DRINKING WATER QUALITY ASSESSMENT IN SELECTED AREAS OF RAWALPINDI BY ANALYSING PHYSICO-CHEMICAL AND BIOLOGICAL PARAMTERS

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A thesis submitted to Bahria University, Islamabad in partial fulfilment of the requirement for the degree of B.S in Environmental Sciences

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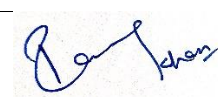
External Examiner	**Ms. Romana Khan**	
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ABSTRACT

In this study, drinking water quality of different densely populated areas of Rawalpindi was analyzed. Since, Commercial Market, Satellite Town, Murree Road, Khayaban-e Sir Syed, Pirwadai are among the busiest areas of Rawalpindi and are densely populated. The main source of water in the area is water from filtration plants and bore tap waters. The filtration plants are installed by WASA under Punjab government and tap water. Since, demand of water is greater than its supply, many locals have installed bores in order to meet their increasing water demand. The main purpose of this study was to determine the quality of drinking water in selected areas of Rawalpindi and to determine whether it is related to gastrointestinal diseases or not. For this, water samples were collected from eleven water filtration plants, this included both government and private plants and nine bore tap waters were selected. The study aimed to analyze both physicochemical as well as microbiological parameters. The physicochemical parameters were pH, EC, Salts, TDS, Turbidity, Total Alkalinity, Total Hardness, Total Chloride, Calcium, Magnesium, Sodium, Carbonates and Arsenic. The microbiological parameters were total bacteria, salmonella, shigella and total coliform. The samples were analyzed by using the standard procedures as listed in APHA. The results from the analysis showed all the parameters were within the permissible limits, except for EC and salts denoting that ions are present in greater amount in water samples. In case of biological parameters, in few samples, growth of salmonella and shigella as well as total coliforms was found to be exceeding permissible limit denoting that water from these sources are contaminated and not fit for use. Tap water samples which were basically bored from groundwater were found to be more contaminated with bacteria as compared to water samples from filter plants.

Keywords: Physicochemical parameters, Biological parameters, Quantitative research method, filtration plants, tap water, Acid-Base titration, Plate count method, Arsenic, Gram staining, distilled water, Agar medium, Autoclave, Incubator, Laminar flow hood, Petri plates, Glass slides.

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ABBREVIATIONS

APHA: American Public Health Association

CDA: Capital development Authority

EC: Electrical Conductivity

EPA: Environmental Protection Agency

NSDWQ: National Standards for Drinking Water Quality

PSQCA: Pakistan Standard and Quality Control Authority

RCB: Rawalpindi Cantonment Board

TA: Total Alkalinity

TC: Total Chlorides

TDS: Total Dissolved Solids

TH: Total Hardness

WAPDA: Water and Power Development Authority

WASA: Water and Sanitation Agency

WHO: World Health Organization

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CHAPTER 1

INTRODUCTION

1.1 Background

Water, chemically a compound made by covalent bonding of two hydrogen atoms and one oxygen atom, but biologically it is what made life possible on this green planet. Water is the fundamental requirement for all life. The existence of every living organism itself revolves around water. Since water is the essence of life and all life on earth depends on it, for this very reason, this resource has been the cause of wars and disputes since the beginning of time. In past, it was priority of every civilization to settle near fresh water source. It can be seen in nature that wherever water is present, life seems to bloom around it, while places that don't have access to water are barren like the deserts. In today's, world although distance has become short due to advancement in technology, still disputes over water continue. Today the disputes exist, but now race is to get access to warm waters, in order to accelerate the import, export and economic purposes (Chaplin, 2006).

Fresh water being one of the most important renewable natural resources on earth holds great importance, be it for humans, animals, plants or any other living organism. Since, all life on earth directly or indirectly depend on water for its existence, so water requirement is an issue that cannot be left unaddressed. Importance of water can be described by the fact that 68% of our bodies are made of water while 55% of blood is made of water, this is how essential water is for life. Water covers 70% of the earth and is present in all forms, liquid, solid and gaseous state. Water is necessary, it is a sentence that can never be underrated, be it for agriculture, aquaculture, for feeding farm animals, for economy, we need water for everything. Even to promote tourism we require water, as clean, healthy environment is a basis for good tourism (Chaplin, 2006).

Water shortage is an issue that is being the cause of distress for many countries and cannot be ignored. When we say shortage of water, we don't necessarily mean that water levels are decreasing but rather that the form in which water is present is not fit for human use. Water throughout its life cycle passes through different phases, but not all these phases of water are useful for mankind. Similarly, when water is polluted by chemicals, toxins or when sewerage waste is mixed with fresh clean water then this alters the quality of water thus making it unfit for drinking purposes, termed as shortage of water (Hadadin et al., 2010).

Water quality holds great importance, mainly due to the impact it has on aquatic life, other animals as well as human health. Today, due to increasing pollution, shortage of water is an issue that cannot be underestimated. Pakistan is also a victim of lack of availability of fresh clean water. Around only 20% of population in Pakistan have access to fresh clean water while the rest 80% rely on polluted water sources. Major source of water contamination is discharge of sewerage water in fresh water resources, this is a practice that is widely done, while the other factor is discharge of toxins and harmful chemicals from industries and pesticides, herbicides, fertilizers runoff from agricultural field (Daud et al., 2017). This discharge of untreated water into fresh water sources is what causes shortage of clean fresh water for human use. Once this polluted water mixes with clean water, it makes it polluted as well, thus causing propagation of waterborne diseases. Due to vast amount of waste discharged in streams, rivers be it industrial or municipal, aided by nature of running water to flow, waste is carried from one place to other, thus polluting far away areas as well. Anthropogenic activities are the main cause for leading to detrimental effects on water. The amount in which industrial effluents and domestic wastes are discharged are so immense that the rivers are unable to cope up with all that waste and are thus unable dilute waste in such amounts. Whereas, increasing human population followed by increasing water requirement puts great pressure on demand for fresh water. Since, demand of water is high then suitability is something that also needs to be in check. For this purpose, we conduct the water assessment by following various techniques to determine water quality. The water quality is based on certain physicochemical parameters, biological parameters and heavy metals which show whether water is fit for use or not. Actually, whenever we talk about quality of water, by it we basically mean the purpose for which water is being is used for, for example, if water is being used for domestic purposes, irrigation purposes, industrial purposes or drinking purposes, depending upon the purpose requirement for water quality also changes. In short, water quality monitoring and analysis form the base for water quality management (Hall et al., 2007).

1.2 Gastrointestinal Diseases

Gastrointestinal diseases are all those diseases that are related to digestive system, which basically consist of number of organs like stomach, small intestine, large intestine etc. Examples of such disorders include, food poisoning, constipation, irritation in bowel etc. (Chan et al., 2019). There are many factors that can irritate or affect gastrointestinal tract like presence of certain bacteria in the body like *salmonella, shigella,* and *E-coli,* indicating fecal contamination in water being ingested. Similarly, excessive presence of salts or acidic substances can also irritate stomach line, causing ulcer. Certain bacteria, chemicals, heavy metals can enter body in any way through food as well as water. But water plays a major role in causing gastrointestinal issues, since in Pakistan, availability and access to clean water is a major issue (Khan et al., 2013).

1.3 Waterborne Diseases

Waterborne diseases, basically, are all those diseases that are caused by microbiological, chemical agents as well as heavy metals that are present in contaminated water and cause harm to human beings. Waterborne diseases are not only caused by microbiological contamination but also by chemical and physical pollutants. Since, whenever quantity of certain chemicals or microbiological agents' increases, then such contaminants deteriorate water quality and cause diseases in human body. Some of the most common examples of waterborne diseases are:

Diarrhea: There are some new environmental bacteria which are capable of multiplying and surviving for longer periods in (WDS) Water Distribution System. Diarrhea is the most common waterborne infection among the developing nations. It is the frequent release of watery defecation from the intestines, sometimes also contains blood and mucus. The significant reason of diarrhea is the consumption of water, contaminated with pathogens from human or animal feces, and without filtration and treatment. The most common victims are young children and individuals with weaker immune systems. Diarrheal illnesses brought about by more than 20 viral, bacterial, and parasitic infections are liable for 2 to 2.5 million deaths every year (Fenwick, 2006).

Cholera: Cholera is a waterborne illness described by extreme loose bowels. Vibrio cholerae is a water bacterium that is normally present in salty water and estuaries, which can contaminate clean water sources as well. The microorganism Vibrio cholerae form a colony in the small intestine and there it produces a toxin called enterotoxin that is responsible for watery loose bowels. Eating food washed with dirtied water can in like manner spread the illness. Cholera dominatingly happens in children, yet it can similarly influence adults. The symptoms include abdominal cramps, vomiting, severe dehydration and diarrhea (Codeço, 2001).

Hepatitis: There are some unique group of viruses, that can be found in the gut of humans. Some of these viruses, for example, Hepatitis A virus (HAV) and Hepatitis E virus (HEV), cause serious illnesses. Hepatitis A virus is the most common and basically impacts the liver. It is basically obtained through the ingestion of water or food, debased with the hepatitis virus. Patients infected with this virus manifest basic symptoms including yellow staining of the skin and whites of the eyes, stomach distress, vomiting, fever, jaundice, and weight reduction. The route of entry is fecal-oral, nevertheless, it is additionally exceptionally contagious (Aggarwal and Naik, 1994).

Typhoid: Typhoid fever is a severe bacterial infection brought about by the bacterium Salmonella Typhi, which normally spreads by the use of tainted water or eating food washed with water debased with the defecation of a contaminated individual in poor cleanliness conditions. When the microbes enter the individual's body, they duplicate as well as spread into the circulatory system via the digestive tract. The symptoms include; abdominal rash, high fever, weakness, diarrhea, constipation, loss of appetite, and enlargement of the liver and spleen. This ailment influences around 12 million people globally every year. It can be lethal if left untreated (Egoz et al., 1988).

1. 3.1 Waterborne Disease-Causing Agents

***Salmonella*:** *Salmonella* disease (salmonellosis) is a typical bacterial infection that influences the intestinal tract. Salmonella microorganisms generally live in human and animal digestive organs and are shed through defecation. Most instances of salmonella originate from ingesting food or water tainted with defecation. The most possible health risks associated with Salmonella bacteria are; Gastroenteritis, typhoid fever or enteric fever. Older people, children, and individuals with compromised immune systems are most at serious risk (Angulo et al., 1997).

Shigella**:** *Shigella* infection (shigellosis) is an intestinal ailment brought about by a group of microscopic organisms known as shigella. This bacterium is spread through tainted water and food or contact with contaminated excrement. The microscopic organisms discharge toxins that disturb the digestion tracts. The most possible health risks associated with Shigella bacteria are intestinal diseases including dysentery (Herwaldt et al., 1991).

Escherichia coli (E. coli)**:** Pathogenic Escherichia coli strains are the significant species in the fecal coliform gathering. It is viewed as the best indicator of fecal pollution and the conceivable presence of pathogenic organisms in water. *Escherichia coli (E. coli)* is a gram-negative bacterium that is normally found in the lower digestive tract of warm-blooded living beings. A few strains of *E. coli* are harmful while some supportive strains of E. coli microorganisms are significant in making a sound intestinal tract. If pathogenic *E. coli* is present in water, it can cause serious intestinal infection (Nabeela et al., 2014).

Giardia lamblia**:** *Giardiasis* is a disease of the small intestine, transmitted through the consumption of water or food debased by the excrement of the tainted individuals. The parasite protozoan liable for this illness is *Giardia lamblia*, which lives in the intestines of infected humans and animals. The route of entry of this microorganism is fecal-oral. In new-born children, immunocompromised people, or those with fundamental diseases, it can cause serious, even deadly diarrhea (Ljungström and Castor, 1992).

Cyclospora Cayetanensis**:** The diarrheal infection brought about by the protozoan parasite *Cyclospora cayetanensis* is known as *Cyclosporiasis*. Individuals become tainted with *Cyclospora* by consuming water or food that has been debased with defecation that contains the parasite. In all probability, fecal dirtied water, utilized for spraying biocides on organic product, can cause indirect contamination (Ortega et al., 1998).

1.3.2 Physicochemical Parameters

Water is a universal solvent as it is capable of dissolving both organic and inorganic compounds in it. To check the quality of water, physical and chemical parameters of water are analysed.

Physical parameters include; Colour, odour, transparency, turbidity, and temperature.

Chemical parameters include; Salinity, hardness, total dissolved solids, total suspended solids, PH, electrical conductivity, alkalinity, dissolved oxygen, sodium, sulphate, nitrate, phosphate, fluoride, chloride, calcium, potassium, and magnesium.

