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# Assessment of faecal contamination and sanitary risk in intermittent piped water systems in rural Nepal

Moa Kenea

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# Assessment of faecal contamination and sanitary risk in intermittent piped water systems in rural Nepal

Master of Science Thesis
by
Moa Kenea

Supervisor

**Prof. Maria D. Kennedy, PhD, MSc (UNESCO-IHE)**

Mentors

**Arnt Diener, MSc (Sandec, EAWAG)**

**Sara Marks, PhD, MSc (Sandec, EAWAG)**

**Jack van de Vossenberg, PhD, MSc (UNESCO-IHE)**

**Giuliana Ferrero, PhD, MSc (UNESCO-IHE)**

Examination committee

**Prof. Maria D. Kennedy, PhD, MSc (UNESCO-IHE)**

**Dr. Patrick W.M.H. Smeets (KWR)**

**Jack van de Vossenberg, PhD, MSc (UNESCO-IHE)**

**Sara Marks, PhD, MSc (Sandec, EAWAG)**

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**Delft**
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# Abstract

Drinking water supply systems in developing countries operate at irregular intervals and several failures occur more frequently than those in developed countries. Waterborne diseases and microbial contamination of water at tap stands have been related with intermittent supply of water. To understand the spatial and temporal dynamics of faecal contamination and the risk factors associated with intermittent supply, a comprehensive microbial water quality monitoring was conducted in intermittent piped water systems (iPWS) located in remote rural areas of Mid-Western Nepal. Three iPWS's fed by improved spring water were chosen for the study and monitored daily for a duration of 35 days. Daily water samples were collected from springs, reservoirs and selected tap stands and measured for the concentration of *E.coli*, total coliforms, pH, turbidity, total dissolved solid and electrical conductivity. Risk factors of contamination were identified by conducting sanitary inspections at sampling points of the supply systems and simple salt tracer tests were conducted to assess the possibility of infiltration into springs. In addition, environmental factors that might influence the microbial quality of water like temperature, precipitation and humidity were monitored daily using sensors and portable devices.

The result of the daily monitoring showed that 97.1% of samples collected from spring of iPWS II had detectable *E. coli* concentration. Similarly, 97.2% of samples from reservoir contained *E. coli* bacteria above the standard of 0 CFU/100 mL. Moreover, the percentage of contaminated samples at taps went beyond 98%. At iPWS III, 94% of samples collected from spring were contaminated with *E. coli* while 63% and 73% represents percentage of samples contaminated at reservoir and taps respectively. A higher percentage (92.4%) of water samples collected from iPWS IV were found to be contaminated with *E. coli*. Water samples tested at sampling points of iPWS IV showed that all samples (100%) from spring, reservoir and distribution chamber were positive for total coliforms. At taps 91% and 99.6% of water samples were found to be contaminated with *E. coli* and total coliforms respectively. The result of the temporal variability showed peak *E. coli* concentrations originating from the spring source. Also, the salt tracer test identified infiltration of contaminants from the external environment as a risk factor for contamination. The sanitary inspection result showed that 96.3% of sampling points had medium to very high risk level of contamination. At all iPWS the springs had medium to very high risk level for contamination. Infiltration of contaminants into spring and poor sanitary condition of the supply system affected the microbial quality of water in the study area.

Key words: Intermittent piped water, sanitary inspection, spatial variability, temporal variability, tracer test

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# Abbreviations

| | |
|---|---|
| CDP | Compact dry plate |
| CFU | Colony forming units |
| DL | Detection limit |
| *E. coli* | *Escherichia coli* |
| EAWAG | Swiss Federal Institute of Aquatic Science and Technology |
| EMPA | Swiss Federal Laboratories for Materials Science and Technology |
| EPA | Environmental protection agency of US |
| GPS | Global Positioning System |
| iPWS | Intermittent piped water system |
| JMP | Joint monitoring program |
| NDWQS | Nepal drinking water quality standard |
| PVC | Polyvinyl chloride |
| PWS | Piped water system |
| RVT | Reservoir tank |
| SDG | Sustainable development goal |
| TNTC | Too numerous to count |
| UNICEF | United Nations Children's Fund |
| WHO | World Health Organization |

## CHAPTER 1

# Introduction

## 1.1. Background

In 2014, the United Nations General Assembly proposed the Sustainable Development Goals (SDG) which by 2030, targets to achieve *"universal and equitable access to safe and affordable drinking water for all"*. According to Goal 6, sub target 6.1 of the SDG the indicators set imply that drinking water should be free from pathogenic microorganisms and hazardous contaminants so that consumption of water does not pose any significant threat to human health and wellbeing (WHO/UNICEF, 2015a).

In developing countries poor drinking water quality is a recognized health threat, as a considerable burden of disease is attributable to unsafe water. According to WHO and UNICEF (2015b), 663 million people still lack access to safe drinking water. Consumption of unsafe water may expose the users to pathogens, which in turn may result in disease: for instance, diarrhoea, typhoid and infectious hepatitis (Howard & Bartram, 2003; Mohanty et al., 2002). In 2012, it has been estimated that 502,000 fatalities were caused by unsafe drinking water, corresponding to 1.5% of the global burden of disease and 5.5 % of the total death was in children under age five (Prüss-Üstün et al., 2014).

In 2015, globally 96% of the urban and 84% of the rural population had access to improved water sources. However, a large gap between urban and rural population exists in terms of water coverage: 75% of urban population has access to piped water on premises as opposed to only 33% in rural areas, where the remaining 51%, 12% and 4% rely on other improved sources (tubewell, rain water), unimproved sources or surface water, respectively (WHO/UNICEF, 2015b). In addition, most improved water sources may not give adequate service in terms of safety and reliability (Onda et al., 2012).The majority of those who do not have access to an improved source live in sub-Saharan Africa and South Asia (WHO/UNICEF, 2015).

In Nepal, access to improved sources of drinking water increased from 66% to 93% from 1990 to 2014, meaning that one individual out of ten obtains water from unimproved sources such as unprotected boreholes and surface water (UNICEF, 2014). Even though the percentage of improved water sources has increased, microbial contamination of drinking water sources and supplies is the biggest threat in the country. Also, a large gap between urban and rural areas exist. For instance, in rural Nepal only 18 % of population has access to improved piped water sources (WHO/UNICEF, 2015b). Among those who do not have access to improved sources only 14% of households treat their water before consumption (NMICS, 2015).

## 1.2. Problem statement

Contamination by pathogenic organisms from human and animal excreta is the most common water hazard in developing countries (WHO, 2010). Nepal is one such country where contamination of water is a major public health concern. The Nepal drinking water quality standards (NDWQS) dictate conducting routine water quality monitoring as a sole responsibility of the water supplier (NDWQS, 2005). In this sense the suppliers are responsible to adhere to national as well as international monitoring schemes. In rural areas a clear picture of water quality is not available due to several challenges and obstacles to implement the proposed monitoring techniques. Rural areas in developing countries lack the infrastructure like laboratory facility, electricity and trained lab personnel to undertake water quality assessment on a regular basis, this makes it difficult to understand the extent of faecal contamination (Sperling & Fattal, 2001). The Nepal Water Law (NWL) points out that the suppliers did not adhere to standards set and water sources were frequently being polluted by microbial as well as chemical contaminants, which reflects that routine water quality monitoring and assessment is not well addressed in the country (NWL, 2005).

The lack of coordinated monitoring and evaluation of drinking water schemes in Nepal could be related with increased faecal contamination of drinking water sources in the country. For instance, according to Nepal's multiple indicator cluster survey (NMICS) (CBS, 2014), 71% of water supply and 82% of household drinking water were contaminated with *E. coli* bacteria. In addition, water quality monitoring conducted by UNICEF and Nepal department of water supply and sewerage (DWSS) on water supply system of Dang and Jajarkot districts indicates increased contamination of the supply system with *E. coli* bacteria. According to the study, 84.4% of samples from Dang and 68% of water samples from Jajarkot contained *E. coli* concentration higher than the Nepal drinking water quality standard of zero MPN/100 mL (DWSS, 2014). Moreover, a study conducted by UNESCO-IHE and Eawag in 2015 showed that 64% of samples from five districts of rural Nepal were faecally contaminated (Daniel, 2015). However, little is known about the risk factors and dynamics of contamination.

In 2012, only 25% of the water supply systems that were properly functional throughout the country, 75% of water supply systems in the country require improvement, maintenance and rebuilding (DWSS, 2014). Thus, poor functionality of water systems coupled with microbial contamination could be attributable to frequent outbreak of waterborne disease and diarrhoea in the country. For instance, in 2009 over 70,000 people in 27 districts have been affected by diarrhoeal outbreaks, resulting in 400 fatalities. In addition, in 2014 waterborne outbreak of Hepatitis E and watery diarrhoea has been registered in two districts (UNICEF, 2014).

## 1.3. Objectives

### 1.3.1. *Objective:*

The objective of this study was to assess the temporal and spatial variability of faecal contamination along an intermittent piped water system (iPWS) in rural Nepal, in order to locate potential contamination hotspots.

### 1.3.2. *Specific objectives:*

1. To describe the spatial and temporal distribution of *E. coli* and total coliform numbers along the iPWS
2. To assess the sanitary condition of several key locations of the iPWS (spring, reservoir, taps)
3. To identify potential correlation between faecal contamination and abiotic water parameters (e.g. pH, conductivity, turbidity)
4. To assess the possibility of intrusion of contaminants into spring

## 1.4. Research questions

1. What is the daily variation in the concentration of *E. coli* at the source, reservoirs and community taps?
2. Does the sanitary risk of the supply system correlate with the microbial quality of drinking water?
3. Which physical-chemical parameters influence microbial water quality at the spring, reservoir and tap stands?

## CHAPTER 2

# Literature review

## 2.1. Water supply system deficiencies

Many drinking water distribution systems in developing countries operate at (irregular) intervals. Besides, several failures along the supply system occur more repeatedly than those in developed countries. Even though, the availability of drinking water distribution systems in developing countries indicates the improved source of water, the quality is still not sufficient (Lee & Schwab, 2005; WHO, 2003).
According to WHO and UNICEF (2000), failure in the distribution pipeline, specifically due to inadequate amount of residual disinfectants, low pressure, and irregular supply cause contamination of supply systems and poor drinking water quality. These reasons coupled with poor sanitation and leakage of pipes may favour pathogen intrusion into the distribution system. Failure in water treatment plants and cross contamination of supply systems may pollute water supply systems prior to distribution. In both of these cases the quality of water will deteriorate and may cause water-borne diseases and diarrhoea when consumed (Craun & Calderon, 2001; Ford, 1999; Lee & Schwab, 2005).

### 2.1.1. *Intermittent supply*

Intermittent piped water systems (iPWS) deliver water for a certain duration of time. Nelson and Kumpel (2015) estimated that iPWS deliver water to around 300 million people globally. Their finding showed that countries located in South Asia were ranked at the lowest level in terms of service delivery hours (7.2 h) when compared with countries in East Asia and the Pacific (16.7 h) (Kumpel & Nelson, 2015).

In developing countries, waterborne diseases and contamination of water at tap stands have been related with intermittent supply of water and deficiencies in the supply system (Lee & Schwab, 2005). In Africa and Latin America approximately one-third of water supply systems provide service intermittently, while in Asia the percentage goes up to half (WHO/UNICEF, 2000). This is mainly due to the discrepancy between increasing demand due to population growth (PI, 2010), slow construction of new water supply infrastructure due to high investment cost (Elala et al., 2011), loss of water due to leakage and extension of the supply system (Kumpel & Nelson, 2015).

iPWS can lead to diminished water quality by facilitating the direct infiltration of pathogens into the pipe network and/or by allowing attachment and growth of microorganism (biofilm) (Coelho et al., 2003). Both mechanisms can cause microbial contamination of drinking water.

For instance, a study conducted in India revealed that iPWS systems are more prone to microbial contamination when compared with distribution systems that provide service continuously. 37% of tap water samples from iPWS were contaminated by *E. coli* bacteria and only 0.7% of tap water from continuous supply contained *E. coli* (Kumpel & Nelson, 2014). Similarly a study conducted in Tajikistan showed a higher percentage (97%) of water samples collected from tap stands were contaminated with faecal coliforms after the water was distributed to pipe networks from treatment plant (Mermin et al., 1999). In Gaza, a study conducted on iPWS revealed the concentration of total coliforms detected at the supply system was higher than samples collected from source (Amr & Yassin, 2008).

### 2.1.2. *Intermittent pressure*

Keeping continuous pressure in a water supply system can prevent the introduction of contaminants into the system. A minimum pressure of 1.4 bar is recommended in distribution pipelines to maintain adequate flow condition (NRC, 2006). A study conducted in India revealed that at pressures below 0.7 bar increased bacterial indicators were detected, even in the presence of residual chlorine in the system. Conversely, when the system operated at pressure above 1.2 bar no *E. coli* and low concentrations of total coliforms were detected (Kumpel & Nelson, 2014).
Low or transient pressure in the supply system can occur as a result of intermittent supply of water, power failure, flushing water for hydrant, damage of valves, installation of household pressure pumps and failure in operation of pumps. As a result, intermittent pressure can cause backflow of water and hence contamination (Besner et al., 2002; Trussell, 1998)

## 2.2. Faecal contamination and disease outcome

Pathogenic organisms such as viruses, bacteria, protozoa and helminth eggs are often found in faeces of humans and animals (Ashbolt, 2004) and may contaminate drinking water sources causing several infectious diseases (WHO, 2011). Table 1 provides examples of disease outbreaks that occurred as a result of failure in supply systems.

**Table 1** Example of disease outbreaks in developing countries

| Supply system deficiencies | Country | Result | Outcome | Reference |
|---|---|---|---|---|
| **Negative pressure** | Tajikistan | 97% of samples collected from tap stands were contaminated with faecal coliforms | Outbreak of typhoid fever and diarrhoeal disease | (Mermin et al., 1999) |
| **Insufficient chlorine** | Trinidad | *E. coli* and total coliforms detected in 80.8% and 67.3% of water samples | Increased concentration of total and thermotolerant coliform | (Agard et al., 2002) |
| **Intermittent supply** | India | 37% of samples from taps stands were contaminated with *E. coli* | Contamination of tap water caused by *E. coli* | (Kumpel & Nelson, 2014) |
| **Intermittent pressure** | Uzbekistan | ---- | Diarrhoea | (Semenza et al., 1998) |
| **Intermittent water supply** | Palestine | 34% of sample in supply systems were found to be positive for total coliforms and 23% of samples contain *E. coli* respectively | Diarrhoea | (Amr & Yassin, 2008) |
| **Leakage in water supply system** | India | --- | Typhoid, GI illness | (Mohanty et al., 2002) |
| **Intermittent supply** | Palestine | Contamination of well water with faecal coliforms | Giardia and Hepatitis A | (Yassin et al., 2006) |
| **Intermittent supply** | India | Association of iPWS with waterborne diseases | GI illness | (Ercumen et al., 2015) |

## 2.3. Indicator organisms of faecal contamination

Direct pathogen monitoring is impractical because pathogens occur generally in small numbers and their detection by routine techniques may be impossible. In addition, detecting pathogenic organisms is often time consuming, costly and poses health risks. As a result, indicator organisms are chosen over pathogenic microorganisms (Leclerc, 2001). Indicator organisms, particularly bacterial indicators, are widely used to assess contamination of water sources by human and animal excreta. *E. coli*, total coliform and thermotolerant coliform bacteria have been used extensively as indicators of faecal contamination to monitor drinking water quality. When compared to detection of pathogenic organisms, detection and enumeration of bacterial indicators remains in the forefront of microbial water quality monitoring. However using indicator organisms has several drawbacks. For instance, they might respond differently to the physico-chemical characteristics of water. Moreover, pathogens can exist in water even in absence of indicator organisms (JMP, 2012).

### 2.3.1. *Total coliform bacteria*

Total coliforms are organisms that are generally found in the environment. The group encompasses coliforms that originate from human and animal faeces along with those coliforms that naturally exist in soil. Coliform bacteria can grow at 37°C in the presence of bile salt, also they are characterized by their ability to utilize lactose and produce gas and acid (Horan, 2003). In many instances coliform bacteria are used to assess the quality of water supplies treated with chlorine, as they are sensitive to chlorination and their presence indicates the occurrence of contamination (JMP, 2012). *However, because of their presence in the environment this group of organisms are not considered as useful indicator of faecal contamination (WHO, 2011).*

### 2.3.2. *Thermotolerant coliform bacteria*

Thermotolerant coliforms comprise coliforms that are able to ferment lactose at 44.5 °C. The group contains bacteria like *E. coli and Klebsiella pneumoniae*. The detection of thermotolerant coliforms indicates contamination of water sources with faecal material (Bitton, 2005). Several studies show that faecal coliforms are not potent indicators of faecal contamination as a result of the presence of species that are found in nature like *Klebsiella* (Alonso et al., 1999; Ashbolt et al., 2001; Leclerc et al., 2001). So, their presence can be used as a secondary indicator to assess the effectiveness of water treatment plants and they are generally easy to detect (WHO, 1997).

### 2.3.3. *Escherichia coli (E. coli)*

*E. coli* is a species of thermotolerant coliform distinguished by producing endole from tryptophan, and it also possess β-galactosidase and β-glucuronidase enzymes. *E. coli* is predominantly found in the gastrointestinal tract of warm-blooded animals (Krieg & Holt, 1984). Nevertheless, some findings show that *E. coli* can also be found, multiply and persist in the environment especially in tropical soils, climates and waters rich with organic matter(Jimenez et al., 1989; JMP, 2012). The majority of *E. coli* strains are non-pathogenic, even though some serotypes, like *E. coli 0157:H7,* can cause serious illnesses(Wilson et al., 2011).

The use of *E. coli* as an indicator organism of water quality dates back to the late of 18$^{th}$ century. However, the procedures were not suitable for periodic detection of *E. coli.* Due to this surrogates for *E. coli* like coliforms were used to detect faecal contamination(Edberg et al., 2000). Multiple tube fermentation and membrane filter methods are most commonly used techniques to detect indicator organisms of faecal contamination in water. After the enzymes β-glucuronidase and β-galactosidase were identified the sensitivity of multiple tube fermentation and membrane filter techniques increased (Annie, 2002).The detection of *E. coli* shows recent faecal contamination of water sources as the bacteria is sensitive to environmental factors due to this the indicator bacteria is widely used to monitor the quality of water.

The detection of *E. coli* in water samples does not prove that pathogenic organisms are present, instead it shows a risk of faecal contamination, and therefore the possible presence of pathogenic microorganisms of faecal origin (Brüssow et al., 2004). As a result the detection and enumeration of *E. coli* is broadly used to monitor water samples for faecal contamination and (Atlas et al., 1993).

## 2.4. Other indicator organisms

### 2.4.1. *Faecal Streptococcus*

This group comprises numerous species of *Enterococcus* bacteria among which *Enterococcus faecalis* and *Enterococcus faecium* appear in large numbers. They are resistant to salt and to action of chlorination. Due to this they tend to live longer than coliform bacteria in water sources. They have limited application in routine water quality monitoring because of their lower abundance in faeces and the long detection (JMP, 2012).

### 2.4.2. *Clostridium perfringens*

The spores formed by *Clostridium perfringens* persist in the environment for a long period of time. This bacterium can stay dormant in soils and biofilms for several years and is therefore unsuitable at indicating recent faecal contamination. Another limitation is that the cost of analysis to detect *Clostridium perfringens* is relatively higher than other indicator organisms due to anaerobic nature of the bacteria it should be incubated in anaerobic condition (Edberg et al., 2000)

## 2.5. Physical parameters

### 2.5.1. *Turbidity*

Water becomes turbid when there is presence of particulate matter, like silt, clay and microorganisms. Turbidity in water stimulates the growth of microorganisms as nutrients and microbes can easily get attached on particulate matter and promote bacterial growth. Moreover, turbidity reduces the performance of disinfection by chlorine (JMP, 2012).

### 2.5.2. *pH*

Water in piped distribution system should maintain a pH below 8, otherwise pipe corrosion may occur. Moreover, the effectiveness of chlorine disinfectant is dependent on pH. When chlorine is added to water it produces hypochlorous acid (HOCl) which subsequently dissociates to hypochlorite ion ($OCl^-$) when the pH of the water increases. Hypochlorite ion is not an effective disinfectant when compared with hypochlorous acid. To prevent microbiological and chemical contamination of water the pH should be checked regularly (JMP, 2012; Yves, 2004).

### 2.5.3. *Temperature*

Temperature in supply systems may affect microbial growth and activity. According to Hallam et al (2001), the activity of biofilm decreases by 50% when the temperature drops from 17°C to 7°C. Also, temperatures above 15°C might increase microbial activity in contaminated distribution systems (Yves, 2004).
In addition, temperature effects the efficiency of treatment plants, the rate of corrosion in pipe lines and dispersion of disinfectants (LeChevallier et al., 1996).

## 2.6. Methods to detect faecal contamination

Routine microbial water quality monitoring plays a major role to assess the safety of drinking-water and to take effective measures in case of contamination. Methods like the membrane filtration, multiple-tube fermentation and presence absence tests have been developed and widely used to qualitatively and quantitatively express the concentration of indicator bacteria as well as pathogenic organisms in drinking water.

### 2.6.1. *Multiple tube fermentation*

This technique involves processing water sample through a series of serial dilutions and replicate of test tubes. The method comprises presumptive test, confirmed test and completed test which occur after each other. This tests differ in the nutrient media they use during inoculation of water sample. In the presumptive test a growth media containing lauryl tryptose broth (LTB) is inoculated with water sample in an inverted Durham tubes and the production of gas is observed after incubating the sample for 24-48 hours at 35 °C. In confirmed test samples which able to produce gas in the presumptive test are further incubated for 24 hours in a media containing brilliant green lactose bile broth (BGLBB). The production of gas or turbidity indicates the growth of total coliforms in the growth media and the concentration of faecal indicator bacteria can be statistically calculated and reported as MPN (APHA, 1998). Finally, the completion test involves simultaneously inoculating of BGLBB for total coliforms and EC-MUG (4-methylumbelliferyl-beta-D-glucuronide) for *E. coli* and incubating at 44.5 °C for 24-48 hours. The advantage of this method is that the results can be interpreted easily by observing for gas production. However, the method requires long processing time and selective growth media for selective group of bacteria and varying incubation temperature. In addition, as the method does not involve counting of colonies it is not as precise when compared with plating methods (Koster et al., 2003).

### 2.6.2. *Presence absence test (P/A)*

The use of P/A tests is recommended in conditions where the concentration of faecal indicator bacteria is very low and to only know whether a faecal indicator bacteria is present or absent. The method involves inoculating water sample (100 mL) in a nutrient media needed for bacterial growth and incubating the sample the change of colour shows the presence of faecal indicator bacteria or contamination. This method does not allow to quantify the density of indicator bacteria (Chigbu & Parveen, 2013 ).

### 2.6.3. *Membrane filtration*

The membrane filtration technique involves filtering a water sample (usually 100 ml) through a sterile porous filter that retains bacteria in the filter. The filter is then transferred to a plate containing growth media/ chromogenic substrate and incubated for 24 hrs at 35±2 °C. The method is recommended for water samples with low turbidity. Turbid water might clog the filter (Chigbu & Parveen, 2013 ).

## 2.7. Sanitary inspection

A broad range of microbiological and chemical contaminants in drinking water can have an effect on human health. The WHO adopts a proactive assessment strategy that includes all component of the supply chain in drinking water distribution system, called Water Safety Plan (WSP). WSP aims at minimizing risks through identification of potential hazards, ranking of risks and active monitoring of appropriate control measures (WHO, 2011).

