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Microbial risk assessment of potential pathogen intrusion during planned maintenance work of drinking water distribution system in Gothenburg, Sweden

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Abstract

Decision makers, scientists, businesses and individual citizens all accept and comprehend that air and water pollution can have negative impacts on human health, but the impacts of microbial pollution of soil or water on human health has had a much lower profile and are not well understood. As such it is time to investigate the interaction between microbial soil contamination and its effects on human health including the various pathways from soil to human body.

Soil is said to be an effective filter for microorganism. However, the existence of micropores, large channels in soil from worm-holes, voids left by decayed plant roots, etc., can easily allow pathogens and fecal indicator organisms to bypass soil filtration and enter into water in the distribution pipe during maintenance works. Water samples were collected from 16 different locations of Gothenburg in Sweden during the pipe repair/maintenance works and then tested to detect the *E. coli* and Coliforms through membrane filtration method. The study provided the evidence of presence fecal indicators in soil or water present outside the drinking water pipe in sampling locations of the Gothenburg city. Results were found to vary in a wide range for *E. coli* (<0 to >240000 CFU/100 ml) and for coliform (<10 to 240000 CFU/100 ml) respectively in the tested water samples. These fecal indicators can act as a contamination source and may further use the intrusion events like pipe repair/maintenance works as a pathway to intrude the drinking water pipe, which may end up in a public health risk situation. Accordingly, the conceptual risk model developed based on worst-case assumptions was used to formulate three different scenarios which were further tested in dose response model of QMRA. The estimated annual infection risk levels for known pathogens *Campylobacter*, *Cryptosporidium* and norovirus significantly exceeded the USEPA target risk levels of 1 person in 10000 per year. The results from sensitivity analysis proved that the pathogen concentration is the most sensitive parameter for the ingested dose. Further research is recommended to include the soil sample testing and couple the final results to perform hydraulic modelling to get the holistic view of the prevailing situation and its related public health risks. Epidemiological studies could be used to validate the results.

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Sammanfattning

Beslutsfattare, forskare, privata företag och enskilda medborgare är medvetna om att luft och vattenföroreningar kan ha en negativ inverkan på människors hälsa Hälsoeffekter till följd av mikrobiell förorening av mark eller vatten har haft en mycket lägre profil och är inte heller helt kartlagda. Därav behövs undersökningar av samspelet mellan mikrobiell markförorening och dess effekter på människors hälsa, inklusive olika transportvägarna från mikrobiell föroreningskälla till människa.

Jord är sägs vara ett effektivt filter för mikroorganismer. Förekomsten av mikroporer, stora kanaler i marken från maskhål, nedbrutna växtrötter m.m. kan emellertid underlätta för patogener och fekala indikatorer att ta sig igenom markens naturliga filtrering. Vid underhållsarbeten kan markvatten ta sig in i distributionsnätet för dricksvatten. Vattenprover hämtades från 16 olika platser i Göteborg vid rörreparations/underhållsarbeten och analyserades för att *E. coli* och koliformer genom membranfiltreringsmetoden. Studien påvisade förekomst av fekala indikatorer i vatten utanför dricksvattenledningen vid provtagningstillfällena. Resultaten visade på stora variationer för både *E. coli* (<0 till> 240000 CFU / 100 ml) och för koliformer (<10 till 240000 CFU / 100 ml) i de analyserade vattenproverna. Fekala indikatorer påvisar möjliga föroreningskällor och vid reparations- och underhållsarbeten kan även patogener associerade med dessa indikatorer tränga in i dricksvattenrören, vilket kan innebära en risk för människors hälsa. En konceptuell riskmodell baserad på antaganden för ett värsta fall användes i tre olika scenarion. Möjliga halter av patogener användes som indata till dosresponsmodellen för QMRA. De uppskattade årliga infektionsrisknivåerna för patogenerna *Campylobacter, Cryptosporidium* och norovirus översteg USEPAs acceptabla risknivå på 1 infektion per 10000 personer per år. Resultaten från känslighetsanalys visade att patogenkoncentrationen är den känsligaste parametern för den intagna dosen. Fortsatt forskning bör inkludera provtagning av markprov samt koppla ihop resultat med hydraulisk modellering för att få en helhetssyn av den rådande situationen och dess relaterade hälsorisker. Epidemiologiska studier kan användas för att validera resultaten.

Nyckelord: *E. coli*, transportvägar föroreningar, dricksvattenrör, utbrott av vattenburna sjukdomar kopplade till dricksvattenrör, membranfiltreringsmetod, intrusionshändelser i dricksvattendistribution, QMRA, acceptabel risk

ACKNOWLEDGEMENTS

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1 Introduction

1.1 Background

The supply of safe drinking water is one of humanity's most successful public health interventions. Nevertheless, the lack of awareness on the associated potential risk areas (like for example, pathogen intrusion during pipe repair work) is often the root cause of many waterborne disease outbreaks. Despite robust drinking-water treatment systems, the aging drinking water distributions system are becoming more vulnerable to higher rates of breaks/repairs events which further allow the pathogens to intrude the pipe. Traditionally, the microbial quality of the drinking water is seen to be often tested by detecting the fecal indicator organisms (like *E. coli*) in water samples which are collected either at the outlet of the drinking water treatment plant or at the consumer's tap. After testing the water sample, the drinking water was declared to be 'safe' when the target *E. coli* was absent. As such, conventional water testing methods indicate that the connecting component of water conveyer from the water treatment plant outlet to the consumer's tap, which is the 'drinking water distribution system' is often ignored or is out of sight. Therefore, such type of traditional end-of-pipe compliance monitoring practices are inadequate as they tend to ignore the potential threat of health risk from several contamination events (like pipe break, repair, leak, cross connections, pressure fluctuations, etc.) that help the pathogens present in the surrounding soil to enter the drinking water pipe. Especially when these contamination events are very close to connected public without any possibility for further treatment. This has been, and still is an important challenge to water supply agencies. Apart from this, unhygienic maintenance procedures may also contaminate the drinking water system. One of the potential sources is through 'soil water'. Contamination of drinking water by fecal sources is a serious problem due to its potential for contracting diseases from pathogens. Investigation of fecal indicator concentrations in local soil water will immensely improve the risk assessment process of drinking water distribution systems. Globally, there are few studies that have investigated the presence of fecal indicators in soil water surrounding drinking water pipes, but hardly any study can be found in Sweden. Therefore, it is of great importance to improve and add to this knowledge.

1.2 Aim and objective

The main aim was to assess the risk of microbial contamination of drinking water during planned maintenance work/repairs. The objective first was to mainly investigate the presence of fecal contamination in the soil water sample collected during the maintenance/repair work of drinking water distribution pipes in the city of Gothenburg. Secondly, to put the findings from microbial analysis in the context of annual probability of human infection risk by use of quantitative microbial risk assessment. Lastly, the objective also included the literature reviews to identify the possible fecal sources, potential pathway and transport mechanisms, hazards in the distribution network, sampling and laboratory process.

1.3 Study Limitations

This thesis was conducted for a specific period from May 2018 to October 2018. During this period the temperatures were higher compared to winter, so the level of microorganisms was expected to be lower when compared to winter. Furthermore, only *E. coli* and Coliforms were considered for the investigation of the water samples but other potentially harmful pathogens (like virus) were excluded and this forms the main drawback of the study. Also, the growth medium, the conditions of incubation, the sample nature, sample age and procedure of the water

sampling can influence the viable count of microorganism causing variable accuracy. More importantly, the occurrence levels of microbes in nature are often event driven (ex. presence of animal host, run-off events, wastewater pipe bursts) and hence there exist a high uncertainty of microbial concentrations over time and in space which adds to the limitations of this study. In addition to this, only water samples (water within the repair pit area) were examined and so the results may not fully represent the true extent of prevailing contamination and its relative human infection risks levels.

1.4 Summary of work plan

In order to achieve the goal of the project, the following steps were undertaken:
- Search of scientific literatures: The choice of literature was focused on those that had made research evidences based on laboratory tests and books which aimed to provide related information on the topic
- Analysis of previous projects with similar aims: To have further progress on the topic, more information was gathered, and an analysis of the latest research findings in and outside Sweden was done
- Collection of soil water samples: The locations for collecting the samples were based on planned shutdowns data received from Gothenburg municipality representatives.
- Prepare samples for membrane filtration analysis in laboratory
- Analyze samples and evaluate the results in the context of risk

1.5 Report Overview

The Figure 1 below guides the reader with the structure of the report and its content.
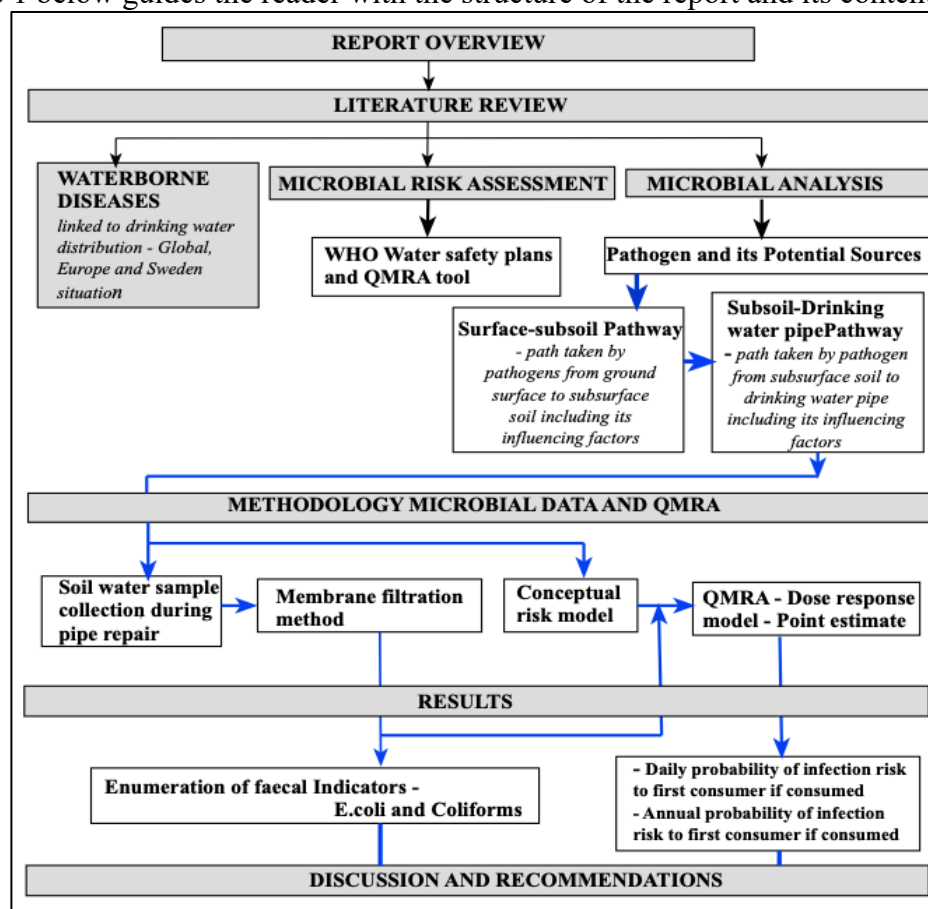
Figure 1 - Overview of report

2 Literature Review

The literature review was aimed at understanding the previous research which had similar objectives as this project and to have a quick overview of the status of research in this field. The reference studies presented below are both from Sweden and abroad (USA, other European countries).

2.1 Drinking water distribution System

According to Geldreich (1996), the primary objective of the water distribution system in early civilizations was to supply sufficient quantity of water to people. As such, the microbial water quality was of little importance. However, in the last century, the microbial safety of the drinking water distribution system has become a major cause of concern for the water supply agencies and the World Health Organization (WHO).

According to the American Water Works Association (NRC, 2006) drinking water distribution system is defined as "*including all water utility components for the distribution of finished or potable water by means of gravity storage feed or pumps though distribution pumping networks to customers or other users, including distribution equalizing storage."* It comprises a system of interconnected series of pipes, storage facilities, and other components. Drinking water distribution systems are also used for fire protection needs for cities, homes, schools, hospitals, businesses, industries and other facilities apart from supply of drinking water. Public water systems normally rely on distribution systems to offer an uninterrupted adequate supply of pressurized safe drinking water to all consumers(NRC, 2006). Distribution systems carry water from treatment facilities (or in the absence of treatment, from the source) to the consumer's tap and so is considered to be an improved source of water supply. Storage facilities provide in-line storage of water, help modulating pressure fluctuations during high water usage times and emergency situations. The 'drinking water distribution systems' is a cluster of the huge complex physical infrastructure with many different components (small and large), and hence indeed is a significant challenge to efficiently manage it both from an operational and public health perspective.

2.2 Microbial hazards in drinking water distribution system

Past researches have shown that drinking water is a potential source of transmission agent, particularly in case of pathogens transmitted by the fecal–oral route (WHO, 2005). Therefore, Bradley in 1974 (Eisenberg et al., 2001) widely classified the infectious diseases transmitted with water as waterborne (e.g. cholera and typhoid), water-washed, water-based or water-related (e.g. malaria and dengue fever). Furthermore, The Protocol on Water and Health (Kulinkina et al., 2016) defines waterborne disease as, "*any significant adverse effects on human health, such as death, disability, illness or disorders, caused directly or indirectly by the condition, or changes in the quantity or quality, of any waters*". This study focuses on assessing the waterborne disease caused in humans by ingestion of fecally contaminated water.

Outbreaks of environmentally-transmitted diseases have been recorded worldwide, both in developed and developing countries (Al Dofour et al., 2012). The importance of understanding the root cause of outbreaks has always been essential, right from the time of Dr. John Snow, who recognized the Broad Street pump to be the main source of cholera infection in 1854, up to the contemporary outbreaks of waterborne Cryptosporidiosis. This pioneering work of Dr. John Snow, later in 1880's, provoked investigations to identify the causative agents that could be further used to associate the enteric disease to fecally contaminated water and to develop water

quality index. Subsequently, in 1884, the German microbiologist and pediatrician Theodor Escherichia discovered that *Bacillus coli* (*Escherichia coli*), present in the human gut was shed in large densities in feces and hence was more frequently associated with the *typhoid bacillus.* Therefore, he further suggested *E. coli* as an indicator of fecal contamination (Blount, 2015). Furthermore, the WHO Guidelines for Drinking-water Quality (WHO, 2011) also recommends to use *E. coli* as an indicator to detect the fecal pathogens to assess the drinking water quality. This is further explained in section 2.6 of this report

Typically, bacteria, viruses, protozoa, and parasites which are found in the intestinal tracts of humans are shed in human feces causing fecal contamination in nature and this forms the main source of infectious microorganisms. Such microorganism present in nature may succeed to enter the distribution pipe and grow further to form biofilms on internal walls of pipe. Eventually, such microorganisms can detach from pipe walls into drinking water due to change in flow rate and contaminate the drinking water, to further increase the likelihood of occurrence of waterborne diseases (WHO, 2014). According to Taylor et al.(2001), a total of around 1415 species of microorganisms have been reported to be pathogenic, among which about 348 were water-associated, causing 115 infectious diseases. Importantly, any microorganism that enters drinking water, can cause human infection only if they are virulent and are in contact with the target organ (e.g., gastrointestinal track, lungs) in large numbers. Moreover, the virulent nature of microorganisms varies. For instance, *Giardia* and *Cryptosporidium* infections are often restricted to one specific target organ but few others like *Salmonella* and enteroviruses are capable of infecting more than one target organ. However, the infection burden on public health relies on many factors like concentration of pathogens, its level of toxicity, its infectivity, minimum infectious dose of ingested pathogen, exposed population and more importantly the immunity of the person (WHO, 2005). Therefore, an official outbreak of waterborne disease is reported only when two or more people get ill and their illness is epidemiologically linked to ingestion of contaminated water (NRC, 2006).

Waterborne diseases associated to failure in water distribution system can often cause large scale infections, which get reported as outbreaks; but in some cases only sporadic diseases are noted(WHO, 2005). At the same time, the waterborne diseases can also be caused from any type of drinking water contaminant (chemical or known pathogen or emerging pathogen) and from any part of the drinking water system (source water, treatment, distribution). As such, it is challenging for any national public health surveillance to pinpoint the 'drinking water distribution system' as the root cause of contamination.

In view of the above, many tools and methods have been developed over time in order to help the national public health surveillance systems to assess and prevent the disease burden (especially those associated with sporadic disease). Moreover, the public health surveillance systems are expected to maintain the set regulatory target infection risk level of 10^{-4} DALY(i.e. disability adjusted life year) per person per at any given time, according water quality guidelines (WHO, 2018). To this effect, quantitative microbial risk assessment (QMRA), a mathematical framework, has been widely used to translate the source of contamination to estimate public disease burden which can further guide the decision makers for choosing the right mitigation measures.

Before moving ahead, an overview the global situation of the waterborne diseases is presented below, as it forms the basis of microbial risk analysis and assessment performed in the interest of public health safety.

2.3 Waterborne diseases linked to drinking water distribution system

According to WHO (2018), the population with regard to drinking water access are classified as using either *improved or unimproved sources.* The improved category is further divided into piped on premises and other improved (which includes standpipes, boreholes and protected wells and springs) sources. Improved sources or safely managed piped water source is said to be used by approximately 56% of the global population and by more than 95% of the population in Europe (WHO, 2018). Despite this progress in coverage of improved drinking water source, the 'drinking-water', is not necessarily free of pathogens and safe for health (UNICEF/WHO, 2011)**.** Evidently, Bain et al. (2014) performed a systematic review to assess the water quality by country and type of water source by combining the data of 345 water quality studies (Bain et al., 2014). His study results suggested that, approximately 26% of people in the world still drink water which is occasionally contaminated with fecal indicator bacteria and contamination was found to be more frequent in improved sources like protected groundwater and rural piped supplies (Bain et al., 2014). Obviously, this situation is different in different regions of world, as in low and middle-income countries, a range from 14% in Europe to over 52% of the population to be exposed to contaminated drinking water. In the same context, another study performed by (K. Yang et al., 2012), on global distribution of outbreaks of waterborne diseases, provides an extensive geographical and epidemiological information on 337 recognized infectious diseases in 231 countries which had occurred between 1991-2008. Majority of the outbreaks studied by Yang et al (2012), were seen to have occurred in west Europe, central Africa, north India and Southeast Asia according to this study. Results of the study showed that, about 70.9% (1,012) of the outbreaks were associated with water-borne diseases and about 46.7% (667) were associated with emerging or reemerging pathogens.

Furthermore, few studies in the past have demonstrated that inadequate management of drinking-water distribution systems have caused waterborne outbreaks in both developed and developing countries. However, the root cause for these outbreaks and the extent of microbial hazards involved seem to be varying significantly. For instance, in the United States of America (USA), 57 outbreaks between 1981-2010 were linked to distribution system faults, resulting in 9000 ill cases. The pathogens which caused illness were suggested to be enteric pathogens like, *Salmonella*, *Campylobacter*, *Shigella*, *Escherichia coli* O157, *Cryptosporidium, Giardia* and norovirus (Ashbolt et al.2014) The Figure 2 below adapted from Water safety in distribution systems report (WHO, 2014), presents the clear classification of waterborne outbreaks linked to microbial quality degradation caused due to distribution system failures in the US between 1981-2010.
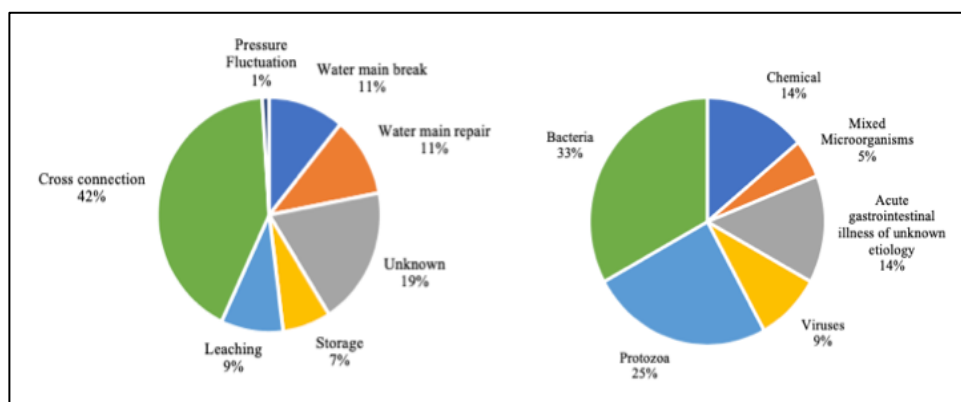
Figure 2 - Waterborne outbreaks from distribution system in the USA, 1981-2010, by (first)system fault and (second) causative agent – adapted from WHO (2014)

Similarly, an increased risk of waterborne gastrointestinal illness was also predicted from a centralized community water supply system in Quebec, Canada, where water was extensively treated by modern methods and met all microbial quality requirement (Ligon & Bartram, 2016). These findings hinted that bacterial indicators used to assess water quality were inadequate as they successfully passed through multiple treatment barriers or intruded the treated water in the community distribution system. Hence, even in developed countries, there are indications that robustly treated community drinking water can still cause community diarrheal illness.

In the European context, maintaining microbial water quality is seen to be more challenging in small water systems compared to large water systems (WHO, 2017a). To elaborate further, data indicate that only 3 countries out of 27 were able to achieve the highest compliance rate (i.e. over 99%), and at least 1 in 10 small systems were found to be contaminated in six countries. Furthermore, the review conducted by Kulinkina et al.(2016), revealed that campylobacteriosis, giardiasis, hepatitis A and shigellosis were the most frequently reported waterborne diseases which summed up to about 18% of investigated outbreaks in the WHO European Region during 2000-2013. Most of the outbreaks were caused by '*contaminated public drinking-water supplies*' which draws one's attention to appreciate the bigger challenge lying in the drinking water distribution system. Other identified sources include recreational exposures (lakes, swimming pools, spas, water parks) as well as heating and cooling towers in the case of legionellosis, however this kind of source is out of this project's scope. Furthermore, a recent study was performed to analyze 175 waterborne outbreaks reported to the national outbreak surveillance systems in Denmark, Finland and Norway between 1998 to 2012 including Sweden between 1998 to 2011 (Guzman-Herrador et al., 2015). Of the total 123 outbreaks, this study reported that 17 were from the municipal waterworks with surface water source, and 42 were from groundwater source. To add on, viruses were causative agent in about 41% of the outbreaks and bacteria (specifically Campylobacter) was causative agent in about 29% of the outbreaks. The level of waterborne infections varies between different European regions based on the capacity of their surveillance system to investigate outbreaks

Considering Sweden in specific, the epidemiological studies conducted by Nygård et al., (2004) based on the national register data of three years (i.e.1998–2000), indicated high risk of *Campylobacter* infection in Sweden. This study further suggested that there could be around 33 000 cases in 4.5 million people getting infected by gastrointestinal disease, which could be directly attributed to the drinking water pipe repair. Subsequently, the outbreak statistics for 1995-2009 showed that the fecally contaminated water in distribution system contributed to about 38% of the total 78 outbreaks (Folkhälsomyndigheten, 2015). Interestingly, pathogens were the causative agents in about 46% of the outbreaks and the causative agents for 54% were unknown (see Figure 3b below). Furthermore, it was noticed that about 35% of the disease outbreaks in Sweden were traced to deficiencies in drinking water distribution system, which was further classified into different causative events (Melle Säve-Söderbergh et al., 2013). Both the data for system faults, (Figure 3a), adapted from Melle Säve-Söderbergh et al.,(2013) and causative agents (Figure 3b), adapted from Folkhälsomyndigheten report (2015) has been presented below. Additionally, about 33 (i.e.58%) outbreaks in Sweden were due to absence of disinfection in drinking water (Folkhälsomyndigheten, 2015). Similarly, an epidemiological study conducted in five municipalities during 2014-2015 by Melle Säve-Söderbergh et al.(2017), also showed that the external contamination of drinking water in distribution network was the potential cause of endemic gastrointestinal illness. Unfortunately, there seems to be a gap in reporting of outbreaks because the cause for about 54% of the outbreaks was unknown as seen in Figure 3b.
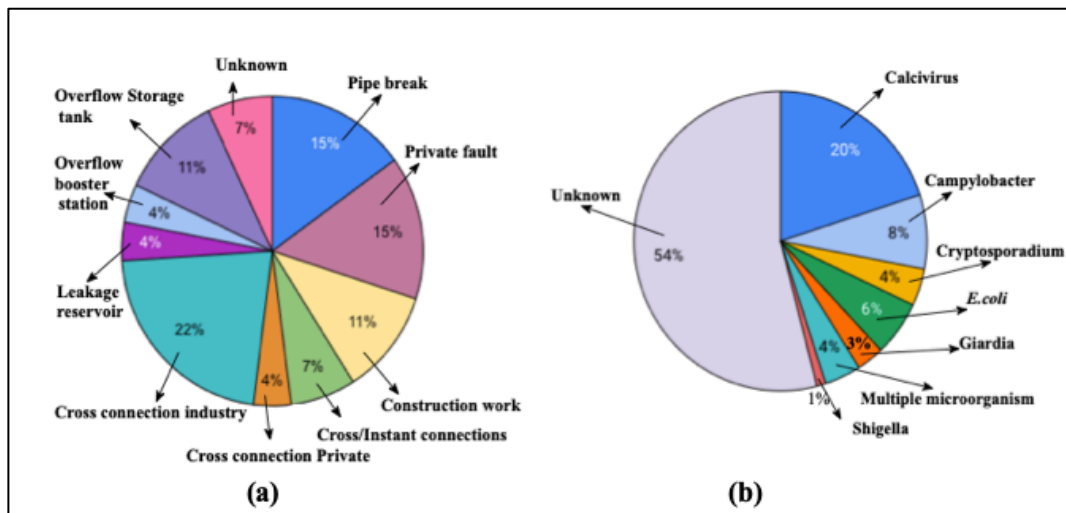
Figure 3 - Waterborne outbreaks associated with distribution system in Sweden, by (a)system fault for period 1980-2009 based on (Melle Säve-Söderbergh et al., 2013). and (b)causative agent for period 1992 -2011 based on report from Folkhälsomyndigheten,2015

Nevertheless, it should be noted that disease outbreaks, although are the most recognizable result of distribution system failures, they indicate very small fraction of contamination events. Routine events like cross-connections, leaks and water main breaks and transient low water pressures do not always lead to reported outbreaks. For example, a survey in North America recorded an average of seven breaks per 100 km of water main per year and in Australia in 2011–2012, the rate was 13 breaks per 100 km of water main, which implies that, in most of the cases, pipe-breaks are repaired without any reported outbreaks. To add more reference to context, in the Netherlands, 50 adverse water quality events, having no link with an outbreak, were reported between 1993 and 2004. However, during this period, only one outbreak was linked to distribution system, and that was caused by cross-connection between a drinking water supply and partially treated river water (van Lieverloo et al., 2007). Conversely, it is more likely that few events which cause sporadic cases of illness can go undetected by standard surveillance systems (Malm, 2015).

