CHAPTER 2

REVIEW OF LITERATURE

Water is the most important element for the existence of life on the earth (universal solvent). Even though it is possible for a human being to live up to a month without food, the same individual can only survive for about a week without water (United States Environmental Protection Agency 2006). Despite the fact that water is essential for human life, about 20% of the world’s population still lacks safe drinking water. With the increasing population worldwide, the problem of lack of access to safe drinking water could worsen the situation (Tibbetts 2000). Water quality is crucial for environmental health, and the problem of water quality is especially acute in developing nations where, up to 90% of the cities discharge the untreated sewage into rivers and streams (Centres for Disease Control and Prevention 2013). Unfortunately, more concern is deployed on the quantity whereas little concern is given to its quality.

2.1 SOURCES OF DRINKING WATER DISTRIBUTION SYSTEM

Drinking water, in a broader sense, can be divided into two types based on its source: groundwater and surface water. Groundwater is found beneath the Earth’s surface in the space between soil particles and rock fractures, and is commonly accessed via wells. Whereas, surface water includes sources such as lake and river water and water bound in the form of snow, ice and glaciers.
Among the water sources on earth, only 3% are good in terms of quality or freshness (Oparaocha et al. 2010). Properly drilled boreholes usually supply pure water at first subsequently, if the water is not treated, the population of microorganisms increases, thus making it unfit for drinking. This can also lead to altered physico-chemistry of the water. India’s growing population and economy are driving rapid urbanization (30% of population now live in urban areas (Census of India 2011a) and exerting increased pressure on surface and groundwater availability. In rural areas ~67% of the population defecate in the open (Census of India 2011b), a practice that poses severe risk to health and safety (Clasen 2010; Mara et al. 2010; Ziegelbauer 2012; Kotloff 2013). While in urban areas ~80% of the population have access to toilet facility (Census of India 2011b), but only ~30% are connected to sewage pipelines and few of them are connected to treatment plants (Narain 2012).

Throughout India, the impact of sanitation problems on surface water quality has been documented for many years (Baghel 2005; Mishra 2009; Central Pollution Control Board 2010).

2.2 SCENARIO OF GROUNDWATER QUALITY: GLOBAL, INDIA AND KERALA

Research concerning the quality of groundwater has been carried out by various investigators from different parts of the world. Microbiological factors of groundwater quality and the status of health indicator profiles of Mexico City was reported by Hiriart et al. (2014). In their study, about 60% of the wells were monitored and complied with Mexican drinking water standards. However based on their microbial evidence, 95% of the wells did not provide good quality water for human consumption.
A water quality analysis carried out in Saudi Arabia reported relatively high levels of nitrate contamination and presence of faecal coliforms in 21.4% of the wells (Alsalah 2015). Industries of various kinds expel several million tons of waste each year. These dumps when come in contact with water generate leachates and percolate inside the sub-soil. Leaching occurs in these disposal sites, landfills, lowland areas and resulted in the mixing of hazardous substances within the surface water and also to the groundwater (Kjeldsen and Christophersen 2001).

Migration of chemicals through ground and surface water in turn affect the human health. Ndolo (2002) determined the bacteriological quality of water drawn from taps, boreholes, unprotected wells and rivers in the Phalula and Kapile areas of Balaka district in Malawi and reported that water from all sources were polluted with faecal coliforms. In a similar study, Mc Quillan (2004) examined the impact of on-site septic systems on groundwater quality, and reported that it could contaminate the groundwater with dissolved solids, nitrate, anoxic compounds, organic compounds and microorganisms. Also, a study conducted on the drinking water quality of Peshawar valley (Pakistan) by InamUllah and Alam (2014) reported the presence of heavy metals and bacteria in water.

Authors from different parts of India have reported about the deteriorating quality of groundwater bodies (Kaushik et al.2002; Rani et al.2003; Paul and Mishra 2004; Balakrishnan and Kurupswamy 2005; Chowdhary and Sastry 2005; Prajapathy and Mathur 2005). Also the number of research works have been published with respect to the use of groundwater for drinking and irrigation purposes (Manjumdar and Gupta 2000; Khurshid et al. 2002; Sreedevi 2004; Rao and Devadas 2005). The impact of land use patterns
on the quality of groundwater in Bulawayo was studied by Mangore and Taigbenu (2004). The authors revealed that water quality parameters were within permissible limits, with the occurrence of 8% faecal coliforms, distributed spatially and temporally.