Water quality monitoring gives information about the quality of water during the period where assessment is conducted. Although the information reflects the current status, it is incomplete in predicting the upcoming events. For this reason, tools that could identify potential sanitary risks and the future trend are useful to maintain the quality of water. Among the tools sanitary inspections and qualitative surveys are recommended by WHO and UNICEF (JMP, 2012) to assess risk factors related with microbial water quality.

Sanitary inspections are conducted to supplement microbial monitoring of drinking water by identifying risks and potential hazards that might influence the microbial quality of water. Data obtained from sanitary inspection inform about the current status of the supply system and help identifying concerns about contamination risk (Howard, 2002). WHO proposed sanitary inspection checklists which include 10 sets of 'Yes' or 'No' questions that are meant to categorize risk factors on a scale ranging from 0 to 10. Where, 0 and 10 representing low risk and very high risk for contamination (WHO, 1997). So far, several studies have been conducted to assess the predictive power of sanitary inspection over microbial quality of water (Howard et al., 2003; Luby et al., 2008; Mushi et al., 2012; Parker et al., 2010). Mushi et al (2012) correlated risk category with microbial quality of tube wells in Tanzania, the result showed that 87% of *E. coli* contamination were associated with the risk score category. Conversely, the finding by Parker et al., (2010), showed weak predictive power of sanitary inspection in predicting the microbial quality of water and no correlation was observed between risk category and feacal contamination of the water sources.

A sanitary inspection conducted in Nicaragua showed that 44.8% of piped water supply systems had medium to higher level of risk for contamination, whereas 15.7 % of supplies had high or very high risk score ranking. According to this study the main risk factor was found to be intermittent supply of water (Aldana, 2010).

## CHAPTER 3

# Description of the study area

Nepal is one of the least developed countries in South East Asia with a population of around 27 million. Since the new constitution was adopted in September 2015, Nepal is divided into 7 provinces by clustering existing 75 administrative districts (CBN, 2015). Districts are further divided into village development committees (VDC) that are located in rural areas and municipalities located in urban areas (MOHP, 2012) Fig 1 shows map of the study area. The major source of drinking water in the country is tap water which serves 48% of households, 35% of households have water from a hand pump, 6% households use stream water and 8 % of households use water from unimproved sources (NPHC, 2011).

The study area was located in remote rural areas of Nepal with an elevation ranging between 1500 m and 1800 m. The lowest and highest temperature during the study period was 7.6 °C and 16 °C respectively. An average pressure of 840 pa and humidity of 71% was recorded in the area (wireless weather station). There is no electricity supply in the study area.


**Figure 1** Map of Nepal showing study site

The supply system providing drinking water to the area is fed by improved spring water constructed by HELVETAS Swiss Intercooperation. All the systems included in the study are driven by gravity and the flow of water is intermittent because the spring sources do not provide sufficient water. The water flows through a PVC pipe to a 10 $m^3$ reservoir tank, where it is stored up to 10 hours. Water from the reservoir tanks are distributed through different branches of pipe networks to private taps which serve individual families and public tap stands which is shared by several households and cluster of families. Figure 2-4 shows layout of the iPWS's chosen for the study.

Taps operate twice a day from 6:30 a.m. to 7:30 a.m. in the morning and 5:00 p.m. to 6:00 p.m. in the evening with around 10 hour gap in between. Village maintenance workers trained by HELVETAS handle the maintenance and operation of the systems. Users pay about 0.5 € per month.

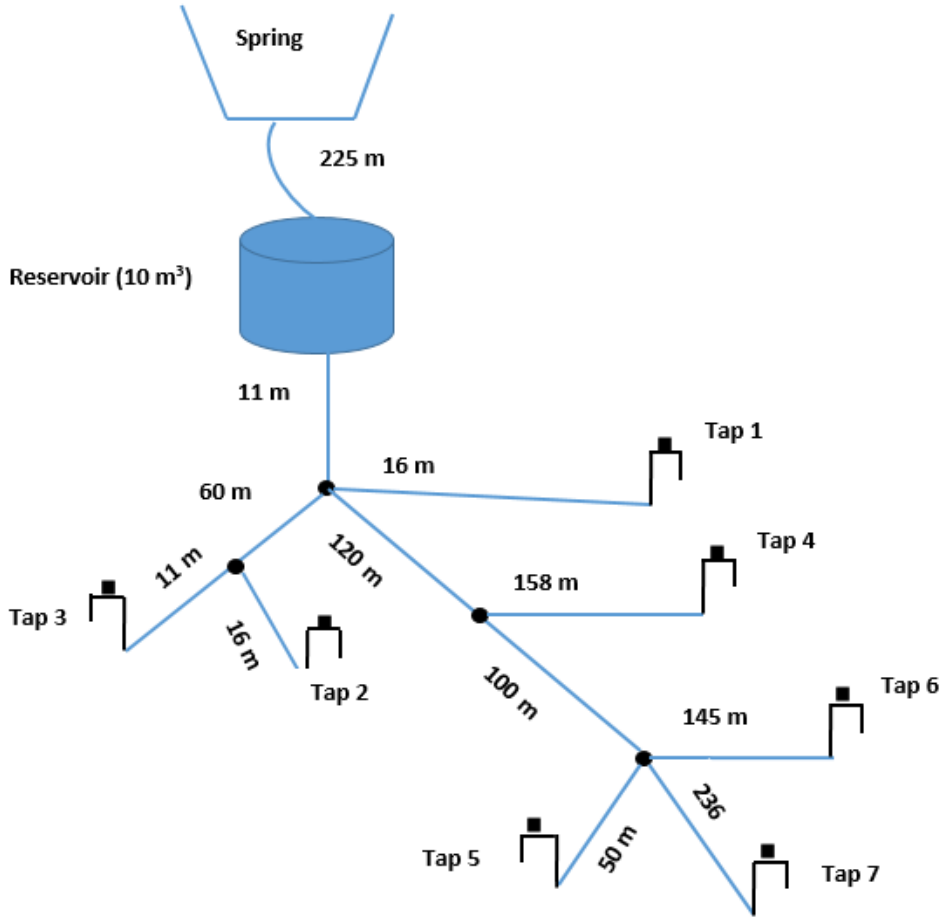


**Figure 2** Schematics of iPWS II. The distribution system is fed by protected spring water in the hills. The water travels to reservoir, where it is stored up to 10 hours. Subsequently, the water is distributed to community and private taps

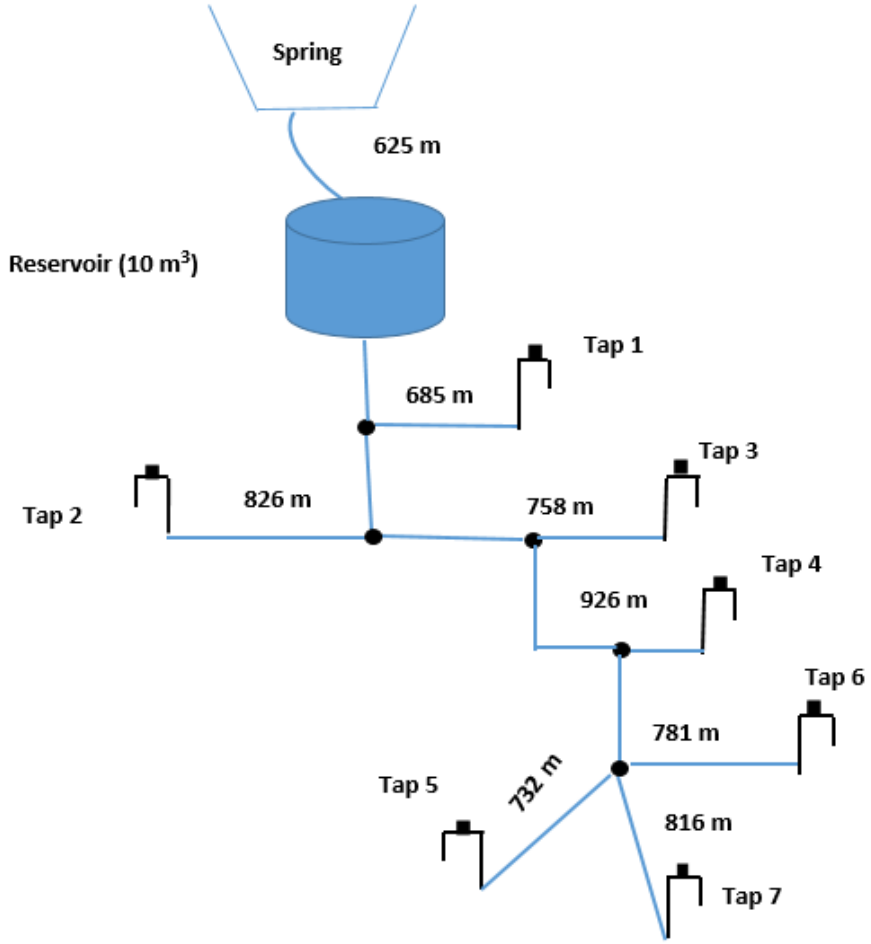


**Figure 3** Schematics of iPWS III. The distribution system is fed by protected spring water in the hills. The water travels to reservoir, where it is stored up to 10 hours. Subsequently, the water is distributed to community and private taps

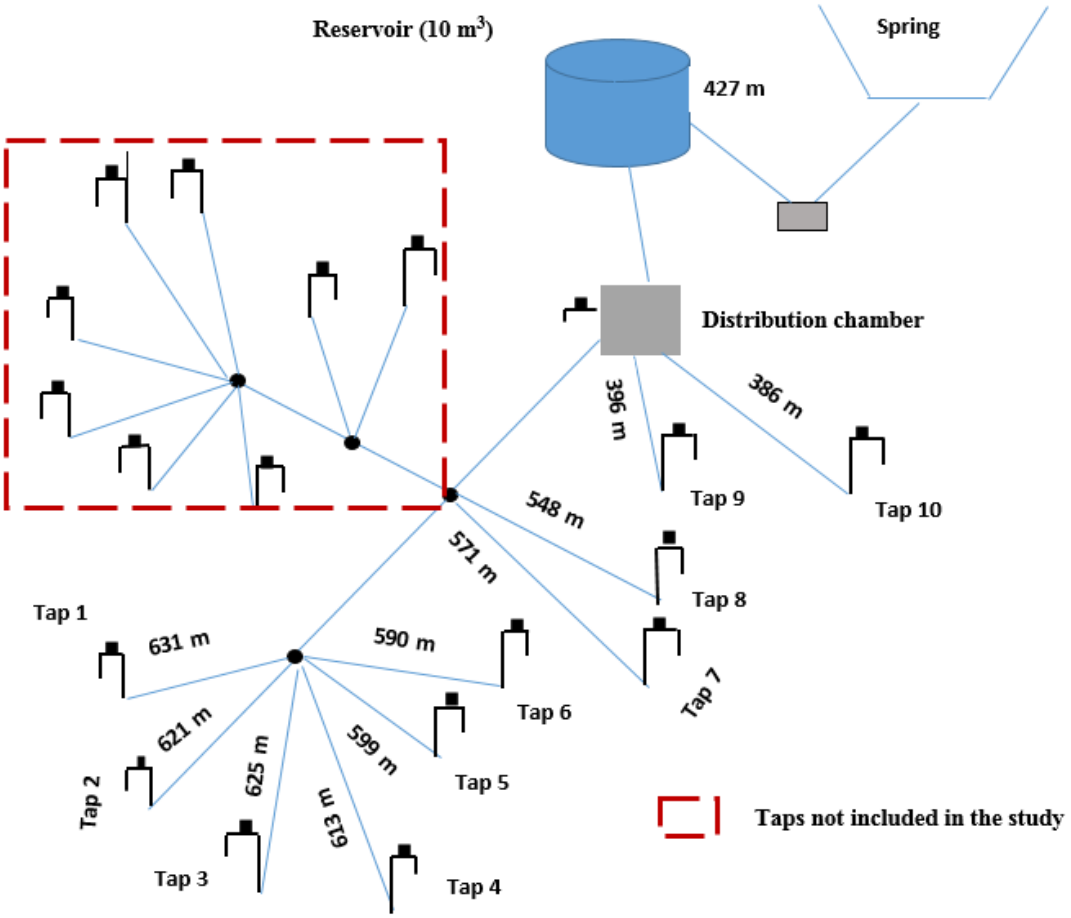


**Figure 4** Schematics of iPWS IV. The distribution system is fed by protected spring water in the hills. The water travels to reservoir, where it is stored up to 10 hours. Subsequently, the water is distributed to distribution chamber and private taps

CHAPTER 4

# Materials and methods

## 4.1. Materials

This sections describes the materials and consumable laboratory supplies used during the field work. The materials are powered either through solar light or batteries.

### 4.1.1. *DelAgua filtration set*

A DelAgua filtration set (OXFAM-DELAGUA, UK) was used to filter water samples. The filtration set comes with plastic funnel marked at 100 mL, manually operated vacuum pump, aluminium base, sample cup and plastic collar which connects the sample cup with the aluminium base (Fig 5). The filtration was done manually and does not require electricity which makes it suitable for rural areas. In addition, the sets can be easily sterilized with methanol or boiled water (DelAgua, 2009). The dimension of the filtration set used for this research was 34.4x14.6x29.7cm.

**Figure 5** DelAgua filtration set

### 4.1.2. *Incubator*

A field incubator was used to incubate compact dry plates to allow the growth of retained bacteria on a filter. The incubator was designed and manufactured at EMPA and Eawag and is light-weight, also the consumption of electricity is very low. The electrical power required for the operation of the incubator is supplied using a 12 V (20 ampere hour) battery which is connected to a solar voltaic array of 30 W (Fig 6). The inner chamber of the incubator is equipped with a fan, two thin-film heaters that are connected to sampling tray and an auxiliary heater as a backup when temperature drops. Optimum temperature was set at 35°C and the performance and inside temperature was recorded daily using iButton (Thermochron, UK) temperature loggers and external temperature regulator box.


**Figure 6** Solar voltaic array, incubator and external temperature regulator box

### 4.1.3. *Portable meters*

The analytical measurements of physical parameters were conducted using handheld HANNA instruments EC/TDS/pH/temperature meter (HI9813-6 multiparameter probe, US) (Fig 7).The instrument was calibrated using calibration fluids on a regular basis.

**Figure 7** HANNA EC/TDS/pH/temperature meter

### 4.1.4. *Rain gauge and wireless weather recorder*

Rain gauges were installed at two springs to record precipitation and rainfall events. In addition, a weather station equipped with a wireless temperature sensor was installed to monitor the outdoor temperature, pressure and humidity level.

### 4.1.5. *SteriPEN*

The SteriPEN operates by battery and uses optical water sensor and UV lamp to disinfect water (Fig 8). SteriPEN was regularly used to prepare sterile and blank water that was used to process sample and negative control.


**Figure 8 SteriPEN**

### 4.1.6. *GPS*

Etrex GPS was used to record the coordinate and elevation of sampling points, leakage points and location of toilets.

### 4.1.7. *Measuring tape*

Measuring tape was used to measure the distance between toilets and springs, reservoirs and taps.

## 4.2. Consumables

### 4.2.1. *Compact dry plate*

Compact dry plates (Nissui pharmaceutical, Tokyo) were used to grow microbial colonies. The plates are enriched with nutrients and chromogenic substrates required for microbial growth and develop colour changes depending on the type of bacterial colony. The chromogenic substrates Magenta GAL and X-GLUC give blue and pink colonies for *E. coli* and total coliforms respectively (Nissui, 2009).

### 4.2.2. *Membrane filter*

A sterile 0.45 µm nitrocellulose membrane filter with a diameter of 47 mm (Merck Millipore, Germany) was used to filter water samples.

## 4.3. Methodology

A comprehensive monitoring of iPWS was conducted for a duration of 35 days. Three intermittent piped water systems (iPWS II, iPWS III, iPWS IV) located in remote rural areas of Nepal were chosen for the study and monitored daily. The methodology involved:

- the detection and quantification of faecal indicator bacteria from water samples
- the assessment of risk factors and potential hazards of contamination through sanitary surveys
- assessing the possibility of infiltration of external contaminants into springs by conducting experimental tracer tests and
- monitoring environmental factors that might influence the microbial quality of water like temperature, precipitation and humidity by using sensors and portable devices.

Monitoring of microbial parameters was done at several sampling points (spring, reservoir and tap stands) of the supply system. Daily 25-30 samples were collected from all systems for microbial and physico-chemical analysis. A total of 691 samples were collected from all systems monitored. The analytical method included lab-based detection of *E. coli* and total coliforms. The sanitary inspections were based on JMP of UNICEF/WHO and addressed the risk factors for contamination and sanitary conditions of the supply system. Experimental salt tracer tests were conducted at springs of iPWS II, III, and IV to assess whether external contaminants influence the quality of water at the source.

## 4.4. Assessment of faecal contamination in iPWS's

### 4.4.1. *Selection of sampling points*

This study was conducted at intermittent piped water supply systems located in Dullu municipality of Mid-Western Nepal. iPWS located in Nauladhara and Koyashidara VDC were monitored daily. Simple random sampling was chosen as sampling approach. Randomly collecting samples from spring, reservoir and tap enables several parts of the network with different extent of risk for contamination to be included in the sampling scheme and allow to identify where along the network the possibility for contamination exists as well as to understand the dynamics of contamination between the sampling points. Based on this water samples were collected by randomly selecting sampling points of the supply system. Springs, reservoirs, and golden taps were monitored daily throughout the research phase, whereas tap stands from each iPWS were monitored on a five day cycle which means 2-4 taps of a one system were monitored for a duration of five day. Table 2 shows the systems and number of sampling points monitored.

**Table 2** iPWS and number of sampling points monitored

| iPWS | Spring | Reservoir | Tap stand | Distribution chamber | Total |
|---|---|---|---|---|---|
| **II** | 1 | 1 | 7 | - | 9 |
| **III** | 1 | 1 | 7 | - | 9 |
| **IV** | 1 | 1 | 10 | 1 | 13 |

### 4.4.2. *Sample collection*

Water samples were collected in the period between 19 November to 24 December 2015 from 6:30 a.m. and 7:30 a.m. Water samples for microbial analysis were collected using sterilized NascoWhirl-pak sample bags. Collected water samples were placed in an insulated heat resistant container. Sampling containers filled with sterilized water were used during sample collection to maintain temperature of the sample after collection and during transportation. Afterwards collected samples were transported to the laboratory site and processed within 6 hours after collection. Duplicate samples were collected every ten samples at one tap equipped with a flow and pressure meter (golden taps).

Sample collection at source was done by taking water with NascoWhirl-pak at the inlet of the springs. Samples at reservoirs were taken by opening the covering lid of the reservoir and taking the sample directly from the reservoir tank. In taps samples were collected after letting the water to flush for 20 seconds.
Samples for physical measurement were taken using a 100 mL glass beaker and the measurement was done onsite. Data collectors and laboratory personnel's were trained on aseptic ways of sample collection and analysis. During the collection process sample collectors used alcohol based Dettol hand sanitizers to reduce the chance of contamination during sample collection.

## 4.5. Analytical methods

### 4.5.1. *Membrane filtration technique*

The detection and enumeration of faecal indicator bacteria was conducted using the membrane filtration technique. DelAgua filtration sets were used to filter 100 mL of water sample through a 0.45 µm pore size filter which retains bacteria that are present in the water sample. The filter was transferred to a compact dry plate containing chromogenic substrate and incubated for 24 hours at 35 °C ±2 °C (EPA, 2002; Nissui, 2009). On the presence of a chromogenic substrate *E. coli* develops blue colonies, whereas pink or purple colonies are total coliforms. After incubation colonies grown on compact dry plate were counted manually and the concentration was reported as CFU/100 mL (See Appendix A). Microbial monitoring of *E. coli* and total coliform had a lower detection limit of 0 CFU/100 mL and upper detection limit of 300 CFU/100 mL. Water samples above 300 CFU/100 mL are reported as too numerous to count (TNTC).

### 4.5.2. *Quality control*

Quality control measures were taken in order to avoid cross-contamination during sample collection and processing. The filtration sets were disinfected using 37% methanol before and after samples were processed. Pipette tips were sterilized in boiling water before processing samples and kept in an insulated container to avoid contamination. The working bench was cleaned using alcohol wipes and swab samples were taken to check if there was contamination on the surface of the benches and analysed.
Swab samples were taken by rubbing surface of the working bench using compact dry swab and transferring the swab into a tube containing substrate solution afterwards the solution was transferred to a CDP and incubated at 37°C. Colonies were counted after 24 hrs of incubation.

Water sample diluted with chicken faeces was used as a positive control and used for daily testing. Likewise, sterilized water sample was used as a negative control and tested every day after processing the positive control sample. Growth of colonies on negative control reflects failure in sterilization process and samples analysed are considered as invalid. In addition, lab blanks and field blanks were tested if there was a possibility of contamination during sample collection and transport.

## 4.6. Sanitary inspections

Sanitary inspection checklists were used to identify potential hazards and risks that might affect the microbial quality of water. 27 sampling points (3 springs, 3 reservoirs and 21 taps) were inspected using sanitary checklists. The checklists address 10 sets of 'Yes' or 'No' questions (Table 3). 'Yes' answers show a higher probability for contamination and score 1 point, whereas 'No' answers indicate that the risk is insignificant and score 0 point. The 'Yes' answers were added together to yield a risk score ranging from 0-10. Risk categories were assigned based on the range of the risk score. A score of 9-10 has a very high risk for contamination, a risk score in the range between 6 and 8 has a high risk category and a risk score 3-5 and 0-2 represent medium and low risk category. The distance between toilets and sampling points was also measured according to the standard set by JMP (JMP, 2012).

Sanitary inspection was carried out using mWater surveyor app. mWater is an open source app intended to monitor and evaluate sub-targets and topics related to water, sanitation and hygiene of the SDG (retrieved from http://www.mwater.co/).The app encompasses three main important features linked to quality of water, sanitary condition and functionality of the water sources. In addition, using the app it is possible to add photos, description of the study site and to insert the corresponding locations using GPS. Moreover, mWater's app works offline which makes the app more suitable for rural areas where access to internet connection is limited.

mWater's sanitary inspection checklists are in line with the WHO/UNICEF JMP for Water Supply and Sanitation (see Appendix B). However, exporting completed surveys into Excel sheets is not supported by the application. Completed surveys were therefore exported manually into Excel sheets. Customizing the sanitary inspection checklist according to the local condition and system under study is also not possible as the checklists are set as a default.

**Table 3** Sanitary inspection checklist for springs [1]

| | | | |
|---|---|---|---|
| 1. | *"Is the spring source unprotected?"* | *Yes* | *No* |
| 2. | *"Is the masonry protecting the spring faulty?"* | *Yes* | *No* |
| 3. | *"Is the backfill area behind the retaining wall eroded?"* | *Yes* | *No* |
| 4. | *"Does split water flood the collection area?"* | *Yes* | *No* |
| 5. | *"Is the fence absent or faulty?"* | *Yes* | *No* |
| 6. | *"Can animals have access within 10 meters of the spring?"* | *Yes* | *No* |
| 7. | *"Is there a latrine uphill and/or within 30 meters of the spring?"* | *Yes* | *No* |
| 8. | *"Does surface water collect uphill of the spring?"* | *Yes* | *No* |
| 9. | *"Is the diversion above the spring absent or non-functional?"* | *Yes* | *No* |
| 10. | *"Are there any other sources of pollution uphill of the spring?"* | *Yes* | *No* |

## 4.7. Salt tracer experiment

Salt tracer experiments were conducted at springs of iPWS II, III and IV to monitor the possibility for infiltration of contaminants into the springs. 50 cm deep holes were dug upstream of the springs in 5, 10, and 15 m distance. Diluted water with a salt concentration of 1.42% was poured into the holes and change in electrical conductivity was monitored at the intake of the springs every two seconds with a portable probe (Fig 9). Water was poured in the holes with a 10 L bucket at 10 minutes interval. The initial electrical conductivity at time t=0 was recorded before injection of the electrolyte solution and the consecutive time series variations were also recorded until the completion of the experiment (Fig 10).