In few other cases, adverse health effects linked to drinking water, have been reported to have occurred (ex. In Australia, Pakistan, USA) due to other reasons apart from physical faults in the distribution system (WHO,2014). For example, pathogens like *Legionella* and the amoeba *Naegleria fowleri* can grow to form biofilms inside the distribution systems to further cause health infection and so not in any way linked to physical faults in the distribution system.

On the other hand, it is unfortunate that there are numerous reports which still continue to highlight the presence of fecal contamination of piped drinking water sources, despite the regulation of 'absence' of fecal indicator (preferably *E. coli)* in any 100 milliliters of drinking water at any given time (WHO, 2011). The principal risk to health is from consumption of microbially contaminated water that causes infectious diseases such as cholera, diarrheal diseases, dysenteries, and other enteric fevers. However, often the disease outbreak information published is normally insufficient and thus it is challenging to assess the prevailing situation by the surveillance system. To add on, recent studies also indicated that global warming creates a favorable environment for the bacteria and hence can also increase the disease burden (WHO, 2015).

2.4 Quantitative Microbial Risk Assessment (QMRA)

Risk assessment forms an integral part of Water Safety Plans (WSPs) for drinking-water systems (WHO, 2016). Assessment of risk in relation to drinking water supplies is undertaken for a number of reasons. According USEPA (2014) microbial risk assessments can be performed for a variety of reasons, but few of them are listed below:

- to assess the human risk potential to exposure to a known pathogen;
- to decide critical points for control, such as watershed protection measures;
- to decide the specific treatment processes to reduce, remove, or inactivate various pathogens;
- to anticipate the consequences of various management options for reducing risk;
- to recognize and prioritize suitable research needs; and
- to facilitate epidemiological investigations.

This study aims to assess the potential for human health risk associated with exposure to a known pathogen.

"Risk" is a notional phenomenon and hence implies different things to different people, but the term can be simplified into the below three questions(Lechevallier & Buckley, 2007):

- What can go wrong? (identification of the hazardous event)
- How likely are the various results? (quantifying the probability of the event occurring)
- How bad are the possible results? (qualifying the possible damage, the event may cause)

In view of the above, risk can be defined as the likelihood of identified hazards causing harm in a specified time frame. With regard to microbial risk assessment, risk is the probability of an adverse effect under a defined set of hosts, microbiological, and environmental factors (Mark Lechevallier and Merry Buckley, 2007). Exposure to pathogenic microorganisms that pose a threat to human health is one of the critical considerations in risk assessment. Risk is a multi-faceted phenomenon, but however it can be represented by a simple equation below:

$$Risk = Hazard \ x \ Exposure$$

 The risk assessment, according to WHO (WHO, 2016), aids in systematic evaluation of:

1) **hazards** – refers to pathogenic microorganisms (pathogens) that may have an adverse impact on the health of the people who drink the water;
2) **hazardous events** – events that may aid pathogen intrusion into the water supply or fail to remove them. These events may occur at almost all the step of the water supply chain – for example, at the source (e.g. rain events that flush human or animal fecal waste into the water supply), in treatment (e.g. failures in filtration or disinfection), in the distribution network (e.g. improper repair work introducing microbial contamination) and in households (e.g. handling storage containers with dirty hands);
3) **the adequacy of the controls to prevent contamination** – control measures that are or can be implemented to prevent these hazards occurrence, to eliminate these hazards from the water supply system or to reduce of these hazards to an acceptable level. The control or elimination or reduction strategies can be done by engineered control systems like water treatment process and non-engineered measures, such as hygiene protocols for repair works on the water distribution network.

As one can imagine, there can be potentially numerous hazards, numerous hazardous events and numerous control measures. Therefore, the objective of risk assessment must be to identify the risks that are critical for the safety of a particular water supply system and to aid in choosing the best steps to increase the safety of the system. For this purpose, it is important to quantify or classify the risks in terms of health impact.
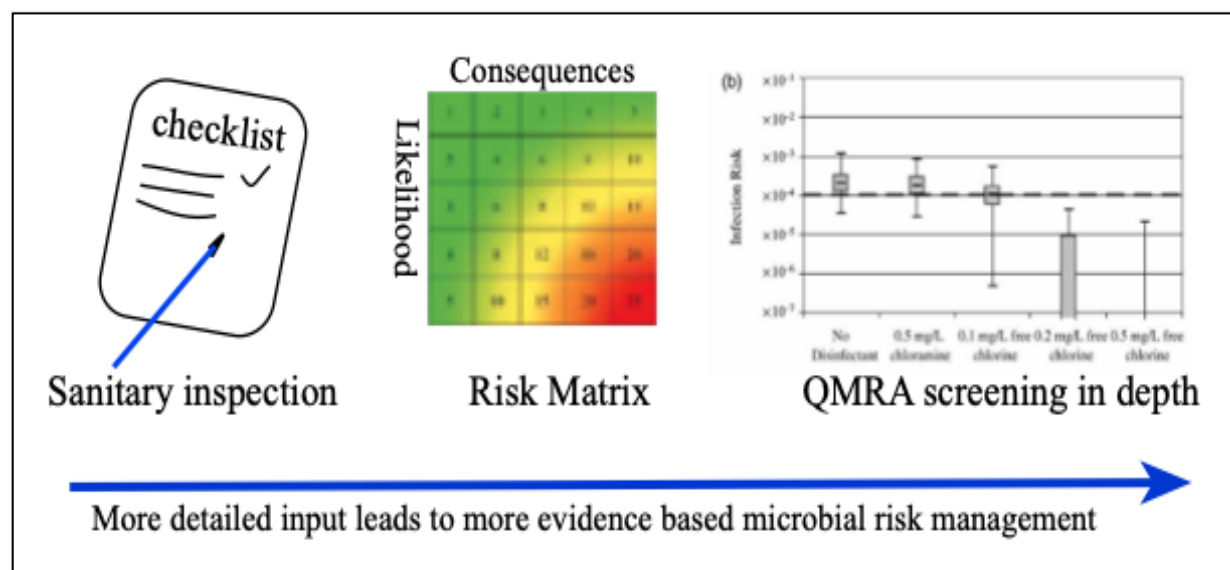
Figure 4 - Approaches of Risk Assessment, adapted from WHO (2016)

A wide range of approaches like sanitary inspection, risk matrix and QMRA are currently available to conduct a risk assessment (WHO, 2016). All risk assessment approaches are valid, but their use is context specific. Their implementation will depend on: human resources (like personnel, skills, access to support institutions) and type of supply system (small community-managed supplies or larger utility-managed supplies). These approaches represent a continuum from simpler to more advanced and from expert judgement to more evidence-based assessment of the risks as shown in above Figure 4 (WHO, 2016). In general, risk assessments should be as simple as possible, and should have the right balance between more precise and evidence base and the assumptions used and expert judgement. Risk management options can be well informed based on the right balance. Complicated assessments may not be required where simplified risk assessments are adequate to help the risk management decisions. Nevertheless, all the above approaches have their own strengths and limitations which can be referred in the WHO (WHO, 2016) report for more information. In summary, the first two approaches are more qualitative and the QMRA is a quantitative approach.

In this study, QMRA- a quantitative approach is chosen to be used as it offers a systematic way to interpret and use the scientific data in the context of estimated health outcomes to support water safety management decisions and prioritize mitigation actions or research effort. The Figure 5 below depicts the framework of QMRA tool.

In addition to the above, QMRA also provides valuable quantitative input into the steps of the Water Safety Plan (WSP) as proposed by Petterson and Ashbolt (S. R. Petterson & Ashbolt, 2016). These inputs were suggested in the form of basic questions like ''what sources are more important?" and so on. The image extracted from WSP, WHO (2016) can be referred in the Appendix 1 for more details on the kind of inputs offered by QMRA for Water Safety Plan (WSP).

In the past, studies extensively relied on the framework developed for chemical risk assessments because the microbial risk framework was still an evolving research area. However, the subsequent studies as mentioned in WHO (WHO, 2016) successfully developed a specific framework for microbial risk assessment which had unique factors (microbial growth, dose response, genetic diversity, host immunity etc.) that differentiated it from chemical risk assessment. Furthermore, WHO (2016) combined all the guidelines and provided a single framework comprising of a formal four-step risk assessment process to harmonize across these guidelines as seen in Figure 6. As can be seen in the Figure 6, the QMRA framework demands explicit quantification of each and every component used in the assessment process.
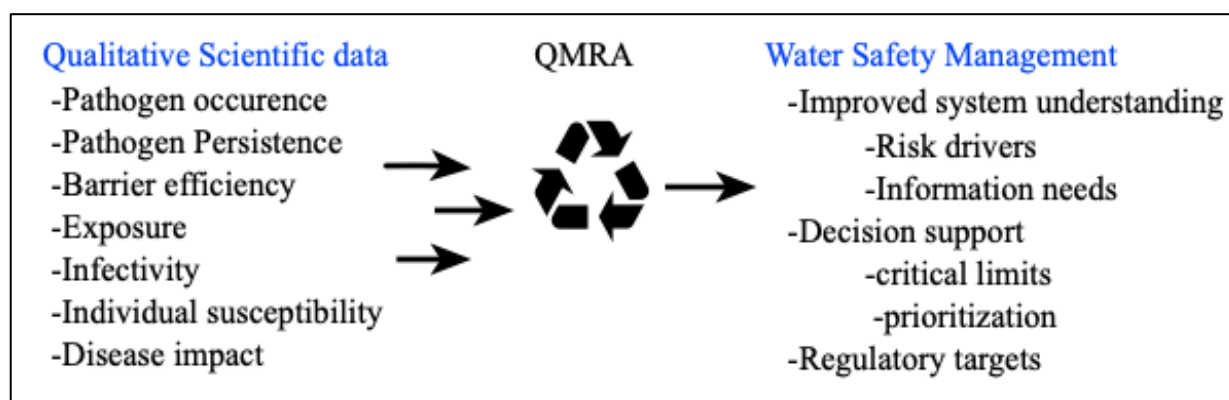
Figure 5 - QMRA framework adapted from WHO (2016)
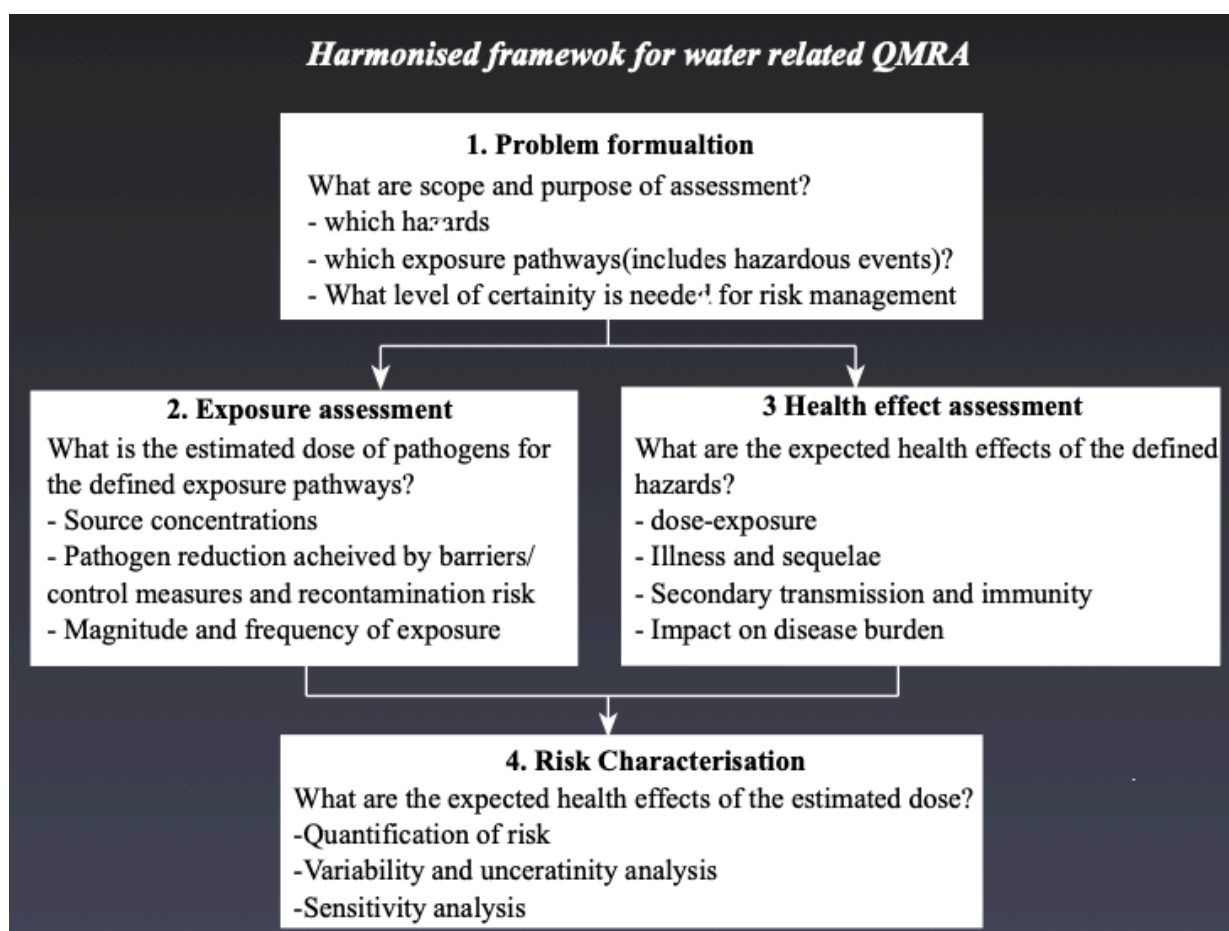
Figure 6 - Harmonized framework for water related QMRA, Source: WHO (2016)

Several studies (M. Blokker et al., 2014; van Lieverloo et al., 2007; J. Yang et al., 2015) have demonstrated and emphasized the specific role of QMRA with regard to distribution network. One study (Yang et al., 2011) also successfully illustrated the method of translating the QMRA output into risk management strategies.

Likewise, another study also used the reverse QMRA approach (Schoen & Ashbolt, 2011) to estimate the *Legionella* concentrations in premise plumbing. However, there is still enormous of scope for improvement in applying the QMRA framework, for example, assessing the risk from other opportunistic pathogens which tend to grow post treatment (apart from *Legionella*).

With regard to microbial risk assessment using fecal indicators, *E. coli* is well-known fecal indicator and can be used in three ways in QMRA(USEPA, 2014). First, it can be used to assess the risk from specific fecal pollution sources to exposed persons, secondly used in assessment of drinking water treatment risks and thirdly, used in "reverse QMRA" process based on estimate of pathogen density from fecal indicator densities and estimated disease burden. However, its poor relationship between *E. coli* and the occurrence of human pathogens makes it inadequate to have reliable assessment of infection risk (Nj Ashbolt et al., 2001; Susan R. Petterson et al., 2016). Despite its limitation, the positive count of fecal indicator organisms within the risk-based approach can give crucial information related to the extent of fecal contamination. Nevertheless, the WHO in 1999 had proposed a predictive risk-based framework (e.g., QMRA) for the management of fecal risks associated with waterborne exposures, including drinking water (WHO, 2016). If adequate data is available, then the statistical distribution methods (ex. Bayesian or Monto carlo method) can be used to account for variability and uncertainties in the risk assessments. Else, assessments will have to rely on point estimates which often do not consider the uncertainty (USEPA, 2014).

In the context of using risk assessment as a tool for decision making, USEPA (2014) quotes that the use of conservative estimates for pathogens in the past have shown to be predicting hyper protective scenarios that questioned the credibility of the results. Therefore, thoughtful balance of tradeoff in being conservative is necessary to make the right decision especially when the risk level approaches the set target levels (WHO, 2016). The decision-making process can be immensely supported defining the degree of uncertainty in findings, because the risk assessors often have a common question: "How sure are the results?" The answer to this question is normally found by performing a 'sensitivity analysis' because it highlights the most uncertain or influential parameter. In microbial risk assessments, USEPA (2014) mentions that there can be two uncertain components: one is exposure assessment with the varying parameter of FIO/pathogen density and exposure volume; the second is the health effects with the varying parameter as dose response and immunity/secondary transmission.

Furthermore, USEPA (2014), suggests that the method of comparing the output results with the epidemiological data could be one way to validate risk assessment findings for a 'reality check'. This method could help to assess the risk estimates in relation to the observed cases of infections and their associated pathogens in real life.

The next section provides overview on different pathogens, their relative importance in drinking water distribution supply system, incubation time, disease caused, duration of illness and its related outbreaks reported specifically in Sweden.

2.5 Pathogens

The human body is said to typically comprise of about 10^{14} bacterial, fungal, and protozoan cells that represent a broad spectrum of microbial species and are often termed as 'normal microflora'. The presence of such microflora is normally restricted to particular areas of body (i.e. skin, mouth, large intestine etc.). Despite living with so close intimacy along with the huge microflora, humans somehow end up in disease or death caused by some pathogenic or harmful microbes (Alberts B et al., 2002).

Given the above overview, a '*pathogen*' is defined as "*any agent that causes disease in animals, plants or human beings*" (Stanwell-Smith et al., 2003). These are broadly classified as bacteria, virus protozoa, helminth (worms) and fungi. Pathogens are very different from the normal flora especially in terms of causing trouble to humans. In simple words, the dedicated pathogens are capable of causing illness irrespective of the immunity status of the host in contrast to the normal microflora (i.e. normal flora attacks only host with weak immunity) (Alberts B et al., 2002). Pathogens can pass through cellular and biochemical boundaries to survive and multiply in order to dramatically destroy the biology of the host. Additionally, there are some criteria that distinguishes a normal flora from a pathogen. For example, a pathogen must be capable to colonize the host, to reduce immunity of host, to replicate, to exit and transmit and lastly to find nutritional niche for its survival in host. However, not all the microorganisms are self-pathogenic (e.g., *E. coli)* but other microorganisms are also found in feces, alongside pathogens, hence these non-pathogenic microorganisms may be helpful in detecting potential fecal contamination in drinking water. They are referred to as fecal indicator organisms which are described in the next section.

More importantly, human migration has brought people into contact with new pathogens and the global environmental change has indeed expanded the range of known pathogens or created the conditions for indigenous microorganisms to emerge as significant human pathogens. Consequently, a trend has been noticed wherein the human pathogens emerge or re-emerge after a long period of inactivity due many reasons (e.g. changes in human behavior and vulnerability) (WHO, 2003). Nevertheless, an infection can occur only when pathogens are present, so emphasis should be given to the 'occurrence' conditions that lead to presence of pathogen in water media. The occurrence level of any pathogen depends on temporal distribution/frequency (USEPA, 2014) and its occurrence density can vary over wide temporal and spatial scale. As in, pathogen occurrence in most of the cases can fluctuate over large range of time scales (i.e. hourly, daily, weekly, monthly, seasonally, or yearly). The main drivers of such temporal variability could be mentioned as seasonal changes, human or animal epidemics, hydrometeorological events, treatment plant operations, animal life cycles, tidal process, solar cycle, etc. Likewise, spatial fluctuations are normally related geographical location of a pathogen source relative to the position of receivers or to site specific mixing process. The drivers for spatial variability could be listed as alignment of source with water of interest, hydraulics and mixing and distribution of sources. These variabilities normally cause high level of uncertainty in detection levels (USEPA, 2014).

In a Swedish context, reports suggests that microbial contamination has been the major cause in the 131 drinking waterborne outbreaks registered in Sweden during the period 1980-1989 and 1992-2003 (Dryselius, 2012). Given these statistics, it is indeed difficult to instantly classify the type of microbes and its relevance level of impact to Swedish drinking water supply. From the few past cases, *Campylobacter, Salmonella,* norovirus*, Giardia* and *Cryptosporidium* parasites seem to be dominant agents in causing the disease outbreaks in Sweden and as such are more focused in this study. Off course, there are other numerous microorganisms which have been

associated with many waterborne diseases elsewhere in the other parts of world (e.g. cholera is caused by the bacterium *Vibrio cholera* and was recently seen in Dominican Republic).

The Table 1 below provides a quick overview of the pathogens that are more relevant to Sweden, including their respective unique characteristics and its associated waterborne disease outbreaks. The Table 1 has been derived based on the review of information available in (Nygård et al., 2004), (WHO, 2005), (USEPA, 2014) and (Folkhälsomyndigheten, 2015).

Table 1-Derived summary of few pathogens of concern in Sweden

Microorganism Type	Pathogen	Relative infectivity	Significance in water supply	Incubation time (in days)	Duration of illness (in days)	Disease caused	Related outbreak examples in Sweden
Bacteria - can exist in spherical (coccus), rod-shaped (bacillus), comma-shaped (vibrio), spiral (spirillum), or corkscrew-shaped (spirochete) and can range from 0.5 to 5.0 μm in size.	*E. coli* O157:H7	High	Moderate	3 – 4	5-10	Diarrhea	reported in 1965
	Campylobacter	Low	Moderate	3–5	2–10	Diarrhea	Söderhamn 2002–2003 with total 6000 people
	Salmonella	High	Moderate	1 - 3	2–7	Typhoid fever	Not found in Folkhälsomyndigheten
Virus - are very small round structured	Norovirus	High	long	1-2	1–2	Gastroenteritis	Lilla Edet in 2008, more than 2000 cases were from Communal water, 400 ill people in Sälen ski resort in 2002
Protozoa - can exist in small, colorless, ovoid to spherical oocyst, containing four sporozoites in cryptosporidium and in the form of cyst in Giardia	*Cryptosporidium*	High	long	2–14	> 30	Diarrhea	In December 2010, >27,000 people infected in Östersund & in Skellefteå in 2011 infecting >20000 people
	Giardia	High	Moderate	1 - 14	1 – 3	Giardiasis	1260 of Giardia infections in Sweden during 2014

References used: (Nygård et al., 2004), (WHO, 2005), (USEPA, 2014) and (Folkhälsomyndigheten, 2015).

2.6 Fecal Indicator Organisms (FIO)

Direct detection of pathogenic organisms in water sample is often difficult because of the cost and its complexity involved in laboratory procedures. Therefore, the concept of using indicator organisms became more prevalent, as they could be easily quantified by use of simple laboratory procedures. The presence of *E. coli* although indicates the presence of pathogens, its absence does not imply that the water is 100% free from pathogens, because past studies show that the outbreaks (ex. cryptosporidiosis and giardiasis) have occurred when water test detected absence of *E.coli* (USEPA, 2013). Thus, relying completely on FIO might be insufficient compliance of water quality and few other reasons for this are discussed further below. Despite this, US in 1914, adopted fecal coliform group as an indicator of fecal contamination of drinking water. The same is being practiced even today by US and all other developed countries. In view of this, WHO (Nj Ashbolt et al., 2001) further recommended a set of the essential criteria for a microorganism to be used as an ideal fecal indicator but however it is not easy to find any one specific indicator that satisfies all the set criteria. Therefore, various groups of microorganisms described below in the Table 2 have been recommended to be used as indicators in different applications. Table 2 has been derived from the information available in (Nj Ashbolt et al., 2001) and (Rosen, 2000).

From below listed indicators, traditionally *E. coli* is seen to have satisfied most of the recommended criteria and so considered to be an ideal fecal indicator. To elaborate on the required criteria, *E. coli* is easy to isolate and affordable to test, it is present whenever the source of fecal contamination is present, its density is greater than the pathogens, it responds to natural environmental conditions and treatment processes, it's a member of human intestinal microflora, it does not grow or multiply in water and lastly it is not a pathogen by itself. On the contrary, there are also some problems of *E. coli* being used as an ideal fecal indicator (Stephen T. Odonkor, 2013). Three such problems could be listed as, one *E. coli* is difficult to find as it dominated by other types of fecal bacteria, second, its poor ability to adapt to environment and the third it can be found in clean tropical environment. Therefore, the tradeoff described between the ideal criterias and problems of *E. coli*, do not make *E. coli* an 'absolute or unbiased indicator' of fecal contamination, especially if detected via culture test method. In addition, the *E. coli* enumeration results from culture tests, could be perceived as 'underestimation' of *E. coli* present in the sample in most of the cases. There can be two main reasons for this scenario to happen, one some healthy coliforms do not grow in prescribed media in spite of being viable and secondly, the coliforms are normally stressed when traced in environment which makes its recovery/isolation more complicated despite its potential to grow in the prescribed media. Irrespective of these drawbacks, the '*E. coli*' tops the list as the 'ideal' determinant of the most recent fecal contaminations and thus often used for water quality assessments.

Conversely, the old assumption that FIO indicates the recent fecal contamination based on its quick die off time in the environment is not valid today, because several studies have reported longer survival time of FIO and even their potential to grow in soils, manure stocks, biofilms, beach sand , storm water ponds (USEPA, 2013). However, with respect to distribution system, *E. coli* is said to be an indicator of fresh contamination because the temperature and nutrient concentrations are maintained to discourage of growth of enteric bacteria in biofilm(WHO, 2017b). As such, the use of FIO may be good only to assert the compliance of water quality standards but does not, however ensure a full pathogen risk assessment. The complete pathogen risk assessment can be accomplished by choosing different control strategies that focuses on true pathogens(USEPA, 2013). In addition to the fecal indicators, there are also other type of indicators like *Pseudomonas* spp., *Aeromonas* and *Staphylococcus* etc. suggested to be used as indicator for certain applications (like recreational water, bathtub water, etc.)

Table 2 - Derived summary on Fecal Indicator organisms

FIO	Description	Use
Coliforms	- Gram-negative, nonspore forming, oxidase negative, rod shaped facultative anaerobic bacteria - Consist members of Escherichia, Citrobacter, Klebsiella, Enterobacter and optimum growth at incubation at 35 °C	Not a fecal pollution indicator as it is also present naturally in environment
Thermotolerant Coliforms	- Subgroup of coliforms which produce acid and gas from lactose - Identified by incubation temperature to 44.5 °C	Fecal indicator
E. coli	- Gram negative, non-sporulating, rod-shaped bacterium, facultative anaerobic portion of human colonic Norma flora with Optimum growth is seen at 37°C	Ideal fecal contamination indicator sourced from warm-blooded animals
Fecal Streptococci (FS)	- Gram positive, belongs to genre Enterococcus and streptococcus - Not abundant in feces, does not multiply in the environment and more rapid death - FC/FS ratio traces the fecal source - Consists of Streptococcus fecalis, S. faecium S. bovis, S. equinus, and S. avium Enterococci	No more used as fecal indicator
Enterococci	- Subset of FS and are over 17 types - Presence correlates well with illness from both fresh and marine water	Used to assess the risk of gastroenteritis for recreational bathwaters, used to test the presence of enteric viruses in environment, also used to identify source of pollution
Clostridium perfringens	Anaerobic, gram-positive, spore-forming rod-shaped bacteria	Fecal indicator as they survive longer, past pollution indicators, indicators of removal of virus or protozoa during treatment
Bifidobacterial	Obligately, anaerobic, non-acid fast, non-spore forming, non-motile, gram positive bacilli	Emerging fecal indicator as they are largest group of bacteria present in feces of warm-blooded animals
Bacteriophages	Bacterial virus is present everywhere in nature	Water quality test and modelling human enteric virus
Coliphages	Viruses that use *E. coli* as hosts for replication	Water quality test to detect virus
Bacteroides fragilis bacteriophages	Belongs to family Siphoviridae with flexible tail	Potential indicators for distinguishing animal or human feces

In addition to the normal harmless *E. coli*, there are also some strains that are harmful to a host, which can cause diarrhea that is often lethal to host (especially children) preferably in developing world. Table 3 provides the summary of pathogenic *E. coli*.

