

**SYNCHRONISED PHYSICAL, CHEMICAL AND MICROBIAL ANALYSIS OF  
WASTEWATER FROM UNIVERSITY OF LIMPOPO FOOD-COURT.**

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**In**

**CHEMISTRY**

**M.G RAPETA**

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**SYNCHRONISED PHYSICAL, CHEMICAL AND MICROBIAL ANALYSIS OF  
WASTEWATER FROM UNIVERSITY OF LIMPOPO FOOD-COURT.**

BY

**MOKGADI GLADNESS RAPETA**

DISSERTATION

Submitted in fulfilment of the requirements for the degree of

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**UNIVERSITY OF LIMPOPO**

**SUPERVISOR: Prof V.L. Mulaudzi**

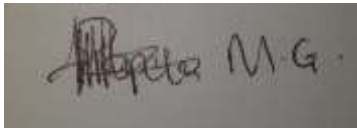
**CO-SUPERVISOR: Prof P. Masoko**

**2021**

## DECLARATION

I **Mokgadi Gladness Rapeta** declare that the dissertation hereby submitted to the University of Limpopo for the degree of Master of Science in Chemistry has not been previously submitted by me for the degree at this or any other University, and that this is my own work in design and execution, and that all materials contained therein have been duly acknowledged.

Signature: Rapeta M.G

A rectangular box containing a handwritten signature in black ink. The signature is written in a cursive style and reads "Rapeta M.G.".

Date: 02/06/2021

## **DEDICATION**

This study is dedicated to my family, my late father Moraka Rapeta for always encouraging me that education is the key to success and a first husband. My mother Kedibone Rapeta, my uncle Joseph Rapeta, sisters (Tebogo and Modjadji) , brothers (Thabiso and Seila) and Aunt Selina Senyolo for endless support. Last but not least my precious son Junior Rapeta along with my nieces and nephews.

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## ABSTRACT

The present research work deals with the study of some of the important physico-chemical parameters of restaurant wastewater effluents collected from Mankweng business centre along the University of Limpopo road. Studies revealed that restaurants, food preparation facilities, engineering, paper mill, fine chemical, dyes, paint, pharmaceutical, petrochemical and textile industries are some of the major industries responsible for polluting the aquatic environments. This research attempts to review the physical, chemical, and microbial analysis of wastewater from food service establishments (FSEs) and extraction of fats, oil, and grease (FOG) and its characterization using different analytical techniques. The elemental analysis revealed that the concentration of elements such as zinc, lead and manganese were 6.44 mg/L, 0.02 mg/L and 0.53 mg/L, respectively. The concentration of these elements was above the required South African National Standards 241 (SANS241) limit, that is,  $\leq 5$ mg/L,  $\leq 0.01$ mg/L and  $\leq 0.4$ mg/L for Zn, Pb and Mn, respectively. The elevated concentrations may be due to corrosion, acidic pH found in some samples and high organic load. High organic content in wastewater influenced the production of oxygen by microorganisms in-order to degrade the amount of waste in the wastewater and this has led to high chemical oxygen demand (COD) level ranging from 1784-7196 mg/L and biochemical oxygen demand (BOD<sub>5</sub>) from 1101.32 to 3222.32 mg/L. The study has demonstrated that the wastewater samples are contaminated with faecal coliforms because the concentration of microbes *Escherichia Coli (E.coli)* with a Most Probable Number (MPN)  $>200.5$  in most samples, *P. aeruginosa*, *Pseudomonas spp.* were too numerous to count (TNC) and total coliforms at the highest concentration of  $9.1 \times 10^3$  CFU/ml which was influenced by change in pH of the wastewater, the FOG content consists of both triglycerides and acrolein. The overall results highlight the discharge of highly polluted wastewater effluent, these FSE have resulted in pollution of nearby rivers thereby affecting the growth of vegetation and aquatic life. The results of the present investigation point out the need to implement common objectives, compatible policies, and programmes for improvement in the FSEs wastewater treatment methods.

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## ABBREVIATION AND ACRONYMS

Al	Aluminium
<i>B. cereus</i>	<i>Bacillus cereus</i>
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
<i>E.coli</i>	<i>Escherichia Coli</i>
EPA	Environmental Protection Agency
FFAs	Free fatty acids
FOG	Fats, oil and grease
FTIR	Fourier Transform Infrared
FSEs	Food services establishments
FSOs	Food service outlets
GI	Grease interceptor
IC	Ion Chromatography
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
LCFA	Long chain fatty acids
LLE	Liquid-Liquid Extraction
NH <sub>3</sub>	Ammonia
NTU	Nephelometric Turbidity Unit
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
SANS241	South African National Standards 241
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
SSMP	Sewer System Management Plan