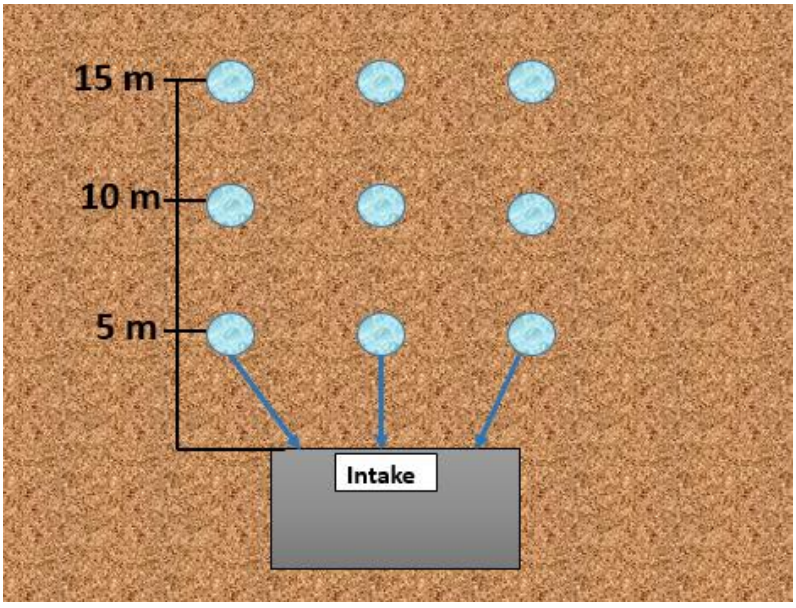


**Figure 9** Schematics of salt tracer experiment

[1] Check list adapted from WHO (1997), See Appendix B for sanitary inspection of reservoirs and taps.

**Figure 10** Recording change in electrical conductivity at intake of springs

## 4.8. Data analysis

Data entry and analysis was carried out using Microsoft Excel and SPSS version 22 to generate test statistics. Normality tests were carried out to understand the distribution of the data. Log transformation was done for variables which deviate from assumption of normal distribution and non-parametric Kruskal-Wallis test was carried out to determine if there is a significant difference between sampling points of the iPWS. Spearman's rank correlation was run to observe the correlation between faecal contamination and physical parameters. One was replaced for *E. coli* and total coliform concentrations below detection limits to avoid negative values during Log transformation. The results were considered significant for a *p*-value of $< 0.05$

## CHAPTER 5

# Results

## 5.1. iPWS II

### 5.1.1. *Temporal variability*

The daily variability result of *E. coli* bacteria at reservoir and spring of iPWS II showed a fluctuation during the monitoring period. A peak *E. coli* concentration of > 300 CFU/100 mL was detected at spring during the 4$^{th}$ week of the monitoring period. The fluctuation also coincides with *E. coli* concentration detected at the reservoir (Fig 11). Most of the observed peak *E. coli* levels correspond to the spring source which implies that contamination occurred at the spring prior to distribution of water to the reservoir tank. The contamination peaks were not associated with turbidity levels at the spring and no rainfall occurred during the monitoring period.

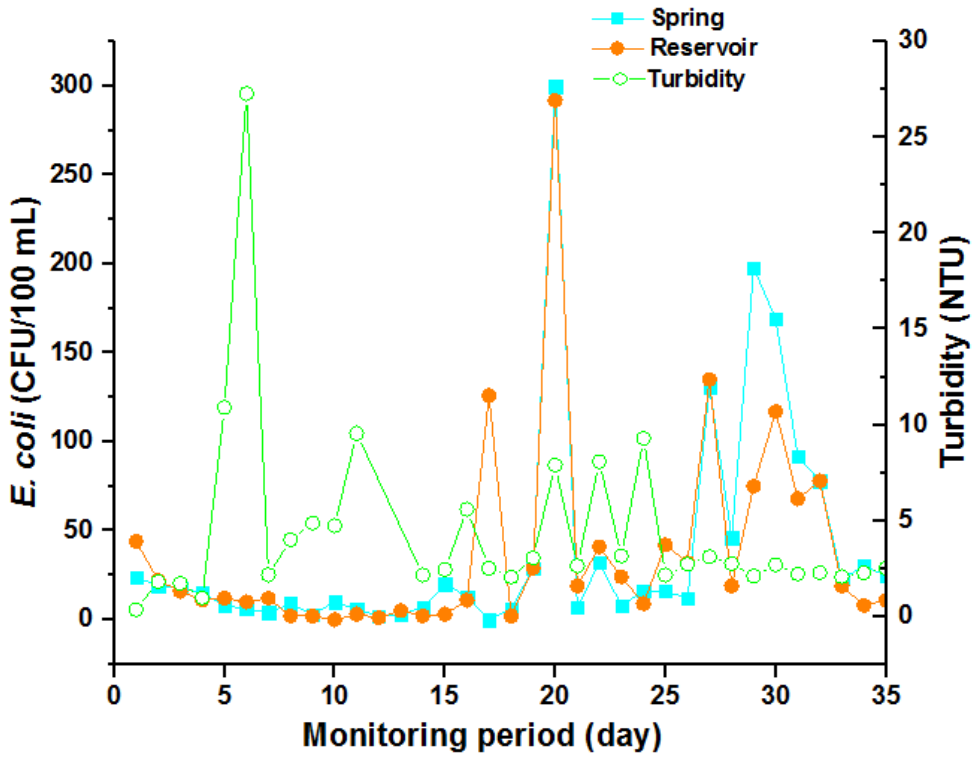


**Figure 11**Temporal variability of *E. coli* at iPWS II

### 5.1.2. *Temporal variability of total coliforms*

The trend of temporal fluctuation of total coliform over the course of 35 days was not consistent between reservoir and spring. At spring the concentration of total coliform fluctuated between 90 and upper detection limit of 300 CFU/100 mL, whereas at reservoir the variation ranged between 55 and 300 CFU/100 mL. Neither of the variations were associated with turbidity at the spring (Fig 12).

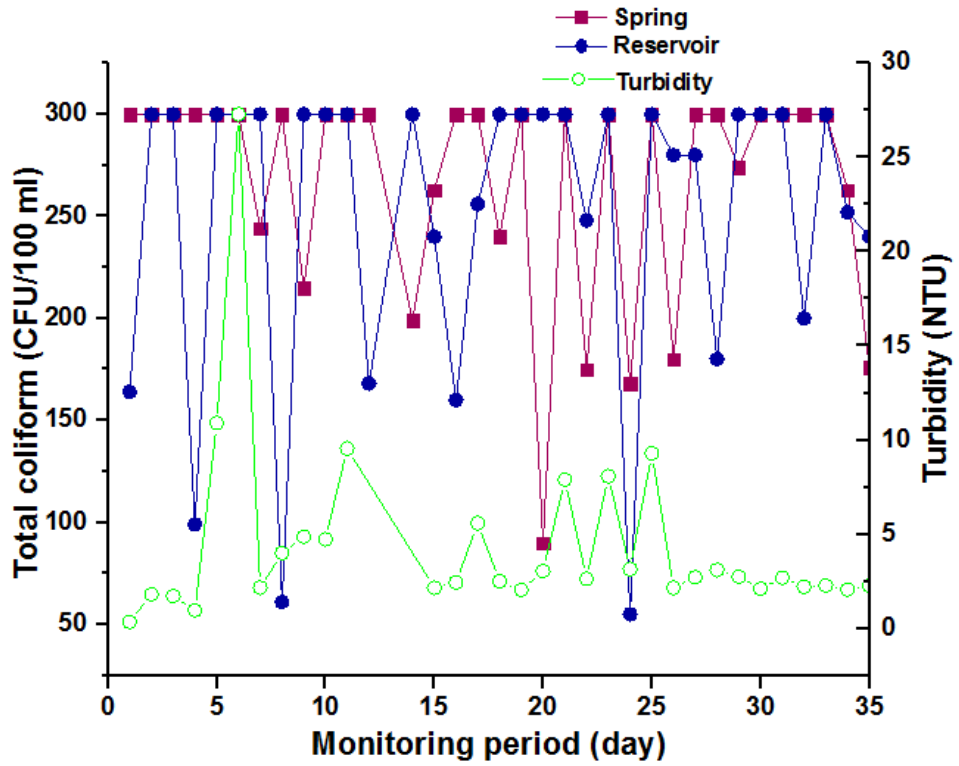


**Figure 12** Temporal variability of total coliforms

### 5.1.3. *Spatial variability of E. coli and total coliforms*

The daily monitoring result at iPWS II showed a higher percentage of samples being contaminated with *E. coli* and total coliforms. At spring almost 97% of samples had detectable *E. coli* concentration in 100 mL. Similarly, 97% of samples from reservoir contained *E. coli* bacteria above the standard of 0 CFU/100 mL. Moreover, the level of contamination at taps goes beyond 98%. Total coliforms were detected in all (100%) of samples taken from this system. Table 3 shows the descriptive statistics.

**Table 3** Descriptive statistics of *E. coli* and total coliforms with mean, standard deviation and quantiles at iPWS II.

| Indicator bacteria | Sampling points | n | Quantiles | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Min | 25% | Median | 75% | Max | % meeting WHO standard |
| *E. coli (CFU/100 mL)* | Spring | 35 | 39.5 | 65 | 0 | 7 | 16 | 29.5 | TNTC | 2.9 |
| | Reservoir | 36 | 38 | 56 | 0 | 6.5 | 17.5 | 43 | 292 | 2.8 |
| | Tap 1 | 20 | 20.4 | 26.5 | 0 | 3 | 8 | 24 | 85 | 5 |
| | Tap 2 | 14 | 57.2 | 82.4 | 3 | 12 | 25 | 60 | TNTC | 0 |
| | Tap 3 | 5 | 62.6 | 39.8 | 20 | 20 | 80 | 89 | 140 | 0 |
| | Tap 4 | 6 | 59.6 | 50 | 19 | 29 | 46 | 63 | 155 | 0 |
| | Tap 5 | 7 | 67.4 | 43.9 | 15 | 43.5 | 59 | 80 | 151 | 0 |
| | Tap 6 | 10 | 28.6 | 31.7 | 4 | 13 | 18.5 | 34 | 113 | 0 |
| | Tap 7 | 11 | 11 | 13.4 | 2 | 5 | 7 | 9 | 50 | 0 |
| Total coliform (CFU/100 mL) | Spring | 35 | 267 | 53 | 90 | 253 | TNTC | TNTC | TNTC | 0 |
| | Reservoir | 36 | 261 | 65.4 | 73 | 250 | TNTC | TNTC | TNTC | 0 |
| | Tap 1 | 20 | 234 | 87 | 66 | 173 | TNTC | TNTC | TNTC | 0 |
| | Tap 2 | 14 | 205 | 95 | 30 | 148 | 226 | TNTC | TNTC | 0 |
| | Tap 3 | 5 | 214 | 105 | 68 | 140 | 265 | TNTC | TNTC | 0 |
| | Tap 4 | 6 | 228 | 60.2 | 149 | 199 | 212 | TNTC | TNTC | 0 |
| | Tap 5 | 7 | 244 | 67.4 | 129 | 207 | 264 | TNTC | TNTC | 0 |
| | Tap 6 | 10 | 179 | 103 | 63 | 81 | 173 | 284 | TNTC | 0 |
| | Tap 7 | 11 | 191 | 79 | 58 | 130 | 218 | 243 | TNTC | 0 |

The data sets at sampling points of iPWS II were grouped into three categories to reduce the effect of spatial variability between the sampling points and to better understand the underlying variability. Based on this, tap 1 and tap 4 were compared in one category against spring and reservoir. Similarly, tap 2 and tap 3 were grouped together and compared with spring and reservoir. Finally, the spatial variability within spring and reservoir were compared against tap 5, 6, and 7. Kruskal-Wallis test was run for each categories of data sets to understand the spatial variability between spring, reservoir and taps. Fig 13 shows layout of the system and sampling points.

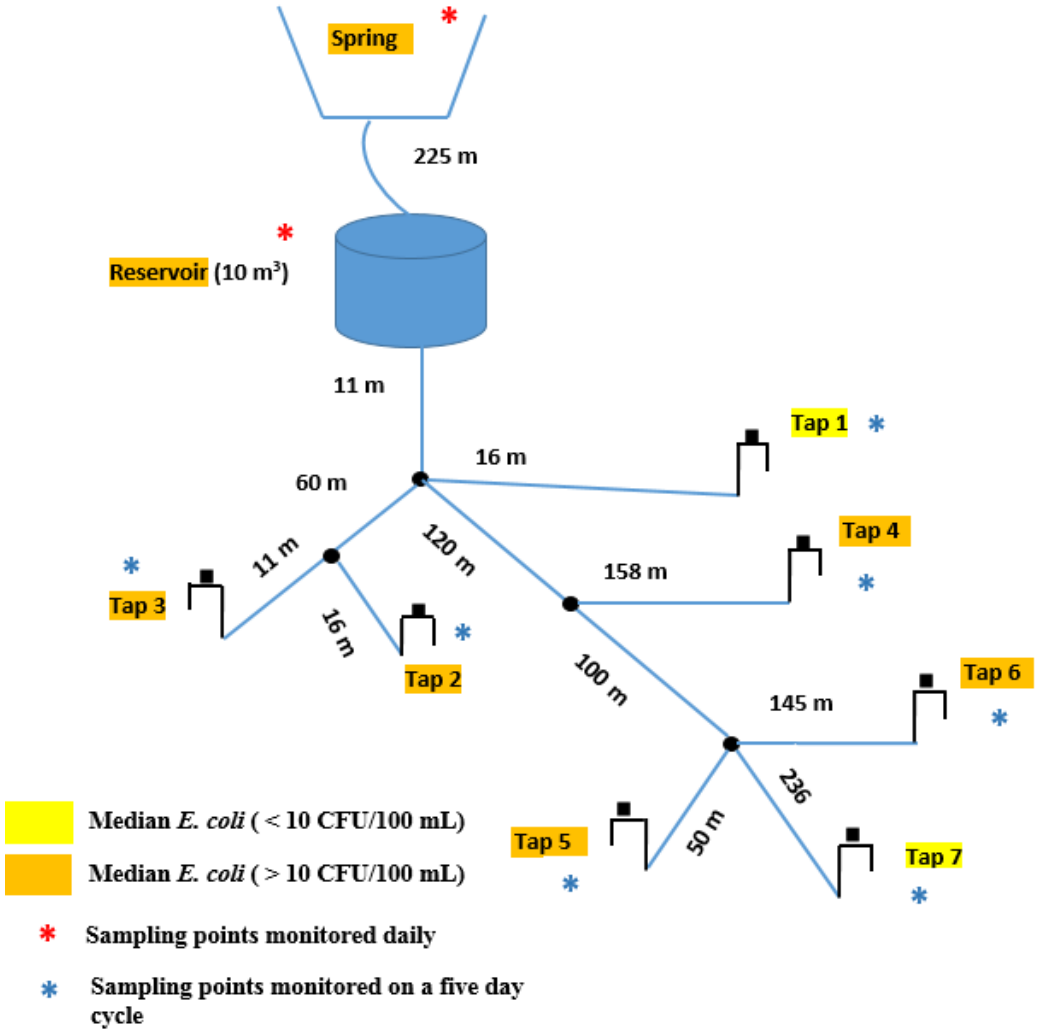


**Figure 13** Sampling points of iPWS II

The result at spring, reservoir, tap 1 and tap 4 of iPWS II showed no significant spatial difference in distribution of *E. coli* between the sampling points of the supply system (Kruskal-Wallis, n= 97, *p=0.07)* (Fig 14). Similarly, the distribution of total coliforms at spring, reservoir, Tap 1 and Tap 4 of iPWS II showed no significant spatial variability between the sampling points (Kruskal-Wallis, n= 97, *p=0.34)* (Fig 15). Likewise, the spatial variability of *E. coli* between tap 2 and tap 3 was found to be not significant (Kruskal-Wallis, n= 90, *p=0.06)* (Fig 16). However, a significant variability was observed in distribution of total coliforms between spring, reservoir, tap 2 and tap 3 (Kruskal-Wallis, n= 90, $p < 0.05$) (Fig 17). Similarly, the distribution of *E. coli* and total coliforms between taps 5, 6, and 7 was found to be statistically significant when compared with spring and reservoir (Kruskal-Wallis, n= 99, $p < 0.05$) (Fig 18 and 19).