Table 3- Derived summary on pathogenic E. coli adapted from (Stephen T. Odonkor, 2013)

Harmful E. coli stain	Disease Characteristics	Host
Enterotoxigenic E. coli (ETEC)	Top bacterial cause of traveler's diarrhea and also diarrhea in children	Human, Pigs, sheep, cattle, dogs, horses
Enteropathogenic E. coli (EPEC)	different molecular mechanism of colonization and etiology cause diarrhea. Shiga toxin can also be present	Humans, rabbits, dogs, cats, horses
Enteroinvasive E. coli (EIEC)	Causes heavy diarrhea and high fever	Only in humans
Enterohemorrhagic E. coli (EHEC) O157:H7	Very popular worldwide, this causes bloody diarrhea with no fever. Hemolytic-uremic syndrome and sudden kidney failure can also be experienced by host	Humans, cattle, goats
Entero Enteroaggregative E. coli (EAEC)	These strains bind to the intestinal mucosa and cause watery diarrhea without fever	Only in humans

2.7 Potential pathogen sources and its pathways

Having gained some insight on pathogens and fecal indicator organisms in the previous sections, it is now time to dive deep into the problem and understand the potential pathogen sources and its pathways. Going by the scope of this study, characterizing the potential sources of fecal contamination and its potential pathway of intrusion into the drinking water pipe apart from the microbial enumeration data, would be the preliminary step towards assessing relative human infection risks on ingestion. As such the upcoming sections provides a brief description on the pathogen source-pathways, in the same order as seen in the Figure 7 below.
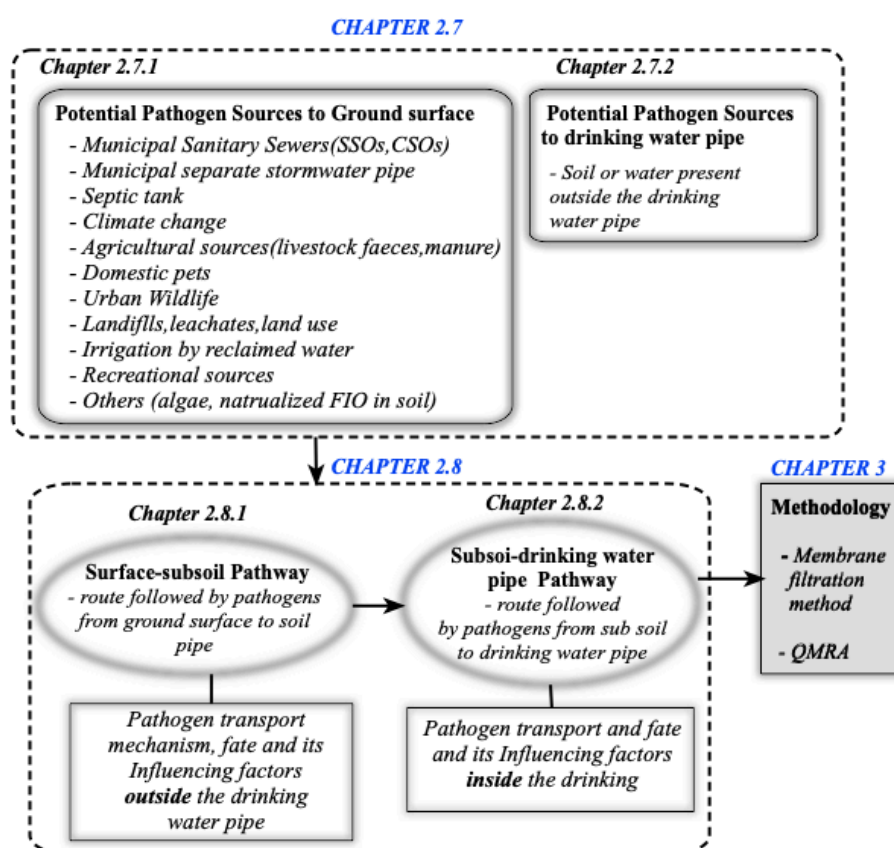
Figure 7 - Source-Pathway model for pathogen intrusion into drinking water during pipe repairs

Water distribution networks are highly susceptible to accidental or intentional attacks due to its complex nature. The potential pathogen sources widely vary from controllable human sources to naturally occurring sources such as wildlife. As such, contamination source identification has increasingly become a main concern in water distribution systems. Various source tracking techniques used in past decades had some or other limitations as reported in literature (Adedoja et al.,2018). Nevertheless, to describe in simple words, contamination source identification technique should include contaminant location, the time of injection and its concentrations in the entire network. In this study context, tracing the source of microbial pollution of water sources is very challenging and is influenced by surrounding land use. Therefore, both point and non-point sources are of equal importance. Examples of point source pollution include industrial effluents (pulp and paper mills, steel plants, food processing plants), municipal sewage treatment plants and combined sewage storm water overflows, resource extraction (mining), livestock farms, and land disposal sites (landfill sites, industrial impoundments) etc. Likewise, some examples of non-point source pollution can be agricultural runoff (pesticides, pathogens, and fertilizers), storm water and urban runoff, and atmospheric deposition (wet and dry though put of persistent organic pollutants). However, it should be noted that the occurrence and persistence of pathogens from any source, absolutely depends on the geological location, soil type, weather conditions, human activities, land use and more importantly proximity of the sources to the drinking water distribution mains.

2.7.1 Potential pathogen sources for microbial contamination of soil

FIO in urban areas can have infinite number of sources (both point and non-point) and hence the backtracking or contamination source identification process is often challenging. However, overcoming this challenge can, to a certain extent, help with the source prioritization process of ranking the various potential sources. With reference to this context, a recent study (Clary et al., 2014) has suggested that the potential fecal contamination sources should be identified by their relationship to human activity and there can be three broad types, as in first human origin (from human body), second non-human origin (from human activities) and third non-human origin (not by human activity). Furthermore, the factors like human health risk, magnitude (or loading), geographical distribution, controllability and frequency of exceedance were considered for ranking the contamination sources. However, the human health risk and magnitude was identified to be the top ranked factor(Clary et al., 2014).

A similar approach has been used to develop the source prioritization for this study. However, factors such as potential to infiltrate into ground water, exfiltration probability, proximity to drinking water pipe (or pipe repair area), etc., have been additionally considered. This prioritization is exclusively done in consideration of the urban areas of Gothenburg, Sweden only. Hence the presented source prioritization will certainly be different in different geographical locations depending on the land use of the city. The pathogen source prioritization criteria (or eligibility criteria) developed to decide the relative importance level (or ranking) of potential sources, in specific to urban areas of Sweden is given in the Table 4 below.

Further on, the potential pathogen sources in specific to urban areas of Sweden, were assessed and prioritized based on the developed eligibility criteria listed in Table 4, on literature reviews (cited under references) and also based on the self-assessments made during sample collection process in different locations. The list of potential sources and their relative importance are presented in Table 5 below. However the Table 5 is adapted from Clary et al.,(2014).

Table 4 - Pathogen source prioritization criteria to assess the relative importance of sources

Level of importance	Factors considered (or eligibility criteria)
High	- easily infiltrates into ground water - can exfiltrate - very close to drinking water pipe/sources - easily transported to receiving waters by runoff - is of human origin and from a human activity - is from non-human activity - frequency of occurrence and loading - frequency of wet and dry weather conditions - involves challenge of changing people's behavior - not always easy to monitor and control
Moderate - High	All the factors listed for 'High' **except** - exfiltration - involves challenge of changing people's behavior
Moderate	- mostly easy to monitor and control - is of non-human origin
Low	- not significant in urban areas - natural

Table 5 - Potential sources and their relative importance level in urban areas of Sweden

Main category	Potential sources/activities	Importance level in urban areas	References
Municipal Sanitary Infrastructure (piped)	Sanitary sewer overflows (SSOs) Combined sewer overflows (CSOs) Leaky sewer pipes (case of exfiltration) Illegal sanitary connections Inefficient Waste water treatment plants	High	*(Clary et al., 2014), (Malm, 2015), (Svenskt Vatten, 2000), (SEPA, 2016)*
Municipal separate storm water pipe	Leaky sewer pipes (exfiltration) Biofilm or sediments in drain pipe	High	*(Svenskt Vatten, 2000)*
Other Human Sanitary System	Septic tanks (Leaking or failed) and contaminated ground water due to sewage seepage	High	*(Bonus Optitreat, 2017),*
Climate change	Rainfall, snowmelt, floods	High	*(SCB,2018)*
Agricultural sources	Livestock feces Livestock manure Livestock feeding stations Slaughterhouses Application of Biosolids	Low	*(Al Dufour, Jamie Bartram, 2012) (Nygård et al., 2004)*
Domestic Pets	Feces of Dogs, cats, etc.	Low	*(Rosen, 2000), (Elvander et al., 2013)*
Urban Wildlife	Feces of Gulls, pigeons, swallows, rodents, rats, raccoons, squirrels, foxes, horses, etc. Grazing (specifically horses)	Moderate	*(Clary et al., 2014), (Poulle et al., 2017), (Elvander et al., 2013)*
Other Urban Sources	Landfills, leachates, land use	Low	*(SCB,2018)*
Urban Non-storm water discharges	Excessive irrigation by reclaimed water	Moderate	*(Raso, 2013)*
Recreational sources	Bathers or boaters	High	*(Clary et al., 2014)*

Further on, the following section provides a general description on some of the above listed potential pathogen sources.

Combined Sewer and Separate Sewer system overflows

Urban areas are normally served with two different types of sewer system, combined system and separate system. In combined sewer systems both wastewater and storm water are carried in a single pipe to the wastewater treatment facility, but in separate systems the wastewater and storm water are conveyed separately with only wastewater undergoing treatment before discharged into surface waters (USEPA, 2004). CSOs and SSOs can contribute to public health risk and water quality concerns, because they comprise of raw sewage and storm water that contains pathogens, solids, debris, and toxic pollutants. Moreover, many beach closures, shellfish bed closures, drinking water supply pollution, and other environmental and public health concerns has been attributed to SSOs. The likely cause of the contamination through overflows can be blockages, hydraulic design (i.e. Structural, mechanical, or electrical) failures, infiltration, inflow, damaged sewer pipes, underestimated conveyance capacity and also vandalism.

According a case study (Gerly Hey et al.,2016) conducted in Sweden, infiltration (especially when sewer pipe is below groundwater) and inflow are mentioned to be the sources for sewage overflows. Further on, the climate change scenario study projections show that the volume of untreated water overflow could increase by 5-10% over the next 30 years, and by 20-40% by the end of the century (SMHI, 2016). Separate and combined sewer systems overflow that bypass treatment during intense storms can be the major sources of coliforms. In Sweden, separate systems have been developed since mid-1950s but still about 20-25% of urban areas are operating with combined sewer systems. To be more specific, Sweden has about 60 200 km separated sewer pipes and 5600 km combined sewer pipes (Malm, 2015). As such this can be one of the potential sources as explained above.

Figure 8 - Separation distance between drinking water and wastewater pipe witnessed in one of the sampling locations in Gothenburg, Sweden

Apart from the above, the inadequate separation distance between the wastewater pipe and drinking water pipe is suggested to be the potential source for pathogen occurrence especially in case of wastewater pipe leakage (Karim et al., 2003). For example, the Figure 8 above shows the location of wastewater pipe and the drinking water pipe in one of the sample collection locations in Gothenburg city and it is visible that the separation distance between the two pipes is less than recommended separation distance of 3 meters. This was seen in most of the sample collection locations of the city. In addition,(Melle Säve-Söderbergh et al., 2013) also indicated that over 80% of the distribution area in Sweden, has both the wastewater pipes and drinking water pipelines present in the same pit and thus obviously poses a great threat to drinking water safety.

Sewage treatment plants

During the wet weather flows, the sewage treatment plants can face operational challenges due to excessive inflow which results in inadequate treatment efficiency. Consequently, the excess wet weather flows result in discharge of untreated wastewater from the plant. Such effluent can contain pathogens and have been found to travel long distances below point of discharges (Rosen, 2000). Wastewater treatment plant effluents have been associated with the fecal contamination of soil. It is a potential source of microbial contamination especially if the drinking water pipes are located in the vicinity of the discharge point downstream. In Sweden (SEPA, 2016) the effluent discharges are normally made to surface water sources. For instance, the discharge point from the Swedish Ryaverket is in Göta älv river which runs through a heavily urbanized and industrialized area. It is not very clear, if the wastewater effluent discharge pipe is always routed through either shallow unsaturated soils or groundwater for additional polishing and diffusion before it discharges to surface water. Additionally, the site conditions (geology or hydrology) and system design may be such that all the effluent would not have discharged to surface waters (Department of Environmental Quality, 2007)

Septic tanks

Modern septic systems are cost effective but can sometime become a health threat because of system failure due to various reasons like inefficient system design, impermeability of soil, improper soil drainage, inadequate vertical distance between the absorption field and the water table, and incorrect slope. If the drain field is not located above the ground water table, then the effluent can travel upward to the surface due to low permeability of soil and get picked up by the rain runoff to further cause pollution. On the contrary, the effluent can move laterally too quickly in coarse soil which further enables the untreated effluent into groundwater. Such inadequately treated wastewater if it reaches the ground water can cause significant health risk to human as it can contain many pathogens. Viruses are the more serious category because they can travel faster to reach ground water. It is also mentioned in a recent study(Curtis, L. and Koopal, 2012), that the infective viruses can move up to 50 meters downwards from septic tanks to drinking wells and can move up to 1.6 kilometers horizontally movement. To add on, Craun in 1985, in his study conducted in United States, mentioned that, the overflow or seepage of sewage caused illness and 63 percent of the reported cases were caused by the use of untreated groundwater in the mid 1980s (USEPA, 2013). Likewise, septic tank could be the potential source in Sweden as well, especially when only septic tanks treatment is used. The reason for this is, in Sweden, around 700 000 properties still have on-site sewage treatment plant, out of which only 60% of the on-site sewage treatment plants are approved according to the National Environmental Code. There are many older on-site sewage treatment plants which are remotely situated have inadequate treatment and therefore it is common that sewage treatment is done only through septic tanks (Ritzman, 2013). As of February 2017, around 130 000 (or 20%) of the small wastewater treatment plants were estimated to have only septic tanks treatment (SEPA, 2016), which is not sufficient according to Swedish legislation. Inventories from the municipalities also indicate that half of the soil-based small wastewater treatment plants are older than 15 years and in need of upgrading (Ritzman, 2013), which clearly makes septic tank a potential source of pathogen.

Municipal separate storm water system

The report on vision of water in Sweden (SWWA, 2014), mentions that rainfall on roofs, roads and other impervious surfaces runs off as storm water. This storm water collects all the contaminants from many sources (buildings, industries, parks, dogs & bird droppings, vehicles etc.) and so can contain several harmful pathogens. Such storm water is further released untreated to the receiving water through separate storm sewer networks and drainage ditches. In the worst scenario like heavy rains or snowmelt, the storm water can lead to leak or break of sewer pipes (either separate or combined) and contribute even more if the storm water pipes are undersized

or located very close to the drinking water pipe. According to Pitt et al. (1999) the highest bacteria and virus concentrations in groundwater found were due to storm water, especially when the water table was near to the land surface. In addition, the Fecal streptococci and *E. coli* were found in about 94% and 95.5%, respectively, in municipal separate storm sewer systems outfalls as monitored in few studies (Weiss et al., 2008). Nevertheless, the groundwater contamination potential from storm water runoff depends on the soil chemical properties, adsorption capability, the ability of the soil to physically strain the pathogens and pathogen survival. Several studies have recognized that fecal indicator bacteria and associated pathogens may be transported in overland flow (Clary et al., 2014; Weiss et al., 2008; Yates & Yates, 1990) resulting in significant contamination of water sources. In addition to this, bacteria and viruses can move through soil media and may be transported to aquifers by infiltrating storm water (Weiss et al., 2008). Moreover, the leaking wastewater pipe located very close to the storm water drain (see figure 9(a)) can be the potential FIO source. Such leaking, old or damaged wastewater pipe can cause exfiltrating flow and infiltrate further into the storm water drain to finally reach the water sources including the soil media as can be seen in Figure 9(b). In addition, the exfiltration is more common when the sanitary lines are above the storm drain, because higher elevation enables the flow by gravity into the storm drain (Clary et al., 2014). In Sweden, there is about 35 100 km of storm water pipes (Malm, 2015) and its challenging to maintain the operational efficiency of the pipe system especially in worst weather conditions. Hence this could be a potential source of contamination in urban areas.
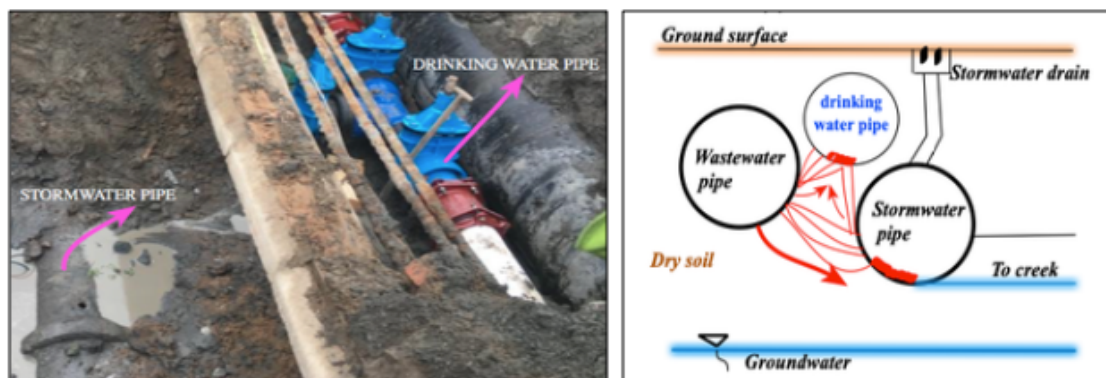
Figure 9 - (a) Separation distance between storm water pipe and drinking water pipe witnessed in one of the sampling locations in Gothenburg, Sweden, (b) Exfiltration from sewage pipe to storm water drain

Climate change effect

According to climate change report of Sweden (SMHI, 2016) the risks of disruption of the drinking water supply can proportionately increase with increased risks of flooding and landslide which will in turn increase microbial pollution of water. The projections show Sweden's annual mean temperature can increase by 2 to 7 degrees Celsius by the period 2071-2100 relative to 1961-1990. In addition, about 0-40% increase in precipitation and around 5-25% increase in runoff is also predicted. However, these projections largely vary across different regions. Such increase in temperature, rainfall and runoff can change the annual rhythm of water supply in most parts of the country and thereby increase the chances of water borne diseases.

Livestock feces (sheep, cattle, pigs, poultry)

Animal feeding operations and grazing lands are all potential sources of pathogens. Many microorganisms shed from animals can affect humans, however only five are widely known to cause illness around the world with high-frequency. They are *Cryptosporidium*, *Giardia*, *Campylobacter*, *Salmonella* and E.*coli O157* (Al Dufour et al., 2012). According to the report (Al Dufour et al., 2012), the excreta of mammals and birds are spread across our planet and they

frequently pollute potable water used for human consumption. Further, the same report presents the fecal pollution caused by domestic animals along with their contribution percentage as, poultry (16%), cattle (57%), sheep (8%) and pigs (5%) which totally accounts for about 85% of the world's animal fecal waste. Under the optimum environmental, hydrological, host and other conditions, human ingestion of water contaminated with low levels of livestock excreta can result in illness. Furthermore, open lots with heavy animal traffic, the chance of direct fecal deposition is higher and thus have high potential for pathogen runoff into surface water or leaching into groundwater or soil. Direct deposit into streams is also a possible source. With regard to Sweden, a study(Nygård et al., 2004) showed a positive correlation between *Campylobacter* incidence and average water-pipe length per person, ruminant density, but did not show any association with the population using the public water supply. Therefore, livestock could be the main source of soil contamination that can be related to sporadic human campylobacteriosis in rural areas of Sweden.

Livestock manure is said to be the reservoir for more than 150 microbial pathogens (such as *Campylobacter*, *Salmonella* (nontyphoid), *Listeria monoctyogenes*, pathogenic *E.coli*, *Cryptosporidium*, and *Giardia*) which can be attributed to about 90% of food and waterborne diseases in humans (USEPA, 2004). A study showed that bacterial pathogens survive for long periods in animal manure under favorable conditions such as low temperature, optimum moisture and nil aeration (USEPA, 2005). For instance, *Salmonella* and *E. coli* O157:H7 have survived for about 4-6 months at 1-9ºC which was about 50 times more than at 40-60ºC. In addition, USEPA (2005) indicated that the survival rate of *E.coli* O157:H7, *Salmonella*, *Listeria*, and *Campylobacter* in dairy cattle, swine, and poultry manures declined with aeration but increased by 88% with increase in dry matter content even when stored at 40-60ºC. Furthermore, in a Swedish context, the main part of the stable manure in Sweden is liquid and the solid part is decreasing(SCB, 2018). Therefore, spreading on land is a potential risk given the fact that most of the microorganisms can survive for a long time in soil and water.

Land application of Biosolids (sewage sludge)
The domestic wastewater produces organic sludge as a by-product after treatment, which is further exposed to aerobic or anaerobic treatment for stabilizing the organic matter and reducing the pathogen content. This byproduct is known as biosolids and it contains nutrients, organic matter, metals, organic contaminants and pathogenic microorganisms (Rosen, 2000). Applied biosolids can further reach ground surface and groundwater either through runoff or leaching causing the occurrence of pathogens. The main risk of land application of biosolids is due to the presence of pathogenic microorganisms (bacteria, viruses and parasites) found in significant concentrations despite the stabilization process because many pathogens can still survive after treatment by adsorbing to sludge (Lewis & Gattie, 2002).The salmonellosis in dairy cows from pastures in Switzerland is an ideal example of inadequate treatment (Rosen, 2000) wherein the cows were infected from the spreading of disinfected sewage biosolids. Land application of biosolids can be a potential source in Sweden as well (especially in non-urban regions), because the Statistics Sweden and Swedish Environmental Protection Agency report in 2016 (SCB, 2018), mentions that the sewage sludge used on farmland was the single most common use of sewage sludge discharged by about 451 Swedish municipal wastewater treatment plants

Direct fecal deposition urban pets and wildlife
Few investigations have suggested that wild birds, poultry and urbanized avian populations (ducks, geese, and gulls) excrete a variety of human gastrointestinal pathogens in their droppings/feces. The feces/droppings from these birds comprised the bacteria *Campylobacter*, *Listeria*, *Salmonella*, *Vibrio cholerae*, *Yersinia* spp. and *E. coli* O157, the protozoa *Giardia* and *Cryptosporidium*, as well as the bacterial indicators of fecal pollution, fecal coliforms and enterococci (USEPA, 2013). As such, wild birds, urbanized birds and poultry may aid as an

expansion environmental reservoir of infection for *Campylobacter*. Furthermore, pets (dogs, cats etc.) are often overlooked as a source of fecal contamination. Yet their large population and the manner in which their excreta are disposed could be a cause of concern. For example, dogs and cats release excreta in yards and walking areas (often adjacent to streams) that are susceptible to direct runoff. Young horses (Rosen, 2000) *and* beavers (Clary et al.,2014) can host *Cryptosporidium* and deer is a host for *Giardia*. In addition to the above, the floors of animal shelters could be a potential collection point for pathogens that leave their animal host. Poor waste management system in these floors can cause contamination. Furthermore, Åström (2013) reported that *Cryptosporidium spp.* was traced in fecal samples from elk, roe deer, red deer and red fox and so wildlife can be one of the fecal contamination sources.

Landfills and Leachate
Leachates associated with landfills of industrial and human waste could be a significant point source of pollutant to both surface water and groundwater according to Miller and Jorgensen studies (Ritter Paul Sibley et al., 2002). It is another potential source of enteric pathogens to soil especially when leachate collection system is not constructed properly. This leachate is suggested to contain viruses and bacteria which can further percolate through soil and contaminate groundwater. In Sweden, third most common use of sewage sludge was for landfill cover, in order to establish a plant layer at 44 500 tones, corresponding to 22 percent of net production of sewage sludge (SCB, 2018). Most of the landfill sites will be capped by 2030.

Land uses
Land use evolution directly changes the biotic and abiotic factors that influence fecal waste production, its survival and transport of fecal coliform bacteria into downstream watercourses. Urbanization produces highly concentrated fecal waste in sewer and septic systems that can overflow during heavy rainfall and release contamination through leaks (St Laurent & Mazumder, 2012). Furthermore, hydraulic modifications or upgrade in urban areas in order increase surface runoff (such as gutters, storm sewers, and pavement), increase the speed and volume of fecal contaminant transport into surface water (Arnone & Walling, 2007). This impact is further enhanced by the removal of vegetation, associated with logging, agriculture, and urbanization, which can reduce infiltration and increase the volume, turbidity, and fecal contamination concentration of surface runoff (Atwill et al., 2002). Considering the huge developmental works for urbanization in Gothenburg (City of Gothenburg, 2014), land use can be a potential source. However, for indicator bacteria, land use for agriculture continues to a major cause of not meeting the water quality standards.

Excessive irrigation by reclaimed water
Reclaimed water can be either used directly or indirectly for irrigation purpose. It is said to be indirectly used when the treated or partially treated or untreated water is released into the recipient waters. However, the health risks are similar for both indirect use and planned wastewater use project. *Giardia, Cryptosporidium*, *Listeria*, *Salmonella*, pathogenic *E. Coli* and multidrug-resistant bacteria all have been found in water used for irrigation purposes. According to a report (USEPA, 2013), *Cryptosporidium* can exist even after chlorine disinfection because of their resistance power and eventually reach the receiving waters with wastewater effluent. In addition, the wastewater reuse report (Raso, 2013), mentions that south eastern region of Sweden despite having rich water resources and low abstraction rate of renewable water resources (only 2%), still are using treated wastewater for irrigation purposes. Thus, reuse of treated wastewater could be potential source mostly in rural areas.

Recreational
Unloading of septic tank from the small recreational boats and leakage from wastewater drainpipes inside the lake can be of potential source of contamination of drinking water.

2.7.2 Potential pathogen sources to drinking water pipe

Pathogens present exterior to the distribution system can enter the drinking water pipes when three conditions exists simultaneously: the presence of contaminant source, a pathway (like pipe leak/break) and a mechanism (ex. pressure surge) (USEPA, 2012). Pipe repair/maintenance works are more often done in open pits or excavations, during which the internal part of pipes can come into contact with soil and water in the repair pit. The likelihood of such soil or water getting in touch with the piping materials during repair activities is said to much higher than it is during storage prior to repair. As a result, AWWA Standard C-651-99 has been set up to assess the probable microbial contamination during pipe repair/maintenance.

