CHAPTER 1

INTRODUCTION

“God created Mankind and Microbes and left them to live happily in the beautiful earthly garden. They all sinned and their Maker was angry. He ordered them to, thereafter struggle for their continuous existence and survival here on earth. In the process, Mankind Exploited and Microbes Revolted, thereby ensuring a continuous conflict or war between them”

-Agbonlahor

1.1 MOTIVATION AND BACKGROUND

Water is the most versatile liquid which plays a pivotal role in sustaining life on the planet as well for its developmental activities. According to Dr. Masaru Emoto, Chairman, International Water for Life Foundation Japan—“Water is almost living and as sensible as we are; good music can positively affect water; water can positively react to good or bad words, peace or war, love and prayer, water can copy what it was shown and as such we should love and respect water because words have vibration and spirits”. Nowadays, these precious natural sources is under direct threat either from over exploitation or pollution, exacerbated by human activities and it is the most poorly managed resource in the globe (Fakayode 2005).

The main contamination sources of water include seepage from waste lagoons, contaminated surface water or subsurface sources such as septic tanks, broken or leaking sewer lines or improperly designed land fill (Howard et al. 2003), livestock and stored manure (Banks et al. 2002). The extent of
groundwater pollution depends on hydro geological and soil condition of surrounding environment, depth to water table and distance between groundwater source and onsite sanitation system. It is estimated that 1.7 million people are affected annually, as a result of diarrhoeal disease and the reasons attributed are unsafe water, poor sanitation and deficient hygiene (WHO 2002; Ashbolt 2004).

Quality of groundwater depends on understanding the subsurface water chemistry and fresh water availability in a region (Achari 2006). In India, groundwater is an important water resource for domestic and agriculture in both rural and urban areas (Krishnakumar et al. 2015). The need to assess the groundwater quality is becoming increasingly important in a global perspective (Unnisa and Zainab 2017). It is only because of unfailing research initiatives and basic treatment techniques adopted, the public health is protected which thereby limit the level of contaminants in drinking water.

1.2 GROUNDWATER

Ground water is an important source of potable water available for human consumption in the world. It is a renewable resource that can sustain various anthropogenic activities, if judiciously used (Shaji 2011). Variation in physical and chemical parameters determines the quality of groundwater and is greatly influenced by geological formations (Subramanian 2000). Likewise, the suitability of groundwater for domestic and irrigation purposes is greatly determined by its geochemical constituents.

Subsurface rock formations control the composition of soil and hence that of water and vegetation. Geochemistry of groundwater explains that
there is a link between the chemical composition of groundwater and the health of plants, animals and human beings (Jayaseelan et al. 2012). Decrease in soil fertility and the groundwater contamination is due to the use of untreated waste water for agricultural purposes (Golekar et al. 2013). It is estimated that 38% of improved water sources globally are contaminated by faecal indicator bacteria (Bain et al. 2014). The untreated groundwater still serves as a major source of enteric disease globally and the proven disease burden is only ‘the tip of the iceberg’ (Murphy et al. 2017).

**Figure 1.1:** Demonstrates how man-made pollutants enter the groundwater supply (Source: Zaporozec and Miller, 2000)

Variation in groundwater quality occurs from place to place, and it is related to factors like seasonal changes and well depth. On the other hand, declining and rising water tables are also found to be unfavorable for groundwater quality (Inderjeet 2005). Moreover, uncontrolled dumping of domestic solid waste on land results in environmental, social and health problems (Figure 1.1). The adverse impact of such landfill contaminates the
underlying soil and groundwater that leads to health hazards. Likewise, the percolation of leachate from uncontrolled landfill site into the soil aquifer is also considered as a serious problem of water environment (Dhage et al. 2005).

About 65% of Indian villagers use well water (groundwater) for domestic activities. On the other hand, rivers, lakes and springs meet the demand of 15% of the rural populations. However, 2% of the villages are supplied with protected water. Eight percent utilize tube-well water, while rest depends on tank, pond and sundry resources (Kapoor 2001). About 10% of the urban population does not have access to regular safe drinking water while 30% of urban and 90% of rural households depend on unsafe water sources to meet their daily needs (Sajjad 2006). It is estimated that diarrhoeal diseases are responsible for approximately 2.5 million deaths annually in developing countries, affecting children younger than five years (Kosek et al. 2003).

The present scenario illustrates the prevalence of water crisis both in rural and urban sectors. The problem of groundwater quality is more acute in the areas which are characterized by dense population, heavy industrialization and shallow groundwater table (Patil and Patil 2010). Another issue related to groundwater bodies is the increasing prevalence of antibiotic resistant bacteria. So, there is a high demand for development of new and improved antimicrobial agents that will be effective against pathogenic bacteria, without any fear of resistance (Ogbodo et al. 2011). Hence, there is always a need for and concern over the protection and management of groundwater quality.