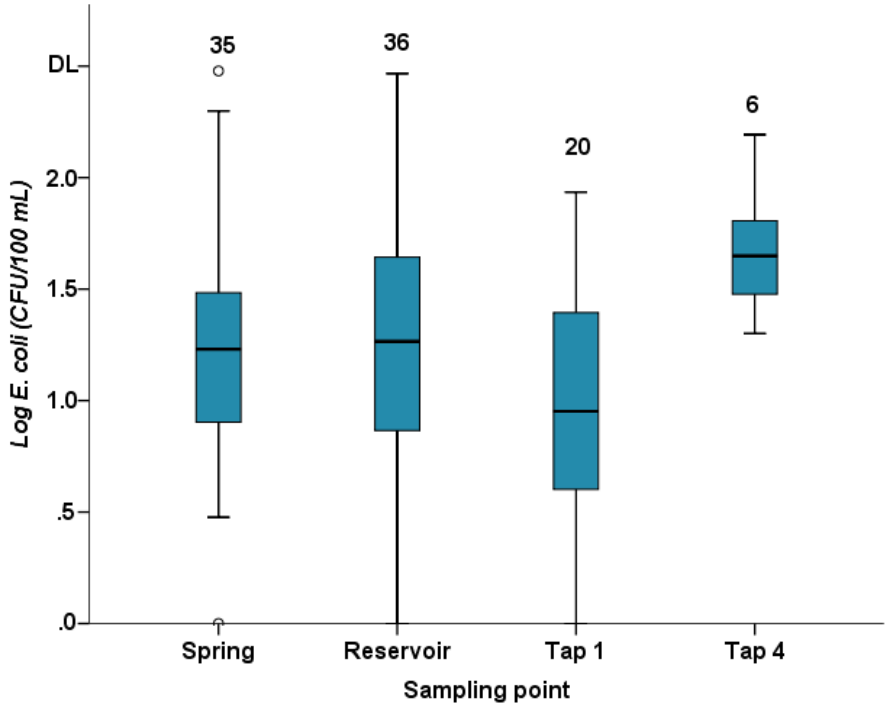


**Figure 14** Box and whisker plot of *E. coli* at spring, Reservoir, Tap 1 and Tap 4 of iPWS II.

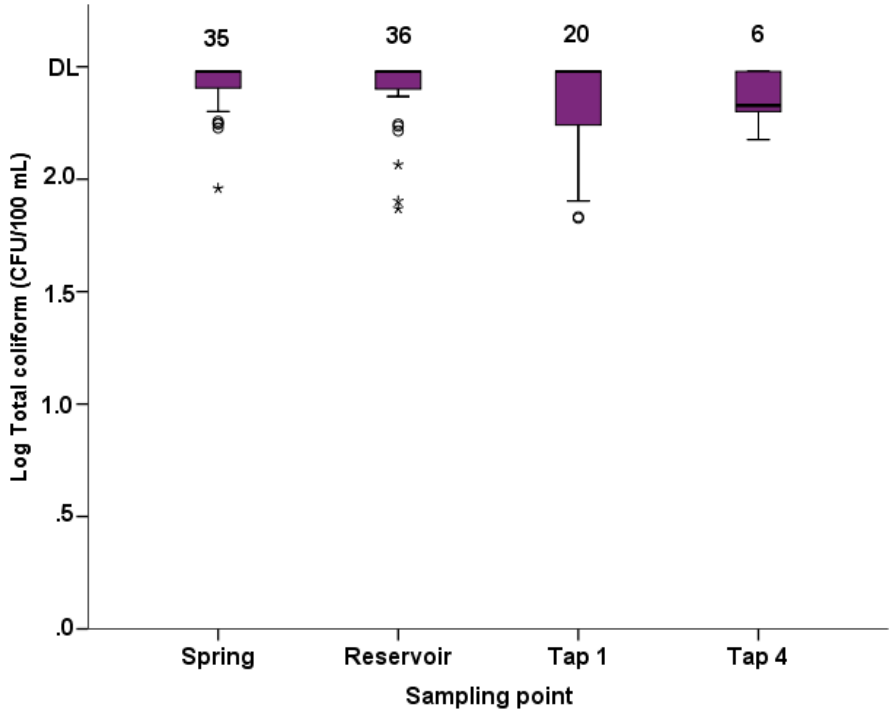


**Figure 15** Box and whisker plot of total coliforms at spring, Reservoir, Tap1 and Tap 4 of iPWS II.

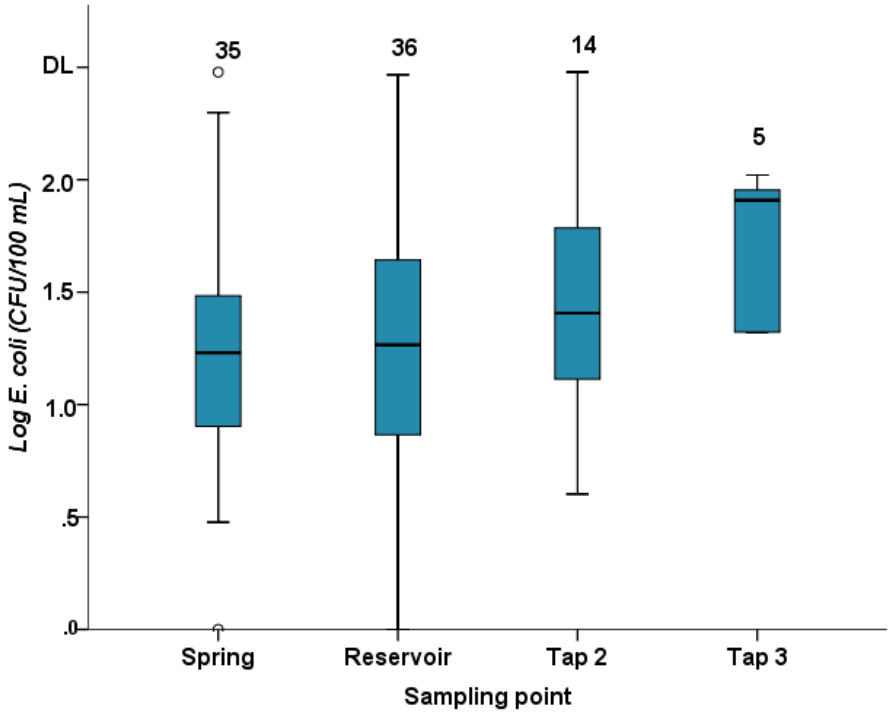


**Figure 16** Box and whisker plot of *E. coli* at spring, Reservoir, Tap2 and Tap 3 of iPWS II.

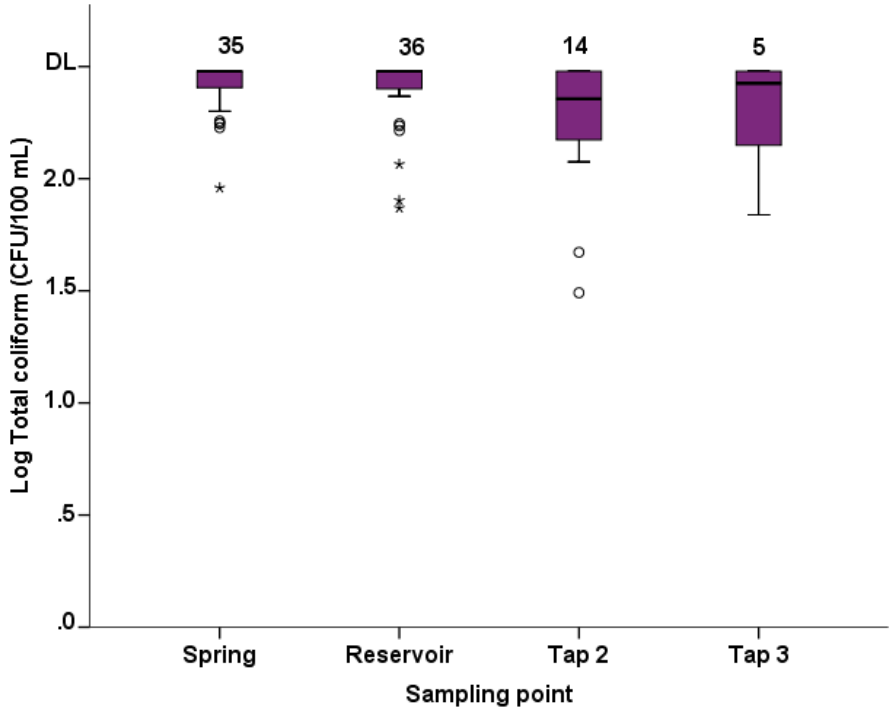


**Figure 17** Box and whisker plot of total coliforms at spring, Reservoir, Tap2 and Tap 3 of iPWS II.

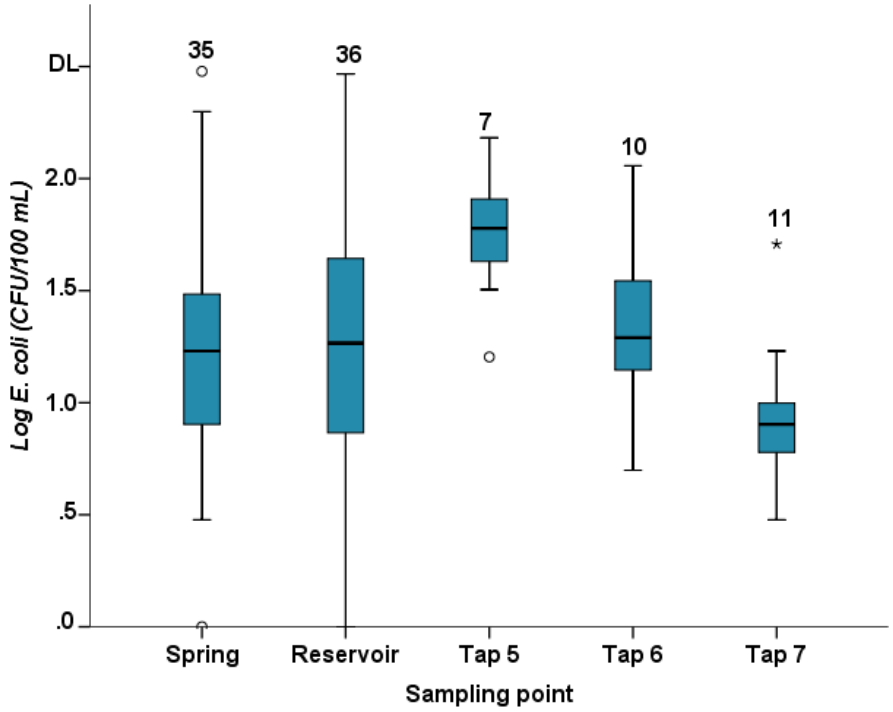


**Figure 18** Box and whisker plot of *E. coli* at spring, Reservoir, Tap 5, 6 and 7 of iPWS II.

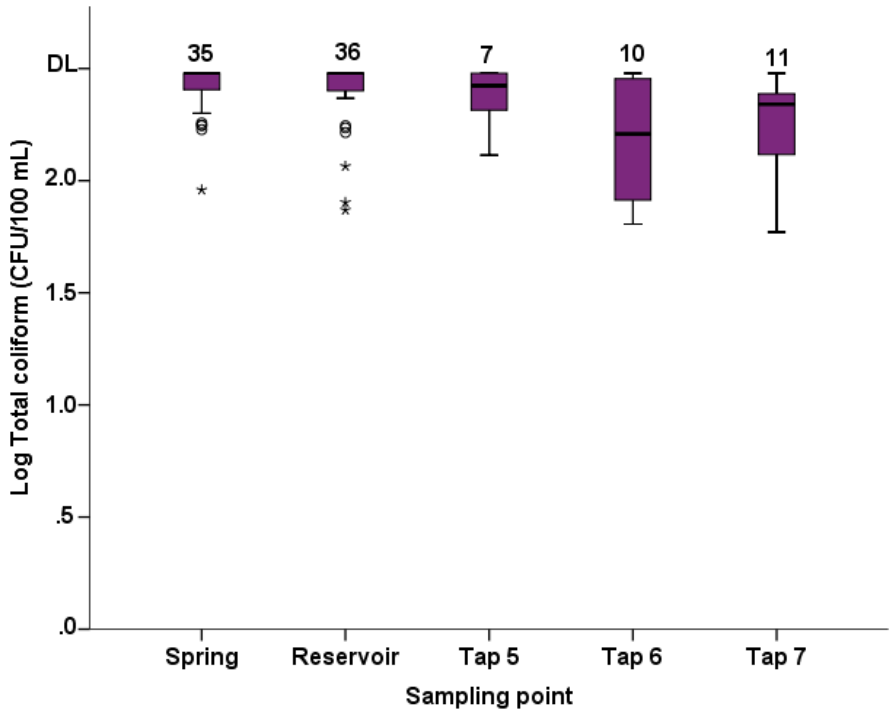


**Figure 19** Box and whisker plot of total coliforms at spring, Reservoir, Tap 5, Tap 6 and Tap 7 of iPWS II.

### 5.1.4. *Sanitary inspection*

Sanitary inspections were conducted to assess the risk of contamination at the sampling points of iPWS II. The results showed that the spring source was not protected and prone to contamination from surface-runoff. Local communities living around the spring use uphill of the area as an agricultural field to plant crops and grains due to this the area around the spring was frequently irrigated. The fence of the spring was absent. Animals could easily access the spring source within 10 m distance (Fig 20). The spring source was not constructed in a way to divert flood or run off from uphill of the spring source during rainfall events and no diversion ditches were observed around the area. No human faeces were seen around 10 m of the spring source, however animal faeces were seen frequently. Except for the reservoir uphill of all the sampling points (taps and spring) of iPWS II were eroded. Out of the seven taps inspected, 6 were located within 30 m range of toilet, the shortest distance being 7 m and 8 m at tap 6 and at tap 3 respectively. Leakage was observed between pipe connecting reservoir with tap 5 and tap 6. The spring of iPWS II and tap 6 had a very high risk category, whereas the risk category of the other sampling points ranged between low (tap 1, tap 3 and reservoir) and medium (tap 4, tap 5 and tap 7).


**Figure 20** Spring of iPWS II and leaking pipe

### 5.1.5. *Tracer test*

The possibility for intrusion of external contaminants into spring sources of iPWS II was monitored by conducting simple salt tracer test at the upstream of the spring. A salt concentration of 1.42% was dosed upstream of the spring and change in electrical conductivity was monitored at the intake of the spring. The initial EC measured at the intake of the spring was 80 µS/cm and no major change was observed until t=60 min. After dosing the electrolyte solution a fluctuation in electrical conductivity was observed at intake of the spring. The measurement of EC changed from an initial value of 80 µS/cm at t=0 to a peak value of 159 µS/cm at t= 120 min.

The electrolyte solution took almost one hour to infiltrate through the ground into the pathway of the spring (Fig 21). The infiltration of the electrolyte solution to the ground could be due to type of soil (loam), pore size of the soil and drainage capacity.

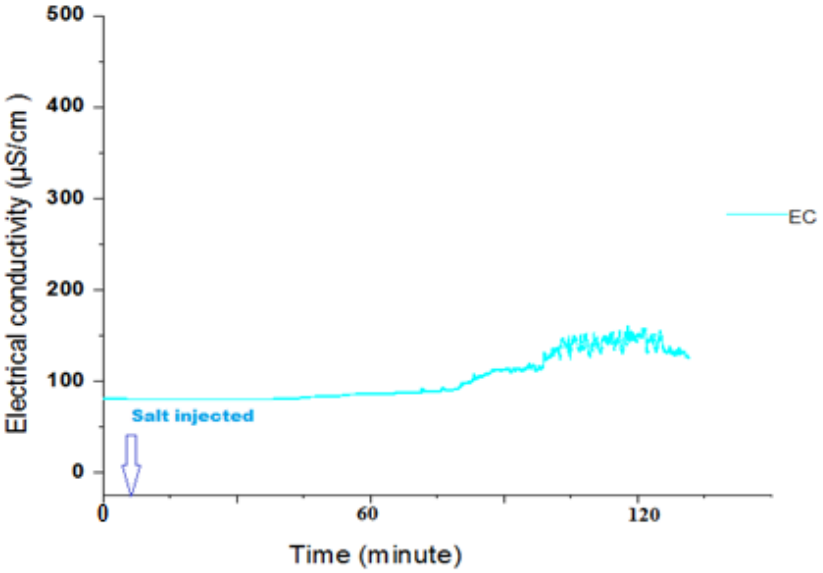


**Figure 21** Salt tracer test at intake of iPWS II

Overall, the results of iPWS II revealed that 97% of samples (n=35) collected from the spring source were contaminated with *E. coli* bacteria. This could be related with poor infrastructural setup and construction of the spring favouring infiltration of contaminants. The result of the sanitary inspection at the spring also showed a higher risk for contamination from external source. Moreover, the salt tracer test explains the underlying mechanism of intrusion into the spring source. The observed spatial variability between spring, reservoir, and taps (5, 6 and 7) might be linked with the leakage detected at tap 5. Also, the median *E. coli* detected at tap 5 was found to be higher (59 CFU/100 mL) when compared with spring (16 CFU/100), reservoir (17.5 CFU/100 mL), tap 6 (18.5 CFU/100 mL) and tap 7 (7 CFU/100 mL).

## 5.2. iPWS III

### 5.2.1. *Temporal variability*

At spring of iPWS III a peak *E. coli* concentration of 42 and 71 CFU/100 mL was detected during the 2nd (day 5-10) and 3rd (day 10-15) week of the monitoring period, while at reservoir the detected *E. coli* concentration ranged between 0 and 5 CFU/100 mL and no major variation was observed. No consistent variation was detected in concentration of *E. coli* at the sampling points (Fig 22). In addition, the trend of fluctuation was relatively stable when compared with iPWS II. The measured turbidity level at the spring was higher when compared with iPWS II and iPWS IV and association between *E. coli* bacteria and turbidity was also observed.

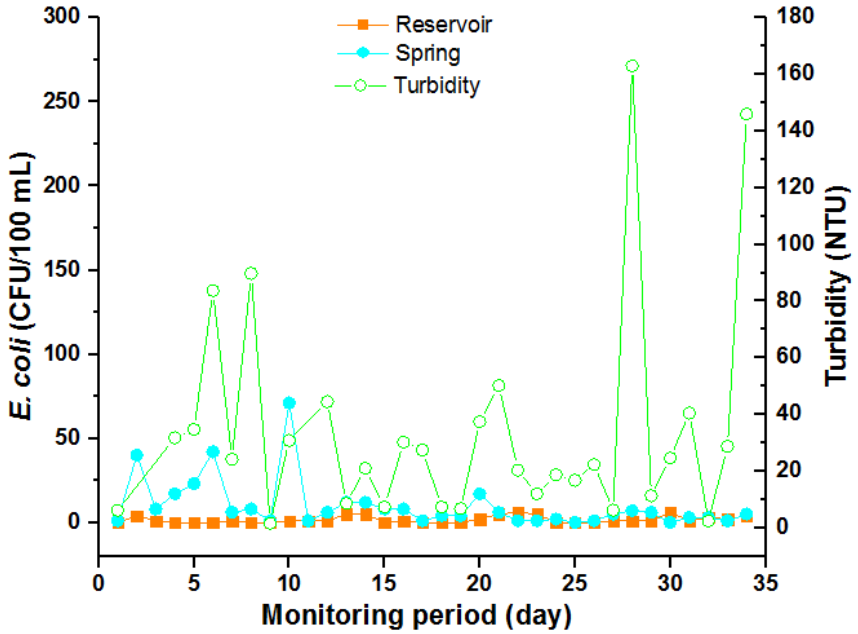


**Figure 22** Temporal variability of *E. coli* at iPWS III

### 5.2.2. *Temporal variability of total coliforms*

The temporal variability of total coliform at spring and reservoir of iPWS III showed non consistent pattern of fluctuation between the sampling points. Frequent upper detection limit of >300 CFU/100 mL was detected at spring of iPWS III than the reservoir (Fig 23). No association in concentration of total coliforms and turbidity was observed at sampling points of iPWS III.

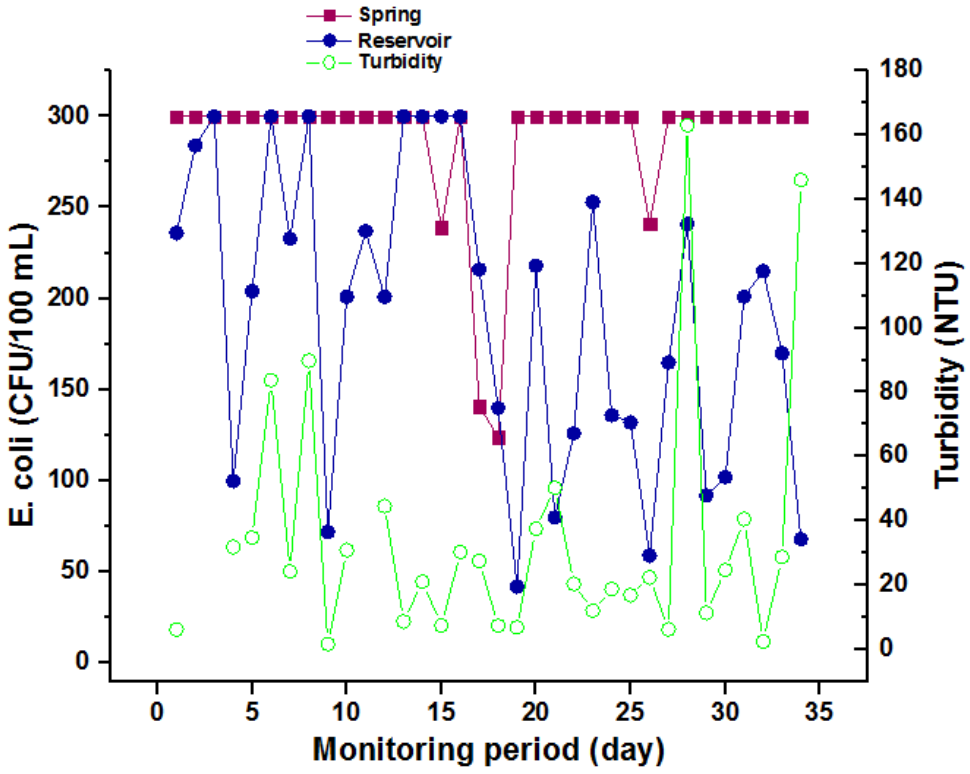


**Figure 23** Temporal variability of total coliforms

### 5.2.3. *Spatial variability of E. coli and total coliforms*

At iPWS III the highest median *E. coli* concentration was detected at the spring source with a median value of 5.5 CFU/100 mL when compared with reservoir (1 CFU/100 mL) and taps (1CFU/100 mL). Table 4 shows the descriptive statistics and percent of samples meeting the WHO drinking water quality standard.

**Table 4** Descriptive statistics of *E. coli* and total coliforms with mean, standard deviation and quantiles at iPWS III.

| Indicator bacteria | Sampling points | n | Quantiles | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Min | 25% | Median | 75% | Max | % meeting WHO standard |
| *E. coli* (CFU/100 mL) | Spring | 34 | 9.7 | 14.7 | 0 | 1 | 5.5 | 8 | 71 | 5.9 |
| | Reservoir | 35 | 1.7 | 2 | 0 | 0 | 1 | 3 | 6 | 37.1 |
| | Tap 1 | 21 | 1.8 | 2.6 | 0 | 0 | 1 | 2 | 10 | 28.6 |
| | Tap 2 | 13 | 1.92 | 1.9 | 0 | 0 | 2 | 2 | 5 | 30.8 |
| | Tap 3 | 7 | 3.7 | 3.5 | 0 | 1 | 3 | 5.5 | 10 | 14.3 |
| | Tap 4 | 7 | 1.5 | 1.2 | 0 | 0.5 | 2 | 2.5 | 3 | 28.6 |
| | Tap 5 | 14 | 1 | 1.8 | 0 | 0 | 1 | 5 | 7 | 42.9 |
| | Tap 6 | 9 | 3.5 | 3 | 1 | 1 | 2 | 5 | 9 | 0 |
| | Tap 7 | 9 | 2.3 | 1.6 | 0 | 2 | 2 | 3 | 5 | 22.2 |
| Total coliform (CFU/100 mL) | Spring | 34 | 286 | 41.6 | 124 | TNTC | TNTC | TNTC | TNTC | 0 |
| | Reservoir | 35 | 188 | 83.8 | 42 | 114 | 201 | 247 | TNTC | 0 |
| | Tap 1 | 21 | 202 | 79 | 63 | 161 | 217 | 256 | TNTC | 0 |
| | Tap 2 | 13 | 139 | 61 | 60 | 89 | 129 | 174 | 238 | 0 |
| | Tap 3 | 7 | 109 | 43.2 | 32 | 95.5 | 101 | 141 | 161 | 0 |
| | Tap 4 | 7 | 140 | 92.8 | 33 | 75.5 | 134 | 181 | TNTC | 0 |
| | Tap 5 | 14 | 202 | 75.3 | 84 | 127 | 221 | 241 | TNTC | 0 |
| | Tap 6 | 9 | 154 | 62.3 | 58 | 137 | 153 | 202 | 255 | 0 |
| | Tap 7 | 9 | 131 | 70.8 | 46 | 85 | 99 | 210 | 231 | 0 |

The data sets at iPWS III were organized in a way to better understand the spatial distribution of *E. coli* and total coliforms among sampling points. Based on this, sampling points were grouped into 3 sets. For the spatial variability tap 1 and tap 2 were categorized in one group and compared against reservoir and spring. Similarly, tap 3 and 4 were grouped in one set and the distribution was compared with spring and reservoir. Finally, tap 5, 6, and 7 were also organized in a similar way and compared with spring and reservoir. Kruskal-Wallis test was performed to compare the distribution of *E. coli* between the sampling points. Fig 24 shows layout of the system and sampling points.

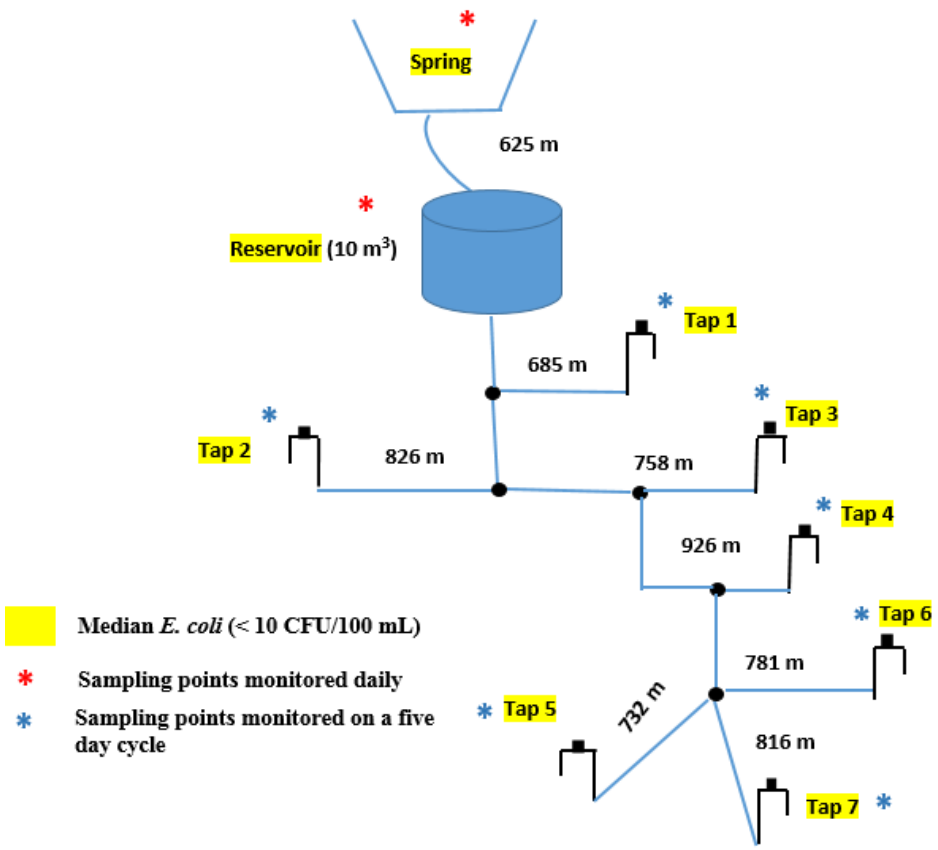


**Figure 24** Sampling points of iPWS III

Statistically significant variability was observed in concentration of *E. coli* and total coliforms at spring, reservoir, tap 1 and tap 2 of iPWS III (Kruskal-Wallis, n= 103, $p < 0.01$) (Figure 25 and 26). Similarly, the distribution of *E. coli* and total coliforms at taps 3 and 4 showed a variation between spring, reservoir (Kruskal-Wallis, n= 83, $p < 0.01$) (Figure 27 and 28). In addition, a significant variability was observed in median distribution of *E. coli* and total coliforms between spring, reservoir and taps 5, 6 and 7 (Figure 29 and 30). At iPWS III the gradient of *E. coli* concentration decreases from spring to reservoir, however the median concentration is the same between reservoir and taps and no significant difference exists between the two points ($p$=0.32). The spring source had the highest total coliform concentration with a median value of > 300 CFU/100 mL, whereas at reservoir and taps the concentration of total coliforms detected was 201 CFU/100 mL and 161 CFU/100 mL respectively.