According to Ramesh (2000), continuous monitoring of groundwater is necessary for assessing pollution and also to ascertain its suitability as a dependable source of water supply. Sivakumar et al. (2000) performed the physico-chemical characteristics of water sources in Ooty, South India and compared with water quality standards and suggested mitigatory measures for improving the same. Pophare et al. (2014) observed that groundwater from more than 50% of the dug wells of the WR-2 watershed of Deccan trap basaltic terrain (Maharashtra) was not suitable for drinking purpose and this was due to the impact of over-exploitation of groundwater.

The drinking water sources in rural and urban areas in desert city Bikaner (New Rajasthan), India was found to be contaminated with fecal coliforms and correlated with source of water (Chhabra and Saxena 2003). As expected, the groundwater in urban centres faced severe threat than in rural areas. Physico-chemical and bacteriological quality of drinking water from Auriya district of Uttar Pradesh was examined by Gupta and Shukla (2004) and reported to have high turbidity and coliforms. In a similar work by Ikbal and Hussain (2004), quality of drinking water in villages situated near river Kothari, Rajasthan was evaluated and found that no source in the study area was suitable for drinking purpose as per standards for drinking water.

Chemical and microbiological analysis of potable water of Patiala city, Punjab was studied by Kumar et al. (2005) and found that water supplied
by the municipal corporation had the poorest microbial quality. Likewise, Jain et al. (2007) analyzed the quality of groundwater in Ludhiana district (Punjab) and found that the water was polluted by industrial effluents. Assessment of groundwater quality in Roorkee town (Uttarakhand), with special reference to seasonal variations was examined by Joshi and Srivastava (2006) and revealed that water pollution of 80% was caused by domestic waste which adversely affected the groundwater quality. Likewise, Rekha et al. (2005) assessed the microbiological quality of drinking water at Satna, (Madhya Pradesh) and observed that the pollution was due to poorly maintained water supply system.

Enumeration of total coliform in groundwater of Pettavaithali, Tamilnadu was performed by Jameel and Sirajudeen (2005) and reported high coliform load making the water unsuitable for drinking. Similarly, the bacterial quality of groundwater was analysed in and around Madurai city, Tamilnadu based on samples drawn from bore-wells. Observations indicated that the bacteriological quality of bore well water had crossed the upper limit prescribed by WHO (Jayanthi et al.2005). Groundwater pollution in and around Tuticorin, Tamilnadu was also observed by Kumaresan et al.(2004) and reported that water was polluted due to the lack of proper sewage and solid waste dumping system.

Significant investigations on groundwater quality of Kerala have been carried out by Pillai and Ouseph (2000) on water quality management in the wells of Pazhaya Kunnummel Panchayath, Trivandrum district. The study revealed that temperature and rain had a decisive impact on bacterial count in the water. They also found that 0.2-0.3 ppm free chlorine was optimal for the disinfection of open wells. An assessment on the bacteriological quality of potable water and an evaluation of water purification techniques in Kottayam
district was done by Panicker et al. (2000). They reported that almost all water samples collected from wells in different parts of the district had *E.coli* and faecal streptococci and the count was found to exceed the WHO standards for drinking water.

Roy (2001) performed the water quality of Kollam municipality and reported that all drinking water sources in the area were highly contaminated with total and faecal coliforms and hence unsafe for drinking as per the stipulations of Bureau of Indian Standards. In a study by Gopalakrishnan et al. (2003) examined the groundwater quality in and around Ponnani, and noticed that water was alkaline in nature with high percentage of coliforms. In a similar study, Valsalakumar (2003) tested the quality of water in Kottayam district (Nattakom panchayath), India and reported the poor quality of water, which was affected by high microbial contamination. Groundwater pollution in areas, adjacent to a water transport canal in Paravur municipality, Kerala, India was studied by Valsalakumar et al. (2005) indicating that water was polluted due to the presence of coliforms and therefore unfit for consumption.

### 2.3 GEOGRAPHICAL INFORMATION SYSTEM (GIS) AND GROUNDWATER QUALITY

Geographical information system (GIS) is a tool used to store, organize, manipulate, analyze, retrieve, display, and output geographically referenced information. It can unify different kinds of data into one manageable system, providing efficient access and mapping of data for environmentalist, engineers, and researchers who use these data for decision making in several areas including engineering and environmental fields (Lo and Yeung 2003).
In connection with information technology, GIS enables new forms of communication between people, not only in the field of research but also in entire community (Goodchild 2000). The use of GIS technology has greatly simplified the assessment of natural resources and environmental concerns, including groundwater quality all over the world (Asadi et al. 2007; Shomar et al. 2010; Ketata-Mouna et al. 2011).

