes sorption was tested with two adsorption isotherms. The Langmuir and the Freundlich equations were used to quantify nematodes sorption behavior in the field soils. The sorption data for the nematodes fitted a linear isotherm (Figures 5) when plotted using the Freundlich equation:

$$\frac{q}{M} = kC^{1/n} \qquad \text{Eq.1.}$$

Where: q = amount of nematodes adsorbed
M = amount of absorbents
C = concentration of equilibrium solution
K, n = are constants

However, the data analysis was fitted to the logarithmic form, which gives a straight line with a slope equal to $\frac{1}{n}$ and an intercept equal to the value of log k for C = 1. The intercept is roughly an indicator of sorption capacity and the slope, adsorption intensity (Corapcioglu and Haridas, 1984). Taking the log of equation (Eq 1) it became:

$$\log \frac{q}{M} = \log k + \frac{1}{n} \log C \qquad \text{Eq. 2.}$$

The R^2 values of the isotherms were close to unity (0.999) suggesting that the Freundlich isotherm can be used to describe the sorption of nematodes to soil. The drawback is that its (isotherm) linear form does not allow it to reach maximum sorption and as a result one can not tell when the surface of an adsorbent is saturated or an adsorption has reached a maximum. Interestingly, Powelson and Gerba (1995) have also used Freundlich isotherm to describe the sorption of micro-organisms (viruses) to soil, although the viruses were smaller in size than the nematodes used in this study. Their isotherms were also linear. Conversely, when our data was plotted with the Langmuir equation, the isotherm was non-linear and the R^2 values were relatively low (Figures 6). This is not surprising because the Langmuir equation is a non-linear model and as such cannot be recommended for describing nematode sorption in soil. The Freundlich sorption parameters are shown in Table 3.

$$R = 1 + \frac{\rho K}{\theta v} \qquad \text{Eq. .3.}$$

Equation (3) was used to calculate the retardation factor, R of the ENs for both soils. Where, ρ is bulk density, θv is volumetric moisture content and k in the equation is the adsorption coefficient and sometimes referred to as the distribution coefficient K_d.

In the case of chemical adsorption to soils, if there is no interaction between the chemical and solid phase the k in equation (3) becomes zero therefore R reduces to 1. Generally, the retardation factor for non-adsorbing chemicals like chloride or bromide is assumed to be 1 (Wagenet and Chen, 1998). In our study the retardation factor is an indication of the slowing down of the ENs transport relative to the bulk of water moving through the media. The value was 4.7 for the AL soils and 5.5 for the TN soils (Table 3). Since our values are grater than 1 for both media, the assumption is that ENs has an affinity for soils and sorption to soils could be an important factor in their transport processes.

Table 2. Mass balance of bromide (applied 3200 mg L^{-1} per column).

Soil Columns	Bromide Recovered (mg L^{-1})	Percent Recovery
Alabama Soil		
1	3145	98.28
2	3153	98.50
3	3140	98.13
4	3149	98.40
Tennessee Soil		
1	3147	98.30
2	3098	96.81
3	3088	96.50
4	3151	98.48

Table 3. Freundlich sorption parameters for ENs by soil type.

Media	K	θv	1/n	R**
AL- Soils	1.032	0.331	0.99	4.74
TN-Soils	1.072	0.287	0.98	5.47

** Retardation factor of ENs in tested soils
Note: Soil columns were packed to a bulk density of 1.2g/cm^3