In this context, a study was conducted by Karim.et al., (2003) wherein they tested 66 samples (both soil and water) from 8 utilities in 6 US states during a pipe repair work. The results of collected water samples showed the presence of total coliform in 18 samples out of 31 samples, fecal coliforms in 12 out of 18 samples, Clostridium perfringens in 9 out of 30 samples and *Bacillus subtilis* were present in 24 out of 30 samples respectively.

In a similar study by Besner et al., (2011), a total of 15 soil samples and 10 water samples from 15 pipe trenches at repair sites, and 30 water samples from 45 inspected air-valve vaults. The frequency of detection of fecal indicator microorganisms was much higher in the examined water samples from the air-valve vaults than in the soil/shallow groundwater surrounding water mains. In addition to this, *E. coli, C. perfringens, enterococci* and *Bacteroidales* fecal bacteria were traced in more than 60% of the samples analyzed from the vaults. However, no culturable human enteric viruses were reported.

In addition to this, USEPA (2012) mentions few studies that showed the largest bacterial densities were seen to be present next to the existing pipe at the bottom of the trench and that the bacterial counts increased dramatically with the moisture content (almost increased by 100 times). Therefore, they concluded that the moist soil of a pipe repair pit to be the potential source of bacterial contamination during repairs.

Furthermore, the study also suggested that the water standing in the repair pit may again re-enter the distribution system if the area next to the pipe repair site was not properly flushed. Subsequently, USEPA (2013) recommended the dewatering the repair pit below the pipe invert to avoid contamination and to use the submersible pumps to eliminate the chance of cross-contamination from nearby sewage water applications.

From the above, it is clear that the pathogens can be present at detectable levels in the soil and water at repair location. In addition, the groundwater, which may contain pathogens if present around the pipe can also enter the drinking water pipe during the repair work and travel through the system to eventually cause infection (USEPA, 2012). Apart from this, a non-sanitized equipment or unhygienic work activities when comes into contact with water can also become a potential source of pathogen entry and is capable of causing illness (USEPA,2002).

In view of the above, it is essential to analyze the different pathways that aid the pathogen intrusion into the drinking water pipe through soil during the repair works. The upcoming section describes the potential intrusion pathways.

2.8 Potential pathogen pathways

According to USDA (Rosen, 2000), water has a major role in movement and transport of microorganisms. The soil gets contaminated by direct or indirect discharges (e.g., from industrial activity, agricultural chemicals, landfills, biosolids, sewage), deposition of contaminants from the atmosphere, erosion of soil and runoff flow. Contaminants deposited on the ground surface can further leach into subsoil or groundwater through various mechanisms, and eventually may also enter the drinking water supply system through pipe leaks/breaks. Accordingly, the pathways can be divided into two phases as follows,

- **Surface - subsoil Pathway:** route describing the transport mechanisms of pathogens from ground surface to subsoil;
- **Subsoil - Drinking water pipe Pathway:** route describing transport of pathogens present in subsoil to drinking water pipe.

The Figure 10 below depicts the two pathways:

Figure 10-Surface and Subsoil pathways (orange- surface and blue - subsoil).

2.8.1 Surface-subsoil pathway -Transport mechanisms, fate and their influencing factors

Various microbial analysis conducted for different purposes like source identification, evaluation of treatment alternatives, public health risk estimation and soil remediation etc(Clary et al., 2014). demand deeper understanding of the pathogen transport and its fate in soil media. The concept by itself is complex and exhaustive to be fully quantified in this report. However, some basic information like transport mechanism in soil, its fate and the influencing factors are discussed below;

Whilst soil is presumed to be an effective self-purifier, it is always subject to complex hydrological, chemical and biological process that occur in varying time periods (short or long term), that declines its purifying power. In this context, many studies have demonstrated the process of preferential flow through macro pores developed by combination of biological activity (like effects of plants and animals and earthworms, etc.) and environmental factors (like runoff, snowmelt, human activity, etc.) which help in long distance migration of pathogens. Therefore, the pathogen transport in soil is normally best explained by four primary processes: advection, dispersion, adsorption and decay which in turn relies on intrinsic pathogen characteristics (like its size, physiological state, and sorption).

In addition to this, the transport processes are often influenced by straining, attachment, detachment, growth(increase in size),starve(decrease in size),decay, sedimentation and chemotaxis (Abu-Ashour et al., 1994). To elaborate, the pathogens movement controlled by water velocity wherein the pathogens follow the direction of the water flow is termed as advection (Yates & Yates, 1990). On the other hand, the dispersion is an effect caused by spreading or mobility of pathogens as they move along the water path and this effect normally attenuates the pathogen concentration peaks. Further on, the other transport mechanisms are attachment (pathogens attach to the solid surface of the soil grains), detachment (attached pathogens are remobilized to again enter the water phase) and straining (the pathogens are too big to pass through a pore). Nevertheless, 'straining due to pore space size and the bacterium geometry' is suggested to be the dominant process for retention of pathogens in soil media (Clary et al., 2014). Furthermore, with regard to biological process, it is seen that the pathogen once entrapped in the soil (normal in clay) may have to compete and fight for nutrients to survive amongst the native microorganisms (present in the soil). In this 'survival of the fittest' drive, some pathogens may adapt (starve and reduce their size) or die, especially when there is decrease in the nutrient levels in the deeper layers of soil. Conversely, some pathogens get protected to survive longer in deeper layers of soil, due to absence of sunlight and consistent humidity including moisture content. Nevertheless, it must be noted that different pathogens are subject to different transport mechanisms (like physical, geochemical and biological) and their dynamics are altered by soil-pathogen interactions. The surface pathways (i.e. pathogen route from ground surface to subsoil) and various transport mechanisms have been illustrated in Figure 11 below.

Moreover, according to Clary et al.(2014) , the fate of the pathogens relies on two components, one the ability of pathogens to attach and second one its sensorial mechanisms (i.e. response to stimulus in environment). The pathogens having these two traits can successfully perceive the surrounding and find the most favorable space (with high nutrients) for its survival. In summary, the fate of pathogens can be related to important components; one survival and another transport. As in, the pathogen can contaminate the drinking water supply only if it survives (in an infective form) for a long time in the soil media and finds a pathway to intrude the drinking water pipe.
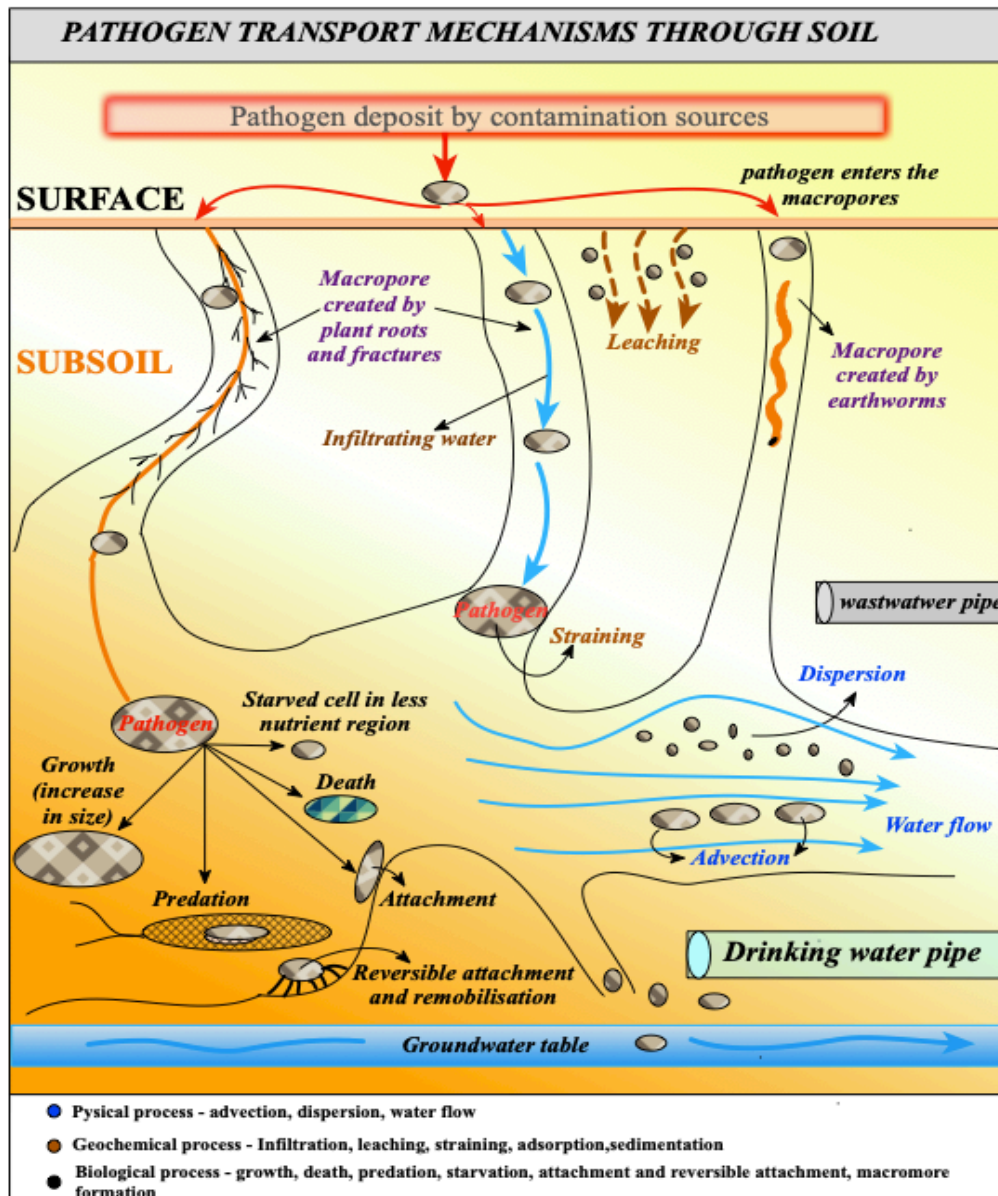
Figure 11- Pathogen transport mechanisms through soil, references used - (Ian L. Pepper, Charles P. Gerba, 2015),(Abu-Ashour et al., 1994),(Yates & Yates, 1990)

With regard to pathogen survival, some pathogens which are shed in huge numbers can have the potential to remain viable in the environment for some time (for ex. *E. coli* can survive up to 200+ days in soil media, (Rosen, 2000)) as against a few in comparison with pathogens shed in small density which often tend to find a new host to repeat its lifecycle. Either which way, the transport and survival of the pathogen through the soil media is influenced by many factors as listed in Table 6 below. However, Clary et al. (2014) suggests that the influencing factors are only indicative of the cause and effect relationships between pathogen and factors because the real-life scenario is much more complex (ex. unknown effects of unknown factors) than tabulated here. Moreover, the influencing factors are also suggested to be interdependent on each other when influencing the pathogen survival or transport (Clary et al., 2014). For instance, transport to deeper layer of soil through water flow causes less penetration of sunlight, which in turn affects the pathogen die-off rates. Thus, the effects of sunlight, flow and survival seem to be interrelated. Similarly, other relationships between other factors can be important.

Table 6 - Factors influencing pathogen transport and its survival in soil media

Factor	Influence on pathogen survival	Influence on pathogen transport
Texture, clay content & particle size distribution	Survival time is high in fine soil with high clay content and humic matter due its water retention capacity *(Abu-Ashour et al., 1994)*	Fine textures soil(clay) with high clay content increases the movement by adsorption *(Santamaría & Toranzos, 2003)*
Organic matter type	Soil rich in organic matter(manure) can increase survival and growth *(Abu-Ashour et al., 1994)*	Increases or decreases the bacterial adsorption depending on its quality, solubility *(Jamieson et. al, 2002)*
Soil pH	pH can increase or decrease survival (Clary et al., 2014), Low pH enhances retention and pH more than 5 increases survival time of bacteria in soil *(Azadpour-Keeley, Faulkner, & Chen, 2003)*	Adsorption characteristics of cells are affected by low pH and hence low inactivation rates in acidic soils *(Santamaría & Toranzos, 2003)*
Sunlight, temperature, water	Pathogens survive less at the soil surface *(Clary et al., 2014) (Abu-Ashour et al., 1994)*	Water increases migration, sunlight protects pathogens and aids in migration to deeper layers. However, temperature effects are unclear *(Santamaría & Toranzos, 2003)*
Nutrient availability	Pathogens survive longer in nutrient rich soil	Presence of nutrients can increase chemotaxis due to sensorial ability
Ionic strength	Increases in cation concentrations affects microbial survival	Increase in the concentration of electrolytes increases the bacterial adsorption and hence their movement
Soil Mineralogy	Clays protect bacterial cells, and viral particles, by creating a barrier against microbial predators and parasites *(Santamaría & Toranzos, 2003)*	Adsorption efficiencies of bacteria increases with increase in the fraction of quartz sand coated with calcite, minerals such as hematite and magnetite increase virus retention
Physiological state	Starved cells can migrate far distances through the terrestrial profile *(Ian L et al., 2015)*	Bacteria are subject to filtration due to big size *(Abu-Ashour et al., 1994)*
Rainfall	Increases survival time *(Santamaría & Toranzos, 2003)*	Increases movement due to dispersion by runoff or by leaching through the soil profile *(Santamaría & Toranzos, 2003)*
Predators / Competition	Can decrease survival time *(Jamieson et. al, 2002)*	Earthworm activity can increase the mixing of microorganisms in soil to move downwards *(Abu-Ashour et al., 1994)*

2.8.2 Subsoil-drinking water pipe pathway, fate and their influencing factors

This section focuses specifically on (re)contamination of treated drinking water because the treated water is often more vulnerable to recontamination based on the construction characteristics, operation, and maintenance of the water distribution system (USEPA ,2002). Drinking water distribution systems are often subject to events like pipe breaks, leaks, repair works, cross connections due to faulty construction, backflow, permeable pipe materials and importantly pressure transients. These events in various literatures are referred in different terminologies like 'contamination events' or 'entry portals' or 'gaps' or 'intrusion pathways' or 'incidents. Therefore, the title 'Subsoil-drinking water pipe pathway' in this section has been coined to describe the route taken by the pathogens from subsoil to drinking water pipe. According to USEPA (2002), pathogens, if present in the surrounding soil, can invade the drinking water pipe through these 'gaps' and eventually compromises the prerequisite pipe integrity (physical, hydraulic and quality) of the distribution network. The Figure 12 below provides a brief overview of common pathways or scenarios which facilitate the contaminant intrusion in a drinking water distribution system.
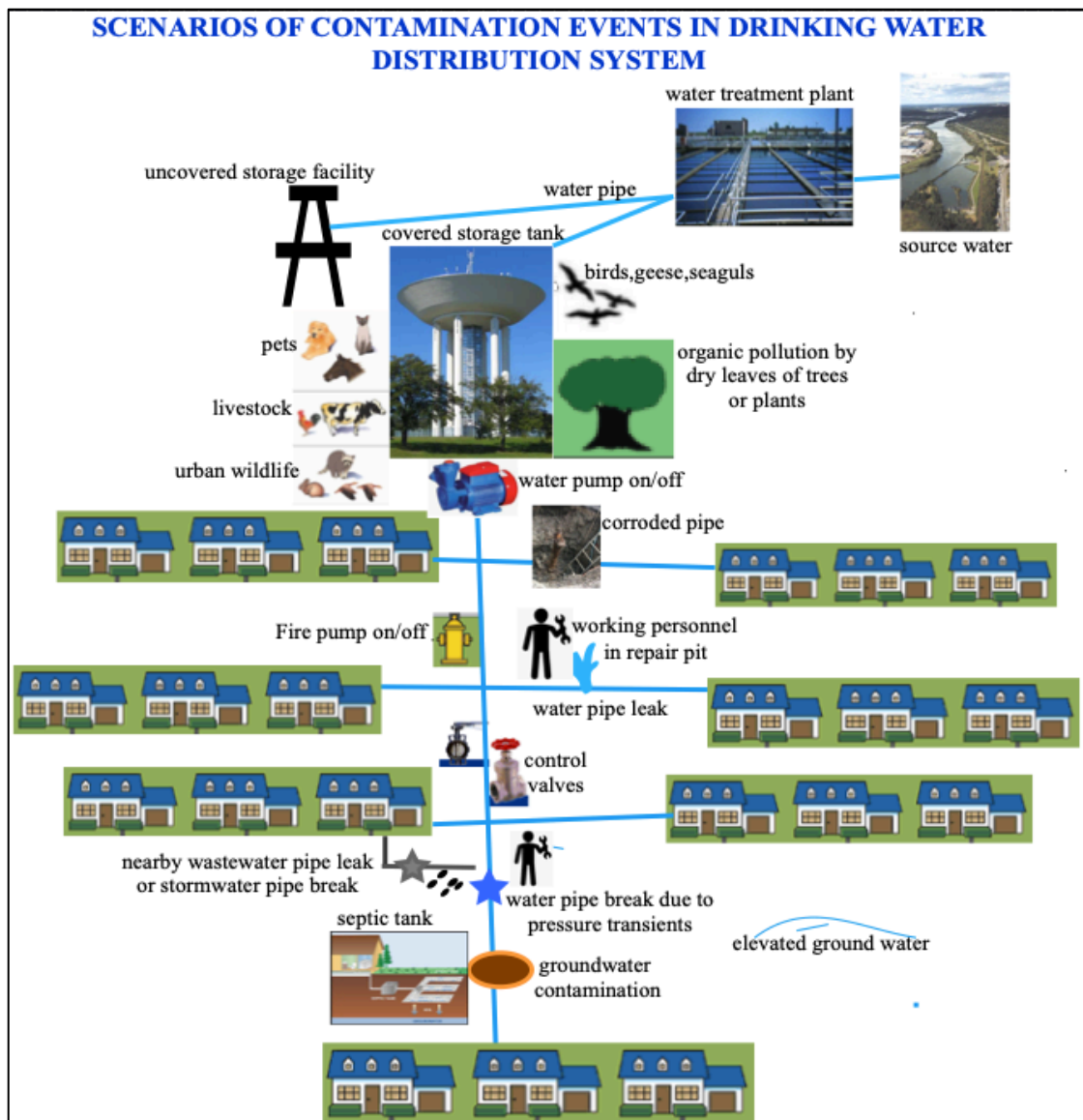
Figure 12 - Common pathways and situations for contaminant intrusion in a drinking water distribution system

In relation to the above-mentioned common scenarios in Figure 12, an example of pathogen intrusion pathway from subsoil to drinking water pipe during the pipe repair event is presented in Figure 13 to support the scope of this report.
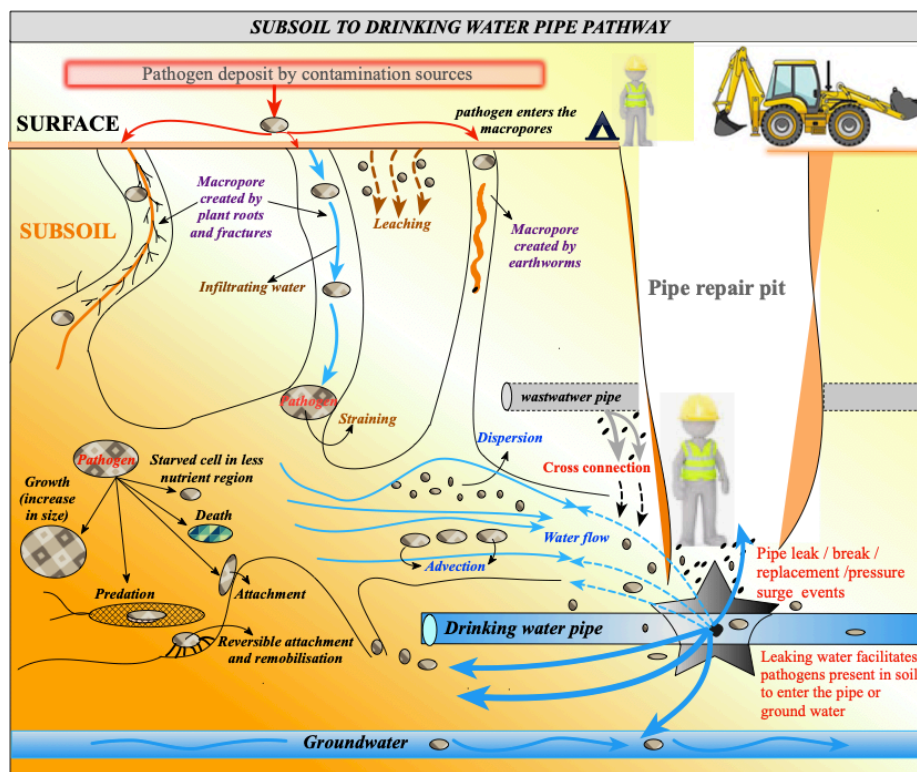
Figure 13- Subsoil to drinking water pipe pathway

From the above Figures (12 & 13), it is clear that the success of safe water supply to the connected consumers relies mostly on efficient maintenance of pipe integrity (physical, hydraulic and quality) along with the treatment at water treatment plants. As such, efficient maintenance of all the three types of integrity are crucial as they all occur due to different causes, have different consequences, have different methods for detecting and preventing, and also have different remedies for regaining the lost integrity (USEPA, 2006). In this context, according to USEPA 2006), the 'physical integrity' refers to the presence of a physical barrier between the distribution system interior and the external environment, the 'hydraulic integrity' refers to constant maintenance of desirable water flow (in terms of water pressure, and water age) and 'quality integrity' means to maintain the finished water quality by prevention of internally derived contamination.

On other hand, despite being different from each other, the pipe integrity can also sometimes be subject to common situations which lead to its loss of integrity (USEPA, 2006). For example, the pipe integrity is often subject to system specificity which in turn rely on particular factors like source water quality, type of water treatment method, type of pipe material, geographical area serviced and its associated population. Apart from pipe integrity, the 'water age' which is the time taken for the water to travel from treatment plant to the customer's tap is said to be an important physical parameter(in addition to pipe diameter and pipe material) contributing to the water quality in the distribution system (Nicholas Ashbolt et al., 2014).

With regard to pipe integrity, a meta-analysis was performed by Ercumen et al.,(2014) on 14 studies that assessed the extent of association between the pipe integrity (all three types) and their related health risk across the globe. The authors concluded that there exist extreme levels of diversity amongst the studies and the related water system characteristics. However, their

study provided evidence that the failure in specific pipe integrity (any of the three types) was linked to high risk of gastroenteritis. To elaborate more on the study results (Ercumen et al., 2014), six studies, suggested a wide range of positive associations between gastrointestinal infection and the different exposures, linked to loss of physical pipe integrity. However, three out of six studies did not show any major associations. Likewise, another study by Blokker et al., (2014) suggested that only 0.07% of the regulatory samples detected with *E. coli*, indicated that the water is more often contaminated due to loss of physical integrity(after pipe break repairs). On the other hand, all the nine studies reviewed by Ercumen et al.(2014), that focused on hydraulic integrity of pipeline, indicated a strong link between high gastrointestinal risk and water outages (i.e. both in continuously and intermittently supply).

Further on, Besner et al. (2011) asserted that occurrence of only physical breaches like cracks/repairs/leaks in pipe is insufficient to facilitate the intrusion of pathogens or contaminant into the drinking water pipe as it is often maintained with adequate pressure. Both physical and hydraulic breaches need to occur simultaneously for an intrusion to occur. For example, negative or zero water pressure during hydraulic breaches allows external contamination to intrude pipelines through the gaps created by physical breaches.

Moreover, with regard to quality integrity, all the three studies reviewed by Ercumen.et.al, (2014) that investigated the water quality integrity, indicated a positive association between the gastrointestinal infection and the inadequate chlorine residual maintained inside the pipe. However only one study out of nine, identified the inadequate residence time (i.e. residual chlorine decreased as the distance from plant increases) as the root cause of significant link between the gastrointestinal infection and chlorine residual.

In addition to above studies, with regard to microbial quality in the drinking water distribution system, Karim et al. (2003) and Besner et al. (2011) exclusively investigated the microbial water quality integrity and provided evidence of detectable level of pathogens (Total coliforms, Fecal coliform, *Clostridium perfringes and Bacillus subtilis*) both in soil or water samples present outside the pipe that may intrude the drinking water pipe during repair and pressure surges respectively.

Apart from breach in single type of pipe integrity, USEPA (2006) also mentions that, there could be some specific scenarios wherein more than one type of pipe integrity can be breached. For example, events like backsiphonage compromises both hydraulic and physical integrity. Likewise, the pipe material quality can breach both physical and water quality integrity. As such, regulations normally enforce maintenance of all three-pipe integrity at any given point of time.

Furthermore, an American Water Works Association Research Foundation sponsored study (Kirmeyer et al., 2001), recognized and quantified pathogen pathways through various events. However, the pathways like water treatment breakthrough, resuspension of sediment or injured organisms, biofilms in the distribution system, and pathogen protection by invertebrates in the distribution system were excluded in this process. The factors like severity of infection, probability of waterborne disease outbreak, contaminated volume and frequency of intrusion were used as the criteria for ranking the different pathways. The expert panel of the study also mentioned that the severity of infection caused from the failure of the distribution network partially depends on the type of pathway used by the pathogens. In view of this, the potential pathways along with their relative ranking as mentioned in USEPA (2002) are briefly outlined in the Table 7 below.

Table 7- Potential pathogen intrusion pathways and their relative risk level

Intrusion Pathway	Causative agent	Risk Levels (USEPA,2002)
Treatment Breakthrough	Primary disinfection or filtration step failure	High
Pipe break/leaks	Aging	High
Pipe break/leak	Seasonal Variations or temperature change effect	High
Pressure transients	Happens during pump startup and shutdown, flushing operations, opening and closing fire hydrants, sudden change in demand, feed tank draining, power failure, main breaks, altitude valve closure, fire flow	High
Cross Connections & Backflow	Happens when pressure inside the distribution system either becomes negative (backsiphonage), or the pressure of a pollutant source becomes greater than the pressure inside the system (backpressure).	High
Uncovered Finished water storage	Fecal waste droppings of infected animals (beaver, muskrat, squirrels, mice, rabbits, birds), Waterfowl, sediment resuspension	Medium
Covered finished water storage	Airborne microorganisms entering through access hatches, overflow pipes and vents, roofs, and poorly constructed sidewall joints, mixing of stagnant rain water with drinking water through covered rips/tears, birds	Low
Pipe storage and handling	Pipes stored at the construction site without protective caps can be easily polluted with dirt, mud, debris and dirty water.	Low
Construction and repair	Gasket seals of pipe joints, soil deposits on new pipes	Low
Natural Hazards	Earthquakes, floods can cause pipes and storage facilities to fail/collapse	Not specified
Improper treatment of equipment's / personnel in contact with finished water	Tank cleaning machines, unhygienic working personnel/area, other equipment used during maintenance works	Not specified
Inadequate distribution system security	unintended or intended contamination may result from unauthorized/untrained users tapping into the distribution system, international security breaches	Low

Each of the intrusion pathways mentioned in above Table 7 is further described below, based on review of information available in USEPA (2016; 2012; 2002). The description below for few pathways includes emphasis on the situation of drinking water distribution system in Sweden as well.