Karthik et al. (2005) studied the spatial variation maps of the groundwater quality parameters that were derived and integrated through GIS. The final integrated map showed three priority classes such as high, medium and low groundwater quality zones and provided a guideline for the suitability of groundwater for domestic purposes. Chowdary et al. (2003) noted that, use of GIS can help to identify all possible combinations of soil type, weather, land and water use, and so forth, and facilitate improving the physical basis of recharge estimation in a much more structured and systematic fashion.

Skubon (2005) and Yammani (2007) used GIS as a database to prepare map of water quality using different concentration values of chemical constituents. In their study, GIS was utilized to locate groundwater quality zones suitable for irrigation and domestic use (Yammani 2007). Likewise, Rangzan et al. (2008) used a GIS approach to prepare layers of map to locate promising well sites based on water quality and availability.

GIS has been used in the map classification of groundwater quality, based on correlating total dissolved solid (TDS) values with some aquifer characteristics (Butler et al. 2002). Nas and Berktay (2010) mapped urban groundwater quality in Konya, Turkey, using GIS. This was also used to map, query, and analyze the spatial patterns of groundwater in North-Central Texas.
that includes large percentages of both urban and agricultural land uses (Hudak and Sanmanee 2003). Babiker et al. (2007) proposed a GIS based groundwater quality index method which synthesizes different available water quality data (like chlorine, sodium and calcium) by indexing them numerically according to WHO standards.

The groundwater quality of Chennai city, India was mapped using GIS by Singh and Lawrence (2007). The authors suggested the use of this technique for testing and improving the groundwater contamination risk. Similarly, nitrate contamination in groundwater of the city of Konya, Turkey, was studied and mapped using GIS software package (Arc View GIS) and a hardcopy of the city map was digitized in the UTM projection system (Nas and Berktay 2006). In a related study, the Rural Development Engineering Department, government of Karnataka, analyzed the quality of water sources in rural villages during 2000-2001 and developed GIS based map showing spatial variation of specific water quality parameters (Ramaraju 2006).

**2.4 TESTING OF DRINKING WATER USING INDICATOR BACTERIA**

The use of indicator bacteria in water quality testing reveals the number of pathogenic organisms that can threaten the safety of drinking water. Indicator bacteria are frequently used as a proxy to assess fecal contamination of drinking water, as it is highly unfeasible to test water for such a large number of potential contaminants. These bacteria are found in fecal material and indicated that fecal contamination of water has occurred, therefore the other potentially harmful pathogens may be present (Yates 2007).
There are a variety of microbial indicators used to assess water quality, in which *E. coli* considered the most ideal (as it is most specific to feces), followed by thermotolerant coliforms (World Health Organization 2004). *E. coli* and other thermotolerant coliforms are most appropriate as they occur in high numbers in human and animal excreta, and are rarely found in nature (World Health Organization 2004). Finally, methods to culture and count these indicators are relatively fast, inexpensive, and require minimal training (World Health Organization 2004).

Waterborne outbreaks attributed to drinking water have been documented by Payment et al. (2004) and demonstrated that it could be dangerous to rely solely on these microbes as indicators of fecal contamination. Despite these drawbacks, total coliforms are still useful indicators of treatment efficacy considering that water leaving a treatment plant should be free of bacterial contaminants like total coliforms may be used as a disinfection indicator, or to assess whether microbiologically clean water has been re-contaminated between source and point-of-use (Lin and Ganesh 2013).

The coliform system is the accepted standard for assessing drinking water safety and has several limitations. A recent systematic literature review found that presence of thermotolerant coliforms predicted diarrheal disease when compared to *E. coli*, indicating its usefulness as a fecal indicator (Gruber et al. 2014). Additionally, presence of coliforms have been scrutinized in a way to accurately predict enteric viruses and protozoa in water, as the growth and susceptibility of bacteria are different than that of viruses and parasites (Lin and Ganesh 2013).
Advancement in research and technology has identified several novel indicators that may have potential for indicating contamination with human excreta, including caffeine and some pharmaceuticals (Daneshvar et al. 2012). These indicators may be advantageous as they are exclusive to human waste and do not occur naturally in the environment; however, further research is required to understand how they behave in aquatic environments before and after treatment, and how to effectively concentrate and detect these compounds in water.