Parameters such as temperature, turbidity, PH, and electrical conductivity are checked immediately after collecting water sample, while, remaining parameters are analysed in laboratory. The results are then compared with standard values of W.H.O guidelines. If these parameters of water exceed their standard limit values, then that water is considered unfit for consumption purposes. Electrical conductivity and total dissolved solids are directly related because, with the increase of mineral salts or TDS, the electrical conductivity increases. The poor quality of water causes many health problems including; kidney problem, low blood pressure, headaches, fatigue, hypertension, nausea, and stomach problems (Reda, 2016).

1.3.3 Heavy Metals

Heavy metals are those elements that have a high density and are toxic even at low concentrations. These metals are naturally present in the earth's crust. Heavy metals include; mercury, arsenic, cadmium, chromium, lead, nickel, thallium, copper, iron, and zinc. Some of these heavy metals are also present in human body as trace elements. These metals such as copper, iron, cobalt, magnesium, chromium, molybdenum, selenium, manganese, zinc and nickel are essential nutrients for maintaining the metabolism of human body. However, higher concentration of these metals lead to poisoning. The poisoning occurs due to consumption of water polluted with heavy metals. The source of these metals into the water body is industrial waste and sometimes acid rain (Singh et al., 2010). After entering into the body via drinking water, they can also bio-accumulate. Water used for consumption purposes must be checked for heavy metals, before consumption. These metals, after entering into human body, cause many gastro-intestinal diseases. They can also disrupt the mucosal lining of gastro-intestinal tract and can lead to cancer. Symptoms of gastro-intestinal infection include; nausea, fever, diarrhea, vomiting, GI epithelial irritation, stomach ulcer, gastroenteritis, colon cancer, and abdominal pain (Wilson et al., 2014).

1.4 Drinking Water Quality

Water quality has always been a grave issue, but in the past few years, the issue has increased mainly due to increasing population, that puts water supply in stress and accelerated by pollution of water. But with the advancement of time and knowledge as well as after seeing shortage in supply of water in Pakistan, people have become aware of the fact that water shortage is a reality and there should be no compromises made when it comes to drinking water. Being a country with high mountain ranges, glaciers, Pakistan does not lack fresh water resources but due to industrialization, weak sanitation practices, aided by a lack of proper water filtration practices, water quality has deteriorated. Increasing population and demand for water result in hindering of water resources while aiding water shortage. Pakistan is also a victim of lack of availability of fresh clean water. Around only 20% of population in Pakistan have access to fresh clean water while the rest 80% rely on polluted water resources. Major source of water contamination is discharge of sewerage water in fresh water resources, this is a practice that is widely done while the other factor is discharge of toxins and harmful chemicals from industries and pesticides, herbicides, fertilizers runoff from agricultural field (Daud et al., 2017). This discharge of untreated water into fresh water sources is what causes shortage of clean fresh water for human use. Once, this polluted water mixes with clean water making it polluted as well thus causing propagation of waterborne diseases.

The country's water capitals have been stressed enormously by rapid population growth and continuous industrial development. The prolonged droughts and population growth have further exacerbated water shortages and contamination. While Pakistan does have both ground and surface water resources, the availability of water per capita has fallen from 5,600 m^3 to 1,000 m^3 per year (Shahid et al., 2015). Drinking water quality in Pakistan is depleting day by day due to the effluent and pollutant released by different industries. Piped water also gets contaminated because the network of the pipeline is not planned properly and laid poorly. This leads to the leachate being seeped into the water sources and cause diseases which are waterborne. According to a monitoring report published by Pakistan Council of Research in Water Resources, they monitored 369 drinking water sources out of which 116 water sources (31%), were supplying safe drinking water and 253 (69%) were determined as unsafe (PCRWR, 2007).

1.5 Situation in Pakistan

Waterborne diseases although a worldwide issue but are more prevalent in developing countries due to a lack of planning. In Pakistan, as well diseases propagating through water are no less either. Due to limitation of access to clean water, Pakistan is also one of those countries where bacteriological contamination of drinking water is a major problem. Since, once water is contaminated be it bacteriological contamination or increase and decrease in physicochemical properties of water it leads to water borne diseases (Nawab et al., 2016). According to a study in Punjab, people suffering from waterborne diseases range up to 90% of the total population. Diseases like, cholera, dysentery, irritation in bowel, diarrhea etc. (Daud et al., 2017). Contaminated drinking water is one of the major reasons as to why there are so many cases of gastrointestinal patients in hospitals and thus increasing hospital load.

1.6 Problem Statement

The study area is different areas of Rawalpindi. The areas selected for study are densely populated and of the main areas of the city. There are two main drinking water sources in Rawalpindi; surface water supplied by Rawalpindi Development Authority (RDA) and ground water (Uzaira et al., 2005). The purpose of the study is to determine the water quality of the water filtration plants located in the selected areas and to check its prevalence with gastrointestinal diseases i.e. whether water plays a role in accelerating gastrointestinal diseases. As, it was reported in June and August of 2019, that in 2019 from May onwards number of gastrointestinal patients and patients suffering from vomiting and so, drastically increased (Qasim, 2019).

1.7 Literature Review

Lack of availability of potable water is an issue that has been prevailing in the world, targeting the third world countries at most. If we look back in time, then this is an issue that's increasing at a very fast pace. For this reasons, water quality has always been a topic under discussion. The topic has been researched upon many times, at various locations. The fact that low quality drinking water can be a cause of increasing number of gastrointestinal cases has been well explained by a project done in suburban area of Montreal, which concluded that 35% of the gastrointestinal cases reported were related to water and were such that could be prevented with proper filtration and management (Payment et al., 1991).

Many water issues can easily be tackled by maintaining filtration plants, ensuring water quality is under drinking water standards frequently. But rate of prevalence of waterborne diseases in such community is especially greater where proper sanitary conditions are compromised. Where literacy rate is also low and people do not take proper measures like boiling water before drinking So, depicts a study conducted in a community residing near River Ravi, Lahore (Qureshi et al., 2011). The sanitary conditions of the community were such that a good number of people lacked proper toilet facility due to which for defecation, they had to go to fields. Due to such unplanned settlements along with poor drainage system and sanitation, groundwater has become contaminated with fecal contamination. And since the river is the major source that recharges the aquifer upon which most of the population of Lahore is dependent, thus increasing the number of people suffering from waterborne diseases like vomiting, diarrhea etc.

In another study of Pakistan, with Rawalpindi and Islamabad as target location, analysis of four different sources of drinking water was done: WASA, CDA, Boring and tanker water. It was derived from analysis that waterborne diseases prevail wherever fecal contamination exists. And the order of decreasing prevalence of waterborne diseases was so; WASA, CDA, Boring water and tanker water (Shoaib et al., 2016). Aside from microbiological contamination like fecal coliforms, higher levels of turbidity in waster also play a role in causing gastrointestinal diseases.

Aside from microbiological contamination, physical parameters like turbidity can also affect water quality. Turbidity basically depicts the clarity of water so when under standard level, it is not an issue but when organic matter, inorganic material, algae, silts concertation increase in water then issues start increasing and thus increases chances of gastrointestinal diseases. As explained in the research article of London depicting that under certain levels of turbidity there exists likeliness of association between gastrointestinal diseases and turbidity (Mann et al., 2007). In another investigation, drinking water of Islamabad from various sources like; tube wells, filtration plants, and water supplies of different sectors were tested. The water was tested for bacteriological contamination and out of fifty-five samples that were analysed, 14.5% of the samples did not meet the drinking water standards set by WHO (Ahmed et al., 2015).

Another study of 2005 indicated that after analysing and comparing drinking water quality of Rawalpindi and Islamabad. Water quality was found to be slightly better in Islamabad as compared to Rawalpindi. But a lot of the samples that were taken were found to have exceeded permissible limits for TDS and microbiological parameters as set by WHO and EPA (Uzaira et al., 2005).

In another study conducted in Pakistan about drinking water quality and its status in Pakistan, it was found out that a lot bacterial growth was determined in water samples collected. If in a sample E. coli is found, then this means that there are traces of fecal contamination. When it comes to drinking water, and fecal contamination then only thing accountable for it is that either sewerage water is being mixed with drinking water or chemical pollution due to discharge of toxins effluents like industrial waste water, dyes, pigments fertilizers run off, pesticides are making their way to the water source and contaminating it (Daud et al., 2017).

Across these studies done at various locations at different time, there exist reliable evidence that waterborne diseases, and poor drinking water quality are related to each other. And certain conditions, like poor sanitation conditions, lack of planning may lead to gastrointestinal diseases. Elements that deteriorate the water quality not necessarily have to be organic matter but in most of the cases fecal contamination, *E. coli* and so are the ones that cause most of the harm. As discussed in one the studies physiochemical parameters like turbidity also plays a role when concentration crosses standards set by WHO. Similar is the case with heavy metals as well. Anything above the standard or below the standard limit can be harmful.

1.8 Scope and Objectives

The aim of this study is basically to determine the water quality status of both the major sources of water in the study area selected in Rawalpindi i.e. areas around Murree Road, satellite town etc. For this purpose, various physicochemical and biological parameters of drinking water in the area will be determined. The result of the analysis will then be compared with permissible limits as mentioned by WHO, EPA, NDWQS. This is important as contaminated water plays a significant role in causing diseases, this will also the fact whether drinking water quality plays a role in causing gastrointestinal diseases.

1.8.1 Objectives

The objectives of this study were:

1) To carry out physicochemical analysis of water samples collected from various water filtration plants and tap water located in study area.
2) To determine microbial counts in water samples

CHAPTER 2

MATERIALS AND METHODS

2.1. Methodology

In order to determine drinking water quality of selected areas of Rawalpindi quantitative research method was used. Quantitative research methods are those methods that rely on measuring variables using a numerical system, analysing these measurements using any of a variety of statistical models, and reporting relationships and associations among the studied variables. In this study waters samples from both filtration plants and tap (bore) water were analysed and then compared. The methodology adapted for the research work is shown in the flow chart below:

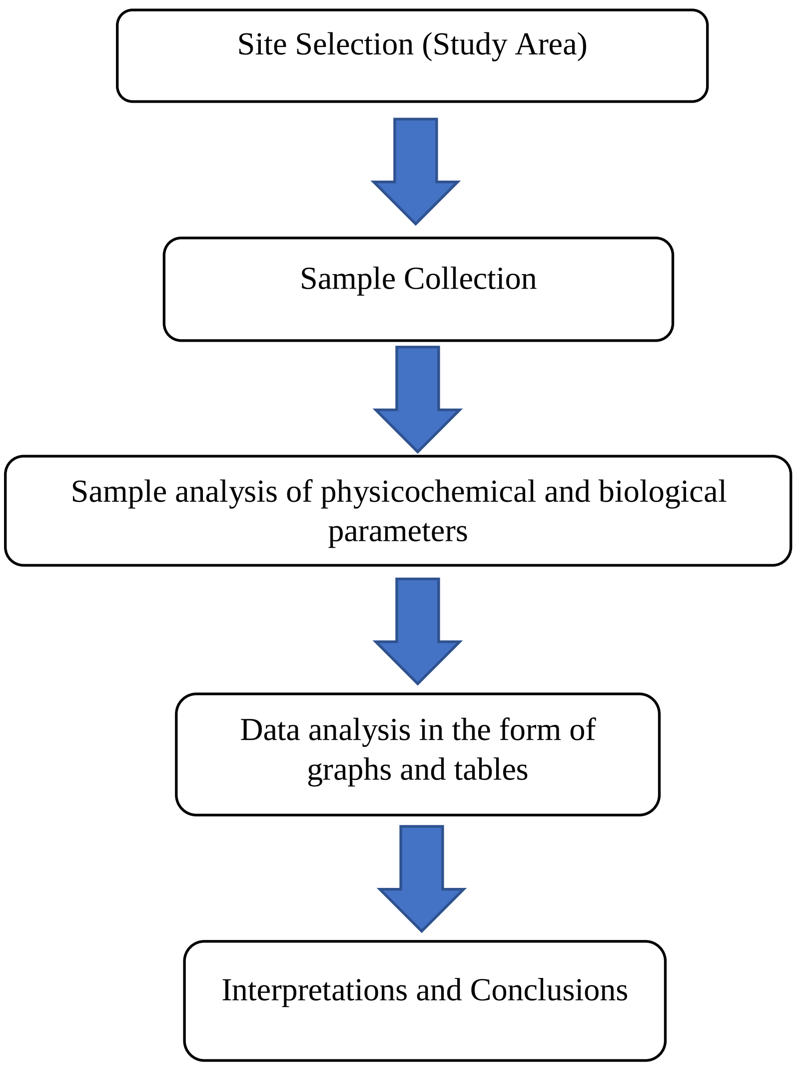


Figure 2.1: Flow chart showing research work methodology

2.2 Study Area

Study area, selected for the study includes densely populated and main areas of Rawalpindi i.e. the capital of Rawalpindi Division, in Punjab, Pakistan. Sites from where water samples for analysis were collected are basically locations near Murree road including, Satellite Town, Pirwadhae, Khayaban e Sir Syed. These are some of the busiest areas of Rawalpindi, being the hub of wholesale shops, businesses etc. A lot of renowned educational institutions as well as residential societies are also located in the area. The main source of water in the study area is surface water and groundwater. Water to filter plants is supplied from Khanpur Dam and Rawal Lake under RDA. But due to increasing demand of water, many residents of the area have installed their own boring wells, and are using it to meet their demands (Mashiatullah, et al., 2010) Map of study area is shown in Figure 2.2 below.