SSOs	Sanitary Sewer Overflows
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
WHO	World Health Organization

# CHAPTER 1

## 1.1 BACKGROUND AND MOTIVATION

### 1.1.1 Background of the study

Water can be viewed as one of the essential life-supporting natural assets and a regular habitat for many animals, an essential driving force for farming, an element essential for both flora and fauna. It serves as a carrier and medium for contamination from both domestic and industrial entities. It is a key element for amphibians, washing proficiency, surface, and drinking water, and plays an important role for effluents rates and distance coverage (Tupper, 2010). There are administrative activities (guidelines) which are intently designed to control the usage of this precious resource. Different countries have their own rules and policies pertaining to the preservation of fresh water. Water is an essential characteristic asset critical to human presence and to the biological framework. In spite of the fact that water is copiously accessible to mankind, just 3% of the waters in the universe are fresh water. Among the fresh waters, only 5% of them or 0.15% of the total world waters are readily available for beneficial use (Usharani *et al.*, 2010). Water has found its way as a recreational medium for many events for example swimming pools and water slides.

In this manner, water goes about as a significant asset for the correct working of manufacturing plants (Owa, 2013), and the vast majority of which end up as modern wastewater (Bhandari and Ranade, 2014). In the course of recent years, anthropogenic exercises combined with fast urbanization and industrialization have heavily weighed on the quality of sea, oceanic and surface waters. This has resulted in aquatic life from oceans, seas, lakes, and rivers being adversely affected, which consequently influences human wellbeing indirectly and directly. Frequently, the animals and plants' health are directly proportional to the degree of ecological corruption and disruption from various anthropogenic exercises (Bhat *et al.*, 2018).

Wastewater is any water whose physical, concoction and natural properties are altered because of the presentation of specific undesirable mostly synthetic concoctions that make it risky for specific uses, for example, drinking. Humans depend on the availability of potable water and this will have a great impact on their well-being. A portion of the constituents incorporate food scraps, fats, oil, clothing powder, texture

conditioners, synthetic compounds, cleansers, family unit cleaners, soil, miniaturized scale life forms (germs) that can cause diseases and adversely harm the environment. A great part of the water referred to ends up as wastewater which makes its treatment significant. Treatment of wastewater is the procedure and innovative skills used to remove a greater percentage of the contaminants to guarantee a healthy water system. Likewise, wastewater treatment leads to overseeing wastewater rehabilitation to secure nature and guarantee general wellbeing, cultural, social, and political adequacy (Metcalf *et al.*, 1991).

Water contamination plays a role within the sight of a worldwide 'water emergency', by raising both the measure of freshwater assets accessible to people and the environment. Freshwater deficiencies are currently occurring in developing nations like India, China, and numerous African nations, just as in some developed nations (Ganoulis, 2009). Approximately 2.1 billion individuals need access to clean water and about 4.5 billion individuals need access to sufficient sanitation (WHO, 2010). An ongoing UN report shows 66% of the total populace will confront water worry by 2025 (Koop and van Leeuwen, 2017). These numbers are rising at alarming rates. Water shortage could be its physical shortage, where water accessibility is constrained and requests are not met, or it could be a financial shortage, where there is no method/framework to give water of the fundamental amount and quality, while water is accessible (Ranade and Bhandari, 2014).

Municipalities face the challenges in setting maintenance requirements in their Sewer System Management Plans (SSMP) based on the definition of fat, oil, and grease (FOG) sediment collection system pipes (Tupper, 2010). FOG deposits are classified as calcium dependent saponified solids and have been implicated in nearly a fifth of the United States-wide sanitary sewer overflows (SSOs). Municipalities are still struggling to specifically connect the creation of FOG deposits in these sewage collection pipes with the wastewater discharge characteristics of food service establishments (FSEs) beyond their complete oil and grease release (Report to Congress, 2004). Clear detection of factors influencing the rate of FOG deposit formations in certain pipe segments will enable municipalities to impose new FOG discharge limits, implement alternate grease interceptor pumping out cycles, or explore new ways of helping FSEs improve their cleaning procedures.



The term FOG includes a number of different materials (liquid and solid) and describes a heterogeneous group of chemicals, including tri-, di- and mono-glycerides, sterols, non-volatile hydrocarbons, waxes, and other complex lipids that occur in a mixture of free and emulsified forms (Tapela, 2015). Suspended and emulsified FOGs are discharged from domestic and commercial premises into sewer systems and are linked to a significant proportion of the deposits (solidated and saponified) that form sewer blockages (Williams *et al.*, 2012). Discharges from FSEs are considered to represent a major source of FOG deposits in sewers, and hence the focus of their discharge management field. Current FSE wastewater management practices include treatment in the form of biological additives to process the FOG material into benign end products or the collection of passive separators. Current challenges associated with the cost and complexity of suspended and emulsified FOG measurement often mean that surrogates are preferred when performance is selected, designed, and confirmed. Passive gravity separators, for example, typically use diesel oil as a substitute in validation tests although the oil's specific gravity is significantly lower than standard FOG, raising questions about its applicability (Barton, 2012).