2.5 INTERVENTIONS TO PROTECT AND IMPROVE DRINKING WATER

Due to the unexpected failures in treatment plants, highly contaminated water sources may pose a risk to public health (Payment et al. 2000). Therefore, it is imperative to ensure a reasonably clean source of drinking water by using multi-barrier approaches (Islam et al. 2013).

Once raw water has been taken from its source, several treatments are often applied to remove physical and microbial contaminants. Physical treatment methods include boiling, heating, settling, filtration and exposure to ultraviolet radiation from sunlight (Centers for Disease Control and Prevention 2013). Boiling is widely used since it is easy and effective in destroying bacteria, viruses and protozoa in all types of water. A further concern is that water is often transferred to storage containers for cooling and can be prone to re-contamination (Sobsey 2002).

Filtration is a widely used method to remove particles and some microorganisms from water samples. However, the effective removal of
microorganisms, the cost and the availability of filter media in developing countries varied from easy to moderate to difficult (Sobsey 2002). Granular type of filters include bucket filters, barrel or drum filters and roughing filters and filter cisterns which can rapidly reduce turbidity and enteric bacteria by >90% and larger parasites by >99% efficiency, and enteric viruses by 50-90% (Clasen and Bastable 2003). A study by Clasen et al. (2004) in Bolivia, demonstrated the reduction of diarrhoea (70%) and thermotolerant coliforms (100%) in households using ceramic filters when compared to the households that do not use ceramic filters.

Chlorination is the most common method of disinfection and has been widely used for over a century due to its efficacy in destroying microbes (Centers for Disease Control and Prevention 2013). However, chlorine is limited in its ability to destroy all waterborne pathogens: bacterial spores, enteric viruses, and certain protozoa including Cryptosporidium and Giardia. These organisms are chlorine-resistant and able to survive in water distribution systems despite the presence of residual chlorine (Painter et al. 2013).

Chlorination though provides residual protection against regrowth of bacteria and pathogens (Zhang and DiGiano 2002), but can result in undesirable taste and odor in addition to the formation of disinfection by-products (DBPs) (Gopal and Tripathy 2007). Some of these by-products are potentially hazardous (carcinogenic and mutagenic) associated with reproductivetoxicity and several types of cancer including bladder, colon, rectal, pancreatic, and brain cancer (Carlsson 2000). Ozonation has been considered to be a less attractive alternative treatment due to its expensiveness and short lifetime. Both ultraviolet (UV) photolysis and ion exchange, though being advanced type of
treatments, but are not feasible alternatives for the removal of microbial pollutants (Adams et al. 2002).

2.6 ANTIBIOTIC RESISTANCE IN WATER BODIES

Alexander Fleming discovered antibiotics in 1928 and since then it has been successfully applied in treatment of human and animal bacterial infections and also in food production. But the emergence of antibiotic resistant bacteria has posed a high challenge in the effectiveness of antibiotics (World Health Organization 2014; Campos et al. 2015). Akinkunmi et al. (2014) has pointed out that most of the available antibiotics, including β-lactams, are becoming less effective and in some cases resistance rates to be very high (almost 98%). Studies done by various researchers suggested varying resistance patterns like E. coli O104:H4 with resistance to 14 different antibiotics (Verstraete et al. 2013), and about 90% of Salmonella isolates to be resistant to one or more antibiotics (Wang et al. 2014; Liang et al. 2015) tested.

More than 14 antibiotics were found to be resistant on Salmonella isolates from poultry (Adesiji et al. 2014; Zhang et al. 2014). Experiments done by National Centre for Health Statistics (2009) observed that 95% of nosocomial infections were caused by resistant Staphylococci spp. Among which the methicillin resistant strains were found to be resistant to more number of antibiotics such as the 4th generation fluoroquinolones, causing systemic infections with a mortality rate of 50% (Kollef and Micek 2005; Rubinstein et al. 2008; Roberts et al. 2013; Chang et al. 2015).