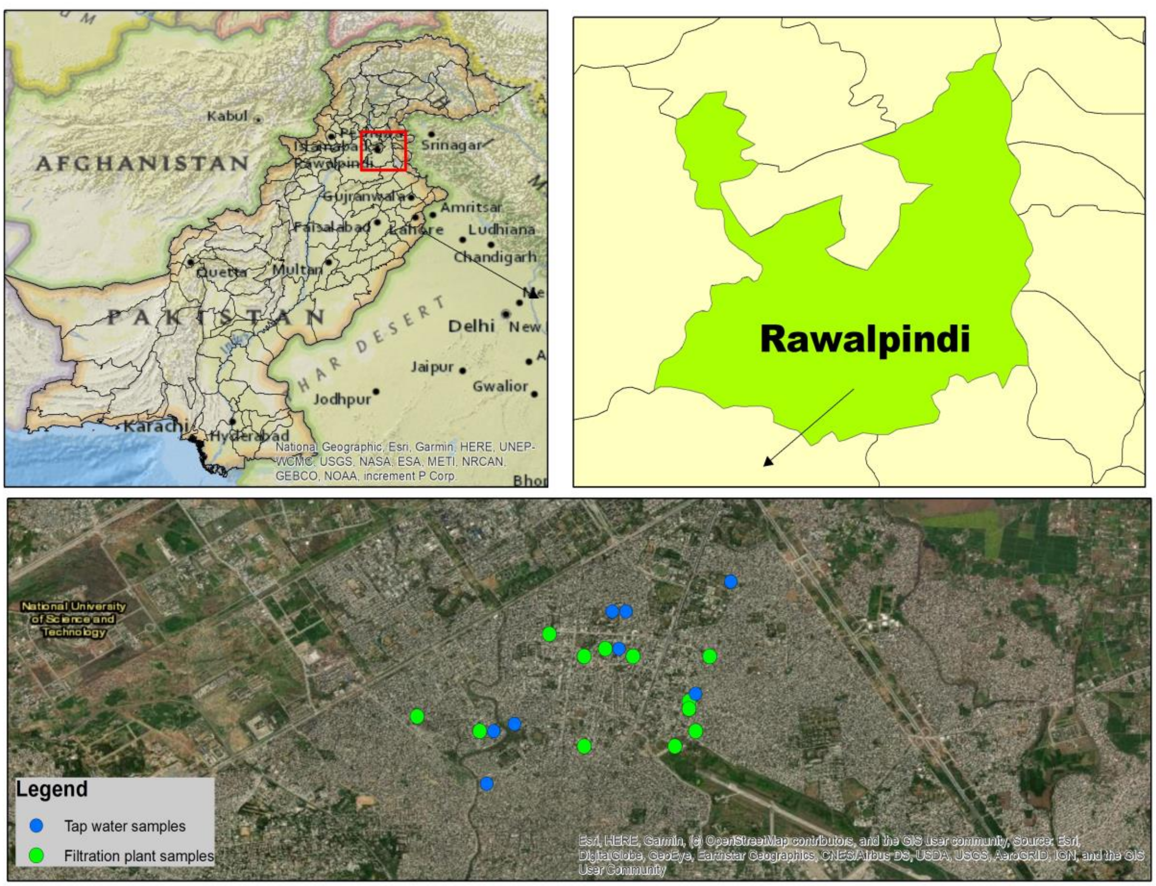


Figure 2.2: Satellite image of the study area along with sampling points

2.3 Description of Study Area

Rawalpindi, being the fourth largest city of Pakistan is densely populated. Rawalpindi city is widespread with a lot of commercial areas, residential communities, businesses, wholesale markets, educational institutions etc. With this number of people to support, water is also a resource that is required in vast amount to fulfil the needs of people. Main water source in Rawalpindi is filtration plants and ground water. Water in filtration plants is supplied by the Rawal Lake which has also been found to be highly contaminated with bacteria (Daud et al., 2017).

The study area selected is one of the most densely populated areas of Rawalpindi. It is the main hub of many businesses and shops. The reason as to why this area was chosen is because there have been researches done in past indicating that water quality has always been an issue in the area (Uzaira et al., 2005).

2.4 Sample Collection

Sampling was done in the month of August 2020. During the study, the samples were taken from different filtration plants and tap water (bore) from different houses located in the study area. Throughout the sampling process, standard methods set by APHA were followed. The samples were collected in a polystyrene bottle for the physicochemical analysis and for the biological analysis the samples were collected in sterilized bottles.

For filtered Water: Eleven water samples were collected from different filtration plants in the vicinity. Some of these were installed by RDA under Punjab Government order while some were by private companies and some were installed in masjids for public use. From each site, samples were collected in two bottles; one for physicochemical analysis and other in sterilized bottle for biological analysis. Samples from filter plants were given code as ‘F1, F2.’ etc.

For tap water: Nine water samples were collected from tap water installed in different houses. People use bore water to fulfil their needs as WASA supply is limited. Samples from tap plants were given code as ‘T1, T2.’ etc.

All the tests for this research were carried out in the laboratory of Bahria University Islamabad Campus.

Figure 2.3: Images from sampling sites

2.4.1 Sample Collection Method and Preservation

i. **Samples for Physicochemical Analysis**

For physicochemical analysis as well as testing for arsenic, water samples were collected in 500 ml polystyrene bottle. Before collecting the water sample in the bottle, we allow the water to flow for a few minutes so that no external factors affect water quality and then the samples were collected in the bottles.

ii. **Samples for Microbiological Analysis**

These samples were collected in sterilized bottles of 150 ml according to standard protocols. For collecting the samples, proper care was taken to overcome the accidental contamination during the sampling. For sampling, proper way was adopted like flaming the tap, before filling the bottle and then the samples were kept in an isolated ice box before transported to the university lab for analysis.

2.4.2 Types and Number of Water Samples

Twenty samples were collected for the physicochemical analysis and biological analysis. There are two types of samples that were collected;

Type-A: 150 ml sterilized sampling bottles for the microbiological analysis

Type-B: 500 ml polystyrene bottles for the physicochemical analysis

2.5 Analysis of Water Quality Parameters

A total of seventeen water quality parameters were selected in order to determine water quality status in the area. Out of these, thirteen are physicochemical while four are biological parameters that were analyzed. Water samples collected from two main types of source i.e. filtration plants and tap water were transported to laboratory for analysis. All the parameters were analyzed in university laboratory using standard protocols (APHA 2012) American Public Health Association standard methods 22nd and 23rd edition (Rice, 2012). Once followed and after determining the various physical, chemical and biological parameters in water samples, the values were compared by WHO and EPA permissible limits. In order to minimize human error and ensure maximum accuracy all the tests were calculated thrice and then taken mean to get average value.

Table 2.1: Methods used for sample analysis

Sr No	Parameters	Equipment & Methods Used
1	pH	Digital Multi-meter
2	Electrical Conductivity	Digital Multi-meter
3	Total Salts	Digital Multi-meter
4	Total Dissolved Solids	Digital Multi-meter
5	Turbidity	Turbidity Meter
6	Total Hardness	Titrametrically
7	Total Alkalinity	Acid-Base Titration
8	Total Chlorides	Argentometric Titration
9	Total Sodium	Argentometric Titration

10	Calcium	Titrametrically
11	Magnesium	Titrametrically
12	Carbonates	Acid-Base Titration
13	Arsenic	Arsenic Kit
14	Total Bacteria	Plate count method on Nutrient Agar
15	Salmonella & Shigella	Plate count method on SS Agar
16	Total Coliforms	Plate count method on EMB Agar
17	Gram Staining	Microscopic Study

2.6 Analysis of Physical Parameters of Water

2.6.1 Temperature

Water temperature of the samples collected was measured in university lab by using a digital multi-meter (Hanna Instrument Model-HI 8424). It is equipped with an electrode which other than temperature, also measures a number of physical parameters including, EC, TDS, pH, salts. To measure temperature, electrode of thermometer was first washed thoroughly with distilled water, and then with sample water, then it was dipped into sample for 1 minute, in order to let the system, stabilize before noting the final reading. Result was then noted in centigrade.

2.6.2 pH

A solution's pH basically depicts concentration of H ions in that solution. Greater the concentration of H ions, the more acidic is the solution and lesser will be pH. Similarly, lesser the concentration of H ions, more basic will be the solution and greater the pH. According to guidelines for drinking water quality given by WHO, exposure to low as well as high pH values can result in irritation to mucous membrane, eyes and skin for humans (Ambica, 2014). As, per WHO permissible limit for pH in drinking water till date is 6.5-8.5 (WHO, 2003).

In order to measure pH of the samples digital multimeter was used. Digital meter used for analysis was (Hanna Instrument Model-HI 8424). This instrument is a multi-meter and has multiple modes to measure, pH, TDS, EC, salts and temperature. First the instrument was standardized as per manufacturer's instruction i.e. by dipping the electrode in a standard solution of high pH and then in solution of low pH. Then electrode was washed thoroughly with distilled water, then with sample water. Then, electrode was dipped into water sample for 1 minute in order to let the electrode stabilize before noting the final reading.

2.6.3 EC

Conductivity in liquid basically measures the ability an aqueous solution has to conduct electric current which is also a useful tool in determining purity of water. This basically depends on the amount of solids dissolved as well as concentration of ions, their valency and mobility in a solution. Greater the concentration of ions in a solution greater will be the electrical conductivity of that solution. If a water sample has found to have high values of conductivity then this indicates that water is polluted as, high conductivity is because of high concentration of salts and ions (Mohsin et al., 2013). TDS and EC are directly proportional to each other, greater the concentration of salts (TDS) in a solution, greater will be its electric conductivity and vice versa. Permissible limit for EC in drinking water according to WHO is 400µs/cm.

To measure EC of the samples again same digital multimeter was used as used to measure pH, TDS, temperature, and salts. Instrument was first standardized according to manufacturer's instruction. Then electrode was washed thoroughly with distilled water, then with sample water. Then electrode was dipped into water sample for 1 minute so that the instrument can stabilize and give correct reading. After 1-minute final reading was noted.

2.6.4 Total Salts

Salts in drinking water are because of the presence of dissolved salts, NaCl mainly. Potassium salts, sodium chloride etc. are what makes water saline. Salts are basically made of ions, for example in case of NaCl, here sodium is a cation having positive charge while chloride is an anion and thus has a negative charge. Presence of salts in any aqueous solution is what makes it conductive for electric current. Greater the concentration of salts greater will be the electrical conductance (Mohsin et al., 2013). According to WHO permissible limit for salts in drinking water is 200mg/l. Salts for the analysis was measured by two methods: physical method as well as chemical method. In physical method digital multimeter, with an electrode was used as used for measuring TDS, pH etc. In this method total salts present in sample were determined. In chemical method concertation of only Na, Cl and NaCl were determined.

2.6.5 TDS

Total dissolved solids primarily represent presence of various kinds of minerals in aqueous solution. It is basically the amounts of solids dissolved in water. Permissible limit of TDS for drinking water according to WHO is 500 mg/l.

TDS in water samples was also measured by using a digital multimeter, same instrument as used for pH, EC, temperature and salts. Mode was changed to determine TDS, then after cleaning electrode thoroughly with distilled water followed by sample water, it was dipped in sample for 1 minute. Once the instrument was stabilized, reading was noted down.

2.6.6 Turbidity

Turbidity basically depicts clarity of a solution. It's a characteristic that is visible to naked eye and thus termed as optical characteristic. It is determined by the fact that how much light is scattered by the particles present in solution when light passes through it. Suspended solids are what majorly cause turbidity. Thus, greater the amount of suspended solids in a solution greater will be the turbidity. It is usually measured in nephelometric turbidity units (NTU). According to both WHO and NSDWQ permissible limit of turbidity in drinking water is less than 5 NTU (PEPA, 2008).

