Analysis of *Escherichia coli* transport through the soil

A Dissertation

Submitted in the partial fulfilment of the requirement for

the award of the degree of

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

By

GARIMA BATISH

301601006

Under the guidance of

Dr. Manoj Baranwal and **Dr. Dwarikanath Ratha**

Associate Professor

DEPARTMENT OF BIOTECHNOLOGY

THAPAR INSTITUTE OF ENGINEERING&TECHNOLOGY

PATIALA, PUNJAB

DECLARATION

I hereby declare that the work which is being presented in dissertation entitled **"Analysis of *Escherichia coli* transport through the soil"** submitted by me for the award of the degree of Master of Science in Department of Biotechnology, Thapar Institute of Engineering & Technology, Patiala, is true and original record of my own independent and original research work carried out under the supervision of **Dr. Manoj Baranwal,** and **Dr. Dwarikanath Ratha, Associate Professor,** Thapar Institute of Engineering & Technology, Patiala. Further, I declare that no part of this dissertation has been submitted to any other University/Institute for the award of any degree in India or abroad.

Garima

Place: Patiala
Date- 21.8.2018

Garima Batish

Certificate

This is to certify that the project entitled "**Analysis of *Escherichia coli* transport through the soil**" submitted by Garima Batish in the partial fulfilment of the requirement for the award of degree of **Master of Science** in Biotechnology to Department of Biotechnology, Thapar Institute of Engineering & Technology, Patiala, is a record of student's own work carried by her. The report has not been submitted for the award of any degree or certificate in this or any other University or Institute.

Dr. Manoj Baranwal

Associate Professor, Supervisor
Department of biotechnology
Thapar Institute of Engineering & Technology
Patiala

Dr. Dwarikanath Ratha

Associate Professor, Supervisor
Department of civil engineering
Thapar Institute of Engineering & Technology
Patiala

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Place: Patiala

Date - 21.8.2018

Garima

GARIMA BATISH

ABSTRACT

Escherichia coli is one of the major bacterial contaminant of groundwater that leads to health ailments. In the present study, effect of different height and soil grain size on transportation of *E. coli* was analyzed by performing column experiments. Experiments were conducted by using 0.25 m long column with internal diameter of 0.06 m. Transportation of bacteria was assessed by comparing relative concentration (C/Co) of bacteria at different time intervals by performing MTT assay and plate count. In this work, the breakthrough curves of *E. coli* were studied for different grain sizes (1.6, 1 and 0.6 mm) and at different heights of soil (10, 15 and 20 cm) at a flow rate of 2.5 ml per minute. The results indicated that the maximum relative concentration of bacteria decrease with increase in height in all cases of different grain size. On the other hand, the maximum relative concentration of bacteria increased with increase in the grain size of soil particle at all heights. Retention of bacteria was more (increase in the height of soil) and less (increase in grain size of soil particle) in soil which suggests that the ground water gets contaminated fast in less height and large grain size.

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CHAPTER 1: INTRODUCTION

Groundwater is one of the major source of drinking water across the globe. In India, most of the rural areas are dependent on groundwater as their source of drinking water drawn from wells. In earlier days, groundwater has been considered as secure for human utilization and does not require any water treatment. Nowadays, waste of municipal, household and industrial are disposed in the landfills that ultimately leads to do contamination in the groundwater. In some cases, the distance between contaminated fields to the wells is less that poses a threat of abstracted water to be polluted with pathogens (Foppen *et al*, 2002). There is a poor management of a groundwater system in terms of both quantity and quality (Dhiman and Keshari, 2003). It is essential to build up a suitable groundwater quality management plan with appropriate understanding of physical phenomena movement of contaminants. Contamination of groundwater occurs due to assimilation of chemical, physical and bacteriological pollutant from different sources.

Contamination in groundwater is due to mixing of inorganic (arsenic, chloride, barium, copper, lead, and nickel) and organic (volatile organic compounds and pesticides) and microbiological contaminants (bacteria, protozoa, and viruses) from different sources. The definition of contaminant is defined as the presence of any objectionable substance in water which makes it unsafe for drinking. According to Freeze and Cherry (1979), groundwater pollutant is explained as "all hazardous particles when goes into groundwater due to human made activities irrespective of concentration, effects the quality of groundwater".

In groundwater, the most commonly used pathogenic indicators are "Coliforms" as they relatively shows a good indication of microbiological pollution. *Escherichia coli* is highly present bacterial strain in the groundwater contamination, which is 2-4 µm in length, 1 µm in diameter and rod in shape (Matthess *et al*., 1991b). A various number of experiments were conducted to observe the behaviour of *Escherichia coli* transportation in the soil (Foppen *et al*., 2004). Caldwell and Parr (1973) studied the breakthrough curve against time and space of "*B. aerogenes"* by collecting daily samples of groundwater from 100 observation wells. They observed that bacteria can transport a hundred of meters and the transport was found to be dependent on the number of factors such as initial concentration of bacteria, the flow velocity of bacterial suspension, survival rate, and grain size of aquifer and dispersion of groundwater. All these factors provide quantitative information about distances over which microbes can

travel and the time required for microorganisms to cover those distances, the ability to calculate actual rate of microorganisms transport through soil in the groundwater.

Cell size has a major effect on microorganism transportation in the soil, although it is usually observed that smaller sized microorganisms are transported faster than larger sized microorganisms. Gannon *et al.*, (1991) studied that cell size was one of the prominent parameter to observe differences in transportation of 19 strains of bacteria through soil by column experiment. Another column experiment was performed by Fontes *et al.*, (1991) where two bacterial strains were taken to understand the difference in mode of transport. In the present study, column experiment was carried by comparing the different grain size and height of soil on the transport of *E. coli* across the soil. Experimental studies supporting the effect of different factors on transport of *E. coli* through the soil is limited.

Hence, the objectives of current study are:

- To investigate the effect of grain size of soil on bacteria transport through soil.
- To compare the bacteria transport across the soil at different height.

CHAPTER 2: REVIEW OF LITERATURE

2.1 Groundwater contamination

At global level, groundwater is represented as the principal stock of freshwater and about one third of groundwater has been abstracted till now for various purposes (Siebert *et al*., 2010; Famiglietti, 2014). It is reported that in 2010, about 37% population of United States was dependent on groundwater as their drinking water source (Maupin *et al*., 2014). In India, the usage of groundwater has increased by ten-fold in the past 50 years (Margat and Van der Gun 2013).

From the pragmatic literature survey, it is observed that with the increase in the usage of groundwater, the level of groundwater is decreasing day by day due to the decrease in its hydraulic level. The impact of decrease in ground water level is increase in the cost of water abstraction from wells. Groundwater contamination and management is one of the major global issues (Zheng and liu, 2013; Gregory *et al*., 2013). Groundwater contamination is also defined as the objectionable compounds present in the groundwater which causes adverse effects to human and animal health.

The source of groundwater contaminants is the wastes generated from municipal, industrial, agricultural and household. Emerging groundwater contaminants are recognized as hazardous contaminants on the basis of properties like physical appearance, chemical characteristics and source of area. These contaminants are known as emerging groundwater contaminants.

2.1.1 Sources of contamination

There are three different sources of contamination which changes the quality of groundwater. The first source is due to natural calamities. Leaching is identified as the prominent natural source of contamination in the arid southeast of United States (scalf *at el*, 1973). Substances, such as iron, manganese, arsenic, chlorides, fluorides and sulphates which are found in rocks or soils are the examples of natural contamination source. The second type of source of contamination is related to man waste dumping activities. The various source of contamination involves dumping of mining wastes, brine disposal from petroleum industries, radioactive, municipal, and industrial wastes. The third type of source of contamination is also linked to human practices but not related to waste disposal management. These sources

involve leakage and accidental spills, de-icing salts, agricultural activities, improper constructed wells, and acid rain.