In Southeast Austria, the rate of ciprofloxacin resistance in E.coli isolate has seen to increase from 4.3 - 16.7% between 1998 and 2013. This was
mainly attributed to the inappropriate use of antibiotics and will lead to increased treatment cost, prolong illness and mortality rate (Van et al. 2007; Bhowmick et al. 2011; Fortini et al. 2011; Pavlickova et al. 2015). Also, bacteria like *Pseudomonas, Campylobacter* and *Listeria* has shown similar antibiotic resistance pattern (Ozbey and Tasdemi 2014; Pobiega et al. 2014; Komba et al. 2015; Obaidat et al. 2015). It is estimated that globally (more than half a million) people die each year due to 17 types of antibiotic-resistant infections (Davies et al. 2013). About 25,000 and 23,000 deaths occur annually due to untreatable bacterial infections in Europe (European Centre for Disease Prevention and Control 2010) and US respectively (Centers for Disease Control and Prevention 2013).

Bogaard et al. (2001) observed antibiotic resistance of *E. coli* with highest prevalence of resistance to ciprofloxacin, flumequine and neomycin. Oliveira et al. (2009) conducted antibiotic sensitivity test of *E. coli* serotypes and observed that 80-90% of the strains were resistant to tetracycline, doxycycline, nalidixic acid and oxytetracycline. Almost 70-75% was resistant to ampicillin and piperacillin, 61.6% resistant to sulfamethoxazole and 47.5% to enrofloxacin. Jakaria et al. (2012) conducted antibiotic sensitivity of *E. coli* isolates and concluded that, out of 10 isolates four were sensitive to ciprofloxacin, gentamycin, chloramphenicol, cephalexin and two were intermediate to kanamycin, cephradine and four were resistant to streptomycin, tetracycline, amoxicillin and nalidixic acid.
2.7 GENERAL FEATURES OF BACTERIOPHAGES

2.7.1 Morphological characteristics of phage

Phages are viruses that infect the bacteria causing cell lysis and can efficiently destroy bacteria which have built up resistance over time to antibiotics and causes life-threatening infections (Biswas et al. 2002). Phages were discovered independently by two different scientists: Frederick Twort, a British pathologist, in 1915 and again by Félix d’Hérelle, a Canadian microbiologist, in 1917 (Salmond et al. 2015). More than 6000 different phage types have been isolated, identified and described morphologically and they measure from tens to hundreds (nm) in size (Ackermann and Prangishvilim, 2012). They have a unique morphology (Figure 2.1) with two protein structure, a DNA filled hexagonal capsid head and a tail (Ackermann and Kropinski 2007).

![Structure of bacteriophage](image)

**Figure 2.1:** Structure of bacteriophage (Graham, 2008)
The main category of phage is tailed ones and they are classified into three groups as *Myoviridae*, *Siphoviridae* and *Podoviridae* and each vary with their tail morphology (Figure 2.2). The next class of phage is the polyhedral phages, followed by filamentous ones. Only a few of these phages have lipid constituents, such as lipid membranes, which make them susceptible to solvents (Poranen et al. 2015). Phages can thrive in any environment where bacteria are present; like the the waste water plants, sewers, soil, outdoor water sources etc. (Lobocka et al. 2014; Mattila et al. 2015).

![Figure 2.2: Taxonomy of bacteriophages (Karthik et al.2014)](image)

### 2.7.2 Life cycle and host specificity of phage

Phages multiply in bacteria by two distinct pathways one is lytic (virulent) and the other is lysogenic (temperate). In the lytic life cycle, phage multiplies in its host and the newly assembled phages immediately escape from the cell using enzymes to lyse the bacterial cell wall (Figure 2.3). In the case of lysogenic life cycle, the phage enters the cell and incorporates its genome to the
bacterial DNA (Canchava et al. 2003). Recently, in a study by Erez et al. (2017) the presence of a small and unique molecule that controls lysis–lysogeny decisions in a temperate phage was identified. Another study reported the assembly of a nucleus-like structure during the viral replication of phage 201Φ2-1 in *P. chlororaphis* suggesting that phage has evolved a specialized structure to compartmentalize viral replication (Chaikeeratisak et al. 2017).

In a lysogenic cycle, the phage genotype can exist indefinitely by being inserted in the bacterial chromosome (Oppenheim et al. 2005; Erez et al. 2017). It is important to select phages for therapeutic uses since they have a reproduction life cycle that is lytic, lysogenic or a combination of both. This can ensure efficacy and prevent adverse effects like bacterial resistance (Merabishvili et al. 2009). Problems of lysogenic phages arise when a phage introduces new genes and coding elements to the bacterial genome in the form of virulence factors through lysogenic conversion (Bae et al. 2006). Due to safety issues, phages with lytic life cycles can only be used in antibacterial treatment.