To measure turbidity of the water samples a turbidity meter was used. It was standardized by running blank solution of distilled water in order to check whether the instrument is giving accurate results or not. Once standardized, sample was filled in vial to the mark i.e. white line marked on the vial. Then to remove fingerprints and water spots the cell was wiped with the help of soft tissue paper. Then the sample vial was placed in instrument cell compartment so that instrument can measure turbidity.

2.7 Chemical Parameters of Water

Following parameters of water samples were tested chemically, by using titration methods.

2.7.1 Total Alkalinity

Alkalinity basically, measures ability an aqueous solution has, in order to neutralize acids. Alkalinity denotes buffering capacity of aqueous solution. Permissible limit for alkalinity in drinking water according to WHO and SLS is 500mg/l and 200mg/l respectively (Memon et al., 2011).

As, alkalinity of a solution is basically the acid-neutralizing capacity of that solution, it depends on the presence of bicarbonates, carbonates, and hydroxide in the solution. In order to determine total alkalinity of the samples, standard methods of APHA were followed. This involved titrating the sample against H_2SO_4, a strong acid, while methyl orange was used as indicator. Reagents used in analysis were:

i. Standardized Acid: 0.02 N H_2SO_4
ii. Indicator: Methyl Orange

First in a flask 50ml of water sample was taken, 2 drops of indicator i.e. methyl orange were added, then flask was stirred well. Slowly the sample solution was titrated against 0.02M H_2SO_4 till solution changed color from yellow to colorless. The volume of acid was noted at which color of solution changed. In order to reduce human error and obtain more accurate results the procedure was repeated thrice for each sample.

$$\text{Total Alkaliniy (mg/L)} = \frac{\text{Volume of acid used x 0.02M x 50000}}{\text{ml of sample}}$$

2.7.2 Total Hardness

Total hardness is described as the amount of dissolved minerals in water mainly magnesium and calcium. Hardness is basically caused by the presence of metallic ions in water but majorly calcium and magnesium cations are the minerals that contribute to total hardness, although other cations like aluminum, iron, strontium barium, zinc and manganese also contribute to an extent to total hardness. WHO depending on concentration of calcium carbonate has categorized water hardness into four groups;

Soft Water: $CaCo_3$ concentration in water is below (60mg/l)

Moderately Hard Water: $CaCo_3$ concentration in water is (60–120 mg/l)

Hard Water: $CaCo_3$ concentration in water is (120–180 mg/l)

Very Hard Water: $CaCo_3$ concentration in water is above (180 mg/l) (WHO, 2010).

Permissible limit set by WHO and Pakistan Standards and Quality Control Authority, PSQCA regarding hardness of drinking water is 500 mg/l.

To determine total hardness in the samples EDTA Titration Standard Method (2012) was used. The reagents used are mentioned below:

i. Standardized Solution 0.01M EDTA
ii. Buffer solution: NH_4Cl
iii. Indicator: Eriochrome Black-T (EBT)

Conical flask was filled with 50 ml of water sample. To this sample 2 ml of buffer solution (NH_4Cl) was added. Then pH of the solution is measured to ensure that pH of buffered solution is around 10. Then 2 to 3 drops of indicator which is Eriochrome Black-T (EBT) are added. After taking 0.01 M EDTA in burette, the sample was slowly titrated against it. The solution in flask during this process was stirred continuously until end point is reached which is from reddish tinge colour to bluish purple colour. This test was first run by using blank solution i.e. distilled water. Formula used for calculation was:

$$\text{Total Hardness (mg/L)} = \frac{\text{A x B x 1000}}{\text{ml of sample}}$$

Where,

A= mL of EDTA used for sample – mL of EDTA used for blank

B= mg of $CaCO_3$ equivalent to 1mL of EDTA titrant (which is equal to 1 mg $CaCO_3$)

Calcium: calcium being one of the major contributors to causing total hardness of water was also analyzed. Calcium is necessary for body as long as under permissible limit set by WHO which is 100 mg/l. Method used for analysis of calcium concentration in the samples was EDTA Titration Standard Method (2017). Reagents used were:

i. Standardized Solution: 0.01M EDTA
ii. Buffer Solution: 1N NaOH
iii. Indicator: Eriochrome Black-T (EBT)

In a conical flash 50 ml of sample water was taken, to this 2 ml of NaOH solution was added along with 2-3 drops of EBT. This solution was then titrated against standard EDTA solution until end point was reached i.e. colour changed from reddish purple to blue. This test was first run for blank solution by using distilled water. The formula used was:

$$\text{Calcium (mg/L)} = \frac{\text{A x B x 1000}}{\text{ml of sample}}$$

Where,

A= mL of EDTA used for sample – mL of EDTA used for blank

B= mg of $CaCO_3$ equivalent to 1mL of EDTA titrant (which is equal to 1 mg $CaCO_3$)

Magnesium: Magnesium makes its way in water when minerals containing Mg ions are dissolved thus contributing to total hardness. Permissible limit for Mg in water as recommended by WHO is 30 mg/l. Here Mg was calculated as by taking difference between total hardness and calcium hardness, resulting into magnesium hardness.

2.7.3 Salts by chemical method

Chloride (Cl): Chloride ions are one of the major inorganic anions present in water. They are the reason for salty taste in water, indicating salinity when present in water (Mohsin et al., 2013). The permissible limits for chlorides in drinking water are 250 mg/l. Total Chlorides were in the samples were analyzed using standard Silver nitrate titration method (2012). Reagents used for this test were:

i. Standardized Solution: Silver Nitrate ($AgNO_3$)
ii. Indicator: Potassium Chromate K_2CrO_4

For this, 10ml of water sample was taken in a conical flask to which 2 to 4 drops of indicator, potassium chromate was added, and it was slowly titrated against 0.01N $AgNO_3$ solution until the colour changed to a pinkish yellow. Amount of chloride present was calculated from the amount of silver nitrate used as a titrant using the formula:

$$\text{Chlorides (mg/L)} = \frac{\text{V x N of AgNO3 x 1000 x 35.5}}{\text{ml of sample}}$$

Where,

V= volume of $AgNO_3$ consumed for sample

N= Given normality of $AgNO_3$ = 0.01 N

Molecular Weight of Cl= 35.5 g/mol

NaCl: In order to calculate concentration sodium ion water samples, first Sodium Chloride (NaCl) concentration in water was calculated using the same titration method which was used as for total chloride. Reagents used were:

i. Standardized Solution: Silver Nitrate Solution
ii. Indicator: Potassium Chromate K_2CrO_4

10ml of water sample was taken in a conical flask to which 2 to 4 drops of potassium chromate as indicator were added and titrated against 0.01N AgNO3 solution until the color changed to a pinkish yellow. Amount of Sodium Chloride present was calculated from the amount of silver nitrate used as a titrant using the formula:

$$\text{NaCl (mg/L)} = \frac{\text{V x N of AgNO3 x 1000 x 58.45}}{\text{ml of sample}}$$

V= volume of $AgNO_3$ consumed for sample

N= Given normality of $AgNO_3$ = 0.01 N

Molecular Weight of NaCl= 58.45 g/mol

This gave the amount of Sodium Chloride. Now from this amount, amount of total chlorides was subtracted giving the concentration of Sodium remaining in the sample.

Sodium (Na): The sodium and chloride are the two important parameters salts concentrations. It was calculated by subtracting amount of chlorides determined from sodium chloride amount present in the water samples. The permissible limits for sodium in drinking water are 200 mg/l.

2.7.4 Carbonates

Carbonates and bicarbonates ions are one of the major contents of water, their concentration in water is found to increase in areas where minerals and rock bodies are found near source of water. Since, weathering of rocks leads to dissolving bicarbonates and carbonates ions in water. When bicarbonates and carbonates are present in water they contribute to hardness as well as alkalinity properties of that water (M. Shahid et al., 2018). About permissible limit of carbonates and bicarbonates in drinking water, no guidelines have been issued by WHO. In order to determine carbonates and bicarbonates in the water samples, standard HCl titration method was used. Reagents used for this analysis were:

i. Standardized Solution: 0.1 M HCl
ii. Indicator: Methyl Orange

10ml of water sample was taken in a clean conical flask, to which 2 to 4 drops of indicator methyl orange were added and the solution was slowly titrated against 0.1 M HCl solution until the color changed from yellow to orangish-red. Amount of carbonates was calculated by first finding unknown molarity of the solution and then multiplying this value with molecular weight of each carbonate. Molarity is calculated by using the formula:

$$M_1V_1 = M_2V_2$$

$$M_1 = (M_2V_2)/V_1$$

After finding molarity, this value is multiplied by molecular weight of the required carbonate, by using the formula:

Amount per dm^3 == M x Molecular Weight of chemical compound

By using this method concentration of $NaHCO_3$, Na_2CO_3, HCO_3 and CO_3 was determined.

2.7.5 Arsenic

Arsenic being one of the most harmful metalloids that exist in water bodies, mainly in groundwater. Its presence in water can be natural source or through anthropogenic source. In nature arsenic make sits way in water bodies through biological activities, weathering and erosion, but major contribution of arsenic is by anthropogenic activities such as mining, petroleum refining, pesticides, semi-conductors. Arsenic is also present in paints, metals, dyes, soaps, drugs, herbicides etc. (Gecol et al., 2004). Whenever arsenic containing fluids are discharged in water, or leached into groundwater, it contaminates the water bodies, and when arsenic concentration exceeds permissible limit then water is considered unfit for drinking purposes. Permissible limit of Arsenic in drinking water as set by WHO is 0.01 mg/l (WHO, 2018).

Materials Required

- Reaction bottles
- Reagent 1
- Reagent 2
- Reagent 3
- Arsenic testing strips
- Distilled water
- Tissue paper/ cotton

Procedure

To perform arsenic test, three reagents and the whole arsenic kit is required. For best results, the temperature of the water sample should be 25-28°C. First of all, the reaction bottles were rinsed with distilled water and then filled to the upper mark line with 50ml water sample. Then 1 level pink spoon of first reagent was added to the reaction bottle and was capped securely with the red cap. Then the sample was shaken vigorously for 15 seconds. After waiting for 45 seconds, 1 level red spoon of second reagent was added to the reaction bottle and was shaken again for 15 seconds. In order to minimize H2S interference, the sample was allowed to sit for 2 minutes before performing the next step. After 2 minutes, 1 level white spoon of reagent 3 was added in the sample and after capping securely with red cap, the reaction bottle was shaken for 5 seconds and then the red cap on the bottle was immediately replaced with white turret cap with test strip inside it. Before recapping the reaction bottle with white turret cap, the cap was dried completely with tissue paper and a test strip was inserted in it. After adding 3rd reagent, if the arsenic is present in the water sample, it is released in the form of gas or fumes. So the indicator present on the test strip indicates the arsenic gas and if the gas is detected, the indicator changes its colour (Powers et al., 2019). After 10 minutes, the test strip was examined, and colour of the indicator was matched with colours on Arsenic Test Kit Colour Chart and results in mg/L were recorded in the form of a table.

2.8 Biological Parameters of Water

In biological assessment of drinking water, the most common methods used for the detection of fecal contamination are Total Plate Count method (Spread plate method) and Gram Staining. In spread plate method a solidified agar plate is basically used and the water sample containing bacteria is spread onto the plate with the help of a spreader (Odeyemi et al., 2010). A total of 20 samples of drinking water were collected from different locations and were analysed bacteriologically.

2.8.1 Total Plate Count Method

In total plate count method, sample is spread on agar for bacterial growth and then analysed. Agar for detection of different types of bacteria is also different. To determine total bacteria, colonies are grown on nutrient agar. For determination *of salmonella* and *shigella*, SS agar is used and for *total coliforms*, EMB agar is used.

Materials required

Materials required in this analysis are mentioned below;

- Reagent glass bottles
- Cotton plug
- Glass cylinder
- Nutrient agar (28g in 1 litre)
- Salmonella Shigella agar (52g in 1 litre)
- EMB agar (37.5g in 1 litre)
- Spatula
- Balance/ weight machine
- Autoclave
- Incubator
- Laminar flow hood
- Glass spreader

- Spirit lamp
- Methylated spirit
- Micropipette tip
- Petri plates

Preparation of Agar Medium

The first step in biological assessment of water quality is the preparation of agar medium for examining bacterial growth and the type of bacteria. Different types of nutrients are used in order to culture different microorganisms.

Nutrient Agar is basically used to cultivate a wide variety of bacteria and also for enumerating bacterial organisms in sewage water, drinking water and many other materials. SS Agar medium is basically used for the isolation of *Salmonella* and *Shigella* species from different pathological specimens. EMB agar medium is used to isolate total coliforms (King and Metzger, 1968). For the preparation of 400ml agar media, 11.2g of N.A, 15g of EMB agar, and 20.8g of SS agar is required.