2.1.2 Types of contaminant present in groundwater and their impact on human health-

Organic contaminants

Pesticides –Pesticides are the biological or chemical substances which retarded the growth of pests and interfere with the crops growth. Pesticides have a major impact on the quality of the groundwater. Pesticide metabolites are the compounds which are biologically active and toxic in nature. These compounds are present in the groundwater at a very high range (Kolpin *et al.,* 2004).

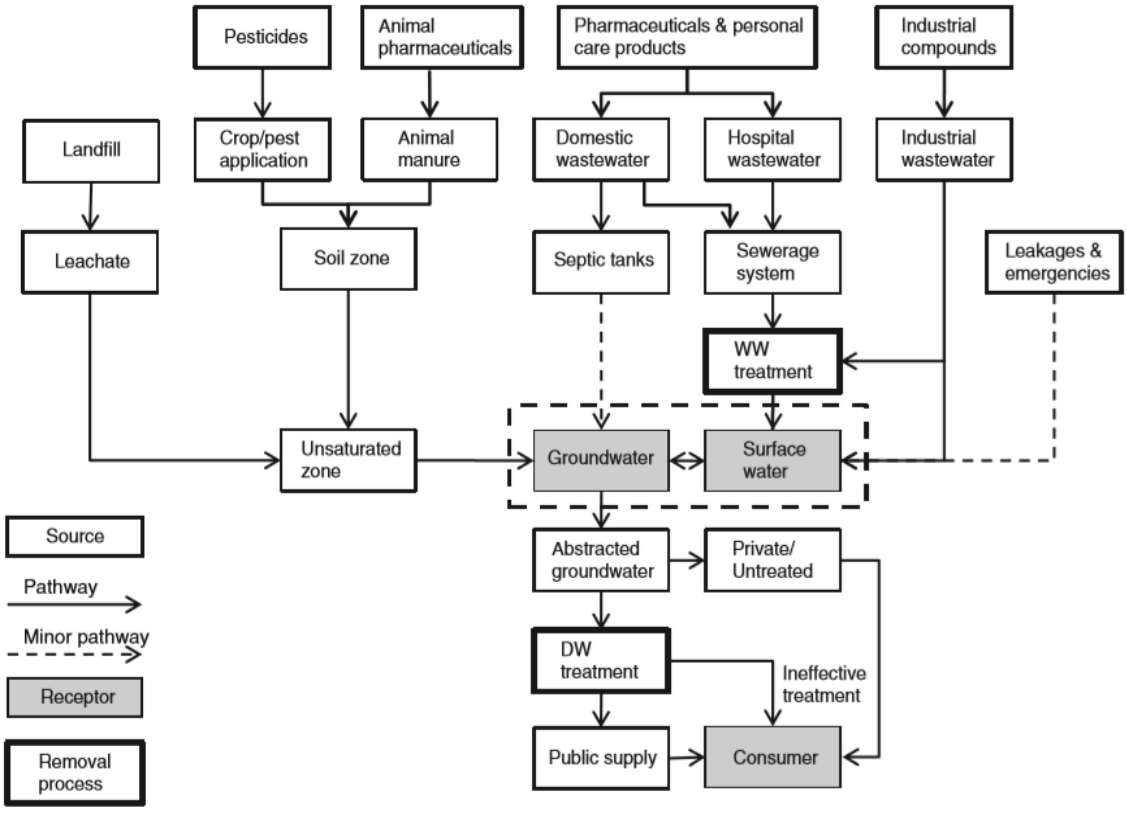

Figure 1: Pathways representing the different source that leads to groundwater contamination (Adapted from Boxall *et al.,* 2002; Jones *et al*., 2002)

Inorganic contaminants

The major inorganic contaminants present in groundwater are fluoride, nitrate, arsenic and heavy metals. Arsenic is one of the major contaminants found in the environment which is

toxic to man and other living organisms. It is generally known that arsenite [As^{3+}] and arsenate [As^{5+}] are the prime groups present in most of the polluted environments. The high range of mercury in the groundwater causes neurodegenerative diseases, mental disorders, autoimmune disorders, disorders during pregnancy and childbirth. Arsenite causes UV-induced skin cancers by effecting DNA repair system in the body.

Microbiological contaminants

The main widespread health related issue linked with drinking water is microbiological contamination from pathogenic microorganisms, such as viruses, bacteria, and protozoa. **Table 1**: Waterborne pathogens found in developing regions (Ashbolt *et al*., 2004)

Micro-organisms	**Major diseases**
Bacteria	
Salmonella typhi	Typhoid fever
Other *Salmonella*	Salmonellosis
Vibrio cholera	dysentery Cholera
Yersinia enterocolitica	Gastroenteritis B
Enteropathogenic *E. coli*	Gastroenteritis
Shigella spp.	Bacillary dysentery
Viruses	
Polio virus	Poliomyelities
Coxsackie viruses A	Aseptic meningitis
Norovirus	Gastroenteritis
Hepatitis A virus	Infectious hepatitis
Adenoviruses	Upper respiratory and gastrointestinal illness
Hepatitis E virus	Infectious hepatitis; miscarriage and death
Protozoa	
Giardia lamblia	Giardiasis (gastroenteritis)
Entamoeba histolytica	Amoebic dysentery
Balantidium coli	Balantidosis (dysentery)
Acanthamocba castellani	Amoebic meningoencephalitis
Cryptosporidium homonis, C. parvum	Cryptosporidiosis (gastroenteritis)

In developed countries, there was a progress in the field of improvement in the quality of drinking water. The bacterial contamination was found to be control but there was no control in the protozoal infections such as cryptosporidiosis and giardiasis (MacKenzie *et al.*, 1994) and viral contamination in the groundwater. On the basis of local analysis in Kanpur, India, it was observed that 80.1% population of Kanpur was suffering from waterborne disease (Trivedi *et al*., 1971).

The different types of microorganisms that were found in groundwater and could be related with waterborne disease are given in Table 2. The impact of microbial contaminant on human health is given in the table.

Bacteria

Escherichia coli are the most common indicator contaminant present in groundwater. Kudva *et al*., (1998) observed that Entero-hemorrhagic *E. coli* O157:H7 had recognised as first pathogen which has an effect on the human health. Gerba *et al*., (1975) studied that the survival rate of *Escherichia coli* in porous media and groundwater ranged from two to four months.

Filip *et al*., (1988) reported that *E. coli* survived more than 100 days at 10°C. *Staphylococcus aureus, Streptococcus faecalis, Streptococcus durans, Streptococcus pneumoniae Streptococcus pyogenes, Klebsiella pneumonia and Salmonella* are other bacterial contaminant present in the groundwater. *Salmonella* are facultative anaerobic bacteria and live under unfavourable environmental conditions i.e. pH 4-8; temperature 8-45 °C and also live for longer duration in soil and water.

Protozoa

Waterborne parasitic protozoa like C*ryptosporidium parvu, Cyclospora cayetanensis, Giardia lamblia* are present as groundwater contaminant in developing countries. *Giardia* cysts are common contaminant observed in surface waters and live for longer time. The infection in humans by protozoa is initiated by a very low infective dose. Waterborne oocysts are usually unaffected to disinfectants. Black, 1993 studied that the cases of persistent diarrhoea, are caused by *Cryptosporidium parvum, Giardia lamblia* and *Entamoeba histolytica.*

Viruses

Viruses such as Hepatitis A and E, various enteroviruses are also present in the polluted groundwater. Hurst *et al.*, (1980) studied that soil had a high adsorption capacity of virus which enhances survival rate of virus in the porous media. Presence of adsorbed viruses was reported by several workers by analysing the samples which was collected from soil columns and in the field area (Duboise *et al*,. 1976).