![Life cycle of bacteriophage](image)

**Figure 2.3:** Life cycle of bacteriophage (Karthik et al. 2014)
As phages have diverse phenotypes and genotypes with different host range, they can recognize the unique receptors, proteins and lipid components of the outer membrane of their host bacteria (Clokie et al. 2011). The host specificity of phage makes them to multiply in the area where the host bacteria are present, and makes them an efficient combat tool. Phages create lesser collateral damage during treatment than antibiotics since they are less harmful for the normal bacterial flora. Also, the risk of opportunistic infections can be reduced to a greater extent by maintaining the healthy flora during treatment (Loc-Carrillo and Abedon 2011).

2.7.3 Isolation and characterization of phages

Sillankorva et al. (2008) isolated 17 lytic bacteriophages from a sewage treatment plant against P. fluorescens by agar over lay method using tryptic soya broth. In a similar study, Mahadevan et al. (2009) used five bacterial pathogen isolates for bacteriophage isolation and found that phage of E. coli and S. typhi were able to infect its original host bacterium, whereas, the phage for P. aeruginosa was able to infect both Pseudomonas and E.coli. Higgin et al. (2007) obtained waste water from local municipal waste water treatment plant and isolated bacteriophages against S. enteritidis from poultry by employing double agar over lay method using tryptic soya agar.

Oliveira et al. (2009) isolated five bacteriophages against E. coli by double agar over lay technique and conducted phage purification from single plaque from higher dilution plates. Jamalludeen et al. (2012) isolated bacteriophages against E. coli from wastewater and fecal samples collected from poultry processing plants by using double agar over lay method and
isolated 11 phages. Similarly, Careysmith et al. (2006) also used soft agar overlay method for the isolation of bacteriophages against *Salmonella* serovars.

Goodridge et al. (2003) extracted bacteriophage DNA from two coliphages AR1 and LG1 and purified it from phage lysates by a proteinase K method, followed by resuspension in Tris-EDTA buffer after isopropanol precipitation. They found that the genome size of phage LG1 as 49.5 kb compared to 150 kb for AR1. Flynn et al. (2006), isolated the nucleic acid of *S. typhimurium* phage and reported that st104a and st104b had a DNA size in the range of 45 kb, typical of *Siphoviridae*, whereas the phage Felix 01 has larger genome size of 86.6 kb. Similarly, Kwon et al. (2008) isolated nucleic acid of phage ΦSG-JL2 against *S. gallinarum* employing standard phenol: chloroform extraction protocol and found the size of nucleic acid to be 38,815 bp in length.

Lappe et al. (2009) prepared DNA using a modification of Qiagen lambda mini kit and reported the genome sizes for the *Podoviridae* and *Siphoviridae* (PFGE-A) as approximately 42 kb. The genome size for *Myoviridae* SETP 2, 4 and 9 was 36.5 kb, and for myovirus SETP6, it was 27 kb. Bao et al. (2011) extracted nucleic acid of phage against *S. pullorum* from the filtered crude lysate and treated it with Ribonuclease A, DNase I, Protease K and phenol:chloroform:isoamyl alcohol (25:24:1). The sizes of the nucleic acid were 58.3 kb in length for PSPu-95 and 45.2 kb in length for PSPu-4-116. Likewise, Jamalludeen et al. (2012) extracted DNA from three coliphages using the Lambda Maxi Kit and observed the genome sizes ranging from 67.06 – 68.04 kb.

Goodridge et al. (2003) observed transmission electron micrographs of LG1 and AR1 phages and revealed that both belong to the family
Myoviridae; phage AR1 belongs to the T4-like genus, while LG1 is unassigned to a genus within the family. Likewise, Careysmith et al. (2006) studied phage FGCSSa1 against Salmonella which was assigned to the family Myoviridae because of the presence of a tail with a contractile sheath. Phage FGCSSa2 was also isolated against Salmonella and assigned to the family Siphoviridae due to the presence of a long tail and the absence of a contractile sheath. Both phages had isometric heads. Also, Atterbury et al. (2009) isolated 232 Salmonella phages from 26 sampling sites on the basis of lytic spectrum data. Three phages with the broadest host range against S. enteric (phage φ151, φ25 and φ10) were examined under electron microscope and indicated that they belonged to Myoviridae and Siphoviridae family of double stranded DNA phages. Electron microscopic studies on 16 phages by Lappe et al. (2009) reported three phage morphotypes as Podoviridae, Siphoviridae and Myoviridae. Similarly, Bao et al. (2011) examined two bacteriophage particles by TEM and reported that the phage1 had a thin tail with round head, a peculiar characteristics of family Siphoviridae, whereas phage2 possessed an icosahedral head with contractile tail which is typical of the family Myoviridae.