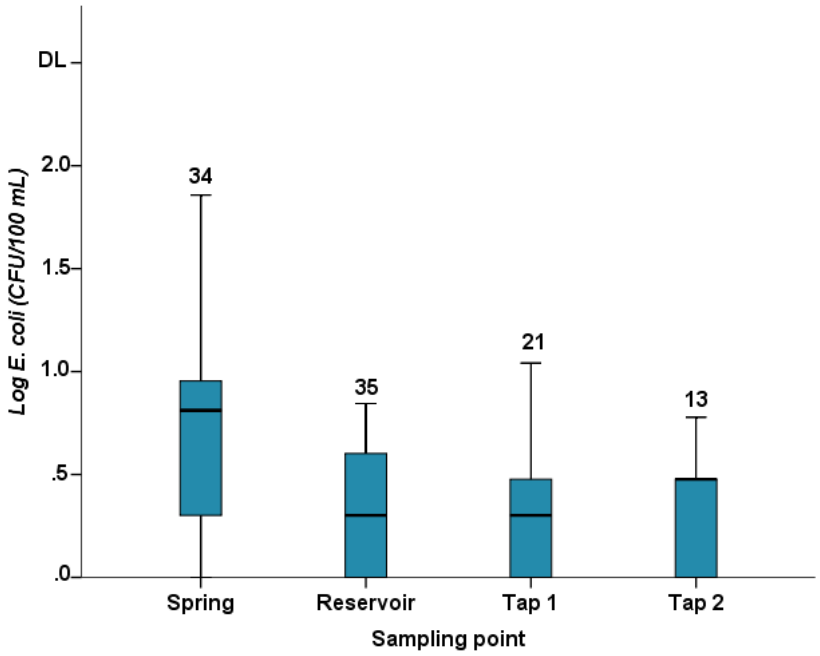


**Figure 25** Box and whisker plot of *E. coli* at spring, Reservoir, Tap 1 and Tap 2 of iPWS III.

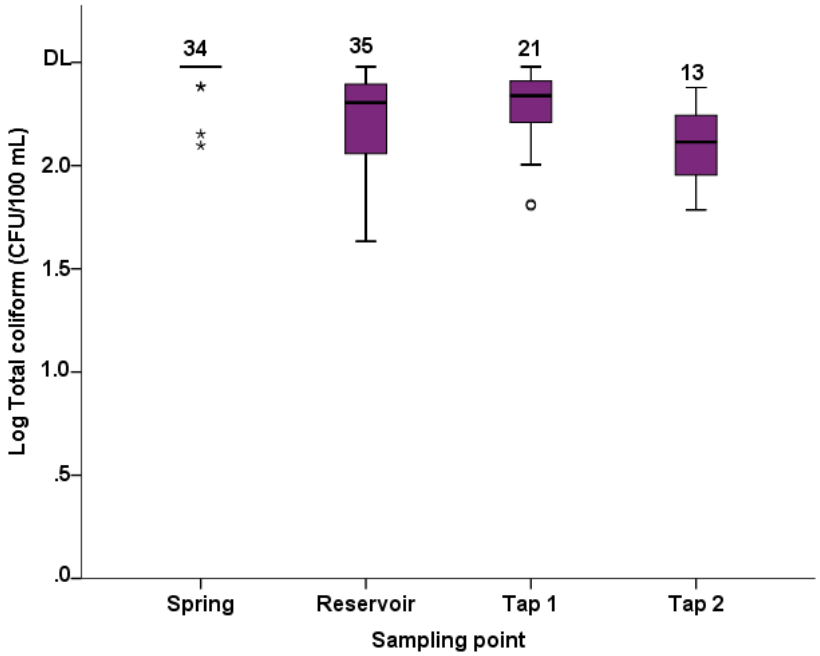


**Figure 26** Box and whisker plot of total coliforms at spring, reservoir, tap 1 and tap 2 of iPWS III.

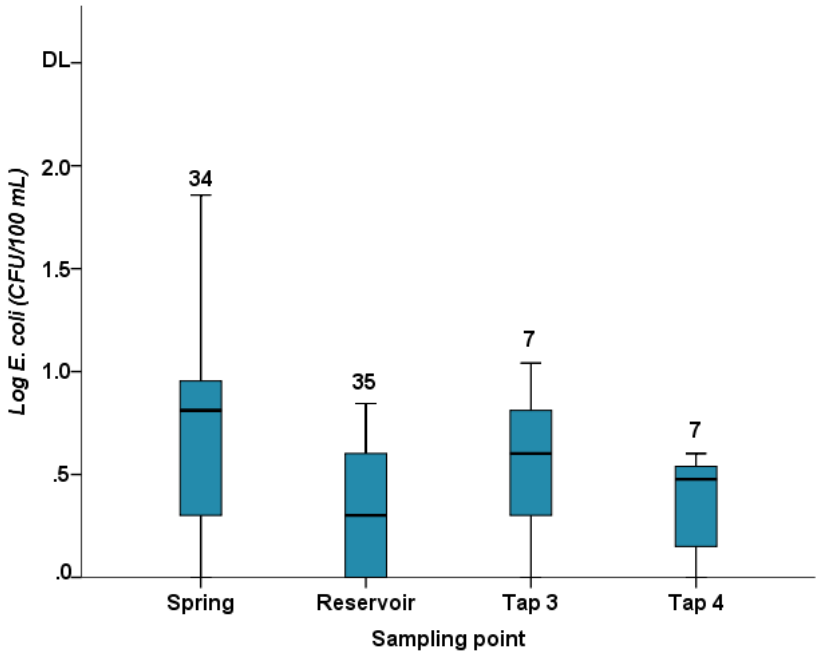


**Figure 27** Box and whisker plot of *E. coli* at spring, reservoir, tap 3 and tap 4 of iPWS III.

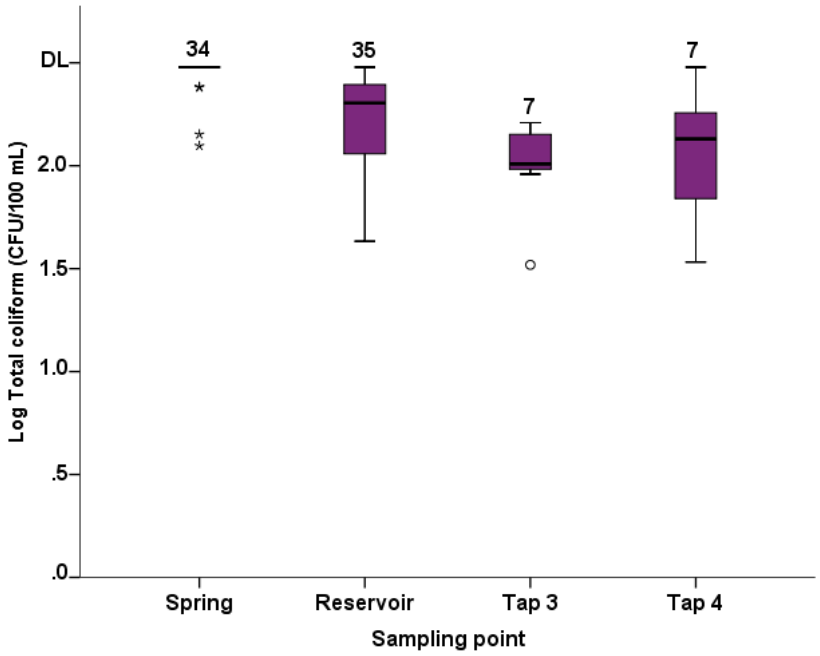


**Figure 28** Box and whisker plot of total coliforms at spring, reservoir, and taps of iPWS III.

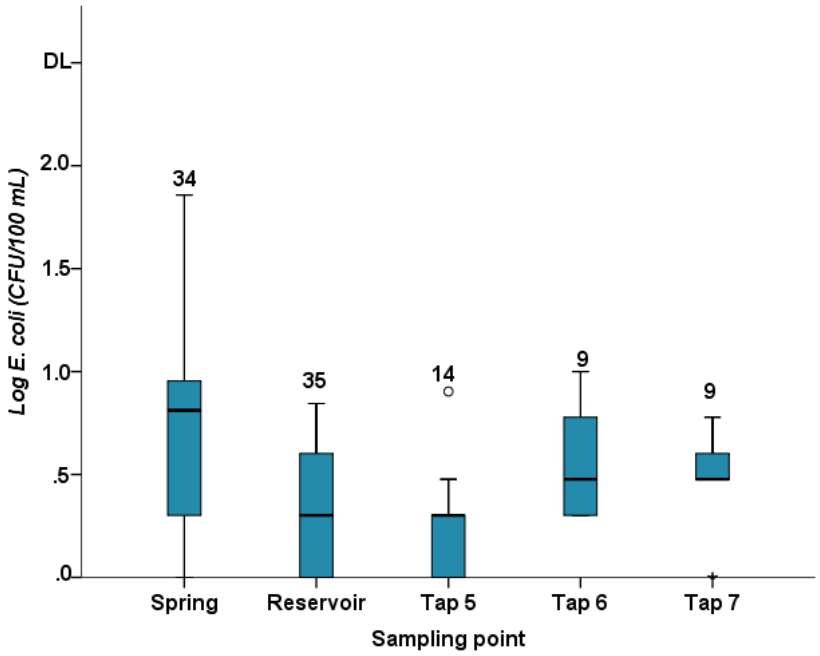


**Figure 29** Box and whisker plot of *E. coli* at spring, reservoir and taps of iPWS III.

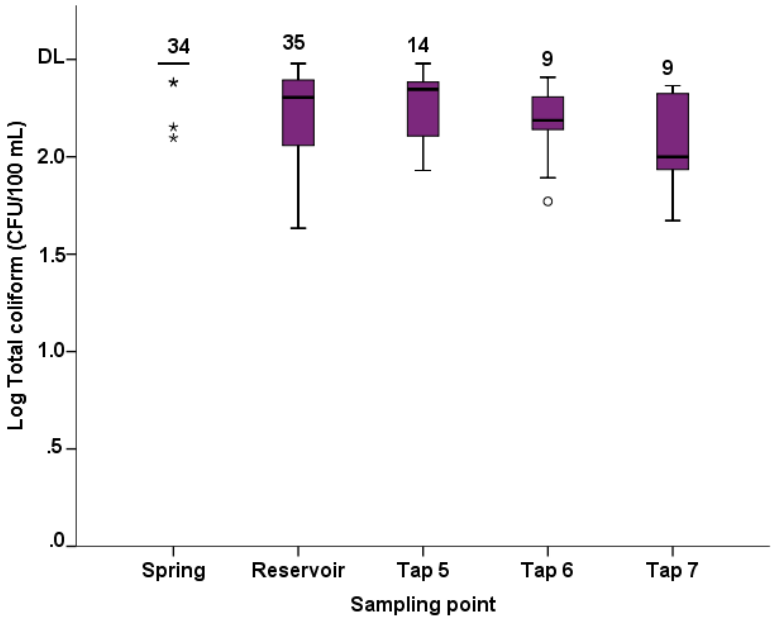


**Figure 30** Box and whisker plot of total coliforms at spring, reservoir, and taps of iPWS III.

### 5.2.4. *Sanitary inspection*

The intake of spring III was not protected with spring box during the inspection and the inlet was covered with decomposed leaves, debris and soil (Fig 31). The fence of the spring was damaged and humans and animals could easily access the spring source within 10 m distance. Also, the area uphill of the spring was used as a plantation field due to these the soil around the spring source was well saturated with moisture and surface water collects around the spring area. No signs of human faeces were seen within 10 m of the spring, while, animal faeces were seen frequently around the spring, reservoir and tap stands. No leakage was detected between spring and reservoir, however leakage was observed after the water was distributed from the reservoir. Uphill of the reservoir was eroded and no sewer or latrine was seen in close proximity with the reservoir. However, at taps toilets were located within 30 m distance from the tap stands. The distance between tap stand of tap 6 and toilet is only 3.7 m and tap 5 was 18 m away from latrine. At tap 6 the main pipe line connected to the tap stand was exposed to the surface and not buried underground. Generally, the risk category at the sampling points of iPWS III ranged between very high (spring), medium (reservoir), and low (tap 1, tap 2, tap 3, tap 4 and tap 5).


**Figure 31** Shows the intake of the spring and pipe connecting the outlet to reservoir

### 5.2.5. *Salt tracer*

The EC before injection of electrolyte solution at spring of iPWS III was 219 µS/cm at t=0. After injection and recording the consecutive time series variations a slight increasing trend was observed from t=35 min onwards. At t=35 min, the EC changed from the initial measurement of 219 µS/cm to 273 µS/cm. Afterwards, an increasing pattern was observed until the EC reached a peak value of 494 µS/cm at around t=120 minute (Fig 32).

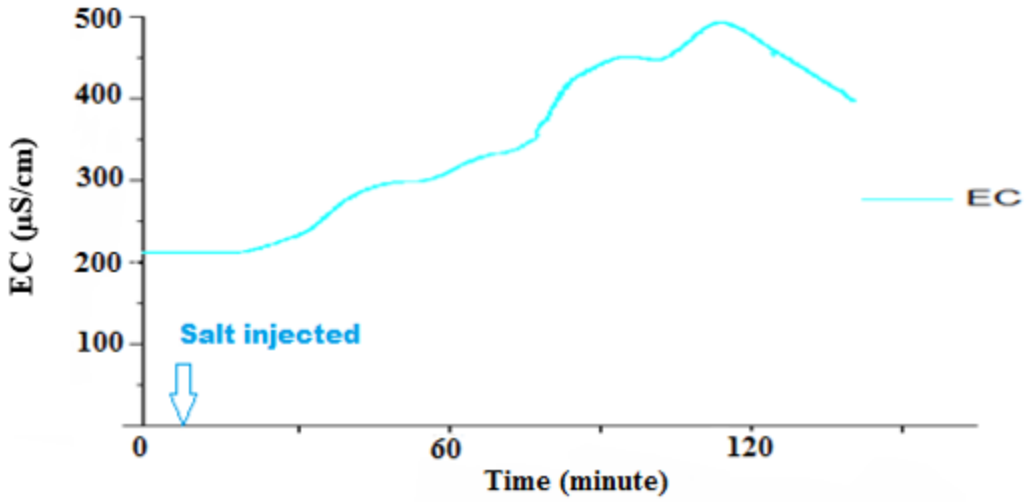


**Figure 32** Salt tracer test at intake of iPWS III

Generally, 94% of samples (n=34) collected from spring of iPWS III contained *E. coli* concentration above the WHO standard. At reservoir 63% of samples (n=35) were contaminated with *E. coli* and at taps the percentage of samples exceeding the standard went down to 27% (n=80). The detected *E. coli* concentration at reservoir and taps was lower than the level at the spring source. This might be due to short residence time of water at the reservoir not initiating increased bacterial activity as the reservoir gets empty between operational cycles. The sanitary inspection result showed high risk level of contamination at the spring which illustrates the spring source was the major risk factor for microbial water quality at the supply system.

In addition, the salt tracer result revealed high risk of contamination at the spring source indicating that infiltration into the source might be the cause for increased level of *E.coli and* total coliforms at the spring.

## 5.3. iPWS IV

### 5.3.1. *Temporal variability of E. coli*

The temporal variation of *E. coli* at iPWS IV showed a fluctuation in the concentration level of *E. coli* which is found to be consistent between spring, reservoir, distribution chamber and tap stand (Tap 1) of the supply system. *E. coli* concentration during the 1st week of the monitoring period (days 0-5) were almost at the lower detection limit, however during the 2nd week of the monitoring period (days 5-10) a slight increasing pattern was observed at all sampling points of the supply system. A highest peak value of 156 CFU/100 mL was detected at spring when compared with tap (114 CFU/100 mL), distribution chamber (93 CFU/100 mL) and reservoir (58 CFU/100 mL). A gradual decreasing trend was observed from the 3rd monitoring period onwards (days 10-15) and resulted in decrease of *E. coli* concentration at all sampling points. However, during the 4th week of the monitoring period *E. coli* concentration increased suddenly until the upper detection limit of 300 CFU/100 mL was reached at spring. Afterwards, the fluctuation dropped gradually where a concentration of 0 CFU was detected at spring (Fig 33). The observed contamination peaks are more likely due to leaching of contaminants into the spring source and increase in turbidity. The detected peak *E. coli* values originate from the spring source which illustrates that contamination occurred at the spring source before the water was distributed to reservoir tank and tap stands.

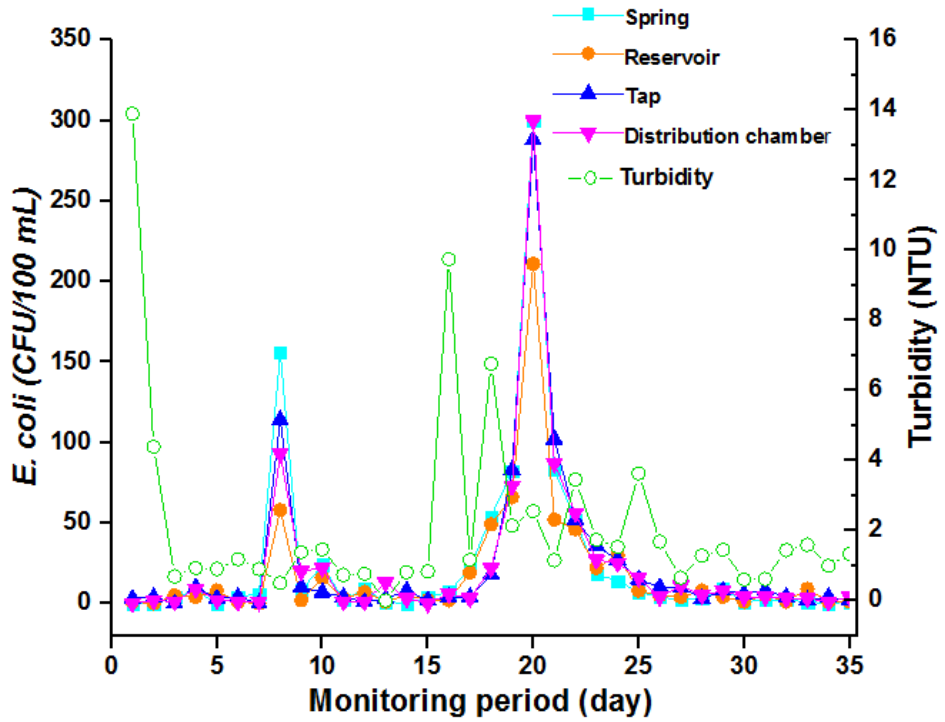


**Figure 33** Temporal Variability of *E. coli* at iPWS IV

### 5.3.2. *Temporal variability of total coliforms*

The pattern of total coliform variability observed at iPWS IV did not coincide between the sampling points monitored. No association in concentration of total coliforms and turbidity was observed at spring of iPWS IV (Fig 34).

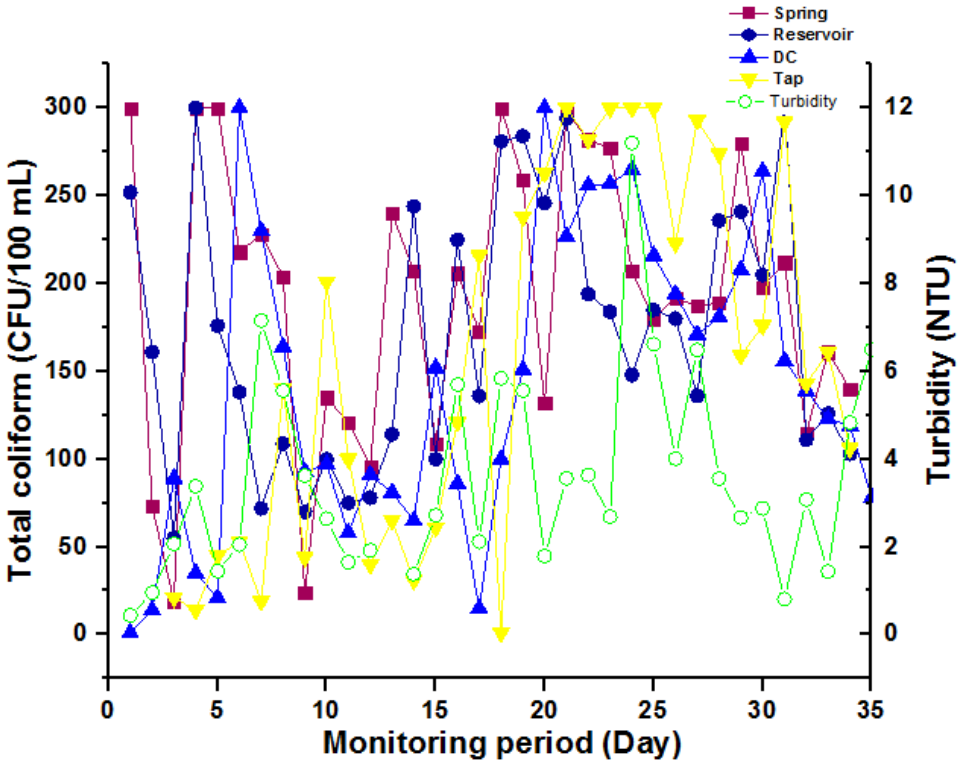


**Figure 34** Temporal Variability of total coliforms at iPWS IV

### 5.3.3. *Spatial variability of E. coli and total coliforms*

A higher percentage (92.4%) of water samples collected from iPWS IV were found to be contaminated with *E. coli*. Water samples tested at sampling points of iPWS IV showed that all samples (100%) from spring, reservoir and distribution chamber were positive for total coliforms and a higher median total coliform concentration of 259 CFU/ 100 mL was detected at spring. At taps only 9% and 0.4% of water sample were found to be negative for *E. coli* and total coliforms respectively. Table 5 shows the descriptive statistics of *E. coli* and total coliforms.

**Table 5** Descriptive statistics of *E. coli* and total coliforms with mean, standard deviation and quantiles at iPWS IV

| Indicator bacteria | Sampling points | n | Quantiles | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Min | 25% | Median | 75% | Max | % meeting WHO standard |
| *E. coli (CFU/100 mL)* | Spring | 34 | 27.2 | 58 | 0 | 2 | 6.5 | 21 | TNTC | 9 |
| | Reservoir | 34 | 19.4 | 38.3 | 0 | 2 | 7.5 | 16 | 211 | 3 |
| | DC | 35 | 23.7 | 53.8 | 0 | 2 | 4 | 21 | TNTC | 6 |
| | Tap 1 | 32 | 23.1 | 52.6 | 0 | 3 | 4 | 16.5 | 288 | 3.1 |
| | Tap 2 | 3 | 1.3 | 0.5 | 1 | 1 | 1 | 1.5 | 2 | 0 |
| | Tap 3 | 4 | 1 | 0.8 | 0 | 0.5 | 1 | 1.5 | 2 | 25 |
| | Tap 4 | 9 | 2.7 | 2.3 | 0 | 1 | 3 | 5 | 6 | 22 |
| | Tap 5 | 11 | 16.4 | 31.1 | 0 | 0.5 | 1 | 10.5 | 94 | 27 |
| | Tap 6 | 10 | 58.5 | 86.2 | 0 | 11 | 25 | 78 | 288 | 10 |
| | Tap 7 | 2 | 8.5 | 6.3 | 4 | 4 | 8.5 | 13 | 13 | 0 |
| | Tap 8 | 10 | 5.3 | 2.9 | 2 | 3 | 5 | 6 | 11 | 0 |
| | Tap 9 | 34 | 23.9 | 48.3 | 0 | 3 | 6 | 23 | 248 | 5.9 |
| | Tap 10 | 33 | 23.1 | 49.2 | 0 | 2 | 6 | 22 | 260 | 9.1 |
| Total coliform (CFU/100 ml) | Spring | 34 | 193 | 78.1 | 19 | 135 | 201 | 259 | TNTC | 0 |
| | Reservoir | 34 | 172 | 75.4 | 55 | 109 | 168 | 241 | TNTC | 0 |
| | DC | 35 | 142 | 86 | 1 | 83.5 | 139 | 212 | TNTC | 0 |
| | Tap 1 | 32 | 155 | 106 | 1 | 49 | 151 | 268 | TNTC | 0 |
| | Tap 2 | 3 | 51.3 | 72 | 2 | 10 | 18 | 76 | 134 | 0 |
| | Tap 3 | 4 | 106 | 141 | 0 | 1.5 | 62 | 210 | TNTC | 25 |
| | Tap 4 | 9 | 145 | 62.9 | 37 | 104 | 157 | 180 | 245 | 0 |
| | Tap 5 | 11 | 98.9 | 68 | 3 | 63.5 | 82 | 145 | 223 | 0 |
| | Tap 6 | 10 | 266 | 61.2 | 112 | 238 | TNTC | TNTC | TNTC | 0 |
| | Tap 7 | 2 | 210 | 37.4 | 184 | 184 | 210 | 237 | 237 | 0 |
| | Tap 8 | 10 | 210 | 68.2 | 103 | 162 | 212 | 263 | TNTC | 0 |
| | Tap 9 | 34 | 141 | 83.2 | 15 | 81 | 137 | 203 | TNTC | 0 |
| | Tap 10 | 33 | 147 | 81.6 | 16 | 85 | 147 | 180 | TNTC | 0 |

The daily monitoring data at iPWS IV was analysed by categorizing the data sets into 3 categories. The spatial difference between spring, reservoir and taps (1-6) was compared in one set and tap 7 and tap 8 were compared against spring and reservoir in one category. Similarly, the spatial variability between tap 9, tap 10, distribution chamber, spring and reservoir was compared in one group. Then, non-parametric Kruskal-Wallis test was run to generate test statics between the sampling points. Fig 35 shows the sampling points.