Table 2: Pathogens present in groundwater (Pedley *et al*., 1997)

Bacteria	**Protozoa**	**Viruses**
Escherichia coli	*Bahmtidium coli*	Hepatitis A virus
Salmonella	*Cryptosporidium species*	Rotavirus
Shigella species	*Entamoeha histolvtica*	Hepatitis E virus
Vibrio species	*Giardia*	
Streptobacillus moniliformis		

2.2 Factors affecting the transportation of bacteria

The two main processes which affect the transportation of bacteria in the porous medium (soil) are adsorption, straining, advection and Hydrodynamic dispersion.

2.2.1 Adsorption

In porous media, when the porosity of soil is larger in size than bacteria, the bacteria withhold in the soil particles. This process is known as adsorption (Sharma *et al*., 1985). Adsorption and affection of bacteria to the soil surface is a two-step mechanism. The first phase of mechanism is reversible adsorption. Reversible adsorption occurs when a bacteria passes through the soil particles that is due to weak forces of interaction (van der Waals forces) between the bacteria and the soil particles.

Reversibly attached micro-organism may detach from soil particle and arrive in the form of effluent. The second phase of attachment of bacteria on the surface of soil particles is the irreversible adsorption and also known as adhesion. Adhesion is a permanent phenomenon of attachment to the soil surface. The bacteria form links between the soil particle and does not pass through porous media.

Parameters that affect the deposition of bacteria on the area of soil particles can be characterized into three groups: chemical, physical and microbiological. Physical parameters involve the porosity of soil particles, organic content in the soil, and flow velocity of bacterial culture and temperature. Microbiological parameters involve bacterial cell hydrophobicity and concentration of bacterial cell. Chemical parameters involve ionic strength and pH.

Porosity of soil

The difference in porosity, charge on soil particles and texture of soil has prominent effect on the adsorption of bacteria on unfiltered medium (i.e. soil) (Marshall *et al*., 1971). Smaller grain size soil particle shows a more result of retention of bacteria in the soil particles (Fontes *et al*., 1991). The texture of surface of porous medium has also a major impact on adsorption of bacteria.

Higher in the variation of the charge between the porous media and bacteria; higher is the attachment of bacteria to the particles (Scholl *et al*., 1990).

The grain size of clay particles are very small, and have higher ion exchange property and surface area per unit volume. Clay particles have more adsorption capacity of bacteria than the course type of soil as the grain size is larger than the size of clay soil particles (Huysman *et al*., 1993). The sticking of bacteria to clay soil particles is high which is due to electrostatic force of attraction among positively charged particles in clay soil and the negatively charged bacteria (Fletcher *et al*., 1979). Accumulation of iron oxides in soil resulted increased positive charge that lead to more bacterial attachment (Tan *et al*., 1992).

Organic content

Organic substance present in the soil media may magnify the retention capability for microorganism (Lawrence *et al*., 1996).

Table 3: Pathogenic bacteria present in manure

Bacteria	**Survival conditions**	**References**
Salmonella	pH 4 to 8, temperatures between 8 and 45 °C, survive for long periods in soil and water	Jones((1980); Calvert *et al*., (1998)
Escherichia coli	Can grow in adverse conditions (low pH and low temperatures) and survive for long periods in soil and water	CDC(1995)
Campylobacter	Sensitive to environment stresses - does not survive in dry environment	Valcour *et al*., 2002
Yersinia	pH value between 4 to 10, temperature range between 4 and 43 °C, survive for long periods in soil and water	Gordeiko *et al*., 1990

Organic substances which are anionic in nature enhances the negative charge on the soil surface and amplify the repulsive forces between the soil and bacteria, resulting in increased movement of microorganism in the soil and reduces the retention capacity of bacteria (Johnson *et al*., 1996). Tate (1978) observed that survival rate of *E.coli* in organic soil has been increased by three-fold value after the addition of manure than in a sandy soil.

Fontes *et al*., (1992) studied the physical and chemical changes by transporting bacteria in fine sand and coarse sand of different sizes. Zhai *et al*. (1995) investigated that fecal bacteria survive more in topsoil than in subsoil. A (table 3) indicates the pathogenic microorganism present in the manure and favourable conditions which increases the survival rate of bacteria in soil media.

Water flow velocity

Smith *et al*., (1985) reported that the absorption of microorganism in soil was inversely proportional to the flow of bacterial suspension supplied to porous media. Many studies have shown that higher flow velocity results in a higher percentage of bacterial can transported through the porous media (Camper *et al*., 1993). Huysman and Verstraete (1993) reported that movement of bacteria in porous media was much higher when water was supplied at a rate of 4.7 than 0.8 cm/h.

Temperature

The survival rate of fecal bacteria was found to be reduced with elevated temperatures. Reddy *et al*., (1981) observed that mortality rate was increased two times with a rise of 10°C in temperature. Kibbey *et al*., (1978) observed that thawing and freezing of soils decreases the bacterial populations.

Filip *et al.,* (1988) reported that *E. coli* survived for over 100 days in water-soil mixtures at 10°C. Hendricks *et al*., (1979) reported that retention of bacteria was significantly larger at elevated temperatures. The decrease in attachment of bacteria with decline in temperature may be due to certain types of physical adsorption and variation in the physiology of the microorganisms (Fletcher *et al*., 1977).

Ionic strength

Huysman and Verstraete (1993) studied that divalent positive ions has increased the adsorption capacity by enhancing the attachment of bacteria to the surface as compared to monovalent positive ions. Peele (1936) found that bacteria were attached to the soil surface more with trivalent than monovalent ions.

Several column experiments was conducted by transporting bacterial cells through soil particles, and reported that the higher ionic strength of the aqueous solution decreases the consequences of repulsive forces, and thus influences adsorption as well as retention of bacteria (Abbott *et al*., 1983; Martin *et al*., 1992; Gross *et al*., 1995; Jewett *et al*., 1995). Several studies reported that the affinity of the bacteria for the surface increases with ion strengths up to 0.1 M (Marshall *et al*., 1971; Orstavik, 1977; Stanley 1983). The reduction in bacterial attachment to the surface of porous media at high salt concentration was linked to the number of cations has been present in the media. (Gordon *et al*., 1984).

pH

Ellis and McCalla (1976) studied that the pH range from 6 to 7 has been considered the most favourable for survival of bacteria (Cuthbert *et al*. 1955; Reddy *et al.* 1981). Sjogren (1994) observed that *E. coli* survived longer at a neutral pH than alkaline pH and then acidic pH in same type of texture of soil. A bacterium has a particular range of pH value, by increasing or decreasing the pH, the survival rate of bacterial get affected (Reddy *et al*., 1981). Sjogren (1994) studied that in neutral-to-alkaline soil conditions, the *Esherichia coli* bacteria survive more than in the acidic soil environment. Cohen (1992) conducted an experiment of transportation of bacteria through by using *S. typhi* and *E. coli* and observed that bacteria survived maximum at pH values of 5–6.4.

Hydrophobicity

Hydrophobicity involves the affection of microorganism to the surface of soil particles. According to Gilbert *et al*., (1991) hydrophobicity means sticking of hydrophobic microorganisms, apart from their ionic surface charge.

In a study of bacteria transportation in the soil, hydrophilic bacteria were seemed to be faster as compared to hydrophobic bacteria because hydrophobic bacteria get attached to porous

media (Huysman *et al*., 1993). However, Gannon *et al*. (1991) studied that there was no relation between hydrophobic nature of bacteria and bacterial transport in soil porous media.

Concentration of bacteria

The rate of deposition of bacterial cell on soil particles is directly proportional to the biomass of bacteria passed through the soil particles for a particular period of time (Escher *et al.*, 1990). A similar result has been also observed by Wollum *et al*., 1978 and Floodgate *et al*., 1966.

2.2.2 Straining

The physical blockage of microorganism transportation due to the pore size of soil (Corapcioglu *et al*., (1984)). Factors that affect straining are grain size of the soil particles, bacterial cell shape and size, and hydraulic capacity.