Nutrient Agar (N.A)

N.A= 28g/1000

= 0.028g (in 1ml)

= 0.028 X 400

= 11.2g

*11.2g of N.A was dissolved in 400ml of distilled water to prepare the required 400ml of N.A agar solution.

Eosin Methylene Blue (E.M.B) Agar

EMB = 37.5g/1000

= 0.0375g (in 1ml)

= 0.0375 X 400

= 15g

*15g of EMB agar was dissolved in 400ml of distilled water to prepare the required 400ml of EMB agar solution.

Salmonella Shigella (S.S) Agar

S.S = 52/1000

= 0.052g (in 1ml)

= 0.052 X 400

= 20.8g

*20.8g of SS agar was dissolved in 400ml of distilled water to prepare the required 400ml of SS agar solution.

Procedure

The solution or nutrient media was prepared by dissolving the required amount of agar medium powder in 400ml distilled water in glass reagent bottles. Then after tightly covering the glass reagent bottles with cotton plugs, all the three bottles containing the dissolved mixture were placed in the autoclave. After closing the autoclave tightly, the agar medium was autoclaved at 121°C for 30 minutes. Inside autoclave, due to high temperature and pressure, glass bottles can burst therefore, cotton plugs were used instead of the lids of reagent glass bottles so that all the fumes could be absorbed by the cotton plugs in order to prevent glass bottles from bursting (Kumar et al., 2018). It took 2-3 hours to release the pressure built-up in the autoclave, and after approximately 3 hours, the solution was ready to use.

After the solutions were autoclaved and cooled slightly, 20ml of each nutrient medium was poured in 20 sterilized Petri-plates and was allowed to solidify. 100µl (0.1ml) volume from each sample was drawn up with the help of micropipette tip and was inoculated on the surface of the pre-solidified agar plates. By using a sterile L-shaped glass rod called spreader, the inoculum was then evenly distributed over the surface of the agar media. While preparing microbiological media, if it was exposed to air, microbial contamination from hands, air, glassware, etc. could enter in the media and reproduce. Therefore, all the petri plates were prepared inside laminar flow hood (Kumar et al., 2018).

After the inoculum was completely absorbed into the medium, the plates were covered and sealed tightly. Petri-plates were then placed upside down inside the incubator and incubated aerobically at 35°C for 18-24 hours. After incubation, the colonies on each plate were counted and results were reported as Colony Forming Units (CFU/ml).

2.8.2 Gram Staining

Gram staining technique is used to differentiate bacteria by physical and chemical properties of the bacterial cell wall. Gram-positive bacteria basically contain a thick cell wall composed of peptidoglycan and therefore are stained purple when crystal violet stain/dye is added, while, gram-negative bacteria possess a thinner layer and therefore, do not retain the purple stain. However, they are counter-stained pink when safranin is added (Gregersen, 1978)

Materials Required

- Glass slides
- Sterile loop
- Spirit lamp
- Distilled water
- Stain 1 (Crystal violet)
- Stain 2 (Gram iodine)
- Decolourizer (ethanol)
- Stain 3 (Safranin)
- Microscope

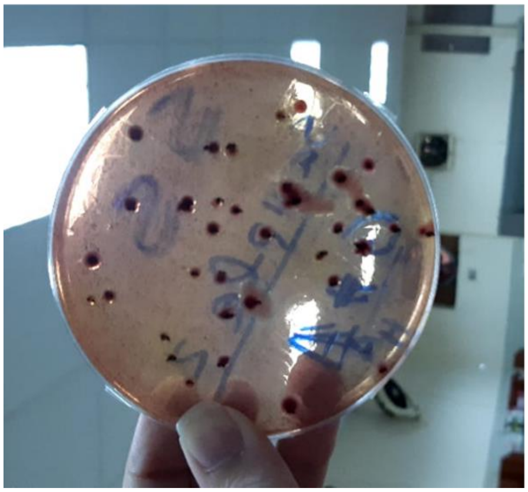
 

Figure 2.4 Microbial growth on S.S and N.A agar respectively

Staining Mechanism

Gram staining is a method of staining used to differentiate and classify bacterial species into two large groups: *gram-positive bacteria* and *gram-negative bacteria*. For gram staining, first of all a drop of distilled water was added on a glass slide. Then a colony of bacteria from the Petri plate containing agar medium was picked up with the help of a sterile loop and mixed thoroughly in that drop of distilled water. After mixing, it was heat-fixed on the slide with the help of spirit lamp. Then 1 or 2 drops of primary stain, Crystal violet were added and washed after 1 minute with distilled water. Then 1 or 2 drops of the second stain called Gram-iodine were added and again washed after 1 minute with distilled water. When gram iodine is added, it binds to crystal violet and traps it in the cell (Moyes et al., 2009). After 1 minute, the slide was washed with decolorizer called ethanol for 5 seconds and again washed with distilled water. When a decolorizer is added, it interacts with the lipids of the cell membrane. A gram-negative cell loses its outer lipopolysaccharide membrane, and the inner peptidoglycan layer is left exposed. After decolorization, the gram-positive cell remains purple and the gram-negative cell loses its purple color (Adams, 1975). Finally, 1 or 2 drops of counter-stain Safranin were added and washed with distilled water after 1 minute. Safranin is applied to give decolorized gram-negative bacteria a pink or red color (Coico, 2001).

CHAPTER 3

RESULTS AND DISCUSSION

The water samples collected from different area of Rawalpindi were analyzed for physicochemical and microbiological parameters. The resultant values obtained after physicochemical and biological analysis were compared with permissible limits set by WHO, PSQCA and NDWQS.

3.1. Physical Parameters

After conducting tests for various physical parameters of water in lab the results obtained were tabulated. The results of the physical parameters of the eleven samples of filtered water have been presented in the Table 3.1 and the results of the samples of tap (bore) water have been presented in the Table 3.2 below.

Table 3.1: Results from the sample analysis of filtered water sample

Sr No	Samples	pH	EC (µS/cm)	Salts (mg/l)	TDS (mg/l)	Turbidity (NTU)
Permissible Limit		**6.5-8.5**	**1000**	**200**	**500**	**<5 NTU**
1	F1	7.48	531	277	376	0
2	F2	7.4	579	303	410	0
3	F3	7.51	567	296	402	0
4	F4	7.61	547	285	388	0
5	F5	7.59	616	323	437`	0
6	F6	7.45	553	290	393	0
7	F7	7.58	561	295	399	0
8	F8	7.64	528	277	375	0
9	F9	7.49	613	321	434	0
10	F10	7.44	775	410	550	0
11	F11	7.36	719	379	510	0
Mean Values		7.5	599	314.18	423.7	0

Table 3.2: Results from the sample analysis of tap water

Sr No	Samples	pH	EC (µS/cm)	Salts (mg/l)	TDS (mg/l)	Turbidity (NTU)
Permissible Limit		**6.5-8.5**	**1000**	**200**	**500**	**<5 NTU**
1	T1	7.58	625	328	443	0
2	T2	7.54	760	403	542	0
3	T3	7.19	514	269	365	0
4	T4	7.58	547	286	388	0
5	T5	7.45	573	300	407	0
6	T6	7.35	602	316	428	0
7	T7	7.73	783	414	556`	0
8	T8	7.73	620	326	440	0
9	T9	7.58	710	374	503	0
Mean Values		7.52	637.11	335.11	452.4	0

The mean values of the physical parameters of filtered and tap water samples are presented in the Figure 3.1. The figure shows the comparison between physical parameters in filtered water and tap water samples.

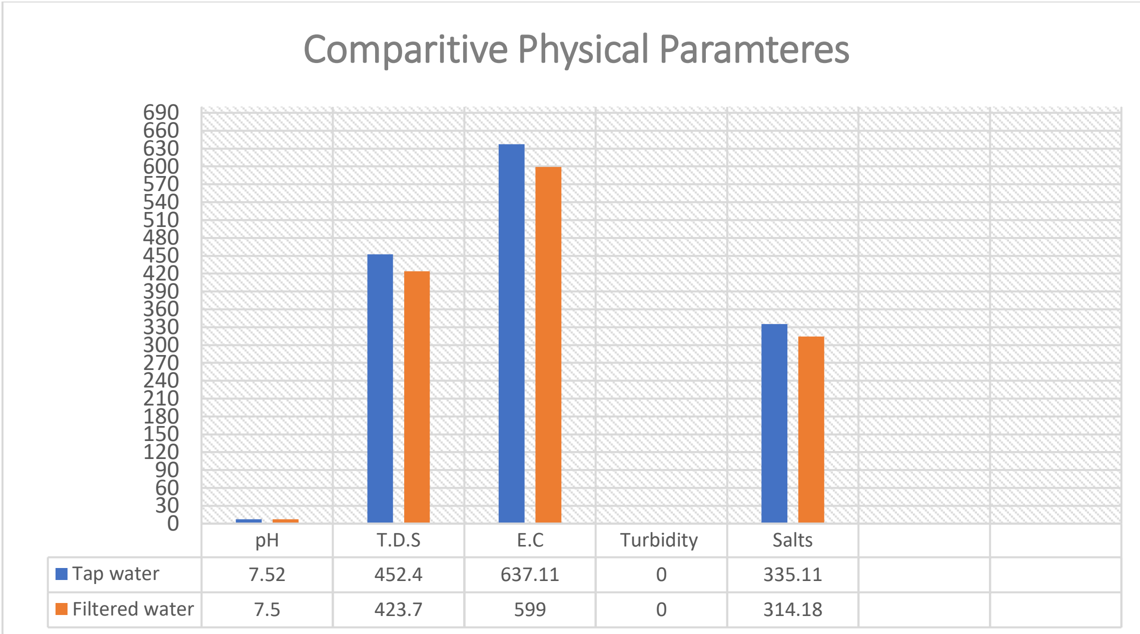


Figure 3.1: Comparative physical parameters in raw and filtered dam water

Water samples collected for the study area were transparent and odorless, that is why turbidity in all samples is 0. It is important to take water temperature into consideration as well, but it is more important in case of surface water bodies like lakes, dams etc. Since, here water samples were taken from filter plants and tap water so average temperature was found to be 22.5℃.

3.1.1 pH: pH is basically measured by amount of hydrogen ions present in a solution. pH value is what indicates alkalinity or acidity of a solution. As advised by WHO the permissible limit of pH in drinking water 6.5–8.5. The value of pH in both filtered water as well as tap water was found to be within permissible limit. In water samples collected from filter plants average pH was found to be, 7.5 with lowest and highest value of 7.36-7.64 respectively. In tap water samples pH ranged from 7.19 to 7.73 lowest and highest with an average value of 7.52. Thus, pH of samples taken from both water filtration plants and tap water is between the desirable range. The average pH values have been computed in figure 3.2.

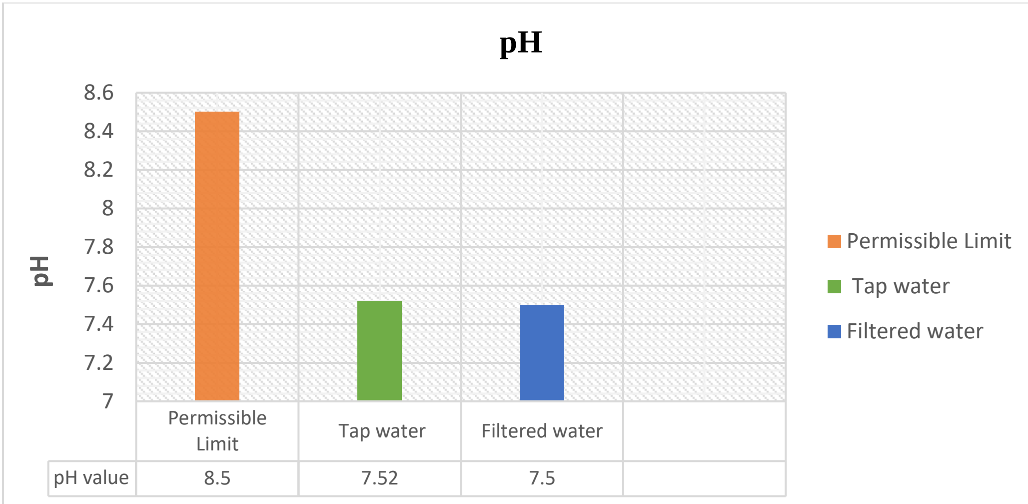


Figure 3.2: Average pH values in study area

3.2.1 EC: Electrical conductivity is basically a measure of water's ability to transmit electric current through it, which is caused by the presence of ions in water. According to WHO, permissible limit for EC in drinking water is 1000 µS/cm. Electric Conductivity in filtered water ranged from 528 to 775 µS/cm, with an average value of 599 µS/cm. while the EC in tap water was found to be ranged between 514 to 783 µS/cm, with an average value of 637.11 µS/cm. All of the samples taken from both filter plants as well as tap waters contained EC, within permissible limit as set by WHO. The average EC values have been computed in Figure 3.3.