The chemicals used to disinfect groundwater include chloramines, chlorine dioxide and ozone. These chemicals are associated with disinfection by-products (DBPs) like trihalomethanes (THMs), which are the most common
and widely measured DBPs (Hwang et al. 2002). Researchers have proved the relationship between DBPs and several forms of cancer (e.g., bladder and rectal cancer) as well as adverse reproductive outcomes. Several epidemiological studies have investigated the association between chlorination of drinking water and risk of birth defects (Hwang et al. 2002). Nevertheless, little is known about the potential health effects on exposure to DBPs (Nieuwenhuijsen et al. 2009). Many researchers who have addressed this topic have been confronted with methodological difficulties, especially with respect to exposure assessment. Typical studies rely on approximate methods of exposure assessment that do not account for individual variability in terms of water consumption (Zender et al. 2001)

1.3 BACTERIOPHAGE

Bacteriophages are type of viruses that infects and replicates within bacteria were discovered by Felix D’Herelle and Frederick Twot (Trudil 2015). Bacteriophages have been identified as a potential alternative to antibiotics (Haq et al. 2012). Bacteriophages also known as phages are bacterial viruses composed of nucleic acid genome enclosed within a protein or lipoprotein coat (Sillankorva et al. 2012). Phages are ubiquitous and almost all the bacteria possess specific bacteriophages that can be isolated from the environment where the host bacteria survive (Kutter 2005). More than 1031 bacteriophages are reported so far from the earth’s biosphere (Endersen et al. 2014). Further, bacteriophages that infect different host bacteria were isolated from diverse sources such as human feces, animal feces, food, water, soil and sewage (Atterbury 2009). However, only limited number of bacteriophages was identified so far and there is a wide scope for the identification of newer phages and their application in various fields (Casjens 2008). Therefore, there is a need
to isolate and identify bacteriophages that could be used as a potential agent to control pathogenic and multidrug resistant bacteria in water bodies.

1.4 BACTERIOPHAGES AS MICROBIAL TRACERS IN SUB-SURFACE SOIL

The communication between phage and bacteria is an integral part of soil ecology, and phages act as an excellent matrix for this interaction. Many factors influence this mechanism but the water content present in the soil is a crucial factor that helps phages to be in contact with resident bacteria and minerals (Chu et al. 2003). Moreover, for the growth and survival of soil microbe’s, water is utmost important, therefore measuring the soil water content becomes vital. Both the moisture content and moisture holding capacity of soil mainly rely on the type of soil and the climatic conditions (Williamson et al. 2005).

Apart from soil water content, many other elements determine the phage-host interaction in soil which includes: pH, temperature, organic matter, water and clay content, ionic strength, hydrophobicity balance, rhizosphere characteristics, etc. Among which the major factor is soil organic matter, present in dual forms (dissolved and particulate) (Armon 2011). The mitigation of phage in sub-surface soil is very important in transport studies and it is mainly influenced by water content and flow through the soil zone. Other parameters like pH, temperature, organic load, and soil type also determine phage transport in saturated soil conditions (Schijven and Hassanizadeh 2000).
A large variety of microorganisms such as bacteria, phage and archae are harboured by vadose zone, the most important part of the sub-surface soil extending from terrestrial surface to groundwater table (Holden and Fierer 2005). The fluctuating capillary forces prevailing in this unsaturated soil supply necessary water content for microbial activity in the vadose zone. Phage movement is impacted by the flow of water and soil porosity/adsorption in dissolved and particulate phase respectively (Armon 2011).

1.5 BIOLOGICAL WATER TREATMENT USING BACTERIOPHAGES

The production of pathogen and toxic chemical free water is the preliminary aim of water treatment. This water should also be maintained safe by regular monitoring, to protect public health from water-borne diseases. Therefore there is high concern and demand in developing an economic, low cost pathogen management system to monitor food and water supplies (Petroman et al. 2010). In the recent past, combinational approach using phage lysins and other natural antimicrobial agents have gained interest in water treatment (Raghu et al. 2012). The potential application of phages in removing MDR bacteria has made them a distinguishing tool in water (Dabrowska et al. 2003).

As in the case of soil a number of factors influence the survival of phage in water environments, some of them includes: density, temperature, pH and host bacteria (Stevens et al. 2001). Among phages, coliphages are preferred as virus surrogates in various disinfection studies as they are found to be free of health risk (Leclerc et al. 2000). Since the removal of clinically important bacteria is of concern in waste water treatment plants, bacteriophages can act as natural antibacterial agent in these systems (Khan et al. 2002). Also, the
occurrence of phage is possible where ever the host bacteria are present making it a natural process of bacterial pathogen removal from environment (Raghu et al. 2012). Therefore treatment using phages can be considered as an effective and alternative strategy in curing problems related to waste water through optimization (Withey et al. 2005).

Stability and infectivity of phage is a crucial factor determining its capacity to be a disinfectant (Ahiwale et al. 2013). This can be achieved by preparing various formulations of phage, which in turn can increase its shelf-life. High titer of bacteriophages were maintained by encapsulating them in biodegradable polyester microcapsules (Puapermpoonsiri et al. 2010), stabilized in freeze-dried cakes of polyethylene glycol plus sucrose (Puapermpoonsiri et al. 2009) and by lyophilising in the liquid media etc.