Grain size

The grain size of soil particle is a major feature leading bacterial transportation (Ausland *et al*., 2002). Coarse and fine soil is the different types of soil that depends upon their pore size and it influences most bacterial cells transport. Straining term can be a used in restricting the bacterial transportation in the porous media (Matthess *et al*., 1985). The level to which bacteria retained in soil particles by straining was inversely comparative to the size of the porous media particles (Hagedorn *et al*., 1981).

Normally, straining term becomes an essential exclusion mechanism when the average cell size of the microorganism is larger by 5% than the size of the soil particles (Updegraff *et al*., 1983). Bouwer *et al*., (1984) observed that straining occurred when the width of the microorganism was larger by 0.2 times than the width grain size of soil particles.

Bacterial cell size and shape

The shape and size of bacteria has a direct relationship between transport through a porous medium (Bitton *et al*., 1974) and straining of bacteria. According to Corapcioglu and Haridas (1984) observed that larger size of bacteria are suppose to be transported earlier by the method of filtration and smaller size bacteria to be retain in the soil particle. Gannon *et al*.,

[1991] observed that there is a positive relation between bacterial transport in the soil particle and straining.

Gannon *et al*., [1991] conducted an experiment with 19 different strains of bacteria and shown that bacterial cells with shorter length (≤ 1mm) were transported faster than bacterial cells of longer length (≥ 1mm).

Hydraulic Capacity

Flow velocity of bacterial suspension has a strong impact on the transportation of bacteria through porous medium. Higher the flow velocity of bacterial suspension, higher is the transportation of bacteria through unsaturated porous medium. This ultimately decreases the straining capacity of bacteria in the soil particles. Gannon *et al*., (1991) had determined the transportation of bacteria (*pseudomonas* sp. strain KL2) by constructing a column experiments. Those experiments were conducted by using 0.3 m long Plexiglas columns with an internal diameter of 0.05 m and the experiments were conducted with a flow rate of 10^{-4} m s^{-1}.

2.2.3 Advection

Yates and Yates, 1999 studied that the transportation of microorganisms in soil was dependent upon the flow velocity of water. In a simple model, advection is equal to the average speed of water which was defined as the product of hydraulic conductivity and hydraulic gradient and divided by porosity of the soil particle (Corapcioglu and Haridas, 1984).

2.2.4 Hydrodynamic dispersion

According to Tim *et al.,* 1988, the hydrodynamic dispersion is defined as "The distribution of microorganisms as they travel along the water path due to both macroscopic and microscopic effects". It is calculated by determining the concentration and time dependence of a tracer with respect to a collecting point of effluent in a particular path of flow.

2.3 Column experiments

To demonstrate bacterial transport across the soil

An example of column experiment set up is shown in figure (2) where the bacterial suspension containing flask was positioned on the top of the transporting column, which was made up of acrylate, the diameter (7.6 cm) and length (10 cm) (Jin *et al.*, 2000). Needles were placed on the top of transporting column to provide uniform distribution of input solution. A stainless steel porous plate was placed at the bottom of column. Column outlet was attached to a vacuum chamber with a fraction collector within. By assimilating the flow rate of the input solution, a uniform run of water has been achieved.

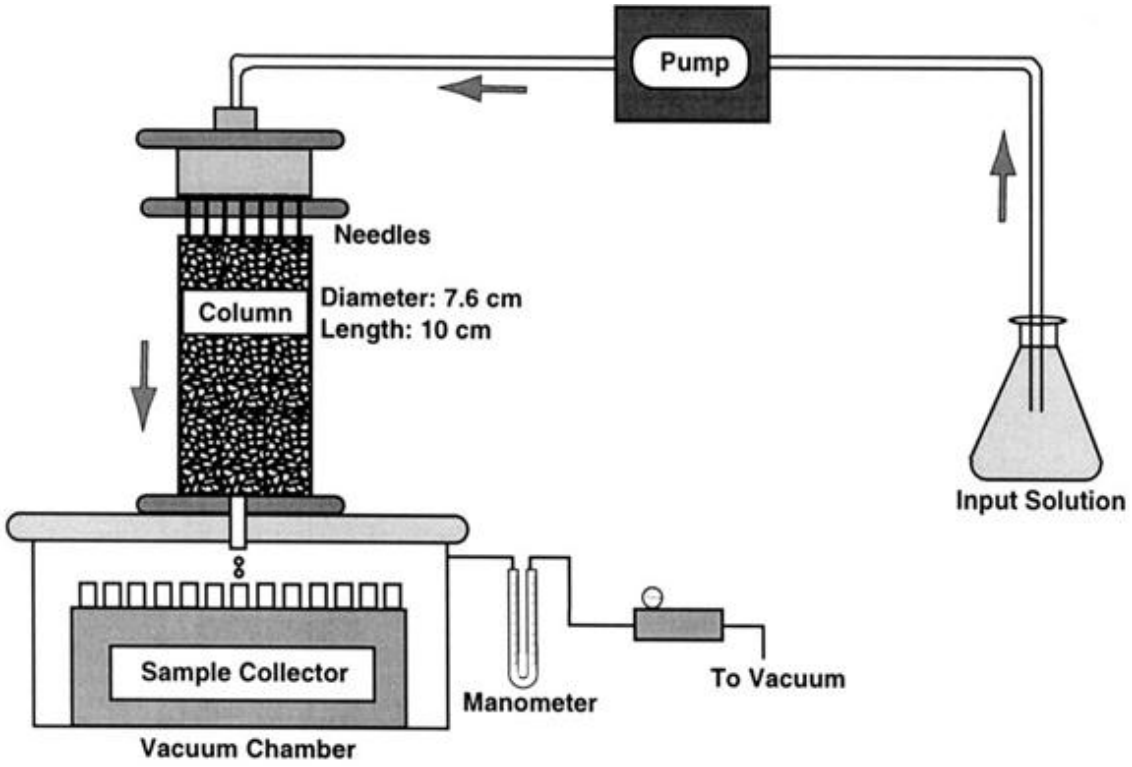

Figure 2: A schematic representation of column experiment (Jin *et al*., 2000)

Gannon *et al*., 1990 conducted a column experiment to demonstrate the transportation of bacteria from porous media by using a column with diameter (0.05 m) and length (0.3 m). In this study, the effect of sodium chloride on the behaviour of bacterial transport was analysed. The experiment was conducted by using two different cell concentrations (10^8 and 10^9 cell per ml) of *Pseudomonas* sp. strain KL2 and the flow rate kept was 10^{-4} m/s. When the flow rate was 10^{-4} m/s and the concentration of *Pseudomonas* used (10^8 cells per ml), the breakthrough was slower in deionised water in comparison with sodium chloride (0.01 M) which indicates

that addition of sodium chloride lead to retention of bacteria in the column. Although increase in cell concentration (10^9 cells per ml) did not show the same effect and the pattern was found to be same in both sodium chloride solution and deionised water. Fontes *et al*., 1991 reported that W6 (coccus) and W8 (rod) bacterial strains collected from the wells of eastern shore of Virginia. Glass chromatography columns were used as experimental column whose diameter was 4.8 cm. The sand sample was divided into two different grain size classes (fine and coarse sand particles). It was observed that peak height and recovery rate was high in the case of homogenous coarse sand than fine-homogenous sand as indicated in table 4.