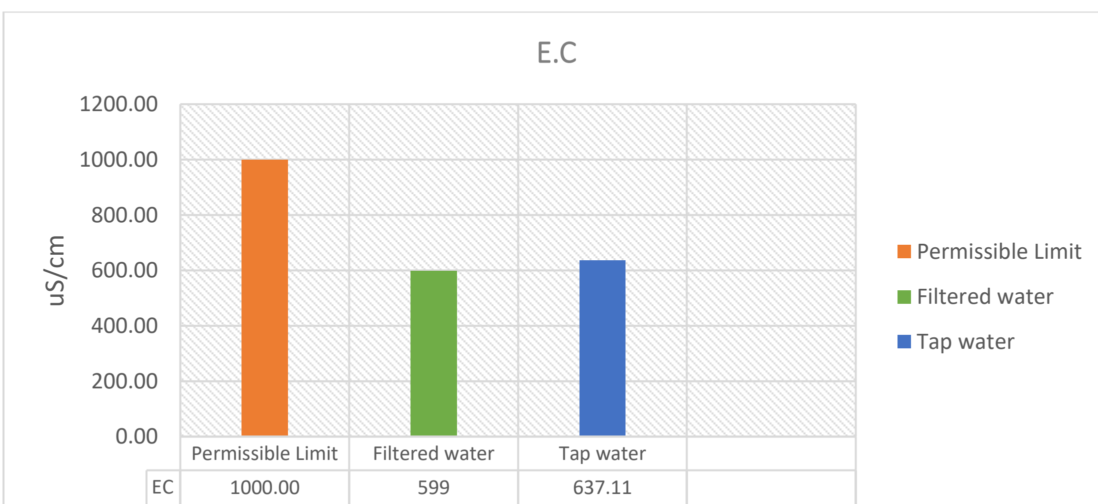


Figure 3.3: Average E.C values in study area

3.3.1 Total Salts

Dissolved salts like NaCl are what causes salinity in water. Salts concentration is directly proportional to TDS in water. Greater the concentration of salts greater will be the electrical conductance and vice versa. According to WHO permissible limit for salts in drinking water is 200 mg/l. Salts concentration in filtered water ranged from 277 to 410 mg/l, with an average value of 314.18 mg/l, while the salts concentration in tap water was found to be ranged between 269 to 414 mg/l, with an average value of 335.11 mg/l. All of the samples collected from both water filtration plants and tap water has salts concertation exceeding the permissible limits. The average concentration of salts has been computed in figure 3.4.

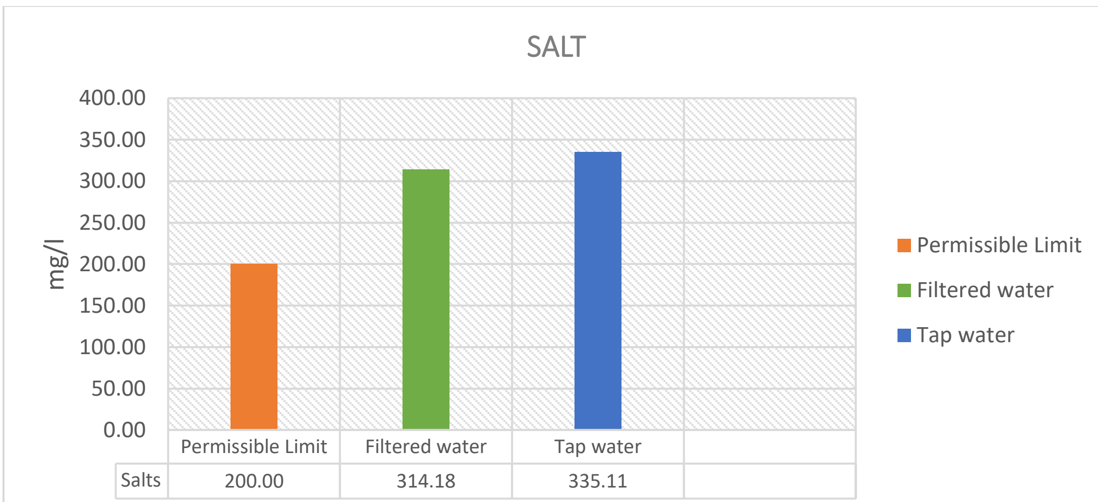


Figure 3.4: Average salts concentration in study area

3.2.TDS

Total dissolved solids primarily represent presence of various kinds of minerals in aqueous solution. It is basically the amounts of solids dissolved in water. Permissible limit of TDS for drinking water according to both NSDWG and WHO is 500 mg/l. TDS in filtered water ranged from 375 to 550 mg/l, with an average value of 424.9 mg/l, while the TDS in tap water was found to be ranged between 365-556 mg/l, with an average value of 452.44 mg/l. 2 samples from filter plants while 3 from tap water exceeded permissible limit for TDS. While the rest of 15 samples were under permissible limit. The average TDS values have been computed in Figure 3.5.

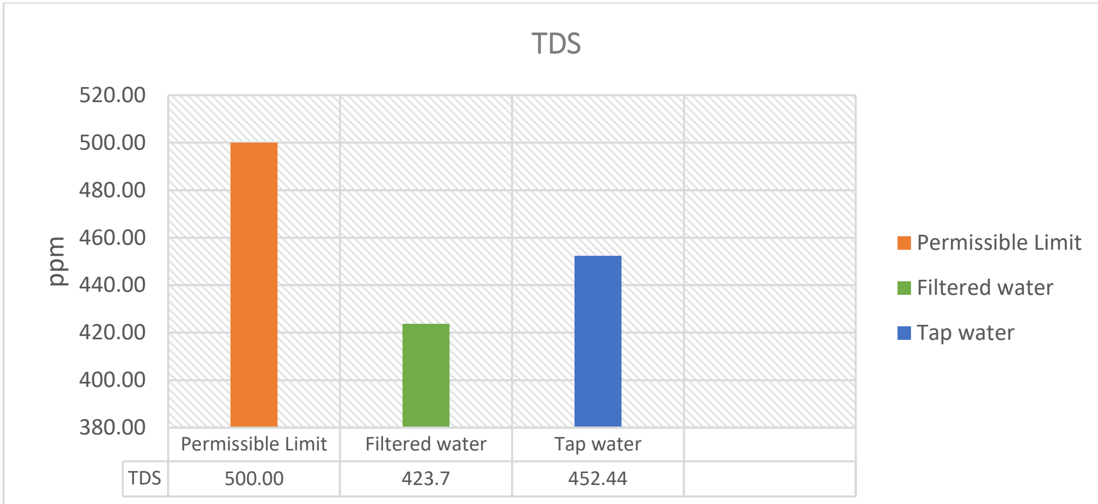


Figure 3.5: Average TDS values in study area

3.3 Chemical Parameters

In order to determine concentration of various elements like Ca, Mg, Na, carbonates etc. Chemical analysis was done in laboratory and obtained results were tabulated. The results of the chemical parameters of the eleven samples of filtered water have been presented in the Table 3.3 and the results of the nine samples of tap (bore) water have been presented in the Table 3.4 below.

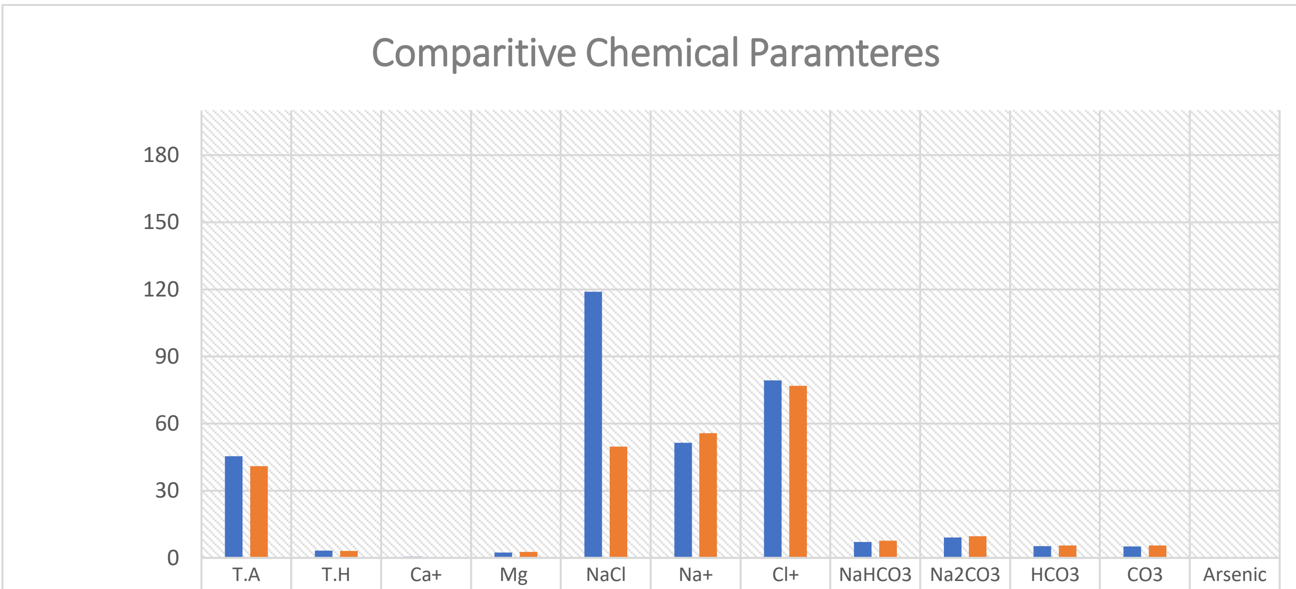
Table 3.3: Results from the chemical analysis of filtered water sample

Sr No	Samples	T. A (mg/l)	T.H (mg/l)	Ca^{+2} (mg/l)	Mg^{+2} (mg/l)	NaCl (mg/l)	Na^{+} (mg/l)	Cl^{-} (mg/l)	Carbonates (mg/l)				Arsenic (mg/l)
									$NaHCO_3$	**Na_2CO_3**	**HCO_3**	**CO_3**	
Permissible Limit		**200**	**500**	**100**	**30**	**-**	**200**	**250**	**-**	**-**	**500**	**-**	**0.01**
1	F1	50.2	2.8	1.12	1.68	68	53.59	82.59	6.97	8.798	5	4.98	0
2	F2	40	3.18	0.32	2.86	51	51	79	7.25	9.145	5.264	6.18	0
3	F3	43	3.12	0.22	2.9	57	46	70.9	8.4	10.6	6.1	5.178	0
4	F4	39	2.68	0.36	2.32	57	46	70.9	7.921	9.99	5.75	6	0
5	F5	43.2	2.82	0.58	2.24	56	55.5	85.08	7.56	9.54	5.49	5.65	0
6	F6	40	3.16	0.22	2.94	44	49.67	76.57	7.98	10.07	5.795	5.4	0
7	F7	35.4	2.66	0.12	2.54	49	45.22	69.69	7.728	9.752	5.612	5.7	0
8	F8	43	2.26	0.34	1.92	55	35.25	54.34	6.972	8.798	5.063	5.52	0
9	F9	41.8	3.48	0.5	2.98	39	62.1	95.71	8.5344	10.7696	6.1976	4.98	0
10	F10	36.8	3.6	0.5	3.1	38	96.6	48.89	8.257	10.4198	5.996	6.096	0
11	F11	39	3.7	0.3	3.4	33	72.68	112.022	7.442	9.3916	5.4046	5.89	0
Mean Values		40.95	41.03	3.04	0.416	2.625	49.727	55.78	76.88	7.72	9.75	5.6	0