All of this waste is directed to the municipality sewage during the preparation and cleaning. The accumulation of this extensive amount of waste presents a great danger to the local community as a whole and creates a problem for the local municipality in coping with the vast generation of gasoline, grease, and animal waste. The FSE impacts the sewage system, as every commercial cooking process creates FOG waste. Heated grease that can block the pipes by congealing in the kitchen pipes and causing water to back into the sink. The same can happen to sewage systems on a larger scale. Most of the blockages can be attributed to FOG in sewer pipes (Johnson *et al.*, 2012). Sewage leaks, manhole overflows or floods into homes and businesses are often triggered by the blockages, and too much grease and oil often raise maintenance cost.

The detrimental effects of FOG concentrations have been extensively documented on sewage collection systems. Each year, the EPA reported that about 23,000 to 75,000 sanitary sewer overflows (SSOs) occur in the United States (US EPA, 2004). Of these large numbers among SSOs, 47% were correlated with FOG accumulation in sewer lines of those induced by line blockages. One water utility company reported in a

recent Australian study that 21% of all blockages were primarily due to accumulation of FOG deposits (Mattson *et al.*, 2014).

### **1.1.2 Economic importance of water analysis**

Given the impacts of physico-chemical contaminants, some of them are intentionally helpful in the businesses for different purposes. A portion of the physico-chemical parameters required for plant and human advancement are self-evident. Phosphates and sulfates, for instance, are utilized in manure and cleanser creation. Phosphates may likewise be utilized in fisheries by assisting with scattering fish by improving food life form development (Sharma, 1999).

pH is utilized in the grouping of water systems, water preparing, synthetic coagulation cleansing, water mellowing, erosion anticipation, and water treatment (Mc Avory *et al.*, 1972). Subsequently, water shading impacts the degree to which light is transmitted, this in turn manages the amount of essential creation liable to be accomplished by controlling the photosynthesis pace of the current green growth. The measure of pollution may be similarly discovered by water shading.

Odour also plays an important role in water quality appraisal since it qualifies it for drinking, poisoning of fish, other oceanic living organisms and also for recreational exercises (Sharma *et al.*, 1999).

Water hardness can be utilized to evaluate the regular amphibian framework. Suspended solids are huge boundaries of wastewater, they are used to quantify the nature of the wastewater influent, to screen many treatment forms, and to gauge the nature of the profluent. EPA has built up the greatest suspended solids standard of 30 mg/L for most rewarded wastewater releases (Comstock, 2014).

All the above have financial implications to the preservation and provision of clean water for human use and agricultural purposes.

### **1.1.3 Physicochemical Characteristics of FOG and Influence on Wastewater Treatment Process**

Due to the physico-chemical attributes of the waste and issues with its handling during cold seasons, high concentration of FOG in wastewater makes it very difficult to achieve. FOG consists mainly of long-chain fatty acids (LCFA) bound to glycerol, esters, waxes, phospholipids, sterols, and esters of sterols, high concentration of

LCFA, leads to a slight acidic in pH value. The content of fatty acids in FOG varies, but waste is typically rich in oleic acid (18:1 cis), elaidic acid (C18:1 trans) and palmitic acid (C16:0 cis) that are difficult to degrade and cause microorganism toxic effects (Cavaleiro *et al.*, 2016).

FOG's chemical composition in conjunction with its non-polar nature and basic gravity (less than 1.0) lets it float over the surface of the water. FOG is in semi-liquid state at room temperature and is readily miscible with liquids. The decrease in temperature, however, results in solidification, and it is barely movable and fully water-immiscible at this state (Seriouie *et al.*, 2010). FOG tends to stick to non-polar surfaces, such as fermenter walls and pipe walls, causing pipe blockage and reducing their lifespan due to corrosive anaerobic processes and formation of hydrogen sulfide gas. Viscosity of FOG correlates with concentration of unsaturated fatty acids in the composition of triglyceride ester' the higher the concentration, the lower the viscosity of FOG' Ineffectively pre-treated food wastewater with a high FOG content raises the organic load rate on the further aerobic treatment process and distresses the oxygen mass transfer in aeration tanks (Brooksbank *et al.*, 2006) as well as sludge dewatering by adhesion on biomass surfaces (Wang *et al.*, 2016).