Table 4: Order of percentage recovery (Fontes *et al*., 1991)

Bacterial strain	**Recovery (%)**	**Ionic strength**	**Grain size**
W6	88.35	Low	Coarse-grained, homogeneously packed sand
W6	79.15	Low	Heterogeneous.
W6	49.30	High	Coarse-grained, homogeneously packed sand
W8	43.55	Low	Coarse-grained, homogeneously packed sand
W8	39.05	Low	Heterogeneous.
W6	19.70	High	Heterogeneous.
W6	14.50	Low	Fine-grained, homogeneously packed sand
W8	11.70	High	Heterogeneous.
W8	4.45	High	coarse-grained, homogeneously packed sand
W8	3.90	Low	Fine-grained, homogeneously packed sand
W6	2.75	High	Fine-grained, homogeneously packed sand
W8	0.34	High	Fine-grained, homogeneously packed sand

The coarse sand was having three times more the diameter of the fine-grain sized sand, and recovery rate was more in the case of columns having coarse sand than the fine sand column as shown in table 4. Due to a 3-fold-lesser sand surface area in coarse sand containing columns than fine sand filled columns, there is a proportionately lesser number of sites

available for bacterial cell attachment on sand particles. There is 5.5-fold increase in the flow discharge in the case of coarse-grain sized columns as compared to the fine-grain sized columns. The recovery of bacterial strain of W6 and W8 in different grain size, homogenous and heterogeneous condition was given in table 4. Increasing ionic power increases the ability of bacteria to attach to the sand surface by raising the accessibility of ions in solution which can form links between charged sites on the sand area and on the bacterial cell surface (Scholl *et al*., 1991).

CHAPTER 3: MATERIAL AND METHODS

3.1 Preparation of media

Nutrient broth is normally used for the growth and development of less fastidious microorganisms. It consists of peptone and yeast extract which provide the necessary amount of carbon, nitrogen, vitamins content and also contain some trace elements for the growth of bacteria. One of the component i.e. sodium chloride is mainly used to regulate the osmotic balance of media.

Media was prepared by dissolving 1.3 gm of nutrient broth in 100 ml of distilled water in 250 ml flask. The mouth of flask was sealed with cotton plug. Then the flask was autoclaved at 121°C for 15 min at 15 psi.

3.2 Revival and maintenance of bacterial culture

The culture of *Escherichia Coli ESS 2231*was obtained from Nicholas Piramal. The *E.coli* sample was at 4°C. Normalised the temperature of bacterial culture was brought to room temperature and then 0.5-1% of bacterial inoculum was inoculated in 100 ml of nutrient broth in laminar air flow and kept the culture flask in an incubator shaker at 37°C overnight.

3.3 Soil sample preparation

Collection of sample- Soil sample was collected from Thapar Institute of Engineering & Technology. Soil sample was dried overnight in the oven at 100°C.

Sieve analysis

Sieve analysis is a technique used to verify the particle size distribution of soil samples. This causes a comparative movement between the grain size of porous media and the sieve, depends on the individual particles size which may move across the sieve mesh or retained on the sieve surface. Different grain sizes of soil particles as shown in the figure 4.

The different sizes of sieves were arranged in descending order, with larger size sieve at the top and the smallest size sieve at the bottom. The dried soil sample was poured from the top of stack of sieves and placed the stack of sieves on the mechanical shaker as shown in the figure 3. The clamp was fixed and the top of the first sieve was covered with lid and adjusted the timer for 10 min. The soil sample was autoclaved at 121°C for 30 min at 15 psi for consecutive 3 days.

Figure 3: Mechanical shaker used for sieve analysis

Figure 4: Different grain sizes of soil particles

3.4 Bacterial culture preparation

The bacterial suspension when reached at absorbance 1 at wavelength 600 nm, the bacterial suspension was centrifuged at 8,000 rpm for 10 min at 4°C and the supernatant was removed and pellet was resuspended in 200 ml autoclaved distilled water which was stored temperature of around 4°C. The resulting bacterial pellet suspension was used to perform the column experiments. The different concentrations of bacterial suspensions were prepared by diluting with autoclaved distilled water.

Plate Method

Preparation of nutrient agar plates- Nutrient agar normally contains nutrient medium for the growth of non-fastidious microorganisms.

Table 5: Composition of nutrient agar

Components	**Uses**
0.5% Peptone	Contains organic nitrogen source
0.3% Yeast extract	Source of vitamins, carbohydrates
1.5% Agar	Solidifying agent
0.5% Sodium chloride	Maintains salt concentration in the medium

Weighed 10.5 gm of nutrient agar and 9.1 gm of nutrient broth and dissolved them in 700 ml of distilled water in a flask. Autoclaved the flask at 121°C for 15 min at 15psi and allowed it to cool but not solidify.

Pour plating

Poured 20-25 ml nutrient agar into each plate and allowed it to solidify by removing the lid of the plate in a laminar air flow. Then wrapped the plates with clean-film and stored the plates in a refrigerator at 4°C.

Serial dilution

A series of sequential dilutions were made to get a countable number of colonies or to reduce a dense bacterial culture by increasing the number of dilutions. In microbiology, 30 to 300 colonies are countable, below or above of this range are not countable.

$$\text{Colony forming unit (cfu)/ml} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume of culture plate}}$$

Prepared nine test tubes containing 9 ml of sterile sodium chloride and transferred 1ml of bacterial culture in the first test tube and marked the test tube with 10^{-1} and then transferred 1 ml from 10^{-1} test tube to next test tube and labeled the test tube with 10^{-2} and so on till $10^{-9.}$

Spreading of bacterial culture

100 µl of bacterial culture was spread on nutrient agar plate with the help of spreader in the laminar air flow and sealed the plate with clean-film. Overnight incubated the plates at 37°C.

3.5 Column preparation

The dropping funnel used as column and was autoclaved prior to each experiment. The autoclaved column whose diameter is 0.06 m and height is 0.25 m long, was filled with soil at different heights (10, 15 and 20 cm) of column in the laminar air flow cabinet. The column was firstly filled with 10-20 glass beads so that soil sample not passed through bottom of the column.

Figure 5: A picture of column setup

3.6 Column experiment

The bacterial pellet culture (200 ml) was passed through column which was packed with soil sample of different grain sizes at different heights of column. The whole experiment was conducted at 4°C to stop the growth and decay of bacterial cells. The experiments were carried out at a flow rate (2.5 ml/min) of bacterial culture. After, the complete bacterial pellet culture was drain out through soil column, 350 ml autoclaved water was run in the same soil column with same flow rate (2.5 ml/min).

Sample collection

The effluent was collected at different time intervals i.e. the first seven samples were collected at an interval of 30 sec and the next seven were collected at an interval of 1 min. After that another slot of next seven samples were collected at an interval of 2 min. In the same way, next effluents were collected at an interval of 5, 10 and 15 min respectively.

3.7 Assessment of viable cells count

MTT

It is a colorimetric assay for assessing the number of viable cells. The assay is based on the principle where the MTT [3-(4, 5 dimethylthiazol-2-yl)-2-5, diphenyltetrazolium bromide)] reduces to a purple coloured product called formazan due to the presence of dehydrogenase enzymes secreted from mitochondria of the metabolic active cells (Figure 4)(van Meerloo *et al.* 2011). The product is produced in the form of crystals which are required to be dissolved by dimethyl sulfoxide (DMSO).