Table 3.4: Results from the chemical analysis of tap water sample

Sr No	Samples	T. A (mg/l)	T.H (mg/l)	Ca^{+2} (mg/l)	Mg^{+2} (mg/l)	NaCl (mg/l)	Na^{+} (mg/l)	Cl^{-} (mg/l)	Carbonates (mg/l)				Arsenic (mg/l)
									$NaHCO_3$	Na_2CO_3	HCO_3	CO_3	
Permissible Limit		**200**	**500**	**100**	**30**	**-**	**200**	**250**	**-**	**-**	**500**	**-**	**0.01**
1	T1	71.4	3.56	0.84	2.72	75.985	29.9	46.08	7.47	9.434	5.429	5.34	0
2	T2	40	3.5	0.56	2.94	59	65.15	100.42	8.65	10.918	6.28	6.18	0
3	T3	39.6	2.94	0.04	2.9	87.675	34.5	53.175	6.82	8.6178	4.96	4.878	0
4	T4	43.6	2.6	0.6	2	97.02	38.18	58.84	8.484	10.706	6.161	6.06	0
5	T5	45.8	2.7	0.42	2.28	70.14	27.6	42.54	9.68	12.224	7.035	6.9198	0
6	T6	42	2.8	0.66	2.14	126.25	49.68	76.57	5.65	7.1338	4.105	4.03	0
7	T7	45	4.3	0.32	3.98	224.03	88.12	135.87	5.376	6.784	3.904	3.84	0
8	T8	42	3.1	0.54	2.56	149.63	58.88	90.75	6.07	7.6638	4.41	4.338	0
9	T9	40	3.84	0.74	3.1	181.195	71.3	109.895	6.493	8.19	4.715	4.638	0
Mean Values		**45.5**	**3.26**	**0.52**	**2.38**	**119**	**51.48**	**79.35**	**7.18**	**9.1**	**5.22**	**5.14**	**0**

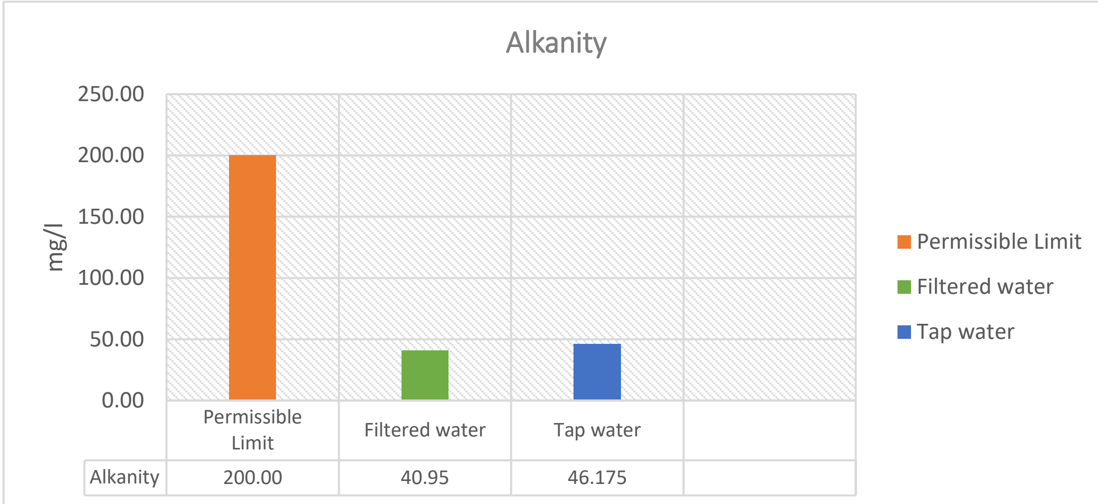
The mean values of the chemical parameters of filtered and tap water samples are presented in the Figure 3.6. The figure shows the comparison between physical parameters in filtered water and tap water samples. While figure 3.6 shows comparative chemical parameters of filtered and tap water samples.



	T.A	T.H	Ca+	Mg	NaCl	Na+	Cl+	NaHCO3	Na2CO3	HCO3	CO3	Arsenic
Tap water	45.5	3.26	0.52	2.38	119	51.48	79.35	7.18	9.1	5.22	5.14	0
Filtered water	41.03	3.04	0.42	2.625	49.727	55.78	76.88	7.72	9.75	5.6	5.52	0

Figure 3.6: Comparative chemical parameters in raw and filtered dam water

Total Alkalinity: Alkalinity of an aqueous solution basically denotes the ability of an aqueous solution in neutralizing acids. The permissible limit for total alkalinity for drinking water is 200 mg/l. The obtained alkalinity in filtered water ranged from 39.6 to 71.4 mg/l, with an average value of 45.5 mg/l. while the alkalinity of tap water ranged between 35.4 to 50.2 mg/l, with an average value of 41.03 mg/l. Samples collected from both water filtration plants and tap water have alkalinity under the permissible limits and there isn't much difference in value of alkalinity of filtered water from tap water. The average alkalinity values have been computed in Figure 3.7.



3.7. Average values of Alkalinity in study area

Total hardness: Total hardness is the chemical parameter which is basically used in order to describe the concentration of dissolved minerals mainly Calcium and Magnesium in water. Since, these two minerals are the major cause of hardness in water. The permissible limit of hardness for drinking water according to PSQCA is 500 mg/l. The samples collected from water filtration plants consisted of hardness ranging from 2.26 to 3.7 mg/l, with an average of 3.04 mg/l. While the tap water showed hardness ranging from 2.6 to 4.3 mg/l, with an average of 3.26 mg/l. The values of total hardness of samples from both filtration plants and tap water is found to be within the standard limits. The average hardness values have been computed in Figure 3.8.

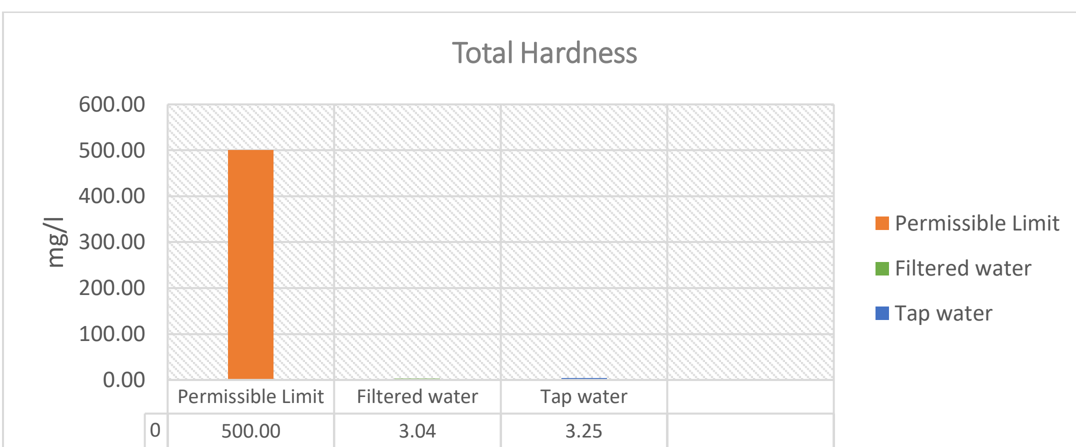


Figure 3.8: Average hardness concentration in study area

Calcium: The permissible limits for calcium in drinking water is 100 mg/l. The calcium concentrations of samples of filtered water ranged between 0.12 to 1.12 mg/l with an average of 0.416 mg/l. While the tap water showed calcium concentration ranging from 0.04 to 0.84 mg/l, with an average of 0.52 mg/l. The values of calcium are within the permissible limits. The average calcium concentration has been computed in Figure 3.8.

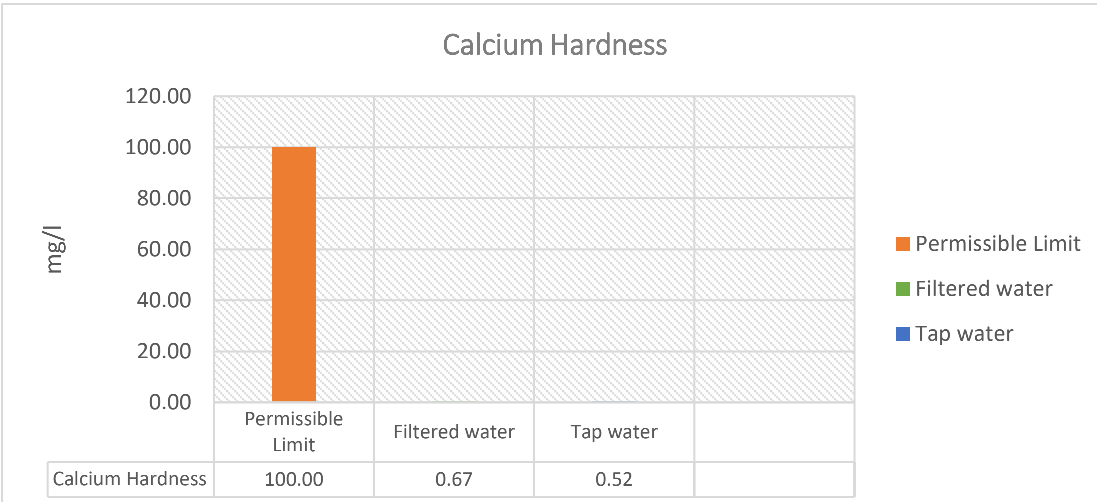
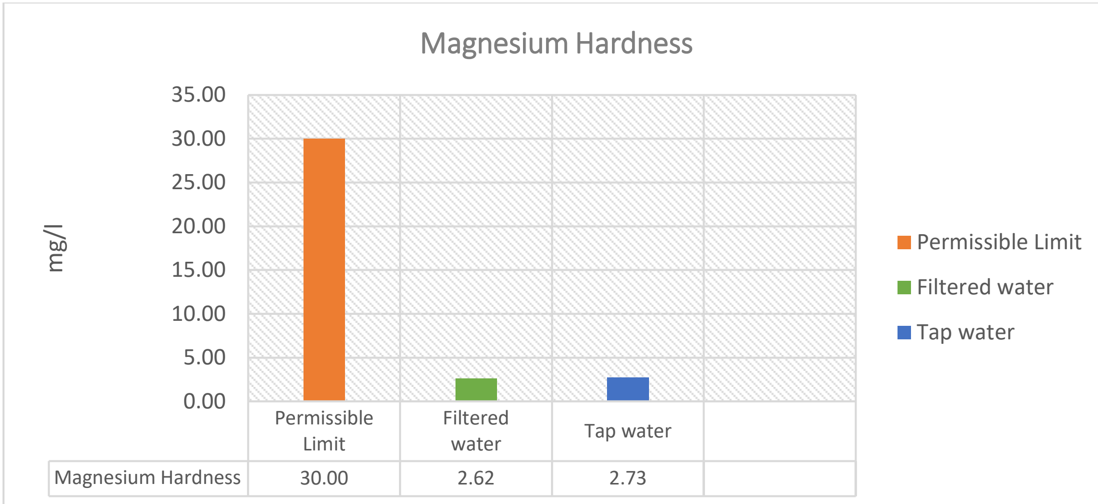


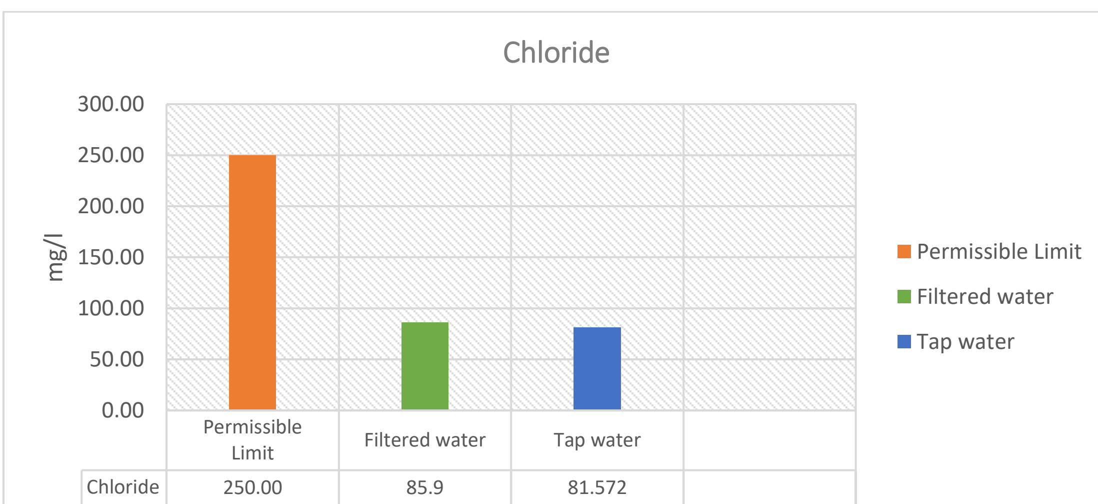
Figure 3.9: Average calcium concentration in study site.

Magnesium: The permissible limit for magnesium in drinking water is 30 mg/l. The samples of filtered water consisted of magnesium concentration ranging from 1.68 to 3.4 mg/l, with an average of 2.625mg/l. While the tap water showed magnesium concentration ranging from 2 to 3.98 mg/l, with an average of 2.38 mg/l. The values of magnesium in both type of sampling sources is within the standard limits. The average magnesium concentration has been computed in Figure 3.10.