Drulis-Kawa et al. (2011) observed phage KP34 of K. pneumoniae with uranyl acetate and observed in electron microscope. It possessed an icosahedral head approximately 57×63 nm connected to short (15 nm) tail common to the members of Podoviridae family, morphotype C1. These dimensions are consistent with T7 type phages. Bacteriophages against Salmonella strains were isolated and their morphology was studied using TEM, revealing that all three phages belonged to the order Caudovirales (Bardina et al. 2012). UABPhi20 and UABPhi78 had an icosahedral head and a non-contractile short tail; both bacteriophages were members of the Podoviridae family. Whereas, UABPhi87 had an icosahedral head but a long contractile tail and belongs to the Myoviridae family.
2.8 BACTERIOPHAGES AS MICROBIAL TRACERS IN SOIL/WATER ENVIRONMENTS

The movement of bacteriophage in sub-surface soil is greatly influenced by the flow and content of water in soil (Schijven and Hassanizadeh 2000). Wu et al. (2016) has emphasised the importance and advantages of phage over chemical tracers due to its non-toxic nature. Schulze-Makuch et al. (2003) determined the transport efficiency of MS2 phage in a model shallow aquifer and reported pH of groundwater and the isoelectric point of mineral matrix to influence phage movement. Similarly, Guan et al. (2008) used a model aquifer and adjusted with various pH (4.6-8.3) in saturated soil zone to study the transport of MS2 and fX174 phages. According to their investigation at a critical pH, which was below the highest isoelectric point of phages, there was a sharp alteration in their behaviour.

The mineral composition of soil is a critical factor which determines the transport mechanism of phages. As per the study conducted by McLeod et al. (2008) three soil types granular, ultic and surface soil were taken for contaminant transport studies. A comparison of transport efficiency of chemical and microbial (phage) tracer was conducted by Hewitt (2009) and the Salmonella phage used in their study revealed a rapid movement and flow in all soils. Xenobiotics can also influence the movement of phages in sub-surface soil. A study by Chattopadhyay et al. (2000) pointed out that, the transport and survival of MS2, T2 and fX174 phages in soil was inactivated in the presence of cationic, anionic and non-ionic surfactants.

Gupta et al. (2009) used pilot scale natural soil column fed with a variety of microorganisms, and the phages MS2 and PRD1 were employed to
assess bacterial removal in Ohio River (United States). The results revealed greater retention of smallest particles namely the phages, when compared with bacteria and protozoa. The study correlated well with the colloid filtration theory (CFT), which predicts the least removal for micrometer size colloids, based on their respective size rather than their surface characteristics. Carlander et al. (2000) examined the transport of phage 28B (host *Salmonella typhimurium*) in two types of willow-cropped field soil containing clay and sand. The transport of phage 28B was highly rapid in clay soil while in sandy soil, it varied with minimal flow and higher retention. Interestingly, the findings suggested that rhizosphere supported the retention of phages rather than the willow plants.

The leaching and transport of MS2 phages from biosolids mixed with soil (93.1% sand, 5.6% silt, 1.3% clay, and 0.01% TOC content) and saturated with water in the ratio (1:1) were investigated by Chetochine et al. (2006). The authors reported about 8% leaching out of coliphages from the biosolid-soil matrix and a minimal retention was observed through the sandy porous matrix. A non-reactive bromide tracer, coliforms and phage 28B (host *S. typhimurium* type 5) transport in different soil core lysimeters of south land (New Zealand) were experimented by McLeod et al. (2003). The authors reported a rapid movement of phage followed by a trail off indicating bypass flow. Wielen et al. (2008) investigated coliphage removal in an anoxic sandy aquifer field, with relatively high pH. But both the phages were removed at lower rate when compared with the laboratory results, due to anoxic redox conditions in the experimental aquifer.