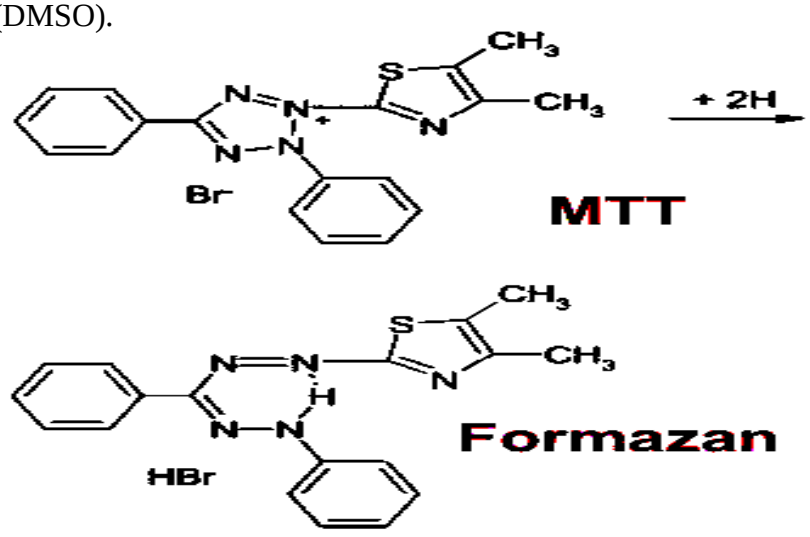

Figure 4: Principle of MTT assay

200 µl of each effluent was poured in the 96 well plates and 20 µl of MTT was added in each well. Then the plate was incubated for four hours. After incubation, 170 µl of media was discarded and 100 µl of DMSO was added in each well. The experiment was performed in triplicates. Absorbance was recorded by taking 570 nm as measurement wavelength and 620 nm as reference wavelength on ELISA plate reader.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Effect of different heights and grain size on *E. coli* transport

Studies have shown that bacterial retention is dependent on several factors such as height, soil pore size, flow rate, pH and temperature. Considering the significance of height and grain size of soil, three different heights (10, 15 and 20 cm) and three different grain size (0.6, 1 and 1.6 mm) were taken and estimated their retention capacity based on relative concentration (C/C_0). The breakthrough curve was plotted which is time versus relative concentration to compare the retention of *E. coli* in different conditions taken in this study. All experiments were carried out at a flow rate of 2.5 ml per min and 10^8 cells per ml. The break curve plot have shown as individual for all conditions and there is clear pattern of variation was observed in *E. coli* transport in different heights and grain size (Figure 6-8).

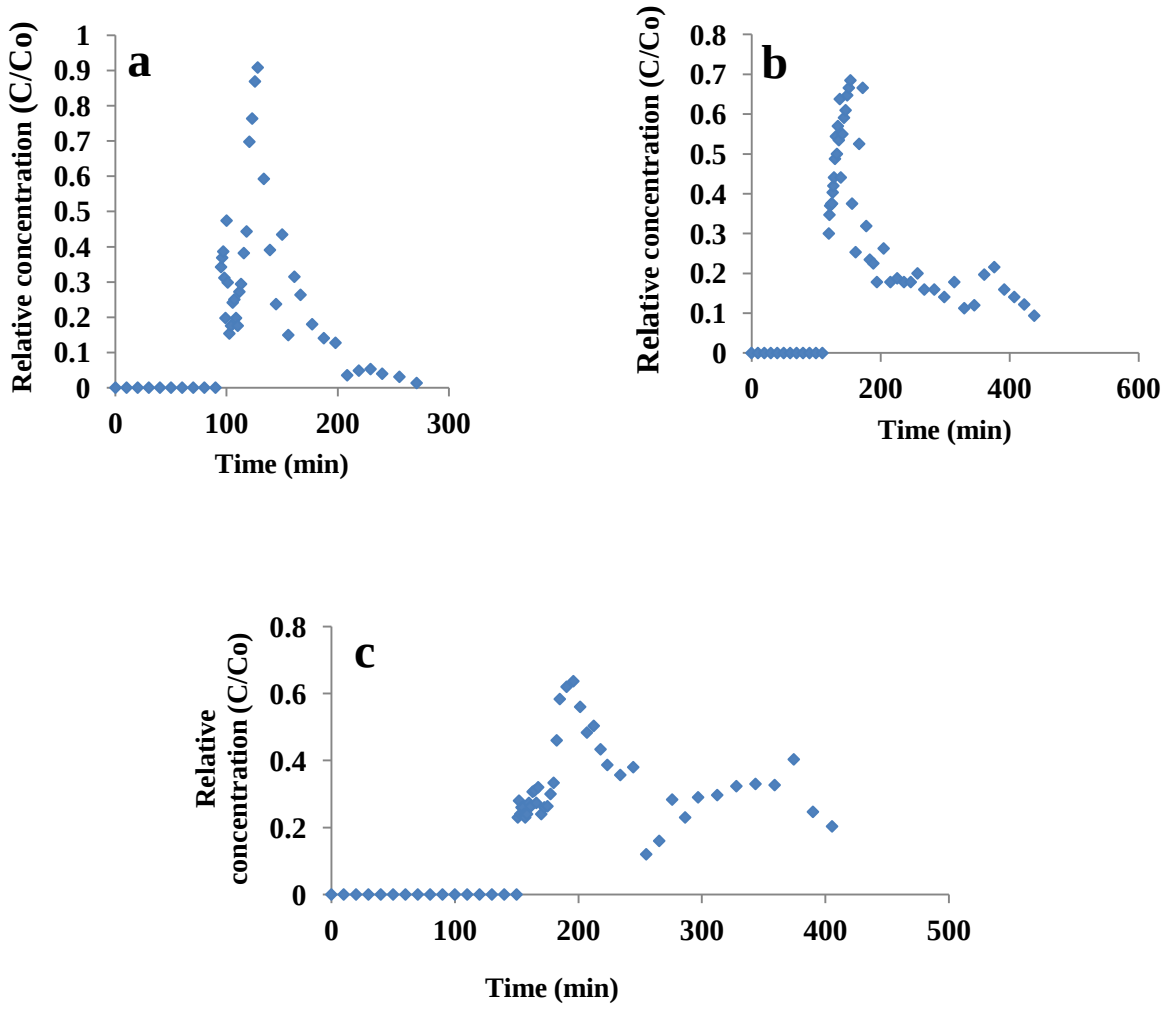

Figure 6: Breakthrough curve of *E. coli* grain size 1.6 mm having **a)** height 10 cm, **b)** 15 cm and **c)** 20 cm.