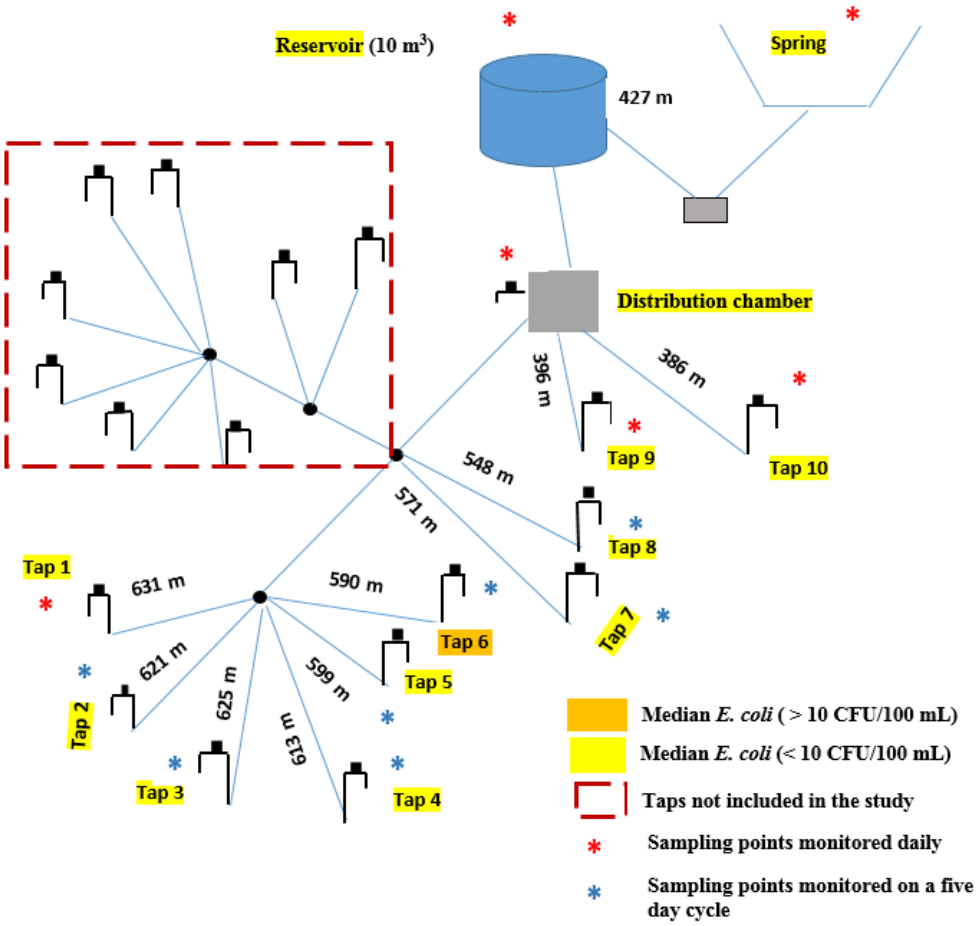


**Figure 35** Sampling points of iPWS IV

At iPWS IV a median *E. coli* concentration of 7.5 CFU/ 100 mL was detected at reservoir and 97 % of samples at reservoir were positive for *E. coli*. Out of 34 samples collected at spring of iPWS IV only 9 % of samples were below the detection limit of 0 CFU/ 100 mL.
The spatial dynamics of *E. coli* concentration at iPWS IV showed a significant variation between spring, reservoir, and taps (tap 1- tap 6) (Kruskal-Wallis, n= 137, $p < 0.01$) (Fig 36). The distribution of total coliforms was also found to be statistically significant (Kruskal-Wallis, n= 137, $p < 0.01$) (Fig 37). However, at tap 7 and 8 no spatial variability was observed in distribution of *E. coli* (Kruskal-Wallis, n= 80, $p = 0.84$) (Fig 38) and total coliforms (Kruskal-Wallis, n=80, $p = 0.4$) (Fig 39). Similarly, no difference was observed in distribution of *E. coli* at spring, reservoir, distribution chamber and taps (9-10) of iPWS IV (Kruskal-Wallis, n=170, $p = 0.98$) (Fig 40). However, the distribution of total coliforms was found to be statistically significant (Kruskal-Wallis, n=170, $p < 0.05$) (Fig 41).

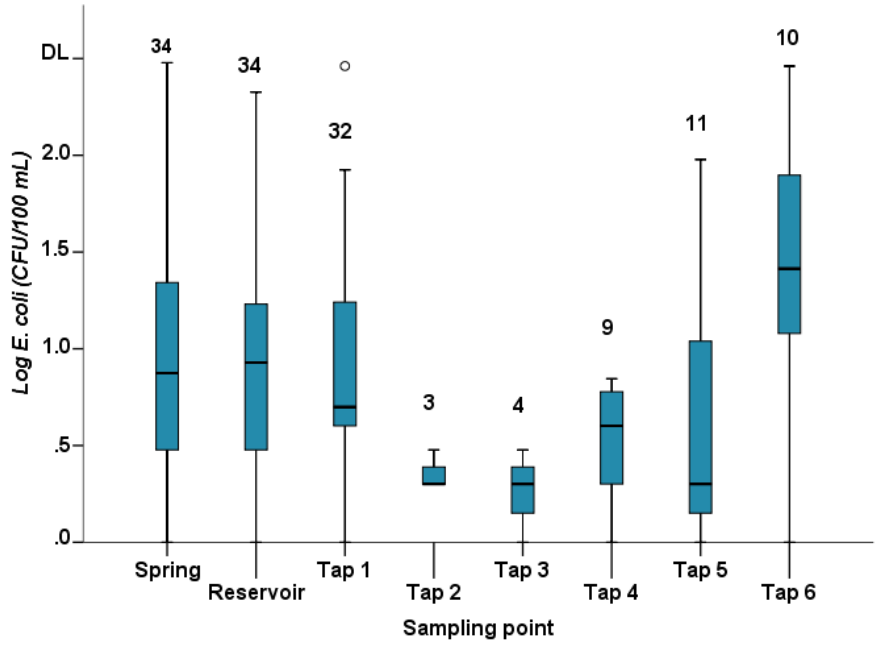


**Figure 36** Box and whisker plot of *E. coli* at spring, reservoir, and taps of iPWS IV.

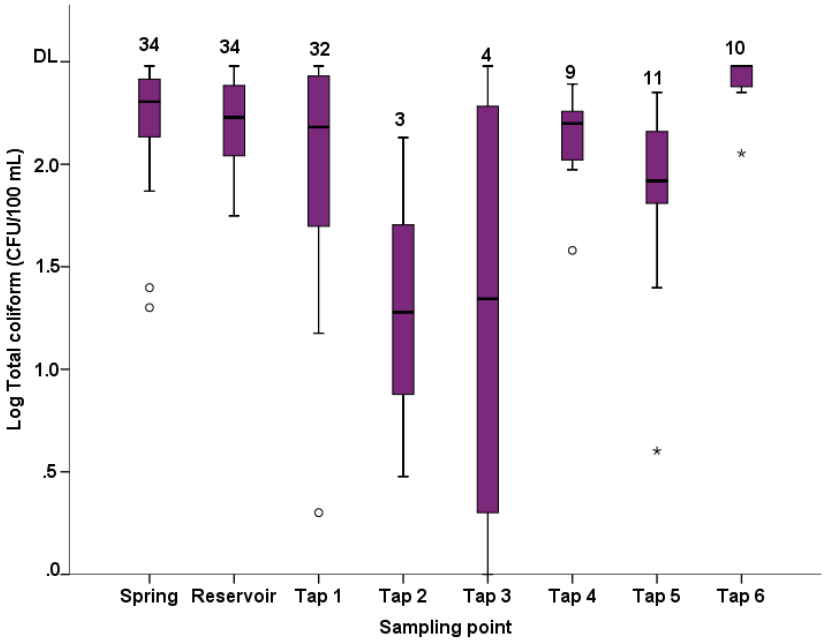


**Figure 37** Box and whisker plot of Total coliforms at spring, reservoir, and taps of iPWS IV.

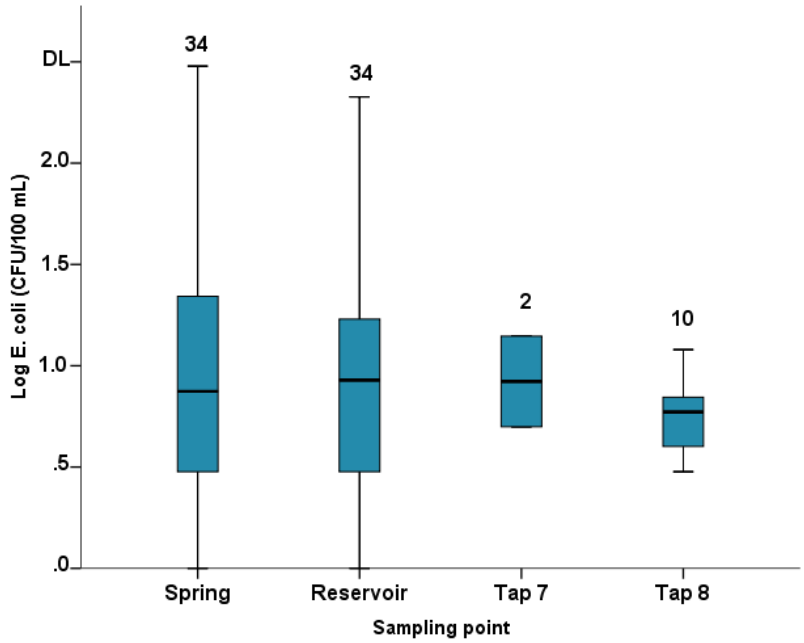


**Figure 38** Box and whisker plot of *E. coli* at spring, reservoir, and taps of iPWS IV.

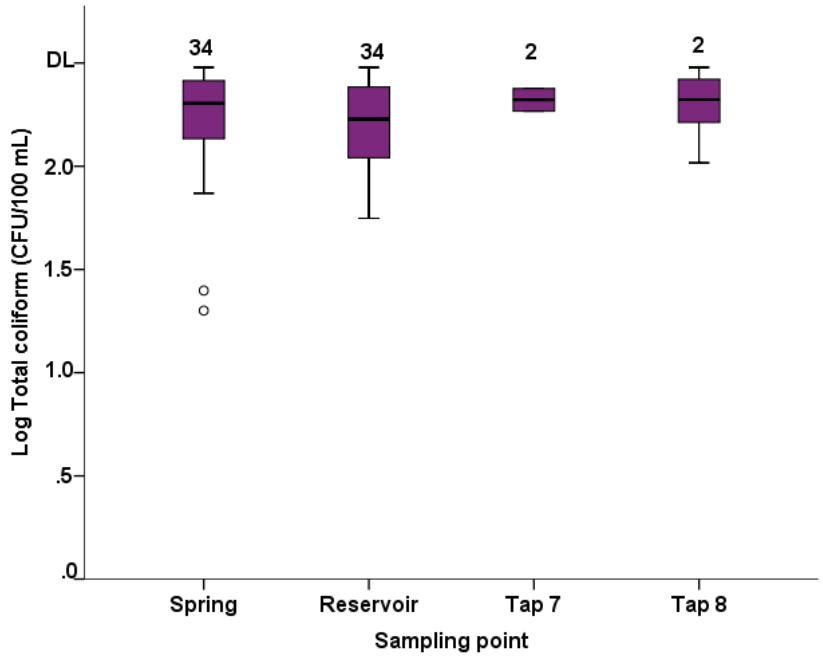


**Figure 39** Box and whisker plot of total coliforms at spring, reservoir, and taps of iPWS IV.

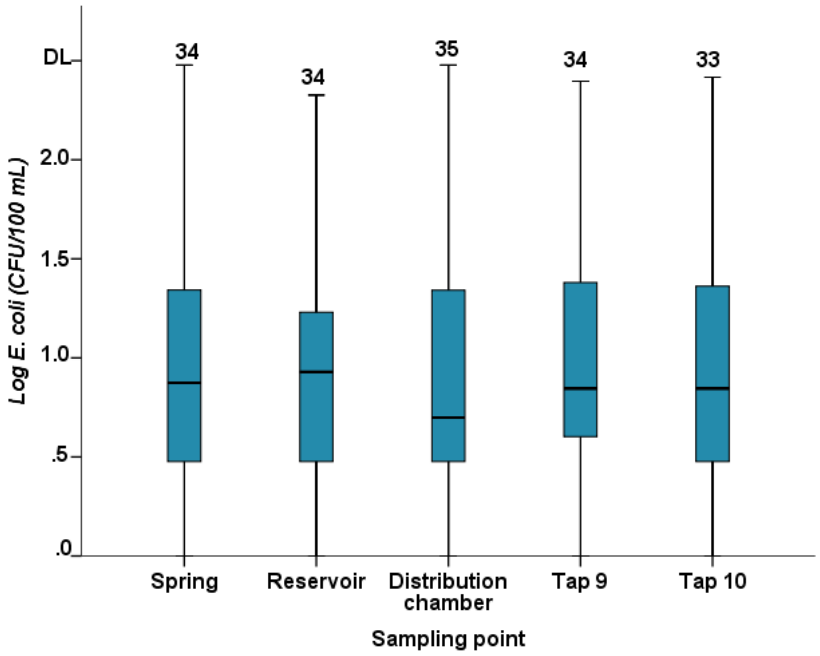


**Figure 40** Box and whisker plot of total coliforms at spring, reservoir, and taps of iPWS IV.

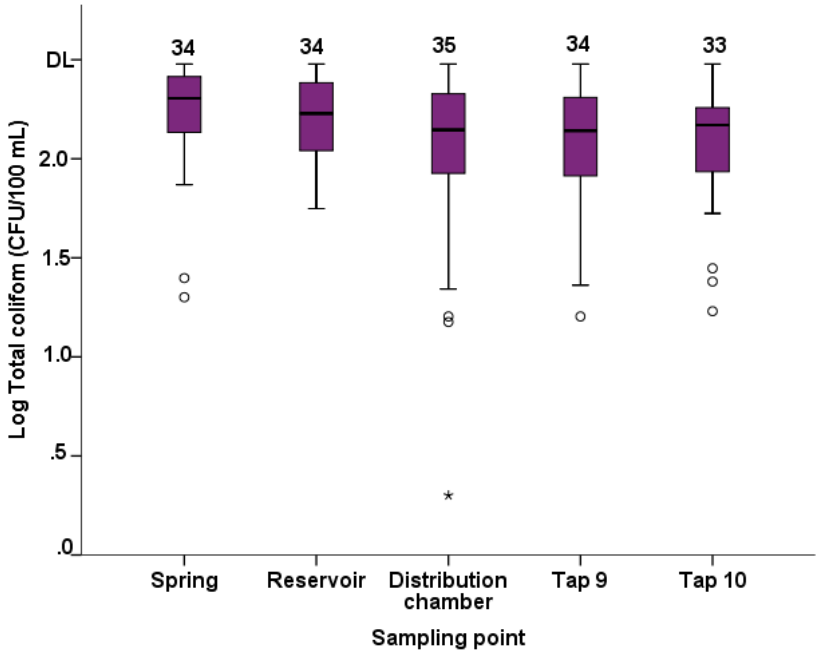


**Figure 41** Box and whisker plot of total coliforms at spring, reservoir, and taps of iPWS IV.

### 5.3.4. *Sanitary inspection*

At spring of iPWS IV the sub-surface water at intake of spring was collected using a concrete spring box before being distributed to the reservoir tank. Unlike spring of iPWS II and III, the intake at iPWS IV was covered with a concrete lid and the water flows through a spring box to reservoir, however the collection point was filled with settled soil particles and leaves (Fig 42). The spring box is located below an eroded agricultural area and prone to surface-runoff from upstream of the hilly area. No physical mechanisms for diverting surface run-off were not constructed and not in place. The spring source was fenced with barbed wire so that animals do not access the spring within 10 m range. Except for tap 5, tap 6 and reservoir surface water collects at all sampling points. Leakage of the main pipeline connecting the spring and reservoir was observed, however no leakage was seen between reservoirs and tap stands. In addition, the pipe line connecting reservoir with tap 1, tap 6, tap 8 and tap 10 was exposed to the surface. Toilets were located within 30 m range at tap 1, tap 3, tap 4, tap 6, tap 8, and tap 10. Overall, the risk category at iPWS IV ranged between low (tap 9, tap 10), medium (tap 3, tap 4, and reservoir) and high (spring, tap 1, tap 6 and tap 8).

**Figure 42** Intake of iPWS IV

In general, the result of sanitary inspection at all systems (iPWS II, III and IV) showed a risk score ranging from 0 to 9. The total risk score of springs was found to be higher when compared with other sampling points (reservoirs and taps). The springs had high to very high risk for contamination. During the inspection the spring sources were not well protected and are likely to be contaminated from the external environment. The masonry covering the intake of the springs were faulty and covered with soil and decomposed leaf. In addition, the back fill area of the springs were also found to be eroded. No latrines and human faeces were found within 30 m and 10 m of all springs, whereas animal faeces were seen frequently within 10 m of all springs. The risk score for reservoirs is between 0-5. The lowest risk score was observed at reservoir of iPWS II. The reservoir was well protected with fence and no crack and leakage was observed during inspection.

However, at iPWS IV the covering lid of the reservoir was damaged and the main pipe between spring and reservoir was leaking during the walkthrough observation. Table (4) shows the risk matrix of sampling points and summarises risk score and risk category of the sampling points. The finding of the study showed no correlation between the level of contamination and the risk category of the sampling points (spearman's rs=0.32, n=27, $p > 0.05$).

**Table 4** Risk matrix

| Median *E. coli* (CFU/100 mL) | Risk Score | | | |
|---|---|---|---|---|
| | 0-2 | 3-5 | 6-8 | 9-10 |
| 0 | | | | |
| 1-10 | iPWSII-Tap 1<br>iPWSIII-Tap 1<br>iPWSIII-Tap 3<br>iPWSIV-Tap 5<br>iPWSIV-Tap 9 | iPWSIII-Tap 5<br>iPWSIII-Tap 4<br>iPWSIII-Tap 2<br>iPWSII-Tap 7<br>iPWSIII-Tap 6<br>iPWSIV-Tap 3<br>iPWSIV-Tap 4<br>iPWSIV-Tap 5<br>iPWSII-Reservoir<br>iPWSIV-Reservoir | iPWSIV-Tap 8<br>iPWSIV-Tap 9<br>iPWSIV-Spring | iPWSIII-Spring |
| 11-100 | iPWSII-Tap 2<br>iPWSII- Reservoir | iPWSII-Tap 3<br>iPWSII-Tap 6<br>iPWSII-Tap 4<br>iPWSII-Tap 5<br>iPWSIV-Tap 6 | | iPWSII-Spring |
| >100 | | | | |

| Low Risk | Medium risk | High risk | Very high risk |
|---|---|---|---|

### 5.3.5. *Tracer test*

Between t=0 and t=45 min EC remained at a steady value of 250 µS/cm. However, EC rose from 250 µS/cm and reached a peak of 509 µS/cm at about t=60 min. Afterwards fluctuation in measurement of EC was observed between different time intervals. At t=60 min EC slightly dropped from the peak value of 509 µS/cm to 300 µS/cm. At t=80 min EC sharply increased to 453 µS/cm which is also followed by a slight decrease to 343 µS/cm at t= 100 min, between t= 110 min and t=130 min an increasing and decreasing pattern was also observed (Fig 43).

The detected peak EC values might be due to the cumulative effects of the salt solution injected. The pattern of fluctuation from t=60 min onwards might be due to the dosing as well as subsequent dilution of the salt solution. Holes were injected at a time interval of 10 minute, so the electrolyte solution might be diluted between time intervals of dosing which in turn results in decreased EC values. Also, the water table of the spring might be high so the salt concentration could be easily diluted once it comes in contact with the underground water.

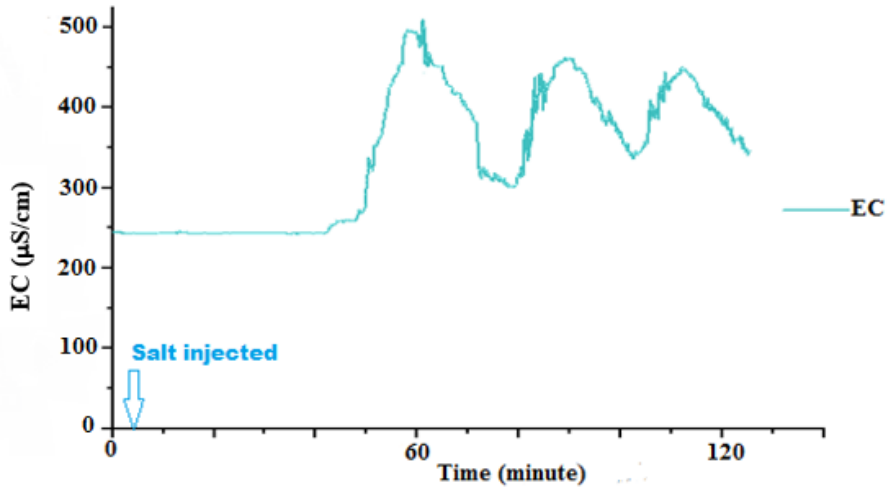


**Figure 43** Salt tracer experiment at intake of iPWS IV

Generally, 91% (n=34) of samples collected from spring of iPWS IV were contaminated with *E. coli*. At reservoir 97% (n=34) of samples exceed the standard for drinking water. At taps 91% of samples contained *E. coli* concentration above the standard of 0 CFU/100 mL. The temporal variability result showed localized nature of the contamination originating from the spring source which also showed the same pattern of fluctuation between reservoir, distribution chamber and tap 1. The sanitary inspection showed a leakage at a pipe connecting the spring with reservoir this might be the reason why the median concentration of *E. coli* at the reservoir was slightly higher than the spring. The tracer test showed increased risk of infiltration from external source.

## 5.4. Physico-chemical parameters

### 5.4.1. *pH*

The measured pH value at sampling points of iPWS II ranged between 6.6 and 7.5. All the samples are within the standard range of drinking water. The spatial variability between sampling points of iPWS II showed no difference between spring, reservoir, tap 2 and tap 3 (Kruskal-Wallis, n=79, $p = 0.06$). However, a difference was observed between tap 1 and tap 4 (Kruskal-Wallis, n=84, $p < 0.05$). Similarly, the measured value between tap 5, tap 6 and tap 7 was found to be significant (Kruskal-Wallis, n=90, $p < 0.05$).