Kennedy (2000) evaluated the use of marine phage as tracers in the heterogeneous (periglacial) granular and fractured-granite aquifers in
Switzerland. Unlike coliphages and phages of other bacterial inhabitants, the advantage of marine phage is that, it does not belong to the regulatory or water quality concerns because of the less harmful host populations. Genetic studies showed that marine phages are closely related to coliphages, although their life histories can be quite different (Rohwer et al. 2000). All tracer experiments using phage have a common drawback of inactivation rate or the rate of loss of infectivity. Although the inactivation rate is often described as a simple function of temperature, there is increasing evidence that inactivation is more complicated and in some cases, loss of infectivity in groundwater systems may be poorly calculated by mean groundwater temperature (Bitton et al. 2002).

2.9 BACTERIOPHAGES AS BIO-CONTROL AGENTS

Phages that infect the bacteria *E.coli* is termed as coliphages. Among which the somatic coliphages are the ones that infect the host cells through the outer cell membrane. The host bacterium and its density, temperature, pH, and other variables affect the incidence, survival, and behavior of phage in different water environments (Stevens et al. 2001). The impact of these variables affects the consistency of data and comparison of bacteriophages in water environment.

Bacteriophages have attracted researchers all over the world as a new bio-control tool against nuisance bacteria. For the successful application of phages as bio-control agents, the phages has to be first isolated, purified and enriched from the natural environment (Ahiwale et al. 2013). Application of phage to control bacterial population has extended from various fields like medicine, agriculture, food industry and water treatment sectors. Nowadays,
phages are developed commercially to remove the pathogens from carcasses and food preparation areas (Thiel 2004).

Bacetriophages play several key roles in the environment mainly in biofilm control and water treatment (Raghu et al. 2012). Zumstein et al. (2000) studied the interactions of bacterial population and bacteriophages in anaerobic wastewater treatment using lab scale anaerobic digesters and suggested that phages dominate bacterial strains. Zhang and Hu (2013) introduced a combined approach of chlorination and phage therapy for biological control of biofilms formed by *P. aeruginosa* on various surfaces. They reported that their combined method could eliminate the bacterial biofilm by up to 95 - 97% in two days (Zhang et al. 2013).

Phages were also used as antifoaming agents in activated sludge as per the research formulated by Thomas et al. (2002). Their study included the isolation of lytic phages from the family *Siphoviridae* which had the capacity to destroy foam producing bacteria. Bio-control studies of sludge bulking in waste water treatment plant using a lytic bacteriophage was applied to remove *Haliscomenobacter hydrossis*, and *Sphaerotilus natans*, which are the causative agents of biomass and filamentous bulking respectively (Choi et al. 2011). *E. coli* and other bacterial pathogens in the hospital waste water was removed using bacteriophages, in which the authors suggested that the *E. coli* in the waste water was completely destroyed within 14 hours of incubation (Periasamy and Sundaram 2013). So far there are no reports addressing the use of coliphages as useful biological control for eliminating or reducing the coliforms load in wastewater treatment plant.
McLaughlin and Brooks (2008) studied the quality of well water by loading them with \textit{S. enterica} (ATCC14028) and then treating with phages alone and in cocktail combinations. These treatments showed high inactivation rate of \textit{Salmonella} bacteria. In another study, river water microcosms were used for testing the potential of coliphages and phages specific to \textit{S. aureus} (Bahadoor 2005). According to Withey et al. (2005), phage has the ability to control environmental waste water process problems such as foaming in activated sludge plant, sludge dewaterability and digestibility. Also, the application of bacteriophage in constructed wetland system is found to be an attractive alternative for storm water management and in the reduction of disease causing viruses (Yousefi et al. 2004).

Beheshti et al. (2014) isolated and identified two novel bacteriophages from Zayandehrood River (Iran) belonging to the family \textit{Myoviridae} and \textit{Podoviridae} and which also showed lytic activity against \textit{E. coli} PTCC1399 and \textit{E. coli} SBSWF27 respectively. The activity of these phages revealed their usefulness in bacterial pathogen reduction along with activated sludge treatment methods. Hagens and Loessner (2007) also suggested the application of phage in detection and prevention of pathogenic bacteria in various emerging fields due to their varied degree of success in the treatments.

Drinking water contamination is a serious problem and the rise of pathogens is mainly influenced by improper treatment methods. Traditionally, water disinfection is done by chemical and physical methods, but this has lead to the emergence of chemical resistant and tolerant bacteria in drinking water. Therefore, phages can be considered as the most reliable method to control bacterial pathogens. Their broad host range and specificity strongly support their
application as an effective disinfectant for prevention of many kinds of water borne bacterial infectious diseases, especially those caused by antimicrobial resistant bacteria.