Intrusion through the source water (e.g., treatment breakthrough)
Modern water treatment methods are robust and mostly efficient, however suboptimal scenarios can occur due to various reasons (like operational errors, human errors, presence of non-viable pathogens etc) which can impair the microbial water quality. Steps like inefficient coagulation, filtration failure (ex. backwash recycling and poor maturation of filters) and poor disinfection (ex. *Klebsiella pneumoniae* is resistant to disinfectants) considered to have more likelihood of increasing the intrusion risk (WHO, 2004) The occurrence of this event is mainly because few organisms have the potential to pass through the treatment barriers (USEPA, 2002). In addition to this, majority of organisms can colonize the pipe materials in distribution systems and they can be found in the system's source water particularly after rainfall events. Ineffective or inadequate treatment can also enable fungi and planktonic diatoms to enter the distribution system. USEPA (2002) reported that the elevated coliform counts in a distribution system in Springfield, Illinois could be attributed to breakthrough due to inadequate treatment.

Intrusion through broken or leaking pipes, valves, joints and seals due to aging
Aging or old water supply infrastructure in the United States and most of the other developed countries make water distribution systems more vulnerable to pathogen intrusion through more frequent pipe breaks and other types of age-related deterioration as pipelines towards the end of their service lives (WHO, 2004). Main breaks can result in contaminant entry even in systems using good sanitary practices. In Sweden an estimated frequency of events / malfunctions at the distribution facility in about 165 Swedish municipalities were compiled in a report (Melle Säve-Söderbergh et al.,2013). This survey revealed that in about 30 of the waterworks, pipe break/leakage is seen at least once a month, and for more than 10 of the waterworks, it takes place at least once a week. The same summary also presents the frequency of malfunctions caused by the human factor (for example fault clutches, opening / closing of valves, etc.) which occurs less often or never for most, but some waterworks had replied that it happens from less than once a month to at least once a year. Nevertheless, the present pipe failure rate is 0.16 failures per kilometer per year which includes both leaks and pipe failure repairs as stated by Malm (2015). To add on, the current Infrastructure Leakage Index (ILI) in Sweden stands at '9' in representing a leakage of about 20% of the total water produced and ILI of '9' is termed as 'very bad' by World Bank Institute Banding system (Seago et al., 2005). As such, there is a high probability of pipe leak scenarios thereby a potential pathway.

Intrusion through broken or leaking pipes, valves, joints due to temperature effect
Temperature effects can cause thermal contraction and expansion resulting in main breaks. A recent study(USEPA, 2016) mentioned that utilities experienced an average of 0.3 main breaks per 1.6 kilometer per year, with a median of 0.18 main breaks per 1.6 kilometer per year and the maximum number reported in the survey was 1.4 main breaks per 1.6 kilometer per year. The authors observed a seasonal pattern wherein the majority of main breaks had occurred during the winter. Pipe breaks were the contributing factor in the Cabool, Missouri outbreak of 1989-1990. This outbreak occurred during unusually cold weather and was reported to be caused by contamination that entered the distribution system through two major pipe breaks and 45 service meter failures (WHO, 2004).

Intrusion through pressure transients or surge
Previous studies have shown that low or negative pressure events are very common in distribution systems (Kirmeyer et al., 2001). Transient negative pressure can suck leaked water (i.e. contaminated water) back into the pipe through leakage point. Such water leakage may represent about 10-20% of the water produced, even in well-operated systems. The causative agents for pressure transients or surges are listed in the above Table 7. In addition to these causes, other reasons of pressure reductions include fire flow, elevation changes, service line breaks and main installation. Moreover, the transient pressure modeling by Kirmeyer et al. (2001) mentioned

that the distribution system analyzed was extremely vulnerable to negative pressures. More recently, Besner et al. (2011) also showed that, around four negative pressure events (out of eighteen), were caused by a sudden shutdown of pumps at the WTP due to power failure along with the closure of a transmission main (Besner et al., 2007). Around 15 000 customers were informed to boil-water during the repair work. Further, according USEPA(2016), a case study by LeChevallier et al. (2014) showed that two pressure events which occurred during the monitoring period, were attributed to the main break. In a Swedish context, pressure reduction is observed in about 52% of all municipalities annually and in about 15% of the municipalities at least once per month (Melle Säve-Söderbergh et al.,2013) and hence poses as a potential pathway.

Intrusion through cross-connections
Cross connection is defined as any connection between the potable water system and any other non-potable water system (e.g. sewer system or storm drains) that can potentially allow the contaminants to enter the drinking water pipes (Kirmeyer et al., 2001). According to USEPA (2002), backflow from cross connections can occur when the pressure inside the distribution system either becomes negative (backsiphonage), or the pressure of a pollutant source becomes greater than the pressure inside the system (backpressure). The degree of contamination in the distribution system partially depends on: the location of the cross-connection, the concentration of the enteric contaminant into the distribution system and the magnitude and duration of the pressure difference resulting in the backflow (USEPA,2002). Some examples of cross connections suggested by Kirmeyer et al. (2001) are air compressors, carbon dioxide beverage dispensers, a leaking hydrant foot valve, garden hose sprayers, cooling systems, irrigation systems and fire sprinkler systems. Likewise, some examples of backflow suggested in USEPA (2002) are pressurized residential, industrial, institutional, or commercial systems which use pumps, including chemical feed pumps or booster pumps, or pressurized auxiliary water systems for irrigation, fire protection, car washes, and cooling systems. In Sweden, cross connection was observed to be the most common cause of disease outbreak as mentioned previously. This could also be supported by information retrieved from the recent survey done by Melle Säve-Söderbergh et al.(2013) for 165 municipalities, wherein none of the municipalities replied that cross-connections are frequently faced. More than 60% of municipalities revealed that back valves in service lines were missing in few parts of the distribution area and 30% of municipalities answered that back valves in service lines are missing in the entire distribution area. Additionally, in more than 30% of municipalities, non-return valves or check valves were informed to be missing from fire posts and fountains connected to the distribution facility (example: irrigation systems, fountains and civil protection vehicles). Another 50% of all municipalities responded that they did not know until back valves were missing.

Intrusion through contamination of uncovered finished water storage facilities
Fecal waste of infected animals in the watershed area such as beaver, muskrat, squirrels, mice and rabbits are said to be non-point sources of contamination in uncovered finished water storage facilities. Apparently, birds are not infected by Cryptosporidium, but they can be a contaminant carrier to finished water. According to USEPA (2002), the samples from six open finished water reservoirs which were examined in New Jersey in US were reported to have Cryptosporidium concentrations sevenfold greater at the outlet than at the inlet, although all oocysts looked to be nonviable. Likewise, waterfowl and sediment resuspension in an open finished water reservoir were noted to be the contributing factors to coliform bacteria recurrences in the New York City distribution system. In addition to this, microbes can also intrude into open reservoirs through windblown dust, debris and algae. Apart from these, organic matter like leaves and pollen are also a cause of concern in open reservoirs (Kirmeyer et al., 2001). Water contaminated by said sources can enter water distribution pipes when the water is drawn from these reservoirs for distribution. This is an obvious risk to drinking water consumers as per (Kirmeyer et al., 2001).

Intrusion through contamination of covered finished water storage facilities

Covered storage are prone to airborne microorganisms entering through penetrations in access hatches, overflow pipes and vents, roofs, and poorly constructed sidewall joints. Also, the microorganisms can invade into ground storage facilities from surface water or groundwater infiltration. Along with these, big tears or rips in floating covers can allow any stagnant rain water containing pollutants from various sources to mix with the finished water. For instance, Vibrio cholerae were isolated from feces of 20 species of aquatic birds in Colorado and Utah (USEPA, 2002). Similarly, the surface water collected on the floating cover had a huge potential for bacterial contamination as it contained fecal coliform bacteria counts as high as 13,000 per milliliter at Philadelphia's Oak Lane Reservoir (USEPA, 2002). Bird droppings were often seen on floating covers because birds are attracted to water which collects on the cover surface. Several reported cases of salmonellosis are said to have been caused by water storage facilities contaminated with bird droppings. Investigations of a *Salmonella* outbreak in the Alamosa municipal water supply concluded that a storage tank that had numerous cracks which was the likely cause of outbreak (USEPA, 2016)

Intrusion through Pipe Storage and Handling

Pipes stored at the construction site without protective caps can get easily polluted with dirt, mud, debris and dirty water. These scenarios can lead to water quality problems such as high turbidity, high heterotrophic bacteria count, and coliform bacteria occurrence as suggested by USEPA (2002). According to USEPA (2002) the new mains were traced with the presence of Enterobacter, *Klebsiella*, *Escherichia*, and *Citrobacter* as well as *Aeromonas hydrophila*. The study also mentioned that *Citrobacter freundii* found in soil contaminated the new mains/pipes. In addition to this, a case study in Philadelphia demonstrated microbial contamination of a new main prior to installation (USEPA,2002). On the other hand, another study mentioned in USEPA, (2002) also examined the survival and transfer of microbial contamination via cloths, hands and utensils, so they concluded that dermal contact with contaminated surfaces or cloths can be potential infection hazard.

Intrusion through Construction and Repair

Gasket seals of pipe joints may be a source of bacterial contamination, from strains such as *Pseudomonas aeruginosa, Chromobacterium strains, Enterobacter aerogenes*, or *Klebsiella* pneumoniae (USEPA,2002). To add on, construction trenches can flood due to street runoff or soiled water resulting in more contamination (Melle Säve-Söderbergh et al., 2013). Apart from this, the shutoff of a water main and the operation of valves during a repair can result in the intrusion of contaminated water due to backsiphonage or the detachment of microbial growth and rust as reported by Burlingame and Neukrug in 1993 (USEPA,2002).

Intrusion through Natural Hazards

Ground movement during earthquakes can cause pipes and storage facilities to fail/collapse which can further result in human health infection risk. Likewise, the flood waters which may be contaminated with untreated sewage and other contaminants, can wash away supporting soil around the pipelines and break the pipes. In addition, the tornadoes and hurricanes can also damage the distribution systems especially when the ground is disrupted due to fallen trees and utility poles (Kirmeyer et al.,2001). SMHI (2016) in Sweden also mentions similar observations.

Intrusion through Inadequate Treatment of Materials, Equipment or Personnel in Contact with water

Materials, equipment and personnel exposed to the distribution system can also provide pathways for microbial contaminants to enter biofilms. The sources may be filter materials, piping, sealing valves, shoes, clothes, etc. Furthermore, equipment such as tank cleaning machines or video

equipment used to inspect pipelines, can be a pathway for contaminant intrusion if not decontaminated prior to use (USEPA, 2002).

Intrusion through inadequate security to distribution system
Inadequate security can be a pathway of pathogen intrusion into the distribution system. This scenario can sometimes be caused from intentional security breaches, such as vandalism or terrorism. Conversely, unintended contamination may result from unauthorized/untrained users tapping into the distribution system and swimmers using storage vessels or reservoirs (USEPA,2002).

In summary, all the above described pathways can facilitate the intrusion of pathogens only if there is presence of contamination source and a mechanism (like pipe leak or pressure surge). If all these prerequisites are present and if the pathogen enters the drinking water pipe, then they lead to biofilm development inside the drinking water pipe. However, pathogen survival depends on many physical, chemical, and biological factors which limit their time of survival inside the pipe after entry. Accordingly, the below Table 8, shows the summary of influencing factors derived based on information available in USEPA (2002) and WHO (2004).

Table 8 - Summary on factors influencing fate of pathogens inside the distribution system,

Factors	Influence on pathogens inside the drinking water pipe
Temperature	Microbial activity increases when water temperature is above 15 degrees centigrade
Presence of Nutrients	Nutrients like assimilable organic carbon concentrates at the solid-liquid interface and creates a comfortable environment for biofilm growth
Microbial Interactions	Biofilm can provide nutrient for pathogen growth. For example, Legionella. Some pathogens can be predated as well
Distribution system components	Bacterial concentration on disinfected iron pipes higher than on PVC pipes
System Hydraulics	Complex design of pipe network can also reduce flows and favor biofilm development. Also, high water velocities can result in collection of microbes in low flow areas
Presence of Residual disinfection	Loss of disinfectant residual can lead to excessive biofilm growth
Sediment Accumulation	Organic and inorganic sediments can accumulate in low-flow areas of the distribution system and enhance microbial activity by providing protection and nutrients. For ex. legionella and mycobacteria can multiply in sediments

3 Methodology

3.1 Case study, sampling and filtration

3.1.1 Study Area

This study was carried out in the city of Gothenburg in Sweden. It is the second-largest city in Sweden and the fifth-largest in the Nordic countries. It is located Västra Götaland region on the west coast of Sweden. The city has a population of 570,000 in the city center and about 1 million inhabitants in the metropolitan area. The citizens are supplied with drinking water from Göta älv river via two waterworks, Alelyckan and Lackarebäck, with a connected distribution network of about 1750 km.

In this study, the collected water samples were examined simultaneously in two laboratories: one sample at Chalmers laboratory and the other one at Lackerebäck water treatment plant. Two different methods were used to test the same samples. The Lackerebäck water treatment plant examined the samples by Colilert test method (see Appendix 4 for method details) and the samples in Chalmers Laboratory were examined by using the membrane filtration method.

3.1.2 Sample collection locations in Gothenburg, Sweden

The below Figure 14 shows the geographical locations of water shut off in the city of Gothenburg, wherein the repair/maintenance works of drinking water pipe was undertaken by Gothenburg municipality. Based on the information shared by the city municipality representative, a total of sixteen (16) locations were accessed to collect the water samples from the repair pits. The images of individual locations are shown in the next section.
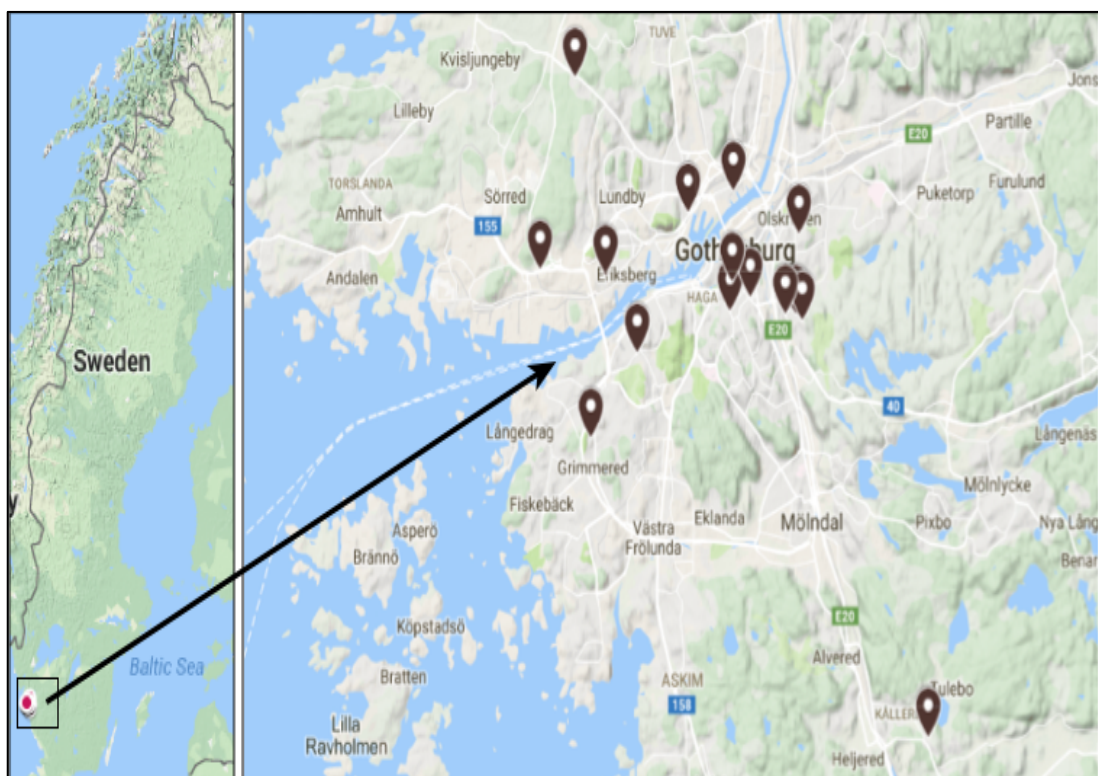
Figure 14 - Geographical locations of water sample collections, Gothenburg, Sweden

The sample collection locations were accessed based on the information shared by the Gothenburg Municipality. The images taken at each sampling locations are shown in Figure 15, 16 and 17 below. The stagnant water from within the repair pit was collected from all the below locations during the maintenance or repair work.

Figure 15- Images of sample collection locations in Gothenburg, Sweden
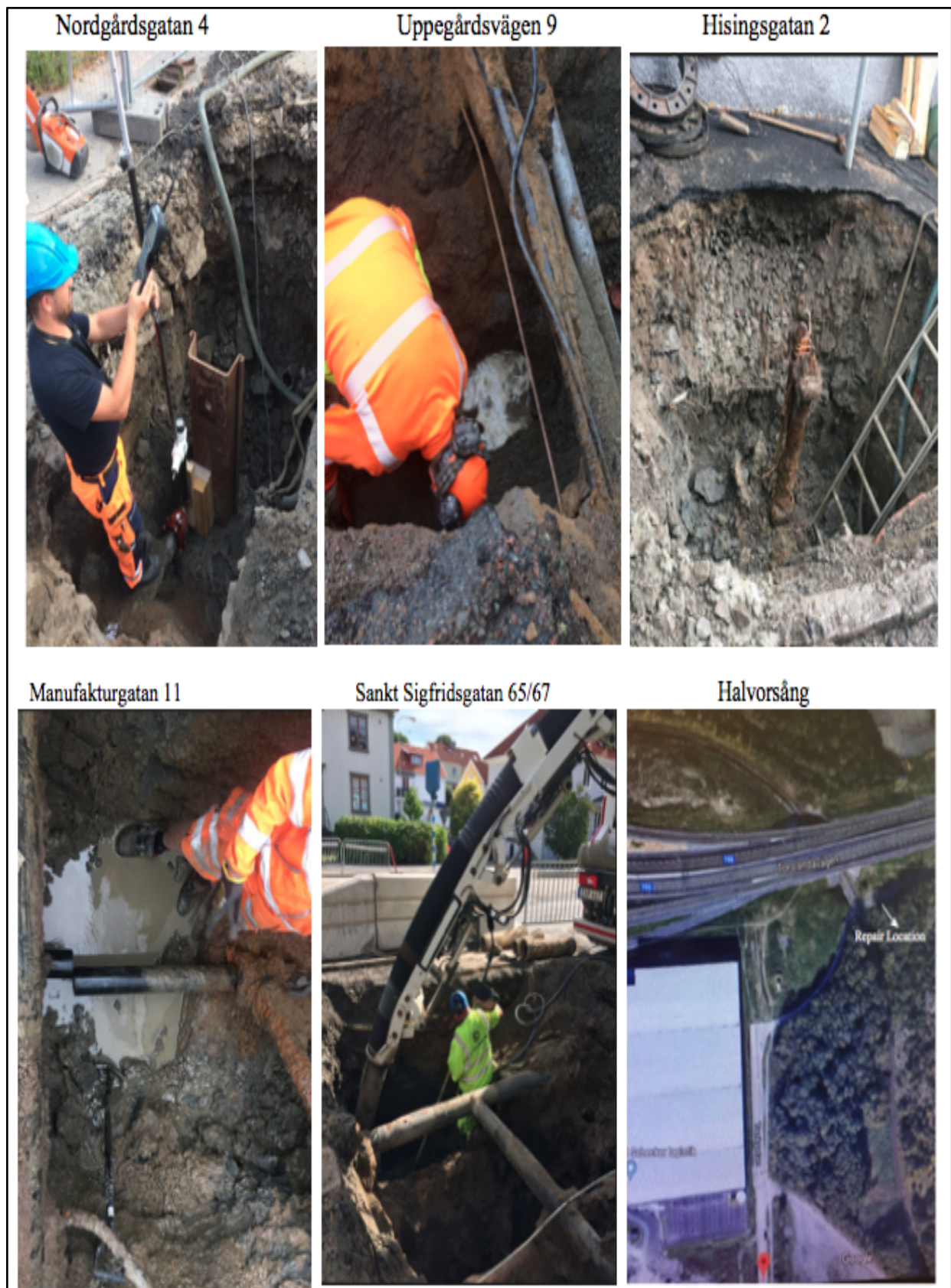
Figure 16 - Images of sample collection locations in Gothenburg, Sweden

Figure 17- Images of sample collection locations in Gothenburg, Sweden

3.1.3 Membrane Filtration Method - Summary

To detect and quantifying *E. coli* and coliforms in collected water samples, the Membrane filtration method, as recommended in method 1604, (USEPA, 2002), ISO 9308-1:1990, and Method 1603 published by the EPA in 2002 was used.

An overview of stepwise procedure is shown in Figure 18 and a more detailed description is provided in the following sections.

This method provides a direct count of bacteria in water based on the development of colonies on the surface of the membrane filter. A 100 ml volume of a water sample (taken separately for coliforms and *E. coli*) was filtered through a 47-mm, 0.45-µm pore size cellulose nitrate membrane filter (for both Total Coliform and *E. coli*) that retains the bacteria present in the sample. The respective filters were inverted (the side through which the water sample passed through) and were placed on a plate of mTEC agar (prepared separately for coliforms and *E. coli*). The plate was then incubated at 37°C for a duration of 24 hours. After incubation, the plates were removed from the incubator and were checked for colonies. The test was positive if the filter contained purple and yellow, yellow-green, or yellow-brown colonies.
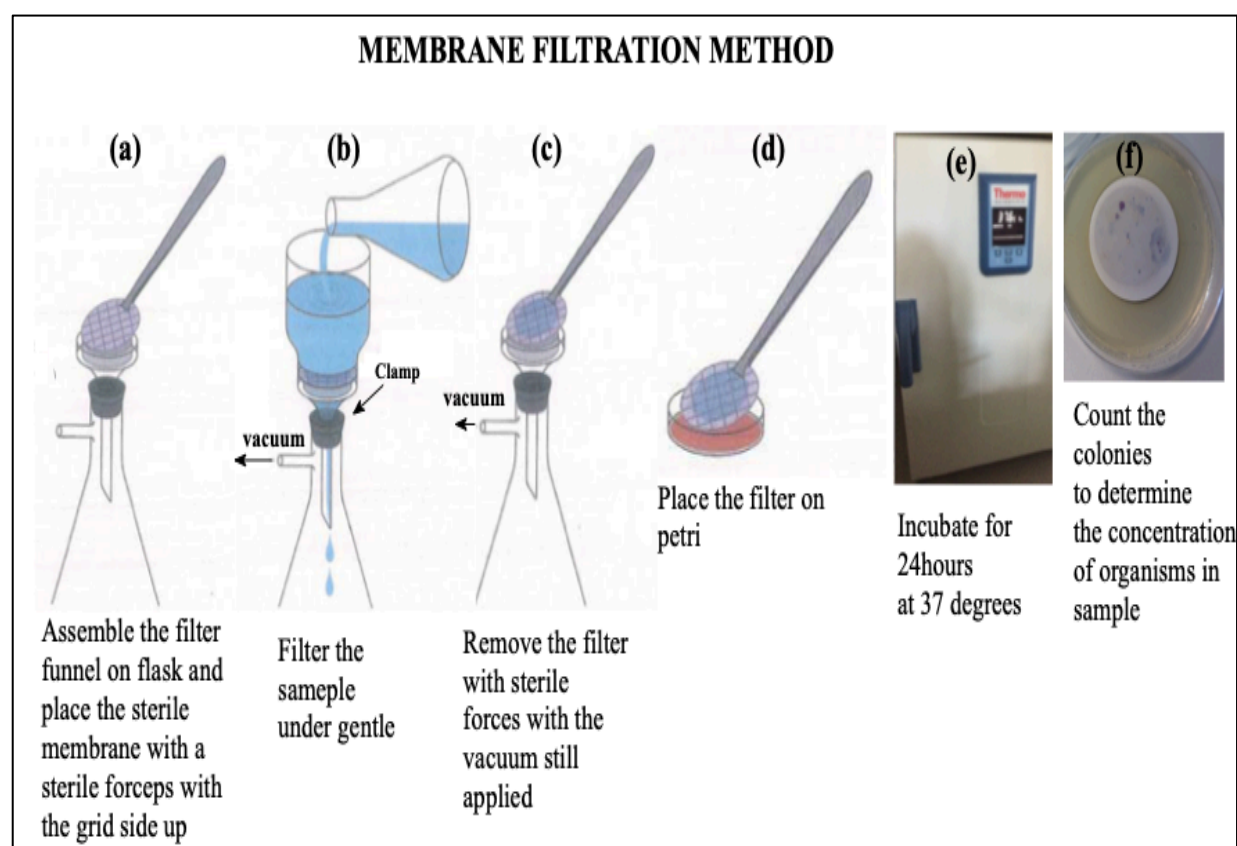
Figure 18 - Membrane filtration method

3.1.4 Equipment and Supplies

A brief list of equipment and supplies that were used while performing different steps of membrane filtration method is presented in Table 9. However, the list of equipment and supplies may vary based on the laboratory set-up and the type of test performed.

Table 9 - List of equipment/supplies needed during each process

Process Step	Equipment/Supplies used for Membrane Filtration Method
Sterilization	- Autoclave (121°C in 15 minutes) - Dip in ethanol and ignite - Spray with ethanol (% dip was 95% and 70% for spray) - UV disinfection unit set for 22 min to sterilize plastic petri dish - Aluminum Foils for wrapping all the equipment
Agar Preparation	- Weight scale, spatula, small plates for measuring - Flasks, borosilicate glass, screw-cap, 250- to 2000-mL volume, - Magnet stirrer for mixing - Vortex for mixing - m-Tec agar powder for *E. coli* and Coliforms
Plate preparation for membrane	- Petri dishes, sterile, plastic, 9 x 50 mm, with tight-fitting lids - Labels and markers for identifying the sample and location - Parafilm for air tight sealing of the agar poured on the plate
Sample collection and storing	- Sterile sample containers - Cool pack and insulated bag for storing while transport - Refrigerator for storing at 4°C both agar plates and samples
Dilution	- Pipet container of stainless steel, aluminum, or Pyrex glass - Graduated beakers, flasks covered with aluminum foil paper and sterilized. - Sterile bacteriological pipets plastic (1mL and 10 mL volumes) - Dilution water: Sterile phosphate-buffered dilution water, prepared in large beakers
Membrane Filtration	- Vacuum filter flask, with appropriate tubing - Filter manifolds to hold several filter bases - Safety trap flask placed between the filter flask and the vacuum source - Forceps with smooth tips to permit easy handling of filters without damage - Glass membrane filtration unit wrapped with aluminum foil and sterilized - Membrane filters, sterile, white, grid marked, 47 mm diameter, with 0.45 μm pore size
Incubation	Incubator set at 37°C for 24 hours

3.1.5 Membrane Filtration Method - Stepwise Procedure

The method can be performed in about nine main steps which are described below;

Step 1. Sterilization
Four options can be used to sterilize the required lab equipment. They were Autoclave, heat in oven, dip in ethanol (95%) and then lit on fire, or spray with ethanol (70%). In order to avoid contamination and for increasing the safety plastic disposable gloves and plastic protective

glasses were used during the experiment. The required equipment was wrapped in aluminium foil and then placed it in the autoclave for 15 minutes in 121°C in. Sterilizing the forceps was performed dipping in ethanol (95%) and ignite for sterilizing the same. In addition to this, UV disinfection unit was used to disinfect the petri dishes for a duration of 22 min. Lastly, before carrying out the activity, spray with ethanol (70%) was used for cleaning the surface.