At iPWS III a minimum and maximum of 6.5 and 8.3 pH values were measured. No significant difference in measurement of pH was observed at spring, reservoir and tap 1, tap 2 (Kruskal-Wallis, $p$=0.36, n=88) and tap 3 and tap 4 (Kruskal-Wallis, n=68, $p$=0.13). Similarly, no significant difference was observed between tap 5, 6 and 7 (Kruskal-Wallis, n=86, $p$ = 0.13)

At iPWS IV the measured pH value ranged between 6.1 and 8.3. The pH value between sampling points of iPWS IV showed a statistically significant difference between spring, reservoir and taps (1-6) (Kruskal-Wallis, n=96, $p < 0.05$). However, no difference was observed between tap 7, tap 8, spring and reservoir (Kruskal-Wallis, n=59, $p$ = 0.1). Similarly, the pH value was found to be the same between spring, reservoir, distribution chamber tap 9 and tap 10.

### 5.4.2. *Turbidity*

At iPWS II a median turbidity of 2.12 NTU was measured and 93.5 % of samples were within the acceptable range of 5 NTU. The turbidity level at taps (6.25 NTU) and reservoir (5.67 NTU) was found to be lower when compared with spring (27.3 NTU). Statistically significant difference in measurement of turbidity was observed between tap 1, tap 4, spring and reservoir (Kruskal-Wallis, n= 97, $p < 0.01$). Tap 3 and 4 also showed a difference in turbidity level when compared with reservoir and spring (Kruskal-Wallis, n=87, $p < 0.01$). Similarly, statistically significant difference was observed between tap 5, tap 6, tap 7, spring and reservoir (Kruskal-Wallis, n=96, $p < 0.01$). Generally, the observed variability between the sampling points of iPWS II might be due to the higher turbidity level measured at the spring source.

A maximum turbidity level of 163 NTU was measured at spring of iPWS III, whereas at taps and reservoir a turbidity level of 4.6 NTU and 4.70 NTU were measured respectively. The measured turbidity between tap 1, tap 2, spring and reservoir differs between each sampling points (Kruskal-Wallis, n=98, $p < 0.01$). Statistically significant difference was observed between spring, reservoir, tap 3 and 4 (Kruskal-Wallis, n=98, $p < 0.01$). Likewise, a variability was observed between spring, reservoir, tap 5, tap 6 and tap 7 (Kruskal-Wallis, n=80, $p < 0.01$).

At iPWS IV, 94.7% of samples are within the WHO standard range of 5 NTU. The highest turbidity value was measured at spring (11.2 NTU) when compared with tap (6.76 NTU), and reservoir (4.05 NTU). The distribution of turbidity across spring, reservoir and taps (1-6) was found to be statistically different (Kruskal-Wallis, n=133, $p < 0.01$), also the turbidity level between tap 7 and tap 8 with spring and reservoir was found to be significant (Kruskal-Wallis, n=78, $p < 0.01$). Similarly, a statistically significant variability was observed between spring, reservoir, tap 9 and tap 10 (Kruskal-Wallis, n=165, $p < 0.01$). The observed variability between the sampling points might be because of the high turbidity value measured at the spring.
Generally, 90.5% of samples measured for turbidity at all systems were below 5 NTU.

### 5.4.3. *Electrical conductivity (EC)*

The measured electrical conductivity at iPWS II ranged between 40 and 79 µS/cm. No statistically significant difference in measurement of EC was observed between taps 2 and 3 (Kruskal-Wallis, n=90, $p$=0.17), also no difference was observed between taps 1 and 4 (Kruskal-Wallis, n=97, $p < 0.28$) when compared with EC value at reservoir and spring and the

median value between the sampling points were found to be 60 µS/cm. However, the measured EC value between taps (5-7) differs when compared with spring and reservoir (Kruskal-Wallis, n=99, $p < 0.05$).

At iPWS III the measured EC value ranged between 100 and 230 µS/cm and the highest value was recorded at the spring. Significant difference was observed in measurement of EC between tap 1, tap 2, spring and reservoir (Kruskal-Wallis, n=102, $p < 0.01$). And no difference in measurement of EC was observed between tap 3 and tap 4 (Kruskal-Wallis, n=82, $p < 0.01$). Similarly, a significant difference was observed in measurement of EC between spring, reservoir and tap 5, tap 6 and tap 7 (Kruskal-Wallis, n=100, $p < 0.01$).

At iPWS IV the measured EC value ranged between 150 and 352 µS/cm. Significant difference was observed between spring, reservoir and taps (1-6) (Kruskal-Wallis, n=137, $p < 0.01$) and tap 7 and tap 8 (Kruskal-Wallis, n=79, $p < 0.01$). Similarly, statistically significant difference was observed between reservoir, spring, distribution chamber and tap 9 and tap 10 (Kruskal-Wallis, n=170, $p < 0.01$)

### 5.4.4. *Total dissolved solids (TDS)*

At iPWS II a minimum and maximum of 37 and 58 mg/L of TDS was measured. Significant difference in measured level was observed between spring, reservoir, tap 1 and tap 4 (ANOVA (F3, 93), n=99, $p < 0.05$). At tap 2 and tap 3 the measured level of TDS was also found to be significant (ANOVA (F3, 86), n=90, $p < 0.05$). Similarly, significant difference was observed between spring, reservoir and tap 5, tap 6 and tap 7 (ANOVA (F4, 94), n=99, $p < 0.01$).

At iPWS III the measured TDS level ranged between 79 and 163 mg/L. Significant difference was observed between spring, reservoir, tap 1 and tap 2 (Kruskal-Wallis, n=102, $p < 0.01$). The difference between tap 3, tap 4, spring and reservoir was also found to be significant (Kruskal-Wallis, n=82, $p < 0.01$). Similarly, significant difference was observed between spring, reservoir, tap 5, tap 6, and tap 7 (Kruskal-Wallis, n=100, $p < 0.01$).

A maximum and minimum of 112 and 189 mg/L TDS values were recorded at sampling points of iPWS IV. Difference in measured value of TDS was observed between spring, reservoir and taps (1-6) (Kruskal-Wallis, n=137, $p < 0.01$). At tap 7 and tap 8 the measured level of TDS was also significant when compared with spring and reservoir (Kruskal-Wallis, n=79, $p < 0.01$). Similarly, significant difference was observed between spring, reservoir, distribution chamber and tap 9 and tap 10 (Kruskal-Wallis, n=170, $p < 0.01$).

### 5.4.5. *Temperature*

The measured temperature at iPWS II ranged between 7.6 and 19.1 °C and the measured temperature level between sampling points of iPWS II was found to be statistically significant (Kruskal-Wallis, n=144, $p < 0.01$). At iPWS III the measured temperature ranged between 8.9 and 17.2 °C and the difference between the sampling points is also statistically significant (Kruskal-Wallis, n=148, $p < 0.01$).Similarly, at iPWS IV the measured temperature between sampling points were found to be statistically significant (Kruskal-Wallis, n=251, $p < 0.01$) and the recorded temperature ranged between 2.3 and 19.1 °C.

## 5.5. Correlation between *E. coli* and Physical parameters

### 5.5.1. *iPWS II* [2]

A spearman's rank-order correlation was run to understand the association between *E. coli* concentration and physical parameters. The result at spring of iPWS II showed that a moderate negative correlation between *E. coli* and temperature ($p < 0.01$). However, the association of *E. coli* with EC, pH, TDS and turbidity was found to be not statistically significant ($p > 0.05$). The negative correlation between *E. coli* and temperature might be due to adaptation of *E. coli* to the cold climatic condition of the area and showing increased growth and activity below 16°C. The temperature of water ranged between 12.2 and 18°C whereas the ambient temperature was between 7.6 and 16°C. No correlation was observed between the concentration of total coliforms and the physico-chemical parameters at spring of iPWS II. Table 5 depicts the parameters, number of samples, correlation coefficient and level of significance.

**Table 5** Spearman's rank-order correlation for *E. coli*, total coliforms and physical parameters at spring of iPWS II

| Parameter | n | Correlation coefficient (rs) | | Significance level ($p$ – value) | |
|---|---|---|---|---|---|
| | | *E. coli* | Total coliform | *E. coli* | Total coliform |
| EC (µS/cm) | 35 | -0.15 | -0.16 | 0.36 | 0.33 |
| pH | 28 | 0.03 | -0.21 | 0.86 | 0.26 |
| TDS (mg/L) | 35 | -0.17 | -0.16 | 0.31 | 0.33 |
| Turbidity (NTU) | 33 | -0.15 | 0.09 | 0.37 | 0.58 |
| Temperature (°C) | 35 | -0.55 | 0.26 | < 0.001** | 0.12 |

***p- correlation is significant at the level 0.01*

At reservoir of iPWS II no correlation was observed between EC, pH, TDS and turbidity ($p > 0.05$). However, a weak negative correlation was observed between the concentration of *E. coli* detected and temperature. No association in concentration of total coliforms with physical parameters were observed at reservoir of iPWS II. Table 6 depicts the parameters, number of samples, correlation coefficient and level of significance.

**Table 6** Spearman's rank-order correlation for *E. coli* and physical parameters at reservoir of iPWS II

| Parameter | n | Correlation coefficient (rs) | | Significance level ($p$ – value) | |
|---|---|---|---|---|---|
| | | *E. coli* | Total coliform | *E. coli* | Total coliform |
| EC (µS/cm) | 36 | 0.13 | -0.98 | 0.42 | 0.57 |
| pH | 31 | -0.1 | -0.35 | 0.55 | 0.053 |
| TDS (mg/L) | 36 | -0.08 | -0.1 | 0.61 | 0.53 |
| Turbidity (NTU) | 35 | 0.32 | 0.09 | 0.056 | 0.59 |
| Temperature (°C) | 36 | -0.35 | -0.03 | 0.03* | 0.84 |

At taps of iPWS II a weak negative correlation was observed between pH, TDS, temperature and *E. coli* ($p < 0.05$). Whereas, a weak positive correlation was observed between *E. coli* and turbidity ($p < 0.01$). However electrical conductivity was not associated with *E. coli* ($p$=0.27).

[2] See Appendex C for scatter plots

Weak positive correlation was observed between total coliform concentration and pH ($p < 0.01$). Table 7 depicts the parameters, number of samples, correlation coefficient and level of significance.

**Table 7** Spearman's rank-order correlation for *E. coli* and physical parameters at taps of iPWS II

| Parameter | n | Correlation coefficient (rs) | | Significance level ($p$ – value) | |
|---|---|---|---|---|---|
| | | *E. coli* | Total coliform | *E. coli* | Total coliform |
| EC (µS/cm) | 73 | -0.12 | -0.09 | 0.27 | 0.43 |
| pH | 67 | -0.25 | 0.32 | 0.03* | 0.008** |
| TDS (mg/L) | 73 | -0.28 | -0.43 | 0.01* | 0.72 |
| Turbidity (NTU) | 72 | 0.36 | 0.19 | 0.002** | 0.09 |
| Temperature (°C) | 73 | -0.3 | 0.02 | < 0.002** | 0.8 |

**p- correlation is significant at the level 0.01*
**p- correlation is significant at the level 0.05*

### 5.5.2. *iPWS III*

At spring of iPWS III *E. coli* was significantly and moderately correlated with turbidity and temperature ($p < 0.01$) However, the association of *E. coli* with EC, pH, and TDS was not found to be statistically significant ($p > 0.05$). Similarly, no association was observed between total coliform concentration and physical parameters ($p > 0.05$). Table 8 shows the parameters, correlation coefficient and degree of significance with *E. coli* and total coliforms.

**Table 8** Spearman's rank-order correlation between *E. coli* and physical parameters at spring of iPWS III

| Parameter | n | Correlation coefficient (rs) | | Significance level ($p$ – value) | |
|---|---|---|---|---|---|
| | | *E. coli* | Total coliform | *E. coli* | Total coliform |
| EC (µS/cm) | 34 | -0.18 | 0.02 | 0.3 | 0.89 |
| pH | 32 | -0.17 | -0.21 | 0.32 | 0.23 |
| TDS (mg/L) | 34 | -0.21 | 0.09 | 0.21 | 0.58 |
| Turbidity (NTU) | 32 | 0.45 | 0.16 | 0.009** | 0.35 |
| Temperature (°C) | 34 | 0.49 | 0.11 | 0.003** | 0.53 |

**p- correlation is significant at the level 0.01*

At reservoir of iPWS III no correlation was found between *E. coli* bacteria and physical parameters ($p > 0.05$) (Table 9). However, temperature was positively correlated with total coliforms ($p < 0.01$).

**Table 9** Spearman's rank-order correlation between *E. coli* and physical parameters at reservoir of iPWS III

| Parameter | n | Correlation coefficient (rs) | | Significance level ($p$ – value) | |
|---|---|---|---|---|---|
| | | *E. coli* | Total coliform | *E. coli* | Total coliform |
| EC (µS/cm) | 34 | -0.009 | -0.06 | 0.9 | 0.7 |
| pH | 31 | 0.16 | 0.21 | 0.38 | 0.24 |
| TDS (mg/L) | 34 | -0.05 | -0.17 | 0.77 | 0.32 |
| Turbidity (NTU) | 32 | -0.007 | -0.09 | 0.9 | 0.59 |
| Temperature (°C) | 34 | -0.28 | 0.46 | 0.09 | 0.006** |

***p- correlation is significant at the level 0.01*

At tap stands temperature was negatively and significantly correlated with *E. coli* ($p < 0.01$). However, the association of *E. coli* with EC, pH, TDS, and turbidity was not found to be significant ($p > 0.05$). Moderate correlation was observed between the concentration of total coliforms and temperature ($p < 0.01$) (Table 10).

**Table 10** Spearman's rank-order correlation between *E. coli* and physical parameters at tap stands of iPWS III

| Parameter | n | Correlation coefficient (rs) | | Significance level ($p$ – value) | |
|---|---|---|---|---|---|
| | | *E. coli* | Total coliform | *E. coli* | Total coliform |
| EC (µS/cm) | 79 | -0.02 | 0.05 | 0.83 | 0.6 |
| pH | 71 | 0.05 | 0.04 | 0.38 | 0.73 |
| TDS (mg/L) | 79 | -0.42 | -0.01 | 0.71 | 0.86 |
| Turbidity (NTU) | 78 | 0.12 | -0.21 | 0.12 | 0.06 |
| Temperature (°C) | 79 | -0.28 | 0.53 | 0.03* | <0.001** |

***p- correlation is significant at the level 0.01*
**p- correlation is significant at the level 0.05*

### 5.5.3. *iPWS IV*

At spring of iPWS IV a weak positive association between *E. coli*, TDS and turbidity ($p < 0.01$) was observed. However, no association was found between *E. coli* and EC, pH and temperature at spring of iPWS IV ($p > 0.05$) (Table 11). Similarly, at reservoir of iPWS IV no correlation was found between *E. coli* and physical parameters ($p > 0.05$) (Table 12). At taps a weak positive correlation was observed between *E. coli* and EC and TDS ($p < 0.01$). However, the correlation of pH, turbidity and temperature with *E. coli* was not found to be significant ($p > 0.05$). Moderate positive correlation was observed between total coliforms and EC ($p < 0.05$) (Table 13)

**Table 11** Spearman's rank-order correlation between *E. coli* and physical parameters at spring of iPWS IV

| Parameter | n | Correlation coefficient (rs) | | Significance level ($p$ – value) | |
|---|---|---|---|---|---|
| | | *E. coli* | Total coliform | *E. coli* | Total coliform |
| EC (µS/cm) | 34 | 0.26 | 0.4 | 0.13 | 0.01* |
| pH | 24 | 0.04 | -0.11 | 0.85 | 0.58 |
| TDS (mg/L) | 34 | 0.48 | 0.02 | 0.003** | 0.87 |
| Turbidity (NTU) | 33 | 0.46 | 0.17 | 0.007** | 0.33 |
| Temperature (°C) | 34 | -0.08 | -0.16 | 0.6 | 0.35 |

***p- correlation is significant at the level 0.01*
**p- correlation is significant at the level 0.05*

**Table 12** Spearman's rank-order correlation between *E. coli* and physical parameters at reservoir of iPWS IV

| Parameter | n | Correlation coefficient (rs) | | Significance level ($p$ – value) | |
|---|---|---|---|---|---|
| | | *E. coli* | Total coliform | *E. coli* | Total coliform |
| EC (µS/cm) | 34 | 0.02 | -0.14 | 0.8 | 0.41 |
| pH | 24 | 0.07 | 0.03 | 0.7 | 0.88 |
| TDS (mg/L) | 34 | 0.3 | -0.15 | 0.07 | 0.38 |
| Turbidity (NTU) | 33 | 0.09 | 0.16 | 0.6 | 0.35 |
| Temperature (°C) | 34 | 0.11 | -0.12 | 0.52 | 0.49 |

**Table 13**. Spearman's rank-order correlation between *E. coli* and physical parameters at Taps of iPWS IV

| Parameter | n | Correlation coefficient (rs) | | Significance level ($p$ – value) | |
|---|---|---|---|---|---|
| | | *E. coli* | Total coliform | *E. coli* | Total coliform |
| EC (µS/cm) | 147 | 0.22 | 0.19 | 0.006** | 0.01* |
| pH | 107 | 0.12 | 0.2 | 0.22 | 0.03* |
| TDS (mg/L) | 147 | 0.3 | 0.17 | < 0.001** | 0.03* |
| Turbidity (NTU) | 144 | 0.16 | -0.08 | 0.052 | 0.3 |
| Temperature (°C) | 148 | 0.1 | -0.23 | 0.21 | 0.004** |

***p- correlation is significant at the level 0.01*
**p- correlation is significant at the level 0.05*

## CHAPTER 6

# Discussion

The finding of this study showed that the iPWS's were faecally contaminated with *E. coli* and total coliforms. 94% of samples collected from spring source of all systems contained *E. coli* concentration above the Nepalese and WHO standard value of 0 CFU /100 mL. Similarly, total coliforms were detected in all (100%) of samples at springs. The finding from this study was consistent with the UNICEF Nepal MICS report which showed that 71.1% of samples collected from sources were positive for *E. coli* (CBS, 2014). In addition a study conducted in 5 development regions of Nepal also showed that a high percentage of the samples (88.5%) were being faecally contaminated with total coliforms whereas *E. coli* bacteria were detected in 56.5% of samples (Rai et al., 2012). The study also reported that a high percentage of samples from tap stands (90.1%) contained total coliforms which is also consistent with the finding of this study; 99.4% of samples were contaminated with total coliforms whereas *E. coli* was detected in 85% of samples from tap stands of all iPWS's monitored.

A slight increasing trend was observed in spatial distribution of *E. coli* at iPWS II which implies that recontamination happened after the water was distributed from spring to taps. The lowest median *E. coli* concentration was detected at the spring (16 CFU/100 mL) when compared with reservoir (17.5 CFU/100 mL) and taps (19 CFU/100 mL). This might be due to failure in the network pipes once the water is distributed from the reservoir to tap stands. Leakage has been detected in pipe line connecting tap 5 and tap 6 with the reservoir. In addition, the supply systems in the study area operate only for two hours per day leaving the pipes under pressurized for more than 10 hours per supply cycle, so, this condition might act as a portal of entry for contaminated water via leaking pipes or orifices. Failure in pipe networks could initiate the ingression of contaminants into the supply system through cracks, leaking joints or during times when the system is off (Lee & Schwab, 2005). A study by Matinshe et al., (2014) also showed intermittent supply of water coupled with low disinfection and long storage time of water in the system as a main contributing factor for deterioration of water quality from water treatment plant to supply network.

At iPWS III, the spring source had the highest *E. coli* concentration, while at taps and reservoirs the median concentration detected was the same (1 CFU/100 mL). The dynamics of *E. coli* bacteria decreased from spring to reservoir and taps. This might be due to the short storage time of water at the reservoir tank. The residence time of water at the reservoir tank was approximately less than ten hours and the reservoir gets empty between operational cycles. So, the short residence time of water in the reservoir might not create a favourable condition for bacterial growth. In reservoir tanks the growth of microorganisms is associated with storage time, where a long residence time favouring microbial growth (EPA, 2002 ).

The temporal dynamics of *E. coli* at spring and reservoir of iPWS III was more stable when compared with the temporal pattern at iPWS II and iPWS IV. This could be related with factors affecting the dynamics and transport of *E. coli* at the source. Studies show the transport of *E. coli* and pathogens in soil and in underground water depends on a number of interrelated factors that either initiates increased activity or affects the microbial load in a negative way due to die-off. Among the factors are characteristics of the soil (moisture, porosity and type), temperature, pH, salt concentration, size and shape of the bacteria (Pedley et al., 2006; West et al., 1998). Thus, due to this reasons the concentration of *E. coli* at the spring of iPWS III might be lower when compared with springs of iPWS II and iPWS IV. Also, *E. coli* might be filtered and removed due to pore size of the soil.

The temporal variability could possibly influence the result of spatial variation on samples which were collected during the period were temporal variations in concentration of *E. coli* were observed. For instance, at tap 6 of iPWS IV the median *E. coli* concentration detected was higher (25 CFU/100 mL) when compared with tap 5 (1 CFU/100 mL). This is due to sampling at tap 5 was done during the period were the temporal variation was stable whereas at tap 6 the sampling was done when temporal variation reached peak value of >300 CFU/100 mL at spring. At some sampling points the observed spatial variability could be due to the temporal variation. Samples which were collected on the same day with spring did not showed spatial variability. For instance, at spring, reservoir, distribution chamber, tap 9 and tap 10 no spatial variability was observed as the temporal variation was consistent between the sampling points. So, the observed spatial variability could be due the effect of temporal variability in addition to leakages that were detected at sampling points.

The variability was not also limited between sampling points, instead the extent of variability at a system level also differs. The highest level of contamination was detected at iPWS II where 98% of all samples were contaminated with *E. coli* bacteria while 75% and 92.4% represents the percentage of samples contaminated with *E. coli* at iPWS III and iPWS IV respectively. The increased detection of faecal indicator bacteria at all iPWS could be related with the intermittent supply of water. A study conducted in India compared supply systems which provide water intermittently and continuously, according to the study a higher concentration of *E. coli* was detected at systems which deliver water intermittently than continuous systems (Kumpel & Nelson, 2014). The Nepal drinking water quality standard state a routine microbial water quality monitoring to be conducted every month for urban water supplies and three times a year for rural areas (NDWQS, 2005). In the study area no monitoring scheme and setup of infrastructure is currently in place to assess the quality of water and to take appropriate remedial measures. This might also be the reason why the supply systems were faecally contaminated.

The underlying mechanism of contamination and possible explanation governing the variability differs within each sampling point and systems monitored. In general poor source protection, sanitary condition of the supply system, distance from the source, intermittency of the system and operational conditions of the supply system affected the microbial quality of water in the study area.

At spring of iPWS II 22% of samples measured for turbidity exceed the WHO as well as the Nepal standard value of 5 NTU whereas at reservoir and taps only 2.9% and 1.4% of samples exceed the standard respectively. The measured turbidity is higher at the spring when compared with the level measured at reservoir and taps. Overall 93.1% of samples meet the standard for turbidity at all sampling points of iPWS II. Conversely, the percentage of samples meeting the standard of 5 NTU is lower at iPWS III when compared with iPWS II; 78% of samples were within the standard range of 5 NTU. However, the measured turbidity in 93.7% of samples at spring of iPWS III was found to be above 5 NTU while at reservoir all samples (100%) and at taps 98.7% of samples were within the standard for drinking water. Likewise, a higher percentage of samples at reservoir (100%) and taps (98.6%) of iPWS IV were within the range of potable water, however the percentage at spring was found to be lower (78%). In general, the level of turbidity at springs of all iPWS's were higher than reservoirs and taps. This might be due to, samples at springs were collected directly at the inlet of the source during collection the suspended particulate matters might not settled to the bottom surface of the spring while at reservoirs the particles might settle prior to distribution into tap stands. Generally, turbidity values at spring of all iPWS's were not driven by rainfall since no precipitation events occurred during the study period. The possible source could be due to particulate matters which cause turbidity coming in contact with spring source from the soil/debris covering the intake of the springs.