1.6 POTENTIAL ADVANTAGES OF BACTERIOPHAGE

Bacteriophages replicate at the site of infection, and no side effects have been reported during or after phage application (Conway et al. 2001). The main advantage of bacteriophage is their ability to target bacteria of certain strains or species, without any harmful effect on the rest of the bacterial flora. Another benefit is their self-limited propagation which is controlled by the availability of a sensitive host (Sankaret al. 2007).

Bacteriophages are environmental friendly and are based on natural selection, isolating and identifying bacteria in a very rapid process and which are very cost effective (Weber-Dabrowska et al. 2000). Bacteriophages get adsorbed on to the host cells and phage entry is mediated by specific receptors such as carbohydrates, proteins and lipopolysaccharides present on the surface.
of host cell (Marks and Sharp 2000). Although bacteria can become resistant to phages, phage resistance is not nearly as worrisome as drug resistance. Like bacteria, phages mutate and therefore can evolve to counter phage-resistant bacteria (Matsuzaki et al. 2005). Furthermore, the development of phage resistance can be forestalled altogether, if phages are used in cocktails (preparation containing multiple types of phages) (Kutateladze and Adamia 2010).

Moreover, unlike antibiotics, phages have the ability to remain in a bacterium in the form of a prophage and increase its adaptive potential, as well as to participate in the horizontal gene transfer between bacterial cells. The factors that influence the application of phages include host range, killing potential, adsorption kinetics, propagation efficiency, ability to penetrate encapsulated cells or biofilms, easiness of purification and stability during storage under natural conditions (Hupfeld and Loessner 2014).

1.7 POTENTIAL DISADVANTAGES OF BACTERIOPHAGE

Bacterial resistances to phage can occur through various mechanisms in a similar manner as bacterial resistance to antibiotics. It can be either by the integration of phage genome to bacterial chromosomal DNA, or by modifying the phage receptors on bacterial cell or due to the loss of genes specific for replication and assembly of phage (Sabouri and Mohammadi 2012). The ability of phages to transfer antimicrobial-resistant genes acquired from host bacterial is the latest concern with regard to phage applications. Many metagenomic studies have shown that gene transfer has occurred among bacterial populations through transduction, because of the direct contact of phage with host in various environments (Kenzaka et al. 2010).
Phages should have a high potential to kill bacteria in combination with a low potential otherwise it will negatively modify the environments to which they are applied. These characteristics can be reasonably assured as long as the phages are obligately lytic, stable under typical storage conditions and temperatures, subject to appropriate efficacy and safety studies, and ideally the absence of undesirable genes such as toxins (Skurnik et al. 2007). Another disadvantage is the narrowness of phage host ranges which is limited to few strains, few species, or much rarer, few genera of bacteria (Gill and Hyman 2010).

1.8 AIM AND OBJECTIVE OF RESEARCH

The quantity as well as the quality of water is deteriorating globally as result of rapid urbanization, population growth and industrialization. Most countries however are currently aware of the necessity of fresh water as a requirement for survival. Potable water includes water from wells and runoff from the land surface. Almost all water in its natural state is impure, because of naturally occurring and anthropogenic sources of pollution. Moreover epidemics arising from waterborne diseases are a global health concern which can be attributed to the faecal contamination of drinking water. Runoff from urban streets is a growing contributor to water pollution, especially after periods of heavy rainfall. As a result, the management of water quality is the source of ongoing concern to the residents of the developing world, where waterborne diarrheal conditions take a great toll in morbidity and mortality.

Waterborne diseases, especially diarrhoea is considered to attribute almost 2 million deaths in children globally. Unpolluted safe drinking water is one of the primary requisites for healthy human life but, the health hazard from
polluted water is evident from the fact that 80% of infectious disease throughout the world is water related. The condition is more serious in densely populated areas with inadequate sanitation and sewer facilities. Unfortunately, the disease burden is high in India due to the contaminated drinking water supply and is further compounded by the limited facilities in curtailting disease outbreaks. Thus, finding solutions to these problems should become a high priority for developing countries and other government agencies.

Research has been focused on probing the development of novel methods and technologies to ensure the safety of water sources with effective quality control. The natural killing property of phages makes them the most prominent tool in eliminating unwanted bacteria making them ideal candidate in water treatment research. So, it is cardinal to understand microbial community dynamics, and the physical and chemical parameters of phages before applying them as treatment tools. On the other hand, substantial scientific findings support the possibility of using phages as microbial tracers and bio-disinfectants without interfering with the natural micro flora.

The present research work aims to determine the safe distance between contamination source and drinking water wells using the transport mechanism of bacteriophages through subsurface soil and also to develop low cost effective technology using bacteriophage as bio-control agents. The research objectives pursued in this thesis work are:

1. Investigate the bacteriological quality of water of urban and rural areas using sanitation mapping and geographical information system.
2. Isolate the bacteriophage and characterize the phages for its application as tracers and bio-control agents.

3. Analyze the soil properties and also to determine the factors affecting the survival of phages in different soil types.

4. Study the transport mechanism of phages in different soil types and to calculate the safe distance between well and contamination source.

5. Development of phage formulations for the reduction of bacterial pathogens in water sources.