Step 2-Agar preparation
To identify the presence of fecal indicators, two different agars were used: M-TEC ChromoSelect Agar was used for the detection and enumeration of *E. coli* whereas Coliform ChromoSelect Agar was used for detection and enumeration of Total Coliform. First step was to measure the amount of lb. agar powder. Based on product information direction, it takes 45.6 gram of powder to make one-liter gel mix for *E. coli* and 27 grams for total coliforms. Since, the quantity needed was not the same every time, the amount of powder demanded (in grams) to prepare the agar, was based on the number of petri dishes required. Then, the required agar and the Milli-Q water was added in a 500 ml bottle and swirl to form a colloid. Afterwards, the agars were sterilized by autoclaving at 121°C for 15 minutes. In order to prevent cracking during the sterilization process, it was of great importance to ensure that the agar mix flask was not screwed tightly. To avoid confusion, every bottle was labeled stating its content. The agar was cooled down to 40-50°C before being poured into the petri dishes.

Step 3 - Petri dish plating of agar
The autoclaved agar was dispensed into UV sterilized 9x50-mm petri dishes (around 5 mL/plate) and to make airtight sealing parafilm was used around the plate. The glass flask had a plastic ring to prevent dripping when pouring agar into petri dishes. The sealed petri dishes were stored in refrigerator at 4˚C until next time use.

Step 4 - Sample collection
A total of sixteen water samples were collected from different locations as shown in map and section 3.3 whenever distribution system pipelines were exposed for repairs or construction. Sampling were taken in 200 ml sterilized containers. Also, at the site, protective gloves were provided to the person in charge, to protect the bottle from contamination. Most of the water samples were collected directly from water within excavation pit except the first location (sample id.2).

Step 5 - Transportation and holding time
The collected water samples were carried in an insulated container to ensure proper maintenance of storage temperature. It is also suggested to ice or refrigerate water samples at a temperature of 1-4°C during transit to the laboratory (USEPA, 2002). Freezing of the samples was not advisable. Therefore, sample analysis was performed within 2 hours of collection. The holding time between sample collection and analysis were around 3 hours. However, it can vary from 6 hours to 30 hours in non-potable water for fecal indicators (USEPA, 2013)

Step 6: Dilution series
According to USEPA (2014), it is recommended that a minimum of three dilutions be analyzed to ensure that a countable plate (20-80 *E. coli* colonies) is obtained. In this study, the dilution level was decided based on the turbidity level of the water sample. The dilution for both *E. coli* and Total coliforms were performed separately. The dilution series were done up to five times depending on the turbidity of the water samples. The first dilution (10^{-1}), was performed by mixing 90 mL of sterile buffered dilution water and 10 ml sample was added by using a pipette in a 250ml glass container. Before and after the sample was added, every glass container was fully mixed. Similarly, the required water sample volume was calculated and added to the sterile water in order to do the next dilutions for 1:100 and 1:1000, whenever required.

Step 7 - Membrane Filtration
First, a sterile membrane filter was placed on the filter base, grid side up, and the funnel was attached to the base so that the membrane filter was held between the funnel and the base. Then the funnel top was removed. Using forceps (sterilized by dipping in ethanol (95%) and then lit on fire) to place the sterile membrane filter in place with the grid side up. The funnel top was carefully placed to ensure not to tear the filter. The complete assembly was held together by using clamp. Then 50 ml of sterile distilled water was poured into funnel and later the sample was poured into the funnel. The sample was allowed to pass through the funnel by applying vacuum gently. Just as the liquid level approached the filter, the sides were rinsed with a small amount of the sterile distilled water, and vacuum was continued to draw all the water through the filter. After the complete sample passed through, the funnel top was removed with the vacuum still applied. Then the filter was removed by use of sterile forceps and carefully placed onto the agar plates. Further, during the experiment checks were made to see if there is any formation of bubbles between the membrane and the agar surface. If bubbles were formed, then the membrane had to be again placed properly on the plates. At that point, the forceps were used to ensure that the filter was properly seated on the agar. The same described procedure was repeated to complete for all the dilution samples.

Step 8 – Incubation
The plates were then placed in incubator for at least 24 hours preset at 37°C. The colonies were counted on the following day in the lab.

Step 9 - Enumerating E. coli and Coliforms
After incubation as stated above, the plates were taken out and checked for contamination. The number of red or magenta colonies were counted and recorded. During the enumeration in order to quicken the process and obtaining more accurate results, pencil mark was used to emphasize each colony.

Step 10 - Calculation of result
The membrane filter with the number of colonies in the acceptable range was selected and calculated based on the following formula:

$$\frac{\boldsymbol{Count}}{\boldsymbol{100ml}} = \left(\frac{Nr.\,of\,colonies\,counted}{Volume\,of\,sample\,filtered\,(ml)}\right) * 100$$

3.1.6 Method Limitations

- Identification may be inaccurate for turbid samples as it can clog the membrane and prevent filtration or cause spreading of colonies
- Quality of the filter can influence the results
- Loss of viability of heat-sensitive organisms coming into contact with hot agar.
- It is not always easy to score the typical colonies
- High scope of human errors (ex. visual count, procedure performance etc.)

The Figure 19 below depicts the steps of membrane filtration method performed in the Chalmers lab.
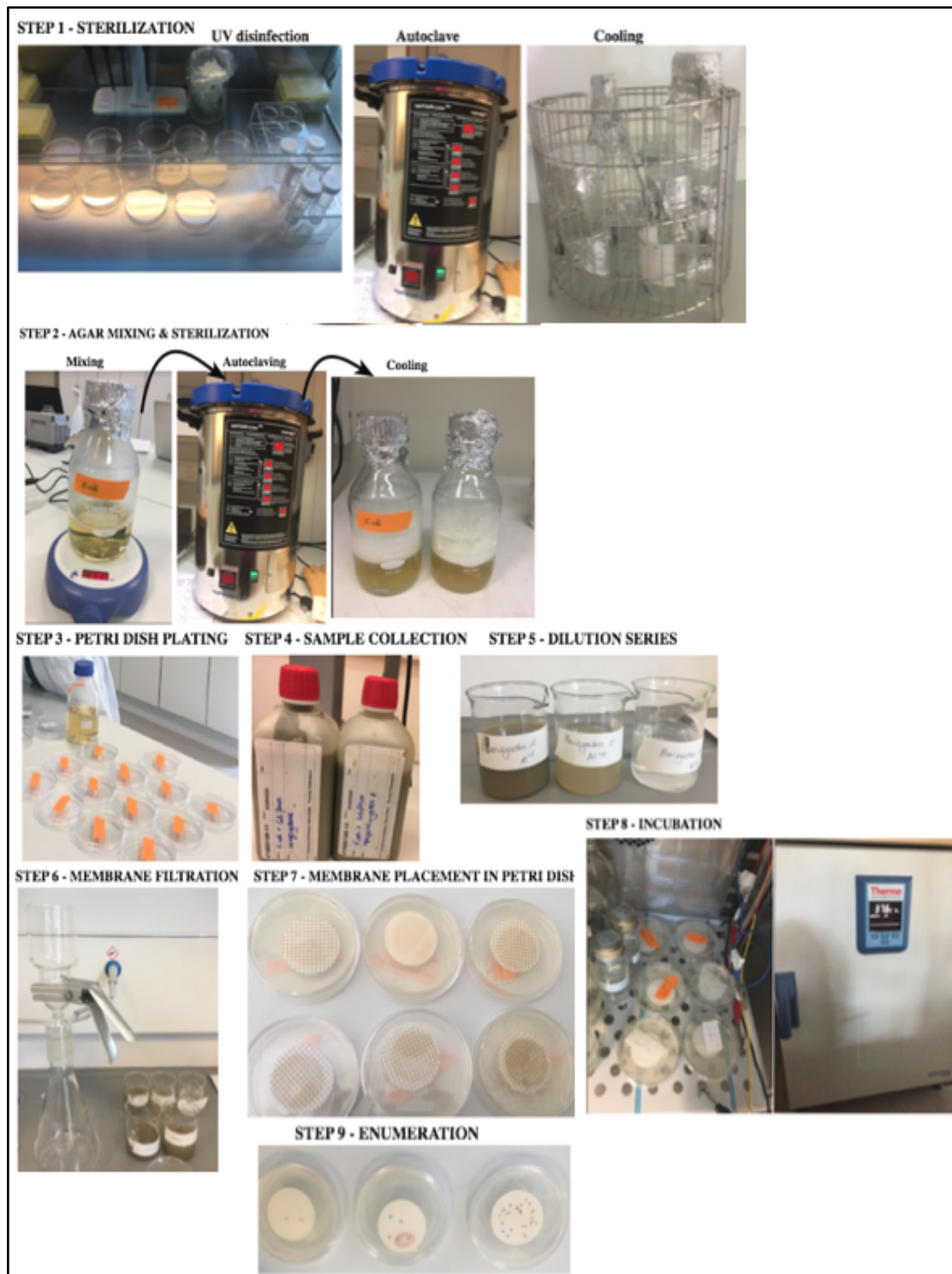
Figure 19 - Membrane filtration method steps performed in Chalmers Laboratory

3.2. Microbial Risk Assessment

3.2.1 Conceptual risk model for risk evaluation and assessment

In this section of the report, the method was developed by creating a conceptual risk model to use the dose response relationship of QMRA module.

The conceptual risk model was developed based on several literature reviews described in previous sections of this report. However, the literature published by Yang et al. (2015) was primarily focused for the prediction of annual infection risk levels. Accordingly, a similar concept but much simplified version of Yang et al. (2015), was adapted to build-up the risk model for this study, where in the annual infection risk to the first consumer was evaluated by use of QMRA tool. Yang et al. (2015) used a hydraulic model to simulate the transport from intrusion to the tap, this was not performed in this study. The developed risk model is presented in the result section. This proposed risk model comprises of contamination sources (wild animals, pet animals, wastewater pipe, septic tank runoff etc.) and its pathway (i.e. from ground surface to subsoil and then further to drinking water pipe) to first consumer who might drink the water immediately after repair. Many assumptions were made to develop and test this risk model in QMRA. These assumptions have been categorically listed down below.

3.2.2 Assumptions

Some key assumptions used to develop the risk model were:
- A zero risk on the normal days when there was no pipe repair/maintenance work because the pipes were not cut opened for any reason other than the repair/maintenance work in all the sampling locations.
- Biofilm was assumed to have no effect on microbial concentration entering after the repair/replacement work
- No soil particles entered the pipe was assumed in order to eliminate the effect of soil texture/size on concentration of microbes
- Raw sewage was assumed to be the fecal contamination source, due to the small distance (less than 46 cm) noticed between the drinking water pipe and wastewater pipe in most of the sample collection locations.
- Only the first consumer downstream of a pipe repair/maintenance would ingest 0.746 liters per day (as per Swedish guideline) of contaminated water immediately after the completion of pipe repair/maintenance work at the same time when the contaminant is passing through (also can imply - the volume (i.e. of 0.746 L/day) is ingested only one time during the day instead of multiple times). The basis for this assumption was to eliminate the further transport and dilution effects (flushing, disinfection etc.) on microbial concentration in the pipe which otherwise would need additional data (pressure, residual disinfection etc.) and computer transport modelling to aid the risk analysis
- The contaminated water would be consumed by the first consumer for only one day since most of the pipe repair works were completed within 24 hours.
- Only one break per year in the same vicinity or location was assumed in order to eliminate effects of recurrence of the pipe break event. In addition, assumed no other contamination upstream
- The leakage location of the pipe is assumed to be somewhere in the middle but not at the end because the pressure surges can influence the intrusion rate and volume
- A very small package volume of soil water (about 10 milliliters, i.e. around 1% of the total volume in a standard drinking water pipe repair length) was assumed to be entering the drinking water pipe at every main break/repair event. This is because the entry of

more than 1% of volume of soil water would need enormous quantity of soil water to intrude and it is very unlikely to happen during a routine repair/maintenance work due cautious working protocol followed by the municipality.

- The duration of intrusion and the distance travelled is however ignored in this study
- The pipe dimensions were assumed to have diameter of 0.15 meter (based on unpublished data) with a length of 2 meters

3.2.3 Quantitative Microbial Risk Assessment (QMRA)

The dose–response relationship for infection model of QMRA was used to estimate the risk levels. This model is suitable for this study because it simulates every ingested pathogen to act independently to cause an individual probability of causing infection (WHO, 2016). To implement this model, the steps shown in Figure 20 below were followed.

QMRA STEPS

- *Find the dilution factor - (E.coli / litrature values of pathogens)*
- *Estimate initial pathogen concentration based on dilution factor*
- *Assume small package volume of contaminant intrusion during pipe repair work*
- *Multiply initial concentration of pathogens with assumed intrusion volume to find intruding pathogen concentration*
- *Assume pipe dimensions(dia and length) and add intrusion volume to find the true volume*
- *Divide true volume by intruding pathogen concentration to find actual intruding pathogen concentration*
- *Input estimated concentration of pathogens as point estimate*
- *Note the output as probability of daily infection risk*
- *Find the probability of annual infection risk by use of formula*
- *Compare the probability of annual infection risk with target risk level of 1 person in 10 000 people to assess the risk levels*
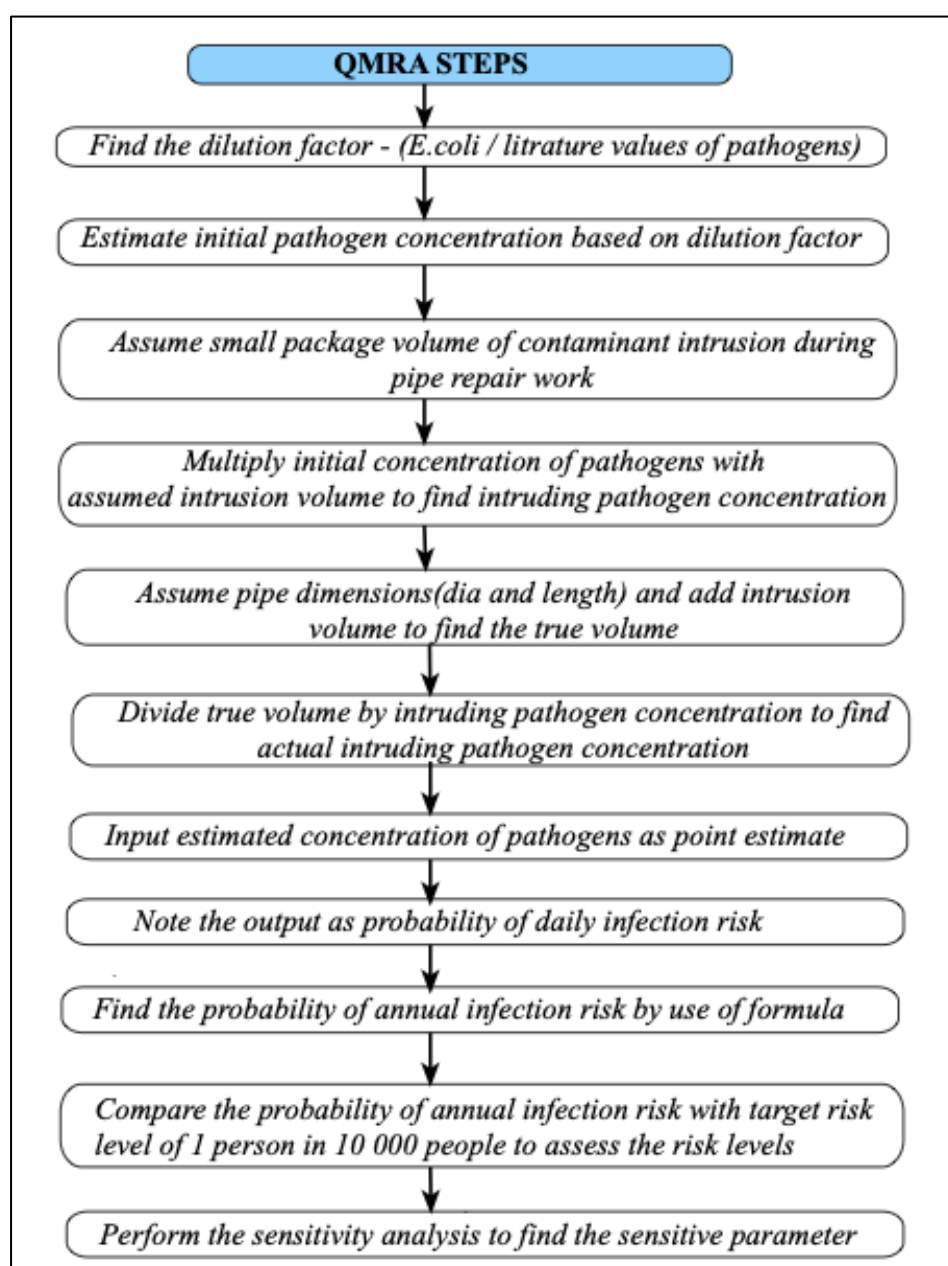- *Perform the sensitivity analysis to find the sensitive parameter*

Figure 20- QMRA steps followed for risk analysis

At first the three reference pathogens (*Campylobacter*, *Cryptosporidium* and norovirus) were chosen for estimating the potential health risks associated with this repair/maintenance event in the drinking water pipe. These pathogens were chosen for two reasons: first, because they have been previously recognized as the cause of many waterborne disease outbreaks associated with the drinking water distribution system in Sweden and also elsewhere. Secondly, these pathogens are seen to survive longer in colder waters and also are very resistant to chlorination.

The annual probability of infection risk was evaluated in the raw water model of QMRA by using Analytica 6 (Lumina Decision System, USA) software. Point estimates were used in the raw water module of the QMRA tool, due to its relative ease of use and simplified risk assessment output. As a first step in this approach, a small package volume of 10 milliliters of soil water was assumed to have entered the drinking water pipe after the repair/maintenance work. Further on, the annual risk levels based on the dilution effects on the intruded pathogen concentration were analyzed in three different scenarios in relation to low, high and too numerous to count (tntc) counts of *E. coli* detected in the membrane filtration method. The low, high and tntc counts of *E. coli* can be referred in Table 11.

Mean concentration values of pathogens (i.e. *Campylobacter*, *Cryptosporidium* and norovirus) in raw sewage (domestic) per 100 milliliters were extracted from the reference literature (Tchobanoglous, G. et al., 2003). These concentrations are now on referred as 'literature values' in this report and these values are presented in the Appendices. Then, the three different dilution factors were calculated for lowest, highest and tntc values of *E. coli* counts detected in water samples, wherein the detected *E. coli* count (cfu /100 milliliters) was divided by the literature values (number per 100 milliliters) of raw sewage. Subsequently, the three different dilution factors obtained for low, high and tntc values were multiplied by the literature values (taken for per liter) of raw sewage to find their respective diluted pathogen concentration. Then each diluted pathogen concentration was further multiplied by assumed intrusion package volume of 10 milliliters in order to estimate the probable pathogen concentration (number per liter) entering the pipe. Lastly, the three different (low, high, tntc) probable pathogen concentrations (number per liter) entering the pipe were divided by the true pipe volume (assumed pipe length of 2 meters with the radius of 0.15 meter plus the intrusion volume) to find the total concentration of pathogens (number per liter) actually present inside the drinking water pipe. The total pathogen concentration in pipe so calculated with regard to three dilution factors were further used as input values in QMRA. Three scenarios were identified to be tested in the raw water model in QMRA and they are described below:

Scenario A - The package volume of 10 milliliters of soil water entering the drinking water pipe for lowest dilution factor of 0.0000006 (implies 0.00006% raw sewage in the package) calculated based on the lowest *E. coli* count (i.e. 6 cfu / 100 milliliters) found in water samples.

Scenario B - The package volume of 10 milliliters of soil water entering the drinking water pipe, with a dilution factor of about 0.024 (implies 2.4% of raw sewage in the package) calculated based on the highest *E. coli* (i.e. 240000 cfu/100 milliliters) count in water sample.

Scenario C - The package of 10 milliliters of soil water entering the drinking water pipe, with the dilution factor of 1 (implies 100% of raw sewage in the package) calculated based on very large number of *E. coli* (termed as 'tntc' in microbial contamination results).

3.2.4 Calculations

The data and formulas used to calculate the input values for QMRA are described in the Table 10 below.

Table 10-Calculations to find pathogen concentration inside the drinking water pipe

Steps	Description	Scenario A	Scenario B	Scenario C
	CALCULATION TO FIND PATHOGEN CONCENTRATION INSIDE DRINKING WATER PIPE			
a	Sampling	6	240000	10000000
	Literature value of *E. coli*	10000000	10000000	10000000
	Dilution factor = Sampling / Literature value of *E. coli*	0.0000006	0.024	1
b	**Literature value of pathogen concentration**	#/L	#/L	#/L
	Campylobacter	500000	500000	500000
	Cryptosporidium	13510	13510	13510
	norovirus	10000000	10000000	10000000
c	**Diluted pathogen concentration = dilution factor * literature value of pathogen concentration per Liter**			
	Campylobacter	0.3	12000	500000
	Cryptosporidium	0.008106	324.24	13510
	Norovirus	6	240000	10000000
d	Small Package volume (assumed 10mililiter)	0.01	0.01	0.01
e	**Pathogen concentration in small package volume = diluted pathogen concentration * small package volume**			
	Campylobacter	0.003	120	5000
	Cryptosporidium	0.00008106	3.2424	135.1
	Norovirus	0.06	2400	100000
f	Total pipe volume (assumed 0.15m diameter, 2m length of pipe) (pipe volume + intrusion volume)	35.01	35.01	35.01
g	**Pathogen concentration inside drinking water (input values to QMRA) = Pathogen concentration in small package volume / Total Pipe volume**			
	Campylobacter	0.00008568980291	3.427592117	142.8163382
	Cryptosporidium	0.000002315338475	0.09261353899	3.858897458
	Norovirus	0.001713796058	68.55184233	2856.326764

3.2.5 Input to QMRA

The input values of pathogen concentration derived from the above calculations are presented in step 'g' of the above Table 10. However, these calculations were based on the assumptions as listed above. As can be seen from the Table 10, the pathogen concentration gradually increases as one moves from scenario A to C. This is because, in scenario C, 100% raw sewage (10 milliliters) is assumed to intrude the drinking water pipe without any dilution effect on pathogen concentrations. Therefore, scenario C could also be seen as the 'worst case scenario', as it binds four prerequisite elements (i.e. presence of hazard, a pathway, exposure and infection) to create human infection risk.

3.2.6 Probability of Annual Infection Risk

The mean value of probability of daily infection risk for each of the pathogen got from QMRA result was further used to compare three scenarios with the recommended regulatory risk levels of 1×10^{-4} persons per year as set by WHO. The annual probability of human infection risk was calculated by inserting the mean value of probability of daily infection risk for each pathogen (*Campylobacter*, *Cryptosporidium* and norovirus) in the below formulae;

$$P_{Annual} = 1 - \left(\left(1 - P_{inf,normal}\right)^{t\,normal} \left(1 - P_{inf,bad\_rawwater}\right)^{t\,bad\_rawwater} \left(1 - P_{inf,suboptimal}\right)^{t\,suboptimal} \right)$$

The below values were assumed and inserted into the above formula to calculate the probability of annual infection risk;

P_{Annual} = annual probability of infection,
$P_{inf,\,normal}$ = daily probability of infection during normal days = 0 (assumed),
$P_{inf,\,bad\_rawwater}$ = daily probability of infection during bad raw water quality days = 0,
$P_{inf,\,suboptimal}$ = daily probability of infection during sub-optimal days = 0 (no treatment received),
t normal = number of normal days = 364 days per year (assumed no repair work for 364 days),
t bad_rawwater = number of bad raw quality day = 1 day per year (most repair works were finished in 24 hours),
t suboptimal = number of suboptimal days = 0 day per year (as this water does not pass through the drinking water treatment plant)

3.2.7 QMRA Limitations

Limited availability of data on pathogen occurrence, fate and its transport form the main drawback of QMRA, as it forces one to make many conservative assumptions (for ex. neglecting biofilm growth effects on concentration, neglecting disinfection or flushing effects, no soil particle intrusion etc.) to simplify the situation when developing the conceptual risk models. Typically, the risk levels estimated on basis of conservative assumptions relate more to a worst-case scenario than a real scenario. As a result, the risk levels often get overestimated and makes it difficult for one to clearly understand the levels of uncertainties while making the decision for the risk mitigation. More importantly, the point estimate when used in QMRA could by itself be the major drawback as it lacks important information on potential degree of uncertainty in the estimated risk levels. Another drawback would be the need of a technical expert for using the QMRA tool as it demands more data, time and in-depth technical knowledge on the subject.

4 Results and Discussions

4.1 Case study, sampling and filtration

4.1.1 Membrane Filtration results

The concentrations of microorganisms detected in water samples from membrane filtration method is shown in Table 11 below.