The finding of this study showed a higher turbidity level being correlated with the concentration of *E. coli* at spring of iPWS III and iPWS IV whereas low turbidity levels at all taps and reservoirs of iPWS III and iPWS IV were not associated with *E. coli*. The association of turbidity in influencing water treatment process and initiating microbial growth have been reported so far (JMP, 2012; LeChevallier et al., 1981). Also, several findings showed the relationship of turbidity with microbiological contamination of drinking water (LeChevallier & Norton, 1993; LeChevallier et al., 1991).

The pH value measured for all samples ranged between 6.5-8.3 and all samples from the iPWS's meet the Nepalese and WHO standard range value of 6.5-8.5. Except for taps of iPWS II, no correlation was observed between *E. coli* concentrations and pH. Although the observed correlation was weak and negative. Negative association of coliforms with pH were reported in a study conducted in US (Sanderson et al., 2005 ). However, the study was not on water sample used for drinking purpose.

A negative correlation between *E. coli* concentration and temperature was observed at sampling points of iPWS II. The finding of this study at iPWS II contradicts with the finding of LeChevallier et al., (1996), the study showed an increased microbial activity when temperature of water was above 15°C. However, the association of temperature with *E. coli* at spring and taps of iPWS III were consistent with the previous study.

The sanitary risk score and risk category were not associated with the microbial contamination of the iPWS's. For instance, a higher level risk category at spring of iPWS II and III fall under low level median *E. coli* concentration. This illustrates that comparing existing microbial contamination with possible risks might not predict the overall microbial safety of drinking water. In addition, observation of the physical integrity and external features of the supply system cannot give a clear picture about the microbial quality of water.

The finding of this study also confirm with other studies conducted so far. For instance, a study aimed to associate microbial contamination of tube wells with risk level of the wells in Bangladesh showed no correlation between the microbiological quality of water and the sanitary condition of the wells (Luby et al., 2008). Similarly, a study conducted by Parker et al., (2010) at improved water sources also showed weak predictive power of sanitary inspection in predicting the microbial quality of water and no correlation was observed between risk category and feacal contamination of the water sources. However, the finding of this study contradicts with a study conducted in Tanzania, according to the study the microbial quality of wells was associated with the risk of faecal contamination (Mushi et al., 2012). The contradiction might be due to the study in Tanzania was conducted for a duration of 3 months and the sample size for the sanitary inspection was relatively high when compared with this study.

The risk of contamination for 96.3% (n=26) of sampling points ranged between medium and very high. Low risk of contamination was observed at only one sampling point (iPWS II reservoir) whereas very high risk categories were observed at spring sources indicating that the springs were the main risk factors for contamination. Moreover, the degree of contamination at the spring sources illustrates the discrete nature of contaminants. This is also supported by the tracer test conducted at the upstream of the springs. The electrolyte solution dosed at upstream of the springs took almost one hour to get through into the subsurface of the springs. However, the overall risk category of the sampling points did not correlate with the microbial water quality of the supply system. The observed increased concentration of *E. coli* and total coliforms at spring sources is more probably due to a wide range of diffuse sources of pollution instead of non-diffuse sources of pollution that were identified by the sanitary checklists and observation of the supply system.

## CHAPTER 7

# Conclusion and recommendation

## 7.1. Conclusion

The study results showed a widespread variability of faecal contamination in terms of spatial as well as temporal dynamics at sampling points monitored. The following conclusions were made based on the finding of the study:

- At iPWS II the percentage of samples contaminated with *E. coli* increased from 97% at spring to 98.6% at taps, implying the variability within space and deterioration of water quality between the sampling points. No variability was observed between spring and reservoir.

- The spatial variability result at iPWS III revealed the percentage of samples contaminated with *E. coli* decreasing from 94% at spring to 63% at reservoir and increasing from 63% at reservoir to 74% at tap stands. The pattern of spatial variability reflects improved quality at reservoir and subsequent re-contamination at tap stands.

- At iPWS IV the spatial variability result showed the level of *E. coli* contamination increasing from 91% at spring to 97% at reservoir and decreasing to 94% at distribution chamber and subsequently to 91% at tap stands.

- The result of the temporal variation showed peak *E. coli* concentrations primarily originating from spring sources of the iPWS's.

- The sanitary inspection result showed that 96.3% of sampling points had medium to very high risk of contamination. Sanitary condition of the iPWS's did not correlate with the microbial water quality of the supply system.

- Sampling points with high turbidity values were associated with *E. coli* contamination.

- The tracer tests showed high risk of infiltration of contaminants from the external environment into the spring sources of the iPWS's.

- The findings of the study give indication that infiltration from nearby agricultural fields into the springs as a major means of contamination.

Generally, leakage, poor source protection and infiltration of contaminants affected the microbial quality of water at the study sites.

## 7.2. Recommendation

1. The finding of this study did not take into account the seasonal variation of microbial water quality in the study area. So, further research needs to done to compare the water quality between dry and monsoon seasons.

2. Understanding the transport of *E. coli* and total coliforms in soil of the springs will enable to take preventive measures depending on the dynamics.

3. Treating the water at reservoir might reduce the level of contamination and the associated water borne illness around the area.

4. Restricting agricultural activities around the spring area might reduce the rate of infiltration of contaminants into the spring source.

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# Appendices

## Appendix A **Laboratory procedure**
## Membrane filtration method

The method described below is based on US EPA and WHO membrane filtration technique.

**Materials and consumables:**

1. Incubator
2. Compact dry plate
3. DelAgua filtration set
4. 0.45 µm membrane filter
5. Adjustable Pipette and 1 ml pipet tips
6. SteriPEN
7. Medical gloves
8. Hand sanitizer
9. Insulated container
10. NascoWhirl-pak
11. Permanent marker
12. Methanol dispenser

**Sample collection:**

- Label Whirl-pak with permanent marker and pour 100 mL water by opening the Whirl-pak
- Whirl the pak 3-4 times and place the sample inside insulated container filled with sterilized water

**Sterilization:**

- Clean working bench with alcohol wipes
- Prepare sterilized water using steriPEN
- Sterilize pipet tips using boiled water
- Dry the inside of DelAgua filtration set with cotton then add 15-20 ml of methanol and ignite with lighter.
- Wait until the methanol burns and then place the filtration head into the sampling cup and wait for up to 15 minute

**Sample processing:**

1. Label compact dry plate with unique sample ID
2. Take 1 mL of sterilized water with pipet and place on a CDP
3. Sterilize tip of forceps/ tweezer
4. Using sterilized tweezer transfer membrane filter into filtration apparatus and place on the filter disc
5. Pour 100 ml of water into filtration funnel

6. Connect the vacuum pump to the aluminium base of the filtration set and pump until the sample is completely filtered
7. Remove the filtration funnel and using sterilized tweezer pick the membrane filter and transfer to CDP
8. Incubate CDP at 35±2 °C for 24 hours
9. After 24 hours take CDP out of incubator and count bacterial colonies. *E. coli* develops blue colonies, whereas total coliforms develop pink/red colonies. Report the concentration as CFU/100 mL.

**Quality control:**

- Daily run positive and negative control sample
- Laboratory blank- test sterilized water used to wet the surface of CDP as a lab blank
- Field blank- prepare sterilized water and transfer to Whirl-pak and place in an insulated container. Test the sample if there is a possibility of re-contamination during transport

# Appendix B **Sanitary inspection checklist**

*The sanitary inspection checklists were adapted from WHO (1997)*
Response ID:
Date:
Sampling point linked to this survey: Tap stand

| | | | |
|---|---|---|---|
| 1. | *"Do any tap stands leak at the sample site?"* | *Yes* | *No* |
| 2. | *"Does surface water collect around any tap stand?"* | *Yes* | *No* |
| 3. | *"Is the area uphill of any tap stand eroded?"* | *Yes* | *No* |
| 4. | *"Are pipes exposed close to any tap stand?"* | *Yes* | *No* |
| 5. | *"Is human excreta on the ground with 10 meters of any tap stand?"* | *Yes* | *No* |
| 6. | *"Is there a latrine uphill and/or within 30 meters of the tap stand?"* | *Yes* | *No* |
| 7. | *"Has there been discontinuity in the last 10 days?"* | *Yes* | *No* |
| 8. | *"Are there signs of leaks in the mains in the area?"* | *Yes* | *No* |
| 9. | *"Do users report any pipe breaks within the last week?"* | *Yes* | *No* |
| 10. | *"Is the supply main pipeline exposed in the area?"* | *Yes* | *No* |

Response ID:
Date:
Sampling point linked to this survey: Reservoir tank

| | | | |
|---|---|---|---|
| 1. | *"Does the pipe leak between the source and storage tank or reservoir?"* | *Yes* | *No* |
| 2. | *"Does water collect around the reservoir?"* | *Yes* | *No* |
| 3. | *"Is the area around the reservoir insanitary?"* | *Yes* | *No* |
| 4. | *"Is there a sewer or latrine within 30 m of the reservoir?"* | *Yes* | *No* |
| 5. | *"Is the supply pipeline exposed around the reservoir?"* | *Yes* | *No* |
| 6. | *"Is the supply tank cracked or leaking?"* | *Yes* | *No* |
| 7. | *"Are the vents on the tank damaged or open?"* | *Yes* | *No* |
| 8. | *"Is the inspection cover or concrete around the cover damaged or corroded?"* | *Yes* | *No* |
| 9. | *"Is human excreta on the ground within 10 meters of the reservoir?"* | *Yes* | *No* |
| 10. | *"Is the area uphill of the tap stand eroded?"* | *Yes* | *No* |

# Appendix C **Scatter plots**

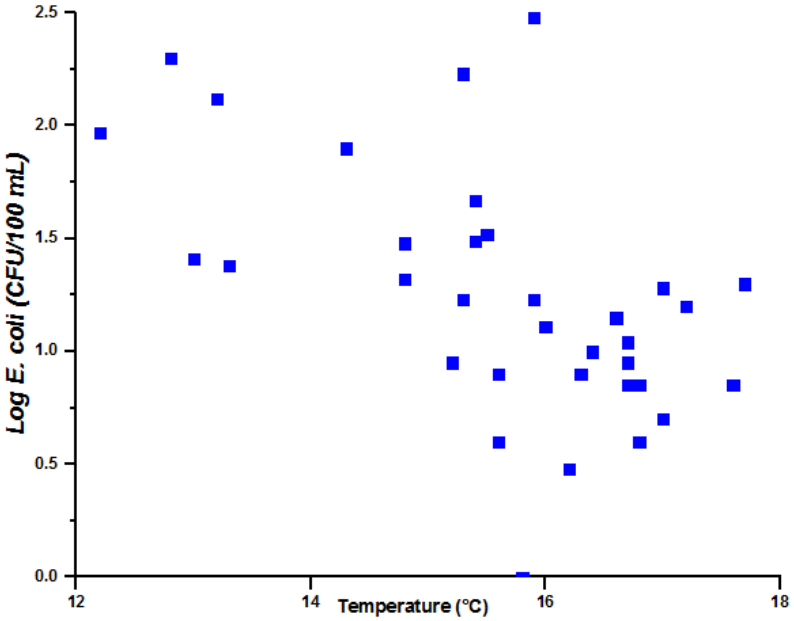


**Figure 44** Scatter plot of *E. coli* and temperature at spring of iPWS II

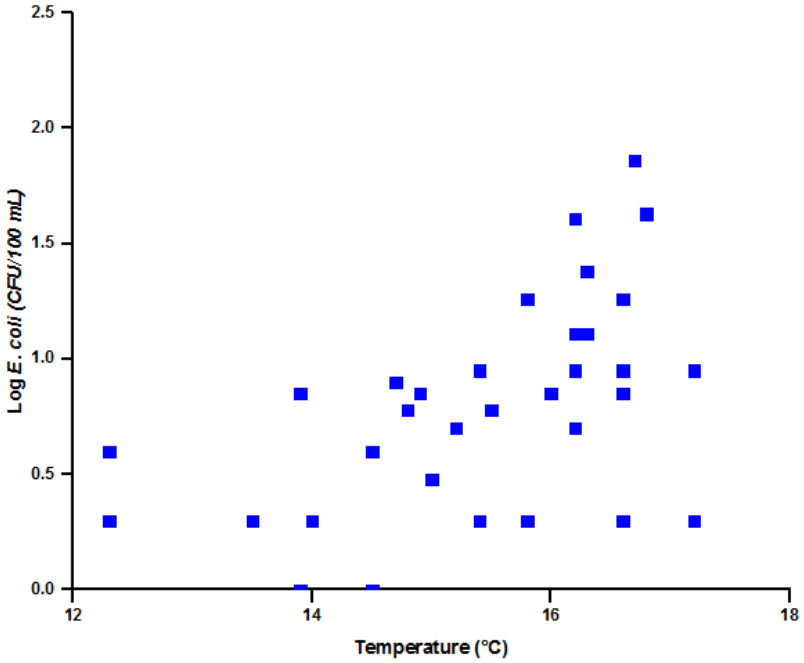


**Figure 45** Scatter plot of *E. coli* and temperature at reservoir of iPWS II

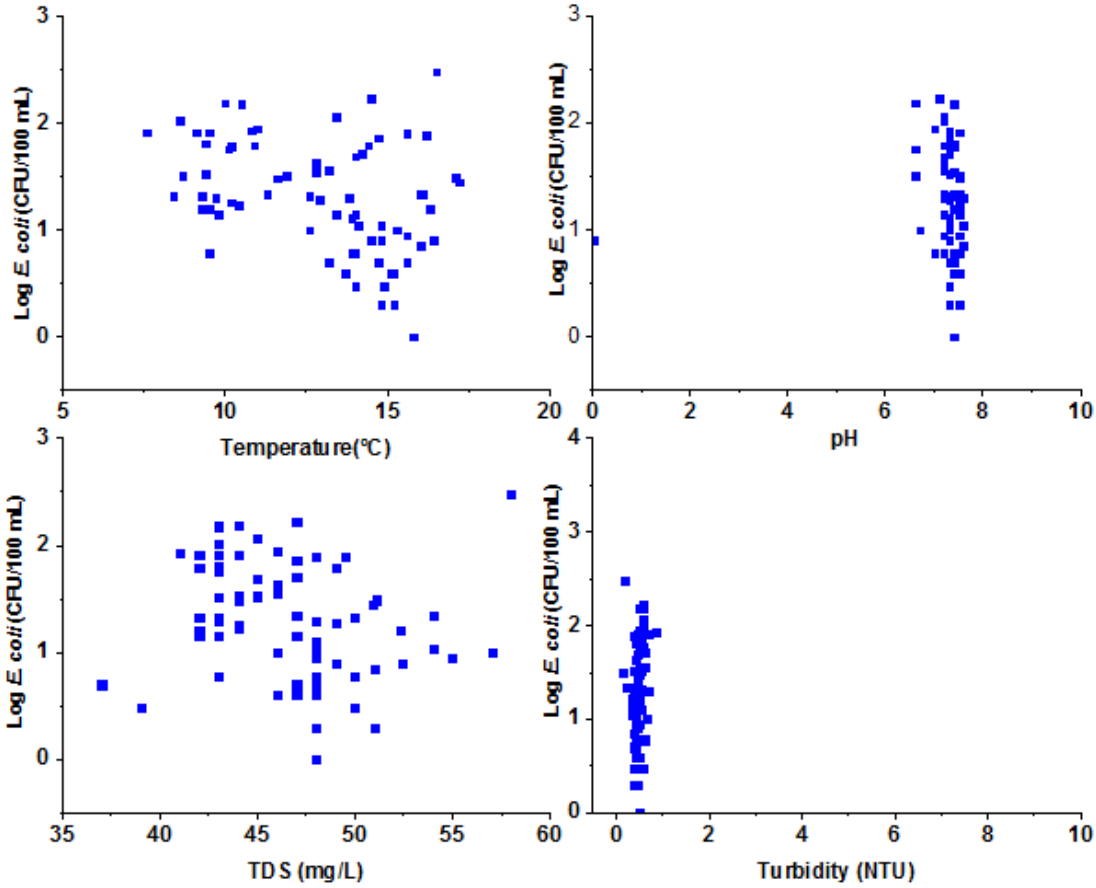


**Figure 46** Scatter plot of *E. coli* with physical parameters at tap stands of iPWS II

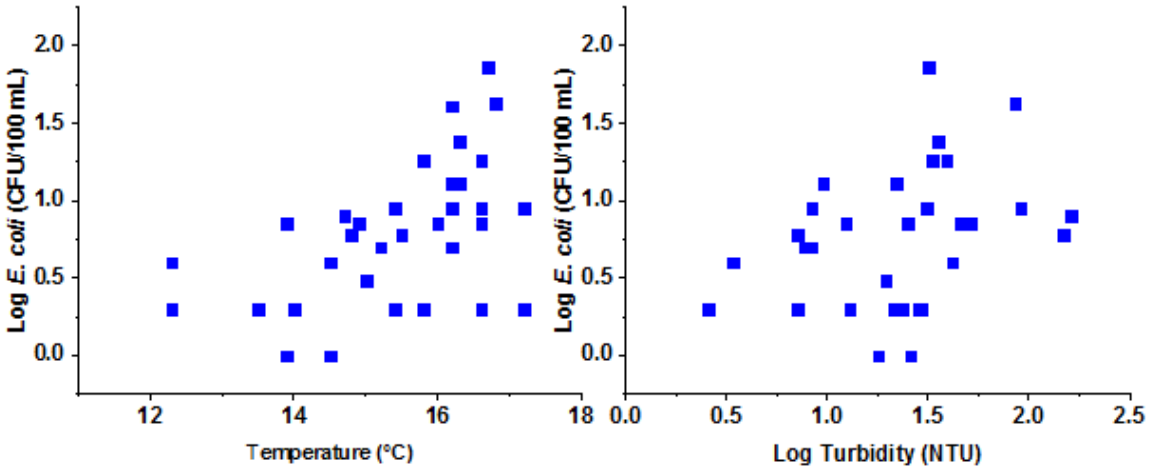


**Figure 47** Scatter plot of *E. coli* with temperature and turbidity at spring of iPWS III

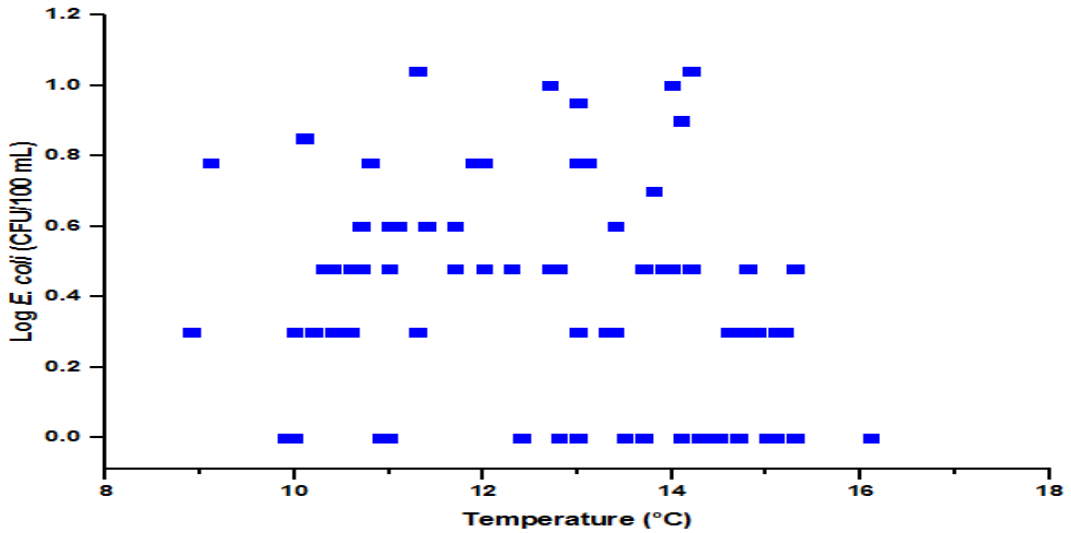


**Figure 48** Scatter plot of *E. coli* with temperature at tap stands of iPWS III

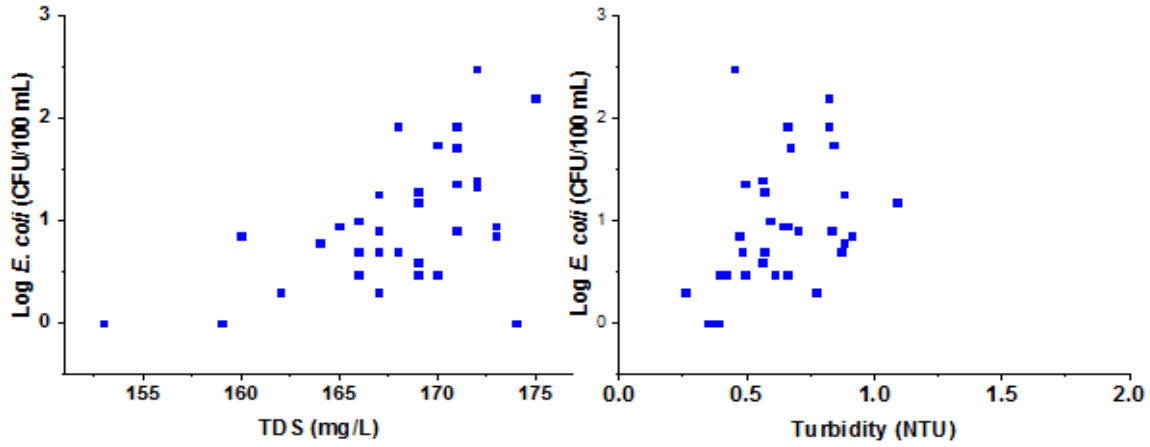


**Figure 49** Scatter plot of *E. coli* with TDS and turbidity at spring of iPWS IV

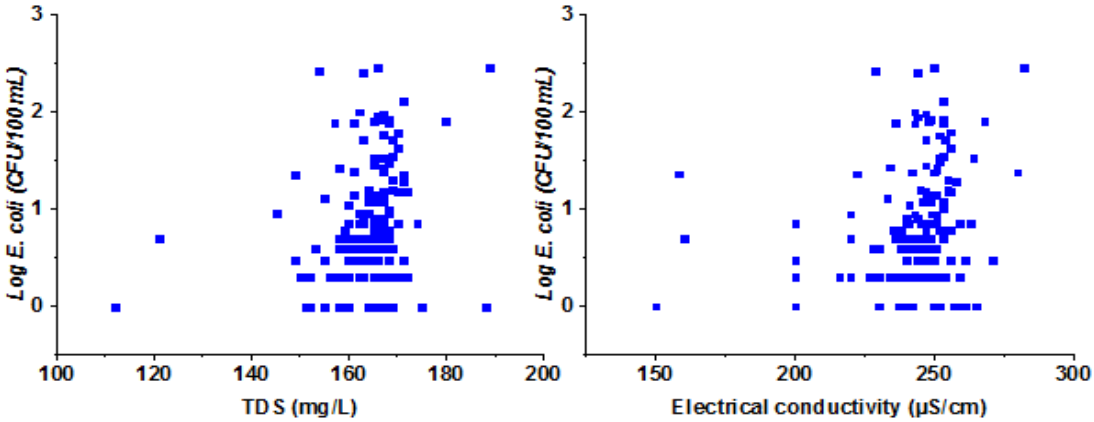


**Figure 50** Scatter plot of *E. coli* with TDS and electrical conductivity at taps of iPWS IV