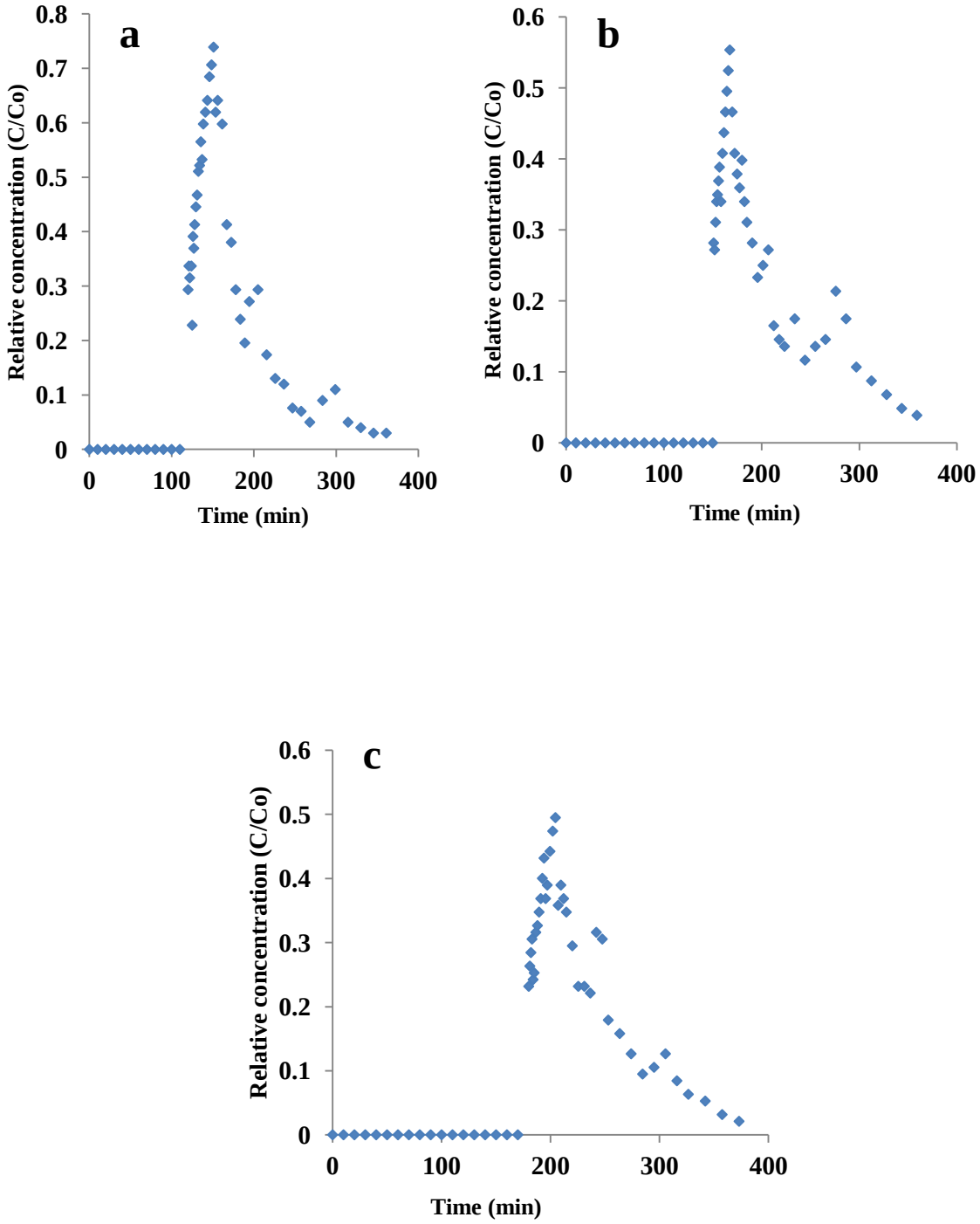

Figure 7: Breakthrough curve of *E. coli* grain size 1mm having **a)** height 10 cm, **b)** 15 cm and **c)** 20 cm.

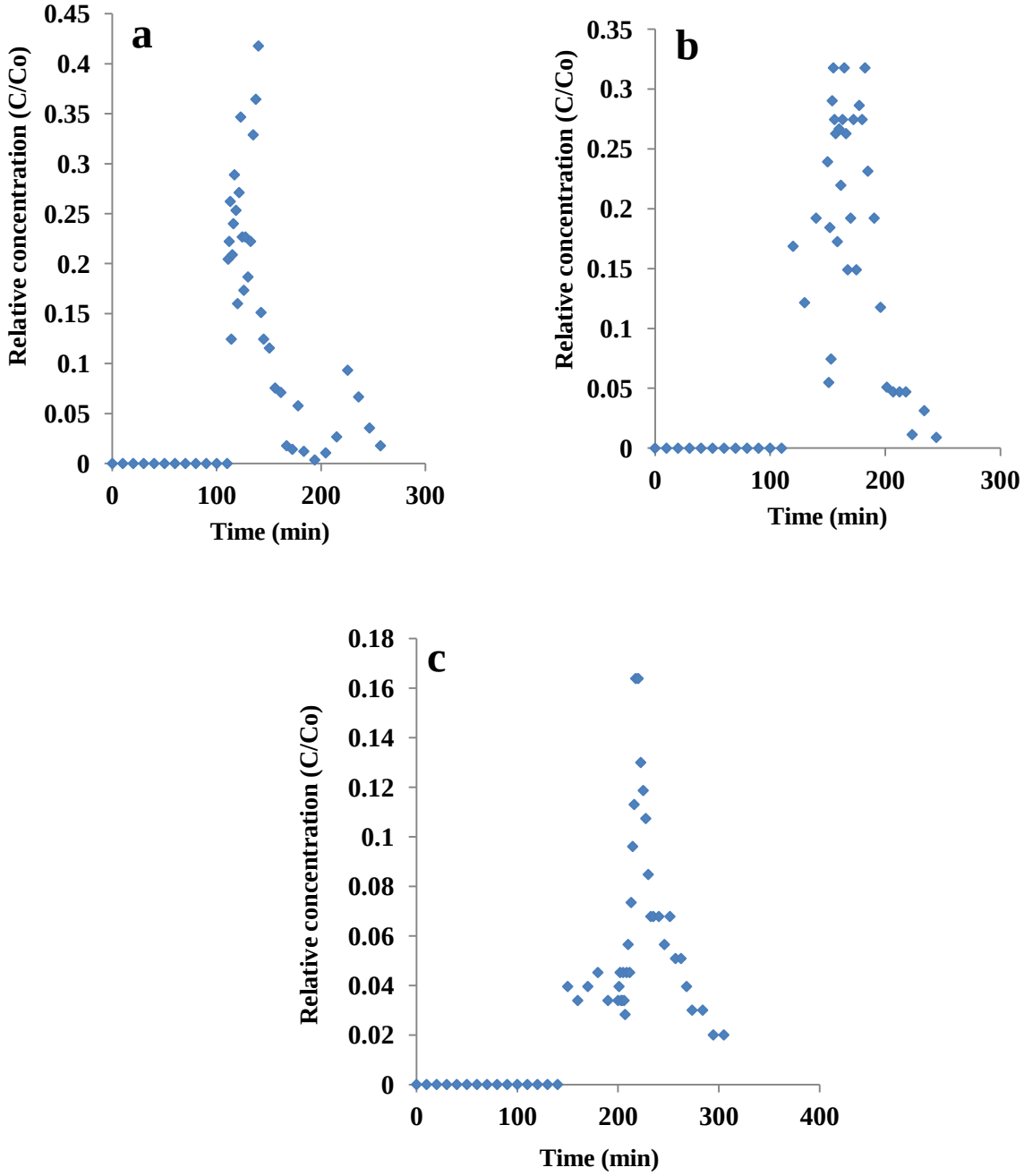

Figure 8: Breakthrough curve of *E. coli* grain size 0.6 mm having **a)** height 10 cm, **b)** 15 cm and **c)** 20 cm.

4.2 Effects of different heights on *E. coli* transport

It was observed the maximum relative concentration decreased in a larger extent as the height of column increases for all different grain size (Figure 9). The time required to achieve the maximum concentration also decreases with increase in height.