Table 11 - Results of E. coli and Coliforms got from Membrane Filtration Method

Sample Location in Gothenburg city	Sample Id.no.	*E. coli* CFU/100ml		Total coliform CFU/100ml	
		Chalmers	Lackerebäck	Chalmers	Lackerebäck
Helgeredsvägen 20	2	N/A	<0	N/A	12
Rangeltorpsgatan 35	**3/4**	N/A	10	N/A	8200
Grönstensvägen 13	*01/05*	*N/A*	*<10*	*N/A*	*34000*
Kummingatan 12	00/06	6	<10	270	440
Runebersgatan 2	7	10	<10	80	<10
Mariagatan 11	8	100000	>240000	tntc*	22000
Nordgårdsgatan 4	9	tntc*	18000	tntc*	100
Uppegårdsvägen 9	*10*	*37000*	*300*	*64000*	*34000*
Hisingsgatan 2	11	20	<10	tntc*	2900
Manufakturgatan 11	12	tntc*	100	tntc*	7000
Halvorsäng	16	10	6	tntc*	16000
Sankt Sigfridsgatan 65	15	100	<100	<2000	<100
Erik Dahlbergsgatan 44	14	NA	120	NA	4600
Södra Vägen 73	13	tntc*	100	tntc*	240000
Kronotorpsgatan 1	17	NA	<100	NA	9900
Kungsportsavenyn 1	*18*	*600*	*<100*	*3000*	*6000*

Note
- If the counts were more than 200 or confluent(full spread) then it is recorded as tntc* (too numerous to count) according to USEPA (APHA, AWWA, & WEF, 2006). An example of tntc* can be seen in sample id.8 & 12 in Appendix 2,
- NA (not applicable) - these samples were not tested in Chalmers Laboratory due to technical reasons
- Italic values (in blue color) are duplicate samples, where one mixed sample was taken in the excavation pit, and then divided into two sample bottles. One bottle was sent do the Lackarebäck laboratory and the other one was analyzed at the Chalmers laboratory. All others were collected in two different bottles

Total 16 water samples were tested to examine the fecal contamination outside the drinking water pipe. The water samples were collected from within the repair pit during the planned pipe repair/maintenance work. The results obtained from both Chalmers and Lackerebäck laboratory were consisted and showed the presence of *E. coli* and coliforms in 15 out of 16. Results were found to vary in a wide range for both *E. coli* and coliforms across different geographical locations. As in the count ranged between <0 to >240000 CFU/100mL for *E. coli* and <10 to >240000 CFU/100mL coliform respectively. The images of the *E. coli* and coliforms detected in membrane filtration method for specific sample id. can be found in Appendix 2.

4.1.2 Discussion & Recommendations

In 15 out of 16 water samples tested proved the presence of fecal contamination by means of viable count of fecal indicator organism i.e. *E. coli* and coliforms. Only one sample collected from location (i.e. Helgeredsvägen 20) did not show the presence of *E. coli* and coliforms, and this was probably because the water sample in this location was collected directly from the drinking water pipe (i.e. water leaking out of the drinking water pipe) instead of collecting the sample from within the repair pit. However, if the water sample in this location was collected from the pit, then there was a high likelihood of getting the higher level of pathogen count here, because the water was already leaking (from about 4-centimeter hole in the pipe) and mixing with the soil for more than one hour until the leaking point was correctly traced.

To add on, the results indicate that the coliforms always occurred in higher levels in all of the locations (except in sample id 8 & 9) of the city when compared with *E. coli* count. The values between *E. coli* and Coliforms do not seem to be strongly correlated, because, coliforms were seen to be present at higher levels like 16 000 cfu/100ml even when *E. coli* was at low detectable count of 6 cfu/100 ml (ex.in sample id 16). Despite this, the coliforms are not recommended to be used as fecal indicator for water quality test unlike *E. coli,* because there are many environmental coliforms (ex. genus Serratia are found in soil, Enterobacter widely spread in nature) which are not of fecal origin(Stevens et al., 2003). Nevertheless, the coliform counts prove its occurrence in nature or environment.

Further on, the count of FIO widely varied from <0 - >240000 cfu/100 mL between 16 sampling locations of Gothenburg and this count is very much lower than the fecal contamination count previously reported by Karim.et.al (2003) in a study performed in United States). Obviously, the results will be different for different geographical locations because of different influencing factors like temperature, rainfall, soil type, land use, sewage infrastructure or drinking water supply system etc. that aid the microbial movement and the difference in microbial sources present for each system. Further on, the results in this case may also imply that the soil in all the sampling locations is microbially contaminated and this could be asserted only if the soil samples were also examined in the laboratory, however, the soil samples were not examined in this study. Therefore, further investigation of soil samples in different geographical locations of the city is recommended in order to judge the extent of microbial contamination of soil. Investigation of soil samples can potentially influence the final results and will immensely help one to get a holistic view of the problem.

Interestingly, the highest detected count of *E. coli (*i.e. up to 2.4×10^5 cfu/100 mL) in one of sampling location seems to be almost close to previously predicted literature values of *E. coli* range in the raw sewage (i.e.10^4 - 10^7 cfu/100mL).So, hypothetically, the presence or occurrence of fecal indicator organisms in all the locations (irrespective of being low, high or tntc), can be linked to a leakage (both past or present) from a raw sewage pipe present in the close vicinity of the drinking water pipe repair location. At the same time, the highest count of *E. coli* seen only in one location, can also mean that the raw sewage pipe leak could be undetected and may still be actively leaking somewhere underground in this particular location.

More importantly, in such a scenario where raw sewage pipe leak is presumed as the main contamination source in the vicinity of the drinking water pipe location in subsoil, then it is obvious that the microbial transport through the soil surface is aided by flowing sewage water. In addition to this, the microbial transport appears to be also favored by the silty or moist clay (Santamaría & Toranzos, 2003), which was present in all the sample locations. The moisture content is much higher in silty or moist clay when compared to coarse grained soil. As such the higher moisture content would have further resulted in increased movement or survival of the

microbes through soil surface media and hence the increase in viable count in few locations (USEPA, 2012). Apart from this the rainfall also can help the pathogen transport but there was no rain during sample collection in any of the sample locations (except in sample id 2- in which sample was collected from wrong place).

Another reason for raw sewage to be seen as a dominant contamination source in this case, is the existing separation distance between the wastewater pipe and the drinking water pipe (Karim et al., 2003). The separation distance maintained in the city seems to be very small and was witnessed to be less than 2 meters (or sometimes even less than a recommended standard of minimum of 46 centimeters) in most of the sampling locations in Gothenburg. In addition to this, both the wastewater pipe and drinking water pipe being located in the same pit in over 80% of the distribution area in the city (Melle Säve-Söderbergh et al., 2013). Thus, there is a very high likelihood that even a negligible amount of leak from the raw sewage pipe can potentially pose a high threat of contamination if intruded into the drinking water pipe during the repair or maintenance work. This can even be more severe if the leak goes undetected for long time because the microbes can survive for up to 200 days in soil media (Rosen, 2000) and so will always have the probability of intruding the drinking water pipe during pipe repair work. However, the extent of microbial intrusion into the pipe depends on factors like the frequency of repairs, nature of repair, location of repair and duration of repair (USEPA, 2002). It is recommended to review the design practices to ensure reasonable safe separation distance between wastewater and drinking water pipe to prevent unforeseen hazards.

On the other hand, contaminated groundwater can also be a potential source of fecal contamination particularly when the drinking water pipes are laid very close above or below the groundwater table. In Gothenburg city of Sweden, about 70% of the municipalities have said to be having the pipes laid below groundwater level during a recent survey and hence there exists an improved probability of microbial intrusion into pipes (Melle Säve-Söderbergh et al., 2013). To add on, there are contamination sources (ex. septic tanks) which can allow the microbes to easily bypass the soil zone, especially in saturated soil conditions and cause further infiltration or leaching into groundwater table. Subsequently, this contaminated groundwater can transport the microbes to long distances in a short span of time and create a strong possibility of contamination intrusion to drinking water pipe. Sometimes this contaminated groundwater along with the urban runoff can increase during high rainfall event and can rush into the repair pit. This event further complicates the possibility to follow the set protocol during pipe repair work because it is often challenging to control the entry of amount of water into the repair area. Furthermore, the different levels of occurrence of microorganism could also partly be associated to the unhygienic working conditions or equipment or practices (especially when reused several times on the same day, one example being the shoes of the working personnel).

Lastly, the results between two different labs (in two different methods) were found to vary. This could be attributed to human errors caused while performing laboratory or sampling procedures or could be also linked to intrinsic characteristics of the microbes in the water media. For instance, when the water sample was collected in two separate sterile bottles then there could be possibility that the water sample were collected from different places (though nearby) that could have resulted in different levels of microbes due to their varying occurrence levels in nature. On the other hand, the results of three locations (marked in italics in blue color in Table 11 above), wherein the samples (id.5, 10 and id.18) were collected in only one sterile bottle instead of collecting in two separate sterile bottles (separately for two laboratories). Theoretically, the results of these specific locations should be the same in two labs but were found to be consistently more in the Chalmers lab than Lackerebäck lab. The reason for this variability could be inadequate mixing of sample where the top clear layer of sample may have been given to Lackerebäck and the bottom layer which may have contained more bacteria (due to its adsorption

and settling properties with the soil particles) was tested in Chalmers. Apart from this, there could be human errors while performing the laboratory procedures which could have caused the variation in results.

Furthermore, one could also associate the errors in results to two different methods used for enumeration of *E. coli* and coliforms. However a comparison study performed in Southern Sweden (Eckner, 1998), which tested about 261 drinking and 77 bathing water samples, mentioned that the Colilert method results were comparable to the reference Swedish multiple-tube fermentation and membrane filtration methods that are used to enumerate the coliforms and *E. coli* in drinking water. Hence, it is unlikely to have significant variations or errors in the viable counts of *E. coli* and coliforms due to difference in methods used in two laboratories for this study.

Although raw sewage pipe and contaminated ground water have been emphasized to be the potential source, it must be noted that the fecal contamination can be derived from various sources listed previously and hence identifying the exact source is challenging which further complicates the human infection risk assessment. As such microbial source tracking systems needs to be efficiently developed and managed in addition to risk assessment procedures.

4.2 Microbial risk assessment

4.2.1 Conceptual model for risk evaluation and assessment

The visual representation of the risk hypothesis used for the study is presented in the Figure 21 below. The conceptual risk model clearly indicates the pathway from source of contamination to the ingestion by the consumer.

The risk model presented below describe the potential fecal contamination sources and their pathways leading to microbial intrusion to the drinking water pipe. The contamination sources are assumed to be the raw sewage (or wastewater) based on the discussion presented in membrane filtration method. Furthermore, a small package volume of contamination, consisting partly of raw sewage (leakage from a wastewater pipe), is assumed to have entered the drinking water pipe immediately after the pipe repair/maintenance work. This assumed small package volume of contamination is further assumed to have travelled in two pathways to reach the destination of drinking water pipe. First, the surface-subsoil pathway and the second subsoil-drinking water pipe pathway as shown in the model (red arrows in Figure 21). In the first pathway, the microbial intrusion is assumed to be from a leaking wastewater pipe which may be present in the vicinity of the drinking water pipe and in the second pathway, the intruded contamination is assumed to enter the drinking water pipe through the pipe repair events during the planned maintenance/repair works. Both the pathways are required to exist for microbial intrusion to occur during the pipe repair works.

The model represents the existence of three conditions that can cause an infection risk, namely, one a pre-existing fecal contamination source, second the microbial survival and transport and the thirdly a pathway including the mechanism (ex; pressure surges) that allows the entry of pathogen into the pipe.
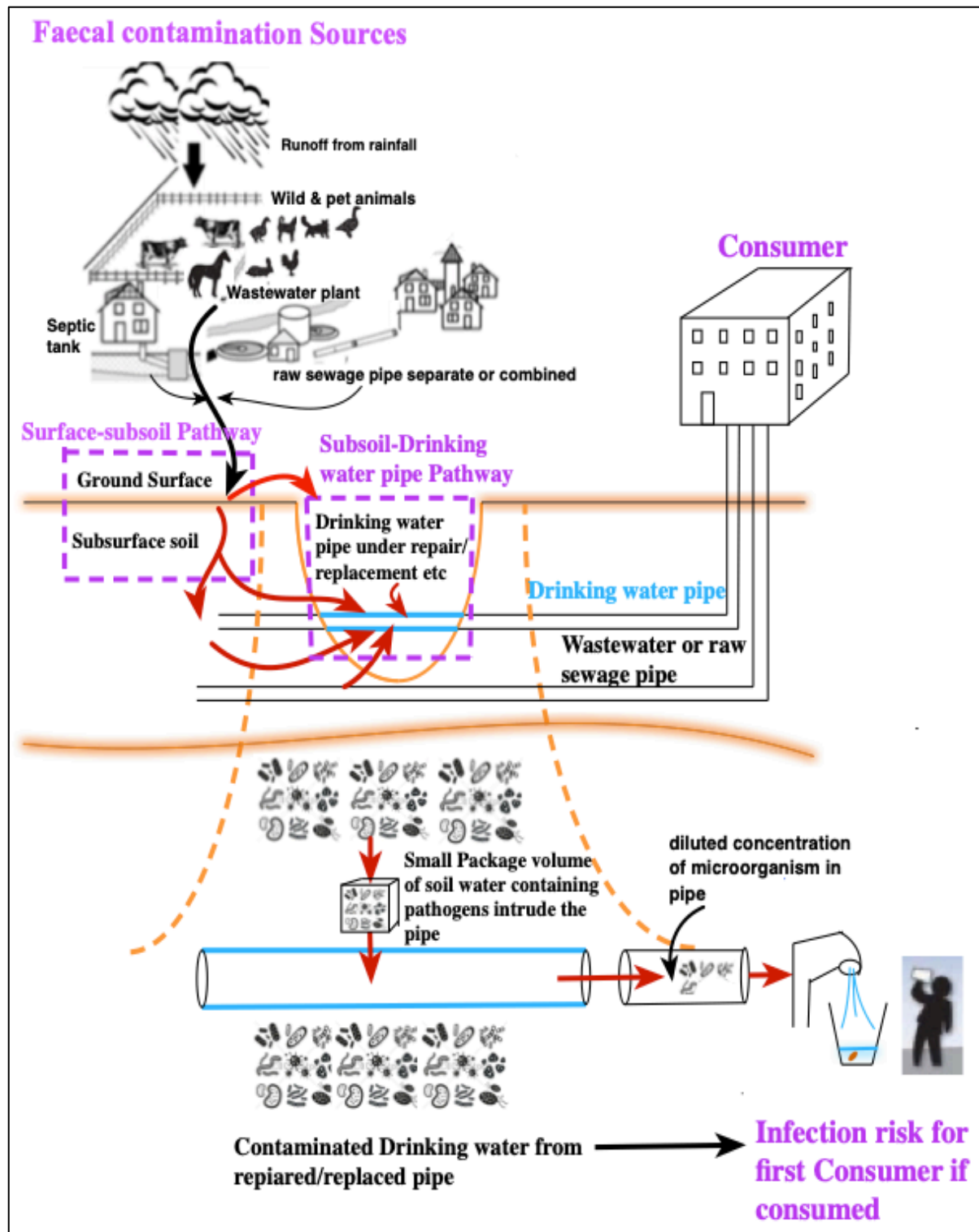
Figure 21- Conceptual risk model

Further on, it was assumed that the first consumer downstream a pipe repair work may ingest the contaminated water, immediately after the pipe maintenance or repair work. Accordingly, the annual infection risk was calculated only in relation to the first consumer downstream to finally compare with the annual regulatory risk limits. As in, a threshold limit of 1×10^{-4} was used to compare the risk levels in relation to first consumer and the USEPA annual regulatory limits of 1 in 10 000 persons per year was used to compare the results if all 10 000 people ingested same pathogen dose.

4.2.2 Annual probability of infection

The daily probability of infection risk was calculated in QMRA and were further used to find the probability of annual infection risk for each pathogen. As can be seen from Table 12 the annual probability of infection risk level is higher for all the pathogens in Scenario C when compared to Scenario A and Scenario B. The trend of significant increase in annual probability of risk levels can be noticed as one goes from Scenario A to Scenario C. However, the annual probability of infections especially for *Cryptosporidium* appears to remain consistently lower than the other two pathogens in all three scenarios. The annual probability of infection risk estimates exceeded the threshold USEPA regulatory value of 1×10^{-4} and hence indicate a high risk of infection to the first consumer.

Table 12 - Probability of Annual Infection Risk to first consumer

P_{annual}	Scenario A	Scenario B	Scenario C
Campylobacter	5×10^{-5}	0.61	0.72
Cryptosporidium	7.9×10^{-7}	0.03	0.43
Norovirus	6.3×10^{-4}	0.51	0.58

The Figure 22 below provides a clear picture of probability of annual infection risk levels exceeding the USEPA regulatory limits of 1 person getting infected in 10000 people per year.
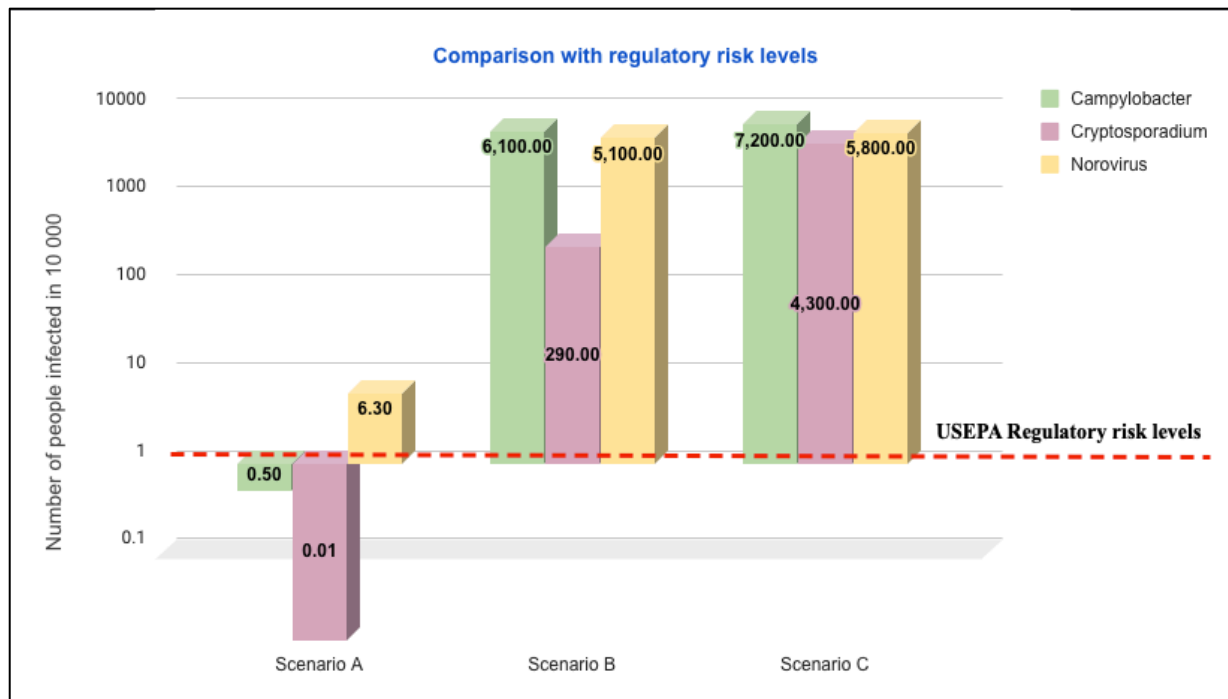
Figure 22- Comparison of probability of annual infection risk to USEPA regulatory risk levels

4.2.3 Sensitivity Analysis

Any model is incomplete without assessing the variability or uncertainties of the results. According to USEPA (2014), it is crucial to test the parameters used in the assessment process in order to clearly define the associated uncertainties which could influence the assessment in various degrees. This requirement is taken care by performing sensitivity analysis as it gives better understanding of the estimated risk levels. Incidentally, the point estimate approach used

in this study, did not provide adequate opportunities to perform the sensitivity analysis. The reason being the use of very minimal parameters (two in this case - pathogen concentration and exposure) and a much simplified overly conservative conceptual risk model. Nevertheless, the sensitivity of the probability of annual infection risk levels was performed by testing the two parameters; one the exposure volume and another by changing the pipe diameter.

Sensitivity in relation to exposure volume

In relation to exposure volume in the three Scenarios (A, B & C) were compared with the different exposure volumes. To perform this step, the two other exposure volume were locally entered in QMRA, one was entered as 0.18 L (median consumption of unheated water, (Yang et al., 2015) and another volume was set to typically used exposure volume of 1L (WHO, 2016). The outputs of these two exposure volumes were then compared with the preset exposure volume of water consumption of 0.7416 L (Westrell , 2006). The annual probability of infection risk levels for three different exposure volumes (0.18 L, 0.7416 L and 1 L) are presented in Table 13 below.

The changes made in the exposure volume parameter did make difference in the estimated risk levels however marginally varied when the exposure volume was increased from 0.7416 L to 1L. The risk levels were found to be consistently increasing with the increase in exposure volume for all the pathogens, when moving from scenario A to scenario C. However, annual infection risk of *Cryptosporidium* remained comparatively low in all scenarios. Moreover, the sensitivity analysis with respect to exposure, proved that the risk will remain at higher levels in presence of higher pathogen concentrations (ex. Scenario C, where 100% contamination entry is assumed) unlike lower pathogen concentration in Scenario A and Scenario B.

Table 13- Probability of annual infection risk in relation to Exposure volume- Sensitivity analysis

Pathogen	Exposure volume (in L) Scenario A			Exposure volume (in L) Scenario B			Exposure volume (in L) Scenario C		
	0.18	0.7416	1	0.18	0.7416	1	0.18	0.7416	1
Campylobacter	1.1E-05	5E-05	5.9E-05	0.32	0.61	0.67	0.71	0.72	0.72
Cryptosporidium	1.6E-07	7.9E-07	9.1E-07	0.007	0.03	0.035	0.21	0.43	0.47
Norovirus	1.3E-04	6.3E-04	7.2E-04	0.48	0.51	0.52	0.56	0.58	0.59

Sensitivity in relation to pipe diameter

Three different pipe diameters were tested to find its influence on the estimated risk levels. The diameters were chosen to be 0.15 meters (median value based on unpublished data), 0.208 meters (average value based on unpublished data) and lastly 0.240 meters (random). The results are shown in Table 14 below.

As can be seen in the below Table 14, the risk levels constantly increased when navigating from average diameter (i.e., 0.15 m) and the random diameter (i.e., 0.24 m). The results infer that the pipe diameter can also contribute significantly in the infection risk because the risk levels mostly reduce with the increase in diameter. However, the reduction in risk levels are dependent on the presence of pathogen concentrations. As in, higher the pathogen concentration, the higher could be the risk irrespective of control measures in exposure volume or diameter.

Table 14 - Probability of annual infection risk in relation to pipe diameter - Sensitivity analysis

Pathogen	Pipe diameter in 'meters' Scenario A			Pipe diameter in 'meters' Scenario B			Pipe diameter in 'meters' Scenario 'C'		
	0.15	0.208	0.24	0.15	0.208	0.24	0.15	0.208	0.24
Campylobacter	5E-05	2.7E-05	2.1E-05	0.61	0.5	0.43	0.72	0.72	0.71
Cryptosporidium	7.9E-07	4.1E-07	3.2e-07	0.03	0.016	0.012	0.43	0.34	0.29
Norovirus	6.3E-04	3.3E-04	2.5E-04	0.51	0.5	0.49	0.58	0.57	0.56

4.2.4 Discussions & Recommendations

This section briefly discusses all the steps involved in arriving the results under specific headings to enable better understanding.

Conceptual model

The derived conceptual risk model is conservative but is very simple to perform especially when available time for risk assessment is limited. The 'point estimate' provides only the single value risk estimate, that does not help much is defining the uncertainties or variabilities. However, the use of point estimate can be useful to get quick and rough idea of the annual infection risk levels under situations which demand immediate risk assessments. Importantly, the model lacks the fundamental information on confidence interval which is often used by the stakeholders to justify the decision taken towards any risk mitigation measures. Additionally, the model results are also not validated in comparison with the reality which adds to the list of limitations. All these drawbacks can be compensated by supplementing this model with the use of Monte Carlo simulations (i.e. use of Beta distribution, poison or fractional models etc.), which would call for collection for more relevant additional data. In addition to this, making assumptions, that closely represent the real-life scenario can immensely help one to eliminate under or overestimation of risk levels that are discussed further.

Model Assumptions

The assumption of zero risk on all 364 days (when there was no pipe repair/maintenance work) is far from reality because the risk if assessed can be present in any situation at any given time due to various hazards (like treatment failure or source water pollution or unhygienic management at user end points etc.)

Moving on to specifics, from Table 12 above, the results indicate that the risk levels significantly exceed the regulatory risk levels for all pathogens both in Scenario 'B' and Scenario 'C'. However, the risk levels are lower in Scenario 'A'. This variation in risk levels could purely be attributed to the extent of presence of pathogen concentrations. As in, the risk levels were always found to be increasing when navigating from Scenario 'A' which had negligible pathogen concentration to Scenario 'C' which had the highest pathogen concentration. However, there is no much variation in risk levels from *Campylobacter* and norovirus when compared between Scenario B and Scenario C. The risk levels from *Campylobacter* and norovirus seems to be levelling down to get close or exceed the regulatory limits, probably after surpassing a certain threshold limit. Moreover, the results imply the importance of variability of occurrence of pathogen levels in the contaminated water. Similar observations have also been mentioned by

two other studies mentioned in parenthesis (Blokker et al., 2018; J. Yang et al., 2015). This observation can also be supported from the results of sensitivity analysis.

On the other hand, the risk levels are overestimated, because of the assumption of zero effects of dilutions, zero sediment entry interaction with pathogens, no flushing or disinfection and no biofilm presence inside the pipe. This assumption can be considered conservative, because activities such as flushing/dilution/disinfection are often used as control strategies to mitigate the risk levels in drinking water distributions system in reality (J. Yang et al., 2015). However, this assumption could also be valid if the control measures fail to perform well (for ex. residual disinfectant may not be maintained at desired level) in real life(van Lieverloo et al., 2007).

Furthermore, the estimated risk levels for Scenario 'C' might seem unrealistically high. This can be justified based on the zero-dilution effect resulting in 100% contamination intrusion into the drinking water pipe as shown in the QMRA calculation section. So, it can be stated that, the dilution factor derived based on *E. coli* count and literature values is indeed governing the pathogen concentration and thereby its associated risk levels. That means, minimum dilution factor of 0.0000006 (i.e. assumed 0.00006% wastewater intrusion) obviously has lower risk levels as seen in scenario A when compared to high dilution factor of 1 (i.e. Assumed 100% wastewater intrusion) having higher risk levels in scenario 'C'.

Whilst scenario 'C' appears to be the 'worst scenario' with the highest risk levels for all pathogens. The results of this scenario also hint at the existence of hypothetical active fecal source scenarios. For example, a wastewater pipe may be still actively leaking (unidentified) in the close vicinity of the drinking water pipe causing fecal contamination or the drinking water pipe may have been pre-contaminated before fixing/servicing during storage/manufacture/transport or due to un-hygienic work conditions during repair and so on. Given the fact, that the *E. coli* often indicates recent fecal contamination(Blount, 2015) , results of scenario 'C' implicitly supports the hypothesis (i.e., active fecal source scenarios) of raw sewage intrusion to the pipe. In view of this, it is good to have regular monitoring and efficient management of fecal contamination sources to reduce the associated public health risks. In addition to this, adequate separation distance from all fecal contamination sources (especially wastewater pipe) must be incorporated while designing drinking water distribution system.