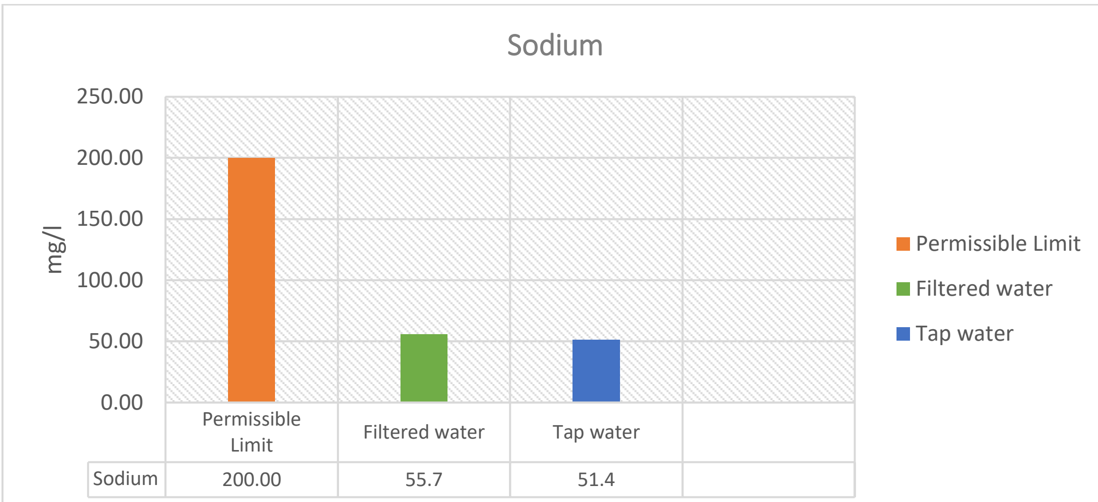
3.10. Average concentration of magnesium in study area

Chloride: Chloride indicates water salinity. The permissible limits for chlorides in drinking water are 250 mg/l. The samples of filtered water consisted of chloride concentration ranging from 48.89-112.022 mg/l, with an average of 76.88 mg/l. While the tap water showed chloride concentration ranging from 42.54 to 135.87 mg/l, with an average of 79.35 mg/l. The values of total chlorides in water samples taken from filtration plants as well as tap water are within the standard limits. The average chlorides concentration has been computed in Figure 3.11.



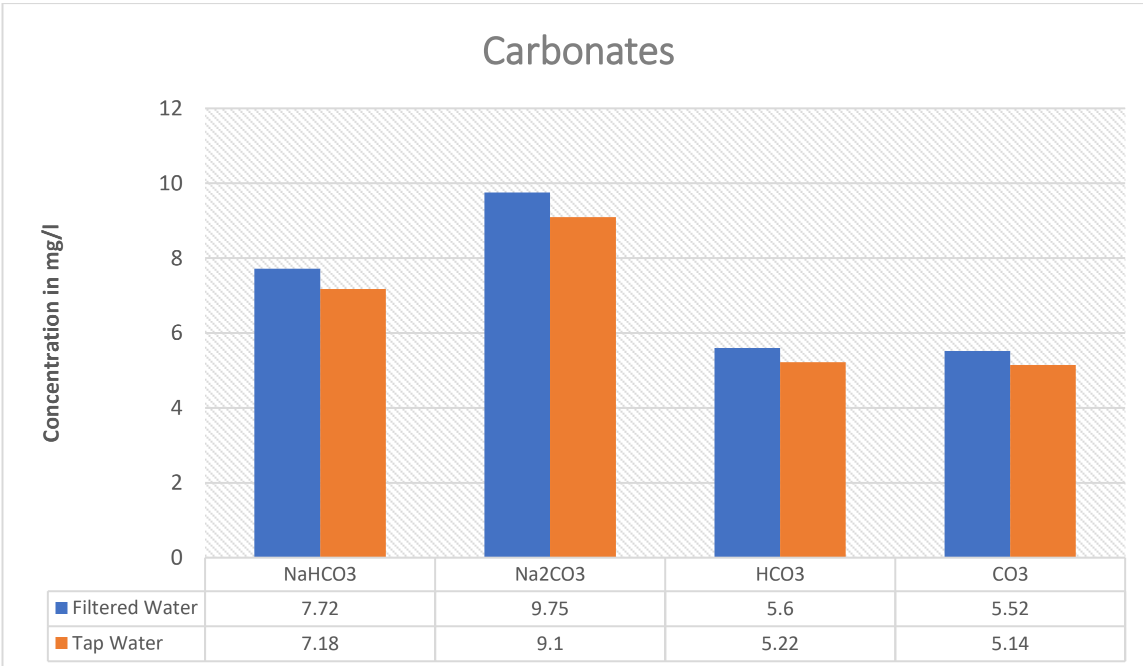
3.11. Average values of chloride in study area

Sodium: The permissible limits for sodium in drinking water is 200 mg/l. The sodium concentration of samples collected from filtration plants ranged between 35.25 to 96.6 mg/l with an average of 55.78 mg/l. While those collected from tap water showed sodium concentration ranging from 27.6 to 88.12 mg/l, with an average of 51.48 mg/l. The concentration of sodium in all the samples are within the standard limits. The average sodium concentration has been computed in Figure 3.12.



3.12. Average values of sodium in study area

Carbonates: Carbonates and bicarbonates are also a reason for hardness in water. The permissible limit for bicarbonate (HCO_3^-) in drinking water is 500 mg/l. $NaHCO_3$, concentration in filtered water samples ranged between 6.97 to 8.53 mg/l with an average of 7.72 mg/l. While in tap water the range was 5.376 to 9.68 mg/l with an average of 7.18 mg/l. For, Na_2CO_3 filtered water samples ranged between 8.798 to 10.7696 mg/l with an average of 9.75. In in tap water 6.784 to 12.224 mg/l with an average of 9.1 mg/l. Concentration of HCO_3 in filtered water samples ranged between 5 to 6.19 mg/l with an average of 5.66 mg/l. And in tap water samples concentration ranged between 3.904 to 7.035 mg/l, with an average of 5.22 mg/l. CO_3 concentration in the filtered samples ranged between 4.98 to 6.096 mg/l with an average of 5.52 mg/l. And in tap water concentration of CO_3 ranged between 3.84 to 6.919 mg/l with an average of 5.14 mg/l. Thus, all the samples contained bicarbonates and carbonates concentrations under permissible limits. There, wasn't much difference noted in concentrations of carbonates and bicarbonates in filtered and tap water samples. The average carbonates concentration has been computed in Figure 3.13.



3.13 Average carbonates and bicarbonates concentration in study area

Arsenic: According to the World Health Organization, the maximum permissible limit for arsenic in drinking water is 0.01mg/l. The arsenic concentration of samples collected from the filtration plants and tap water was 0mg/l. Thus, the values of arsenic in both type of sampling sources were within the standard limits.

3.5 Biological Parameters

Microbial counts

On performing gram staining it was found that all the samples contained gram positive bacterial colonies. The CFU count on Nutrient Agar (N.A) for all the samples was overall higher than the other media (SS and EMB). In samples taken from filter plants, the highest number of colonies grown was found in sample 'F6', the values for total coliform and S.S was found to be 208 and 57 respectively. While 5 colonies growth was found on EMB agar belonging to sample ‘F4’. In samples collected from tap water, 139 colonies for total coliform, 26 colonies of SS, 36 on EMB colony growth was found in sample ‘T2’. On average water from tap water was found to be more contaminated as compared to filtered water. Bacterial growth of samples collected from filtered samples and tap samples are shown in Table 3.4 and 3.5 respectively.

Table 3.4: Results from the biological analysis of filtered water sample

Sr #	Sample No	Total Bacteria on Nutrient Agar (CFU/ml)	Salmonella Shigella Agar (SS) (CFU/ml)	Total Coliform on (EMB) agar (CFU/ml)
Permissible Limit		**<500 CFU/ml**	**0 CFU/ml**	**0 CFU/ml**
1	F1	7	0	0
2	F2	4	0	0
3	F3	15	0	0
4	F4	54	0	5
5	F5	2	0	0
6	F6	208	57	0
7	F7	5	1	0
8	F8	44	0	0
9	F9	1	0	0
10	F10	7	0	0
11	F11	31	0	0

Table 3.5: Results from the biological analysis of tap water sample

Sr #	Sample No	Total Bacteria on Nutrient Agar (CFU/ml)	Salmonella Shigella Agar (SS) (CFU/ml)	Total Coliform on (EMB) agar (CFU/ml)
Permissible Limit		**<500 CFU/ml**	**0 CFU/ml**	**0 CFU/ml**
1	T1	12	0	0
2	T2	139	26	36
3	T3	18	0	9
4	T4	4	2	0
5	T5	103	2	0
6	T6	65	0	0
7	T7	21	0	4
8	T8	18	0	0
9	T9	42	0	0

On average tap water was found to contain more microbial contamination as compared to filtered water. Since, in samples collected from filter plants only two samples showed growth of *total coliforms, salmonella* and *shigella* while in case samples collected from tap water three samples showed growth of *total coliforms*, *salmonella* and *shigella* while the rest showed high bacterial count. As there are two main sources of water in Rawalpindi; surface water and groundwater. Water to filter plants located in the study area is supplied by Rawal dam and Khanpur dam, in a study Rawal Lake was found to be contaminated with total coliforms as well as fecal coliforms, indicating fecal contamination. Reason for this contamination was found to be dumping of poultry waste as well as improper management of sewage water. This can be a possible source of bacterial contamination as when such contaminated water is supplied to filter plants along with improper sewerage pipelines. The pipelines are neither maintained nor checked for leakage, while mixing of sewerage waste with clean water pipeline can also a possible reason for the contamination (Mashiatullah et al., 2010).

In case, of groundwater the main recharging source in the area Nullah lai, which once a freshwater stream has now due to dumping of sewerage waste and wastewater has become contaminated with various microbial species. Due, to over extraction of groundwater along with increasing contamination levels, ground water is depleting at a fast pace. According to a study 0.545 million m^3 /day wastewater is dumped into Nullah, and this number has only increased in the past few years. So, along with recharging groundwater, the Nullah also cause seepage of contaminants in groundwater. Another possible reason for groundwater contamination can be drastic decrease in groundwater causing exposure of upper aquifer to contaminants. In the study it was also highlighted that 220 tube wells revealed that 50% of the tube wells in the areas were contaminated water against 33% in the year 2003 (Haque et al., 2007).

Gram staining: In order to differentiate and classify bacterial species into two large groups; gram-positive bacteria and gram-negative bacteria, a method called gram staining is used. After performing the gram staining, the slides were examined under the microscope to check whether the colonies picked up by us were of gram-positive or gram-negative bacteria. All the slides under the microscope showed dark purple stained cells. This indicated that all the colonies were of gram-positive bacteria.

CONCLUSION

In the present study, water samples were collected from various filtration plants as well as (bore) tap water from different areas of Rawalpindi to be utilized in obtaining water quality information. The physiochemical and biological analysis result of the filtered and tap water samples were compared with the WHO, PSQCA, and NSDWQ 2010 drinking water standards.

Following conclusions are drawn from the study:

1) All the physical parameters were not exceeding the permissible limits of given standards except for the fact that TDS and salts were found in high concentration.
2) While in case of biological parameters most grave fact is that a lot of the samples exceeded the permissible limits for *total coliforms, salmonella* and *shigella* whose permissible limit is '0'. While total bacterial count was also high in many water samples.
3) Also, it was noted that samples taken from tap waters (bore) had higher microbiological growth as compared to those taken from filter plants. It means that the tap water when compared to filter water is less suitable for drinking when compared for biological parameters.
4) The overall result of the study showed that the concentration of all the physicochemical parameters were far below the permissible limits while concentration of biological parameters exceeded the permissible limits.

RECOMMENDATIONS

The recommendations are as following:

1) In future, when evaluation of water quality of Rawalpindi is conducted, then it is recommended that tests for heavy metals should also be tested along with physio-chemical parameters and biological parameters in order to monitor water quality.
2) Since, a lot of samples were found to exceed permissible limits for bacterial parameters, proper checking of pipelines for leakage should be done, as there might be a source of mixing of human or animal waste with drinking water nearby since some of the samples showed growth of total coliforms.
3) To improve water quality filters installed in filtration plants should also be checked.
4) To improve groundwater quality, it is necessary that seepage of contaminants from contaminated streams that recharge groundwater is stopped.
5) To improve quality of water there should be continuous monitoring of pollution level which could arise from the nearby residents of area.

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