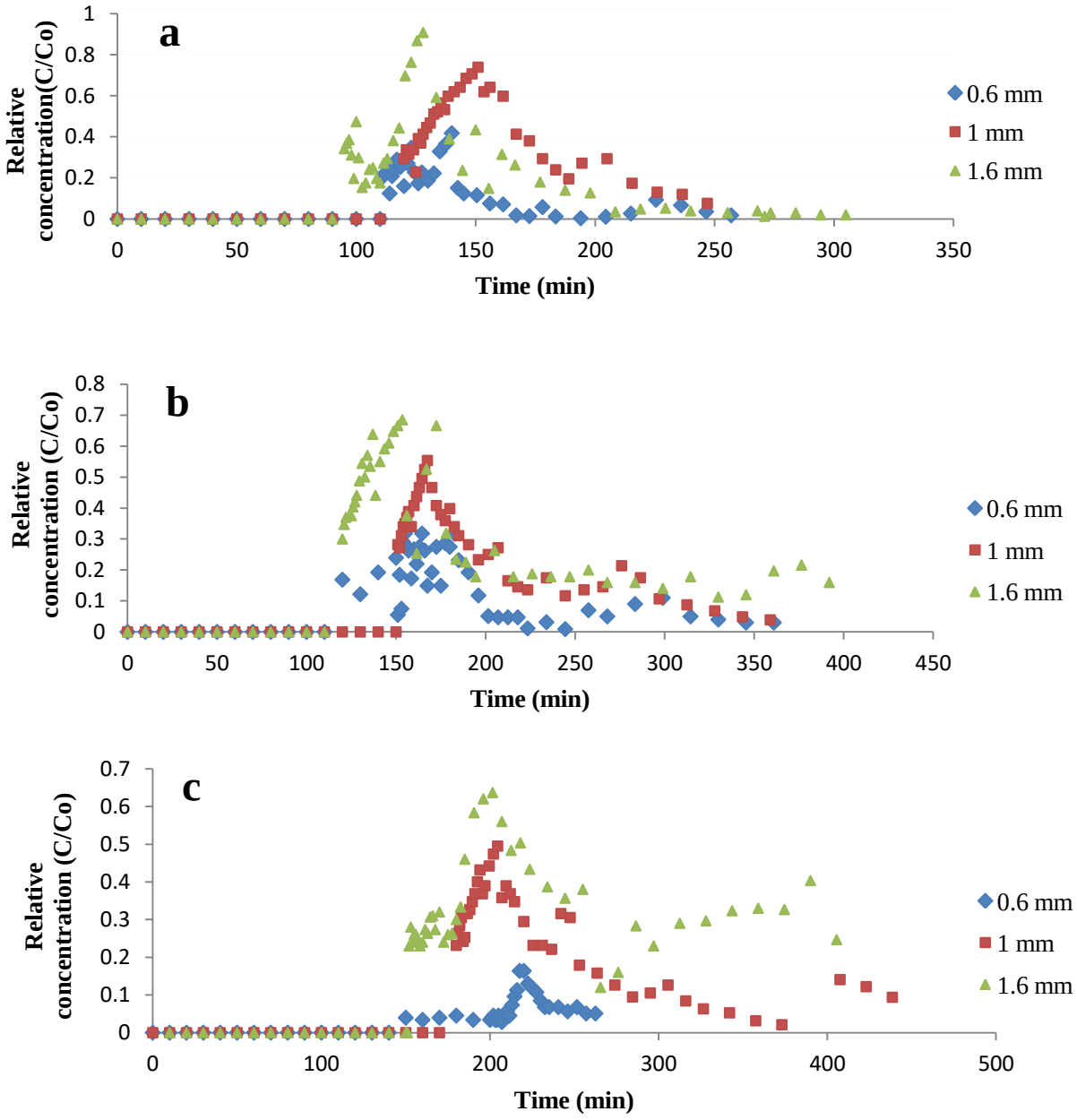

Figure 9: Breakthrough curve of *E. coli* at height a) 10 cm b) 15 cm and c) 20 cm with different grain size (0.6, 1 and 1.6 mm).

4.3 Comparative Effect of different grain on *E. coli* transport

When the grain size decreases, the relative concentration is decreases in a larger extent and also the time required to achieve the maximum concentration increases as the grain size decreases as shown in (figure 10). This indicates that in silt, clay type of soil, the concentration at the farther end will be less in comparison to sandy soil.

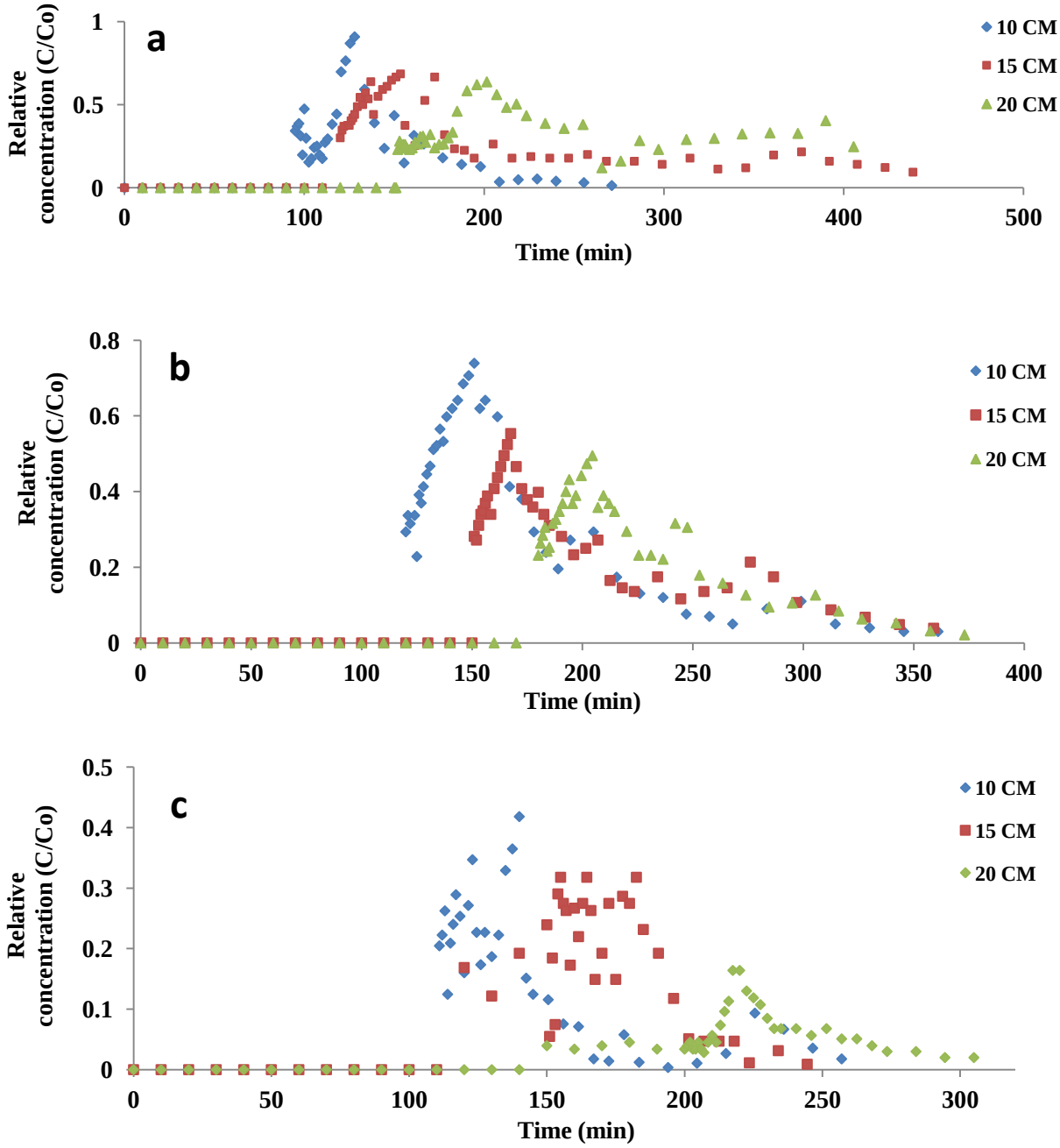

Figure 10: Breakthrough curve of *E. coli* having grain size a) 1.6 mm, b) 1 mm and c) 0.6 mm at different height (10 cm, 15 cm and 20 cm).

Table 6: Maximum relative concentration of *E. coli* obtained at different height of column and grain size of soil.

Grain Size (mm)	Height (cm)		
	10	15	20
0.6	0.42	0.32	0.16
1	0.74	0.55	0.49
1.6	0.91	0.68	0.64

Table 7: Time required for achieving the maximum relative concentration of *E. coli*.

Grain Size (mm)	Height (cm)		
	10	15	20
0.6	140	182.5	220
1	151	167.5	209.5
1.6	128	153.5	196

The time required to attain the maximum concentration as given in (table 7) was increased as the grain size of soil decreases and the height of the column increases. The maximum relative concentration of different grain size (0.6 mm, 1 mm and 1.6 mm) at different height was given in the table 6.

CONCLUSION

Ground water contamination is one of the global challenge for human survival especially the biological contamination leads to several health ailments. Hence, the study on different factors such as height and grain size on bacterial transport across the soli will give insight in management of ground water contamination. From the current study it was concluded that as the height of column increases, the maximum relative concentration of *Escherichia coli* decreases and opposite is the case with grain size was observed. This happened due to more adsorption occurring in the fine type of soil than the coarse type of soil.

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