Furthermore, the assumption that only 10 milliliters of fecally contaminated soil-water enters the drinking water pipe in every pipe repair activity is certainly challenging to justify. Under such circumstance, it is likely that the estimated risk levels can be perceived to be either an underestimation or overestimation, depending on the pipe repair location and prevailing site conditions during the work. Furthermore, as mentioned previously in this report, the pathogen occurrence is typically event driven and so its concentration can often increase with occurrence of natural events like rainfall, runoff or burst of wastewater pipe. As such, there is a high likelihood where the contaminant intrusion volume can uncontrollably exceed the assumed volume of 10 milliliters in real life scenario. Obviously, the significant increase in contaminant intrusion volume can potentially increase the risk levels, making the current risk estimate to be perceived as a case of 'underestimation'. Conversely, the intrusion volume could be even smaller than assumed 10 milliliters if well-structured protocols are practiced during the repair events and sometimes no intrusion occurs at all. Theoretically, lesser contamination volume intrusion may have a lower risk levels than the current estimate, however, there is still a likelihood of infection risk that can be caused due to other hazards (like treatment failures, source water, unhygienic management at the user-end point etc.)(WHO, 2004). Moreover, only *'one'* pipe break/maintenance work in the break vicinity adds up to the unrealistic assumption, because increased frequency can alter the intrusion volume and its consequence. In summary and in reality, the assumption on volume of contamination intrusion into the pipe becomes valid only if

both the leaking raw sewage pipe and the leaking drinking water pipe were coexisting in the close vicinity during the repair work; else the estimated risk levels would be just a 'hypothesis'(J. Yang et al., 2015). Nevertheless, effort should be made to prevent or eliminate the possibility of contaminant intruding the drinking water pipe by continuous maintenance of pipe integrity (physical, hydraulic and quality).

Lastly, assuming that the first consumer would drink only 0.7416L only once in a day could also be treated as unrealistic scenario. Simply because, there may be situations wherein the person may not drink any water during the whole day. Conversely, there could be a likelihood that the first consumer may drink more than or less than 0.7416L per day. As such the exposure volume may have to be coupled with the number ingestions per day before using it in QMRA to get more realistic risk levels (Blokker et al., 2018). However, this may not be possible in the currently available QMRA tool. Hence, an advanced hydraulic modelling could be of help in accounting such scenarios.

Sources of variability and uncertainty

The consideration of only three pathogens for risk analysis is yet again a concern of 'underestimation'. The heterogeneous persistence of wide spectrum of known and emerging pathogens at varying degrees of occurrence levels in the environment matrices in a way justifies the claim of 'underestimation'. As such there is a likelihood of exposure to more than just three organisms assessed in this study (ex. *Legionella*). Nevertheless, this simplification would be still valid only if negligible or low concentrations were present. On the contrary, if high concentrations existed, these then needs to be accounted in the assessment, which would further increase the need for additional data. In the same context, the use of mean pathogen concentrations instead of the maximum concentrations for estimation of risk levels would also underestimate the real situation. Moreover, it is not possible to quantify the wide range of pathogens present in wastewater to further use them in QMRA. As such, choosing the reference pathogens can help the risk control strategies. It implies that, if the QMRA is aimed to control reference pathogens (consist of major group of organisms), then all other pathogens will also be controlled. Nevertheless, all these described reasons could be partly taken care by investigating more advanced water treatment methods and efficient management of pipe integrity (USEPA, 2006).

Comparison of probability of annual infection risk with target risk levels

From the results, it is evident that the estimated probability of annual infection risk levels for all the pathogens clearly surpassed the USEPA annual regulatory limits in all the three scenarios except for *Campylobacter* (0.5 people) and *Cryptosporidium* (0.01 people) in Scenario A. The results appear to be overestimated but also partially underestimated mainly because of the reasons described above under model assumptions. Seeing the risk levels, it is sure that there is high probability of first consumer getting sick if water is consumed immediately after pipe repair as assumed in this study. Given the fact that, it is only a single value risk estimate with no data on uncertainty, the probability of annual infection risk results of the study should be viewed merely as an indication of the level of safety, rather than an absolute prediction of health risk. However, the results could be used to choose the most suitable control measures. In addition to this, it is recommended to warn possible first consumer to boil the drinking water before ingestion at least for 24 hours. Apart from this, USEPA(2014), suggests that whenever such risk assessments are made, the results should be reported to the relevant authorities for further investigations (like soil testing) to assess the prevailing situation.

Sensitivity analysis

The sensitivity analysis performed by testing the exposure volume and the pipe diameter parameters, did showed that the pathogen concentration is the most 'dominant' parameter that influences the risk levels in relation to the conceptual risk model of this study. As in, the risk levels increase with increase in pathogen concentration and will remain at higher levels at higher concentrations (as seen in Scenario C). However, the two perspectives of under and overestimation can be noticed in this section also. For example, the risk levels especially in Scenario C is a sign of overestimation, if the first consumer ingests the total volume (for ex. 1L/day) of boiled water instead of contaminated water. On the other hand, if consumer drinks the contaminated water (i.e. with high concentrations as in Scenario C) several times (i.e. more than one glass or 1 Liter) during the day for any other reasons such as (like tooth brushing scenario mentioned in the paper (Blokker et al., 2018), then the estimated risk levels is an underestimation which can be directly attributed to the assumption of single ingestion per day per event. Therefore, in this case, sensitivity analysis considering only the exposure volume without accounting the number of times of consumption, can make one skeptical about the results. As such, it can be inferred that both exposure volume and the number of times of ingestion must be coupled to accurately assess the sensitivity of either of them. Else, it may not be of any importance in the analysis.

With regard to the pipe diameter, as one moves from 0.15 m diameter to 0.24 m diameter, it is seen that the risk levels tend to reduce (though not substantially) and the reduction indicates the importance of pipe diameter in the distribution system. The result directly hints that the bigger diameter pipe could probably reduce the risk levels. However, on the contrary, it is not always easy to reduce the risk levels just by using the larger diameter pipes, because the larger diameter pipes are vulnerable to stagnation effects which results in deterioration of drinking water quality (Ji, Parks et al., 2015). A similar observation was also mentioned in a recent study (Ling et al., 2018), wherein the team developed a size-effect model to simulate the stagnation effects of tap water and their study indicated an increased bacterial cell count from 103 cells per milliliter to 7.8×10^5 cells per milliliter in the inbuilt environment system. The study asserted that pipe diameter was the driving factor in increasing the cell count by mobilizing the kinetics of hypochlorite decay, cell detachment, migration, and demographic stochasticity. As such, it is important to design the optimum diameter of pipe for drinking water supply that accounts the effects of water stagnation or age, in order to ensure safe water supply to the consumers. Additionally, an hydraulic analysis through EPANET models could also be done to predict the water age (Nicholas Ashbolt et al., 2014) in order to secure water quality. However, this design aspect still seems to be a grey area in the field of research and hence has scope for improvement.

Immunity Aspects

The crucial part of this complete situation is the 'human perspective and individual immunity status'. Risk is notional and is perceived differently by different people. For instance, the first consumer, in spite of being warned may ingest the contaminated water out of mere ignorance and end up in a health risk situation to further transmit the infection to other people. On the contrary, there could be scenarios wherein the first consumer can coincidentally get sick at the same time of the pipe repair work from other sources, even if he abides by the warning given to him to drink boiled water. With regard to immunity status, on one side, the first consumer could get sick from an intrusion event (like pipe repair) if he has weak immunity strength. On the other hand, he may not get sick even after being exposed, if his immunity is strong enough to fight the infection. Therefore, making a balanced judgment of dose-response relationships is challenging and hence more data needs to be collected to reduce the related uncertainties.

5 Conclusions

The study provided evidence of presence of examined fecal indicators *E. coli* and coliforms in water samples collected from within the pipe repair pit from different locations of Gothenburg, Sweden. Further research should include the investigation of soil samples from the repair pit and the final results should be coupled with the hydrodynamic models to assess the true situation.

The derived conceptual risk model, though is conservative, could be used to get a quick idea of the risk levels especially when time is limited. The study also supported the previously recognized concept of the coexistence of three factors (contamination source, pathway and a driving mechanism) that are necessary for any intrusion to happen. The estimated risk levels significantly exceeded the target regulatory risk levels for *Campylobacter*, *Cryptosporidium* and norovirus. However, the risk levels can even be higher, if the distribution system integrity fails more frequently. Therefore, the risk assessment must be based on more realistic data that represents reality not merely based on worst-case assumptions. One way of doing it is by use of Monte Carlo simulations which accounts for variabilities and uncertainties in the results by incorporation of confidence limits. In addition, an epidemiological study is recommended to validate the risk estimates.

More importantly, the estimated risk levels in this case calls for immediate recommendation of issuing warning to the first consumer downstream the pipe repair location, to consume boiled water for at least 24 hours whenever a pipe repair/maintenance work happens in the close vicinity. This can reduce or eliminate the infection risk to the first consumer due to intrusion during pipe repair works.

In a broader perspective, more advanced water treatment methods and control strategies should be investigated in order to translate the estimated risk levels to operational efficiencies/mitigation measures. The focus should be more on controlling or maintaining the optimum pressure inside the drinking water pipe system in order to avoid pipe breaks/leaks that eventually allow the intrusion to happen.

In addition to this, timely information of the estimated risk levels should be given to the decision makers to appropriately plan the risk mitigation measures which can avoid public health risk.

Lastly, the major responsibility mostly lies with the water supply authorities to ensure safely managed drinking water to the consumers rather than merely issuing warnings. In addition to this, the national surveillance systems or public health welfare system should be more cautious while reporting, investigation and documenting the waterborne disease outbreaks in order to avoid oversight or misinterpretation of the crucial causative agent of the outbreaks.

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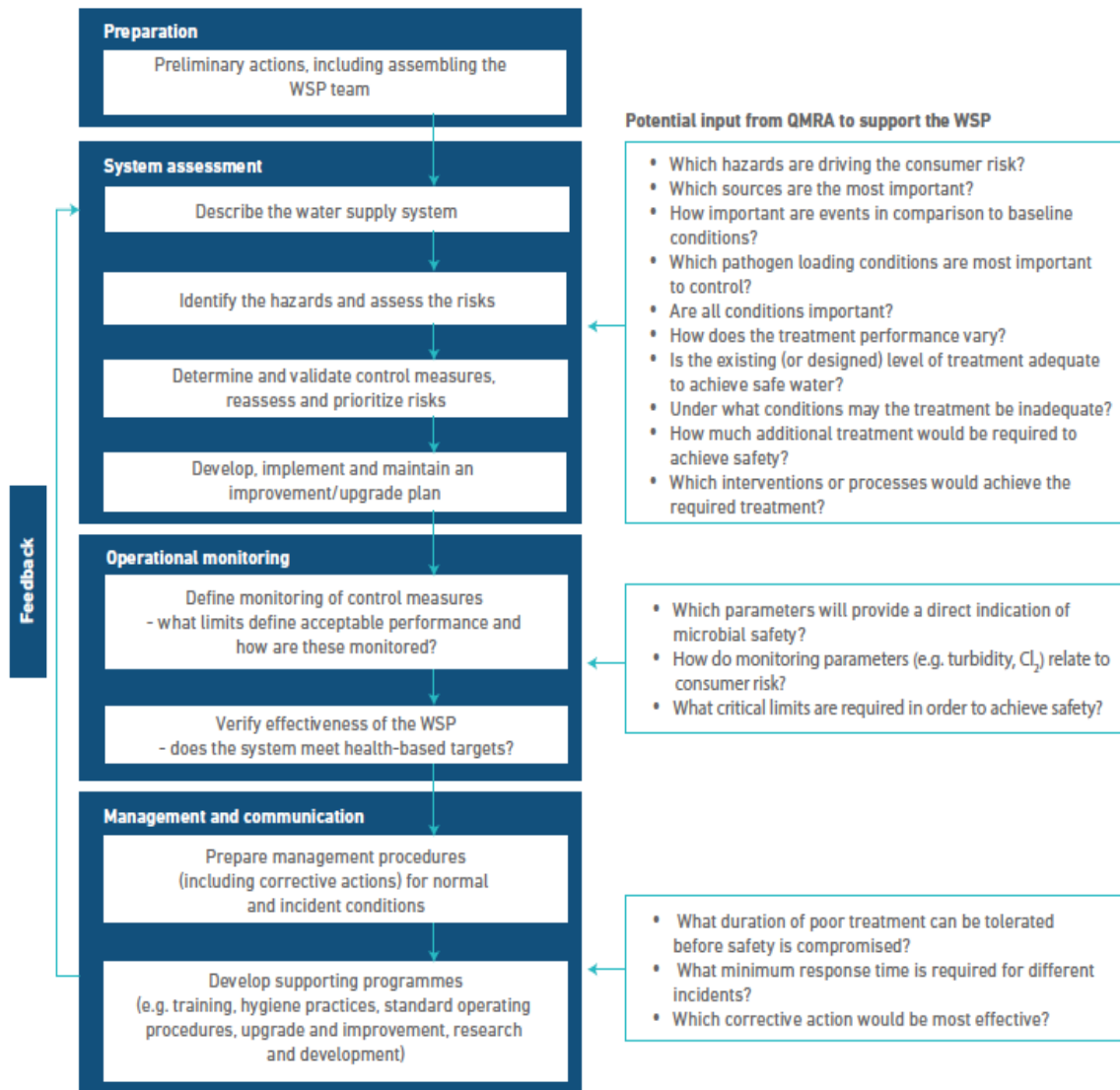
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APPENDICES

## APPENDIX 1- Inputs from QMRA to WSP

Preparation

Preliminary actions, including assembling the WSP team

Potential input from QMRA to support the WSP

System assessment

Describe the water supply system

Identify the hazards and assess the risks

Determine and validate control measures, reassess and prioritize risks

Develop, implement and maintain an improvement/upgrade plan

- Which hazards are driving the consumer risk?
- Which sources are the most important?
- How important are events in comparison to baseline conditions?
- Which pathogen loading conditions are most important to control?
- Are all conditions important?
- How does the treatment performance vary?
- Is the existing (or designed) level of treatment adequate to achieve safe water?
- Under what conditions may the treatment be inadequate?
- How much additional treatment would be required to achieve safety?
- Which interventions or processes would achieve the required treatment?

Operational monitoring

Define monitoring of control measures - what limits define acceptable performance and how are these monitored?

Verify effectiveness of the WSP - does the system meet health-based targets?

- Which parameters will provide a direct indication of microbial safety?
- How do monitoring parameters (e.g. turbidity, Cl_2) relate to consumer risk?
- What critical limits are required in order to achieve safety?

Management and communication

Prepare management procedures (including corrective actions) for normal and incident conditions

Develop supporting programmes (e.g. training, hygiene practices, standard operating procedures, upgrade and improvement, research and development)

- What duration of poor treatment can be tolerated before safety is compromised?
- What minimum response time is required for different incidents?
- Which corrective action would be most effective?

Feedback

APPENDIX 2 - Images of E-coli and Coliforms detected in collected water samples

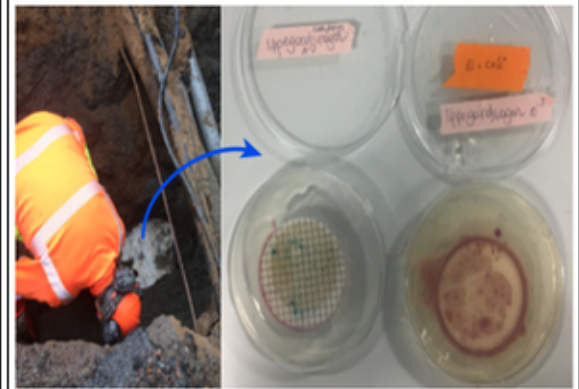The individual images of sampling locations along with their respective results obtained from membrane filtration method can be seen below.

(id.00/06) Kummingatan 12

(id.01/05) Grönstensvägen 13

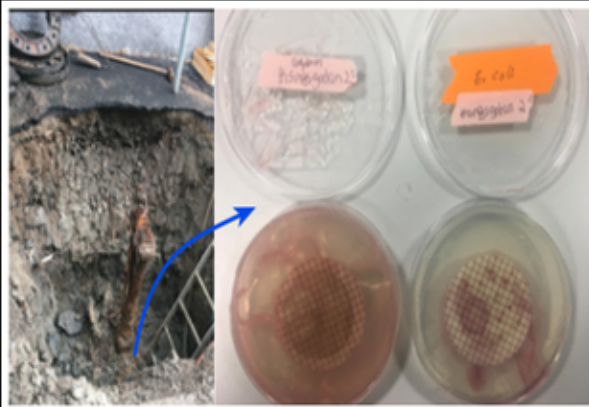(id.7) Runebersgatan 2

(id.8) Mariagatan 11
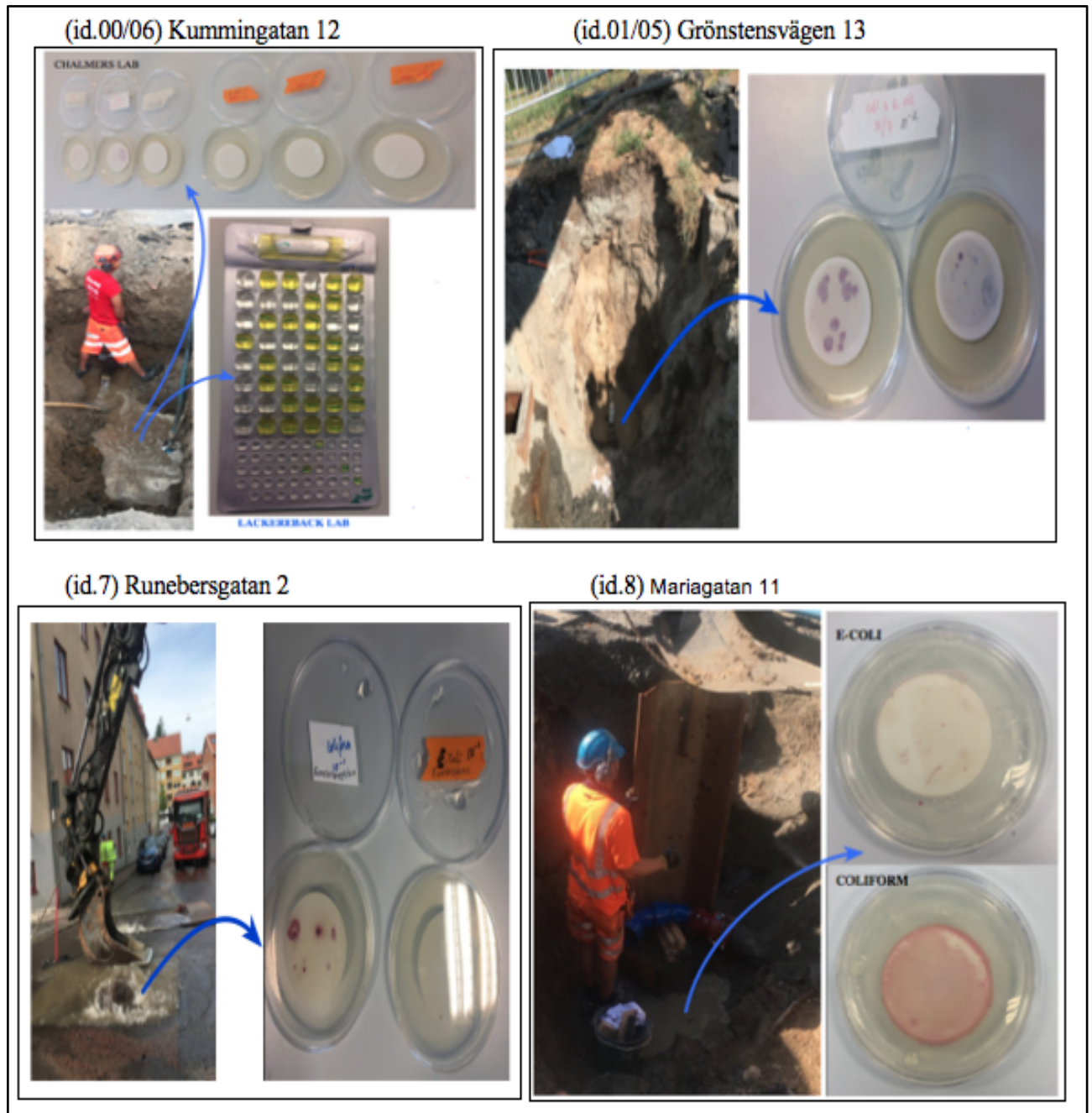
(id.9) Nordgårdsgatan 4
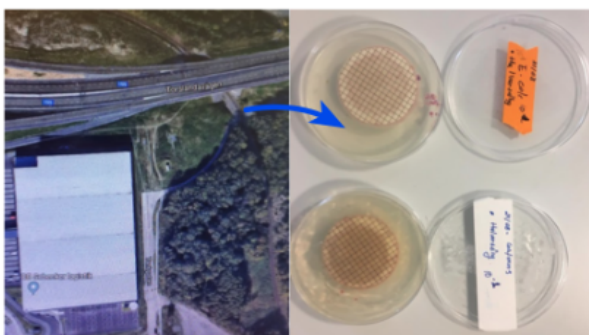
(id.10) Uppegårdsvägen 9

(id.11) Hisingsgatan 2

(id.12) Manufakturgatan 11

id.16. Halvorsäng

id.15 Sankt Sigfridsgatan 65

id.18. Kungsportsavenyn 1

id.17 Södra Vägen 73

APPENDIX 3 – Literature values for pathogens in raw domestic sewage

	Raw sewage	Biol. treated
E.Coli	10^7	10^4
Cl.perfringens	10^4	$3 \cdot 10^2$
Fecal streptococcae	10^7	10^4
Salmonella	200	1
Campylobacter	$5 \cdot 10^4$	$5 \cdot 10^2$
Listeria	$5 \cdot 10^3$	50
Staphyllococcus aureus	$5 \cdot 10^4$	$5 \cdot 10^2$
Coliphages	10^5	10^3
Giardia	10^3	20
Roundworms	10	0.1
Enterovirus	5000	500
Rotavirus	50	5
Susp. matter (mg/100 ml)	30	2

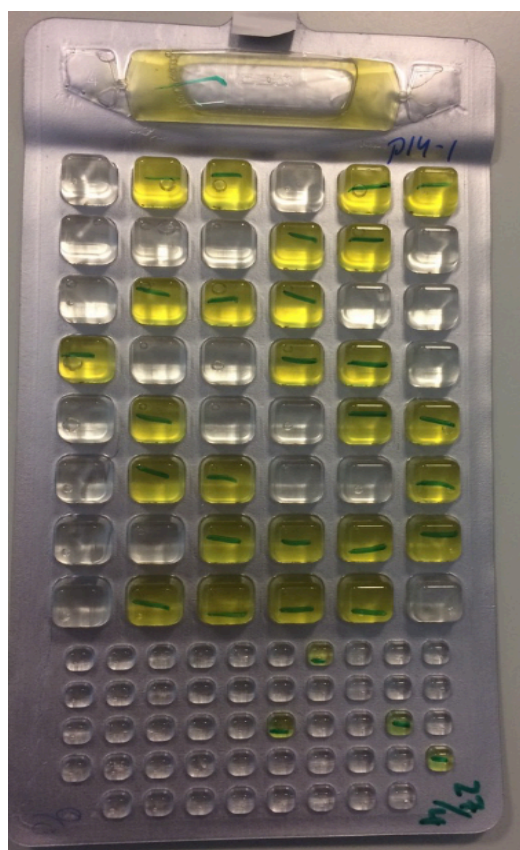| Sample type	no. of samples	no. positive samples	Oocysts min – max, presumptive[1] (#/10 L)	Oocysts min – max, confirmed[2] (#/10 L)	Time period, positive samples
Raw water	18	10	0.2-3.1	0.1-0.7	101127-110209
Drinking water, WTP	7	7	0.047-1.4	0.02-1.3	101127-110120
Drinking water, distribution net	9	9	0.063-0.36	0.05-0.05	101129-110131
Sewage, WWTP inflow	21	13	200-270 000	*-160 000	101129-110217
Sewage, treated WWTP	15	14	30-21 000	30-10 000	101201-110124
Receiving water (Lake Storsjön)	14	8	2-21	1-18	101129-110322
Tributary (stream)	8	5	1 400-5 000	950-3 500	101130-101214
Total	102	68	0.047-270 000	0.02-160 000	101127-110322
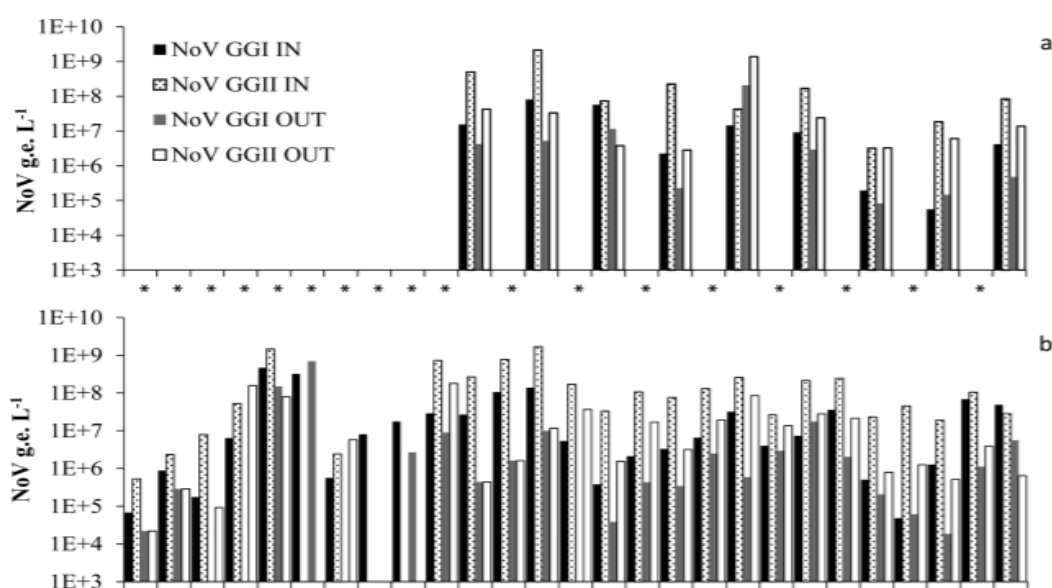
APPENDIX 4 – Colilert test procedure

The following is the general description of the Colilert test procedure. However, the procedure can vary based on the laboratory set up and type of test standards.

1. Collect the water sample
2. Perform the dilution required based on turbidity of sample
3. Add Colilert powder to the sample and mix it to dissolve
4. Then gently pour the prepared sample into a Quanti-Tray
5. Seal the Quanti-Tray tightly and incubate it at 35°C for 24 hours
6. Identify the yellow colored wells in Quanti-Tray and count them
7. Refer to MPN table for quantifying the coliforms
8. Then quantify *E. coli by counting the* Blue Fluorescence wells by placing the Quanti-Tray under a 365nm UV Light
9. Finally, represent the result in relation to dilution used

An example of Quanti-tray with positive count of coliforms (yellow color) taken from Lackerebäck lab is shown below for quick reference

More details can be accessed in the link (https://azdhs.gov/documents/preparedness/state-laboratory/lab-licensure-certification/environmental-laboratory/facilities/ww-presentation-idexx.pdf)