#### **1.1.4 Negative effects of FOG deposition**

FOG tends to stick to the drain and sewer pipes surface, causing clogging that limits sewage flow and can lead to SSOs. The SSOs cause hostile odors, infestation of insects and rodents and sewage may enter water sources that cause pollution of the soil and surface water (Suratman, 2011). These are very inconvenient and allow the municipalities to act quickly to clear the deposition in order to alleviate public concerns. In addition, FOG deposition can cause sewer line corrosion under anaerobic conditions, thereby reducing the pipe's lifespan and requiring earlier repair and pipe replacement. On the other hand, the biological treatment of wastewater with a high concentration of FOG suspended on the surface may be hampered by adhering FOG to the pipes and clogging the strainer and filters, thus, affecting the operations of the treatment unit (Jameel *et al.*, 2011).

## 1.2 Significance of the study

FOG blockage is a worldwide concern. For example, the American Environmental Agency (EPA) estimated that at least (10,350–36,000) SSOs occur per year in the USA, approximately 47% of which is related to FOG (EPA, 2015). Similarly, in the UK over 25,000 flooding incidents per year are due to sewer blockages, of which, 50% is due to FOG (Williams *et al.*, 2012). Moreover, up to 70% of the SSOs that occur in Malaysia are due to FOG. In 2010 only, the wastewater municipality in Malaysia, Indah Water Konsortium (IWK), received a total of 22,184 blockage enquiries (IWK, 2010).

Reddy and co-workers (2003) studied water issues in the South African context, the proliferation of FOG, chemical species, and microbial influence with interest on edible oil effluent. The current study will provide valuable information on the types and quantities of FOGs, chemical and microbial constituents in wastewater associated with food preparation and serving. Key findings will aid in updating South Africa's guidelines of effluent/wastewater quality and policy development. This problem is not only unique to South Africa but has affected some other African countries like Cameroon where a similar study was conducted with modifications (Porwal *et al.*, 2015).

Recently, extensive efforts have been expanded to investigate the possibility of treating and reusing FOG to reduce the amount disposed of at landfills and into the municipal sewer system (Aziz *et al.*, 2010). However, understanding the chemical, physical and microbial properties of FOG from wastewater and its deposit-formation mechanism is essential for developing more environmentally and economically efficient methods of controlling, treating, and reusing FOG (He *et al.*, 2013; Keneer *et al.*, 2016).

The current study was focused on water effluents from Mankweng food court at the University of Limpopo. Furthermore, it was specifically focussing on the physico-chemical and microbial characteristics of the wastewater particularly FOG. The analytical method of distillation was applied to specifically isolate the oil. In addition to the aforementioned, carbohydrates, organic nitrogen, and the following ions:  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ,  $\text{PO}_4^{2-}$  and  $\text{NO}_3^-$  were also be analysed. Also, organic, and inorganic phosphorus and the following elements were quantified, Na, K, Ca, Mg, Fe, Co, Ni and Mn. Microbes such as *Pseudomonas*, *total coliform*, *Salmonella* and *Shigella*, *Bacillus cereus*, *Escherichia coli*, *Enterics*, *Staphylococcus* were also be analyzed.

### 1.3 Problem statement

FOG is an ever-growing environmental concern, it is usually produced at food service establishments or other food preparation facilities (Aziz, 2010). The by-products and wastes from these food services include meat, sauces, gravy, dressings, deep-fried food, baked goods, cheese, and butter. All these wastes are considered FOG and may build-up in the sewer system when discharged directly into the facility's plumbing system (Garza, 2006). Recently, more people are using fast-food outlets which leads to proliferation of FOG deposits and increasing the frequency of blockages of the local sewer system (Burton *et al.*, 2014). Investigations of FOG is important because it tends to stick to the surface of drain and sewer pipes causing clogging that restricts the flow of sewage and may lead to SSOs. These SSOs cause unpleasant odours, insect and rat infestation, and the sewage may make its way into water sources causing ground and surface water pollution (Suratman *et al.*, 2011).

They are very unpleasant and require quick action from the authorities to clear the deposition to allay public concerns. Moreover, FOG deposition can cause corrosion of sewer lines under anaerobic conditions, thus reducing the lifetime of the pipe and demanding earlier repair and replacement of the pipe (Jameel *et al.*, 2011). Quantity and quality of FOG requires a good managing strategy from commercial food preparation to avoid problems such as odours and minimize pre-treatment equipment installation and ongoing maintenance cost (Barton, 2012). This study entails an in-depth qualitative and quantitative analysis of FOG, microbes and chemical species associated with fast food preparation from the wastewater. It further investigates the physical parameters of the wastewater to be used as the sample. A study of this nature and magnitude has not as yet been carried out for the Mankweng area located in South Africa.

**Hypothesis:** Quantitative/qualitative analysis of FOG and microbial analysis lead to total wastewater analysis from food establishments.

## **1.4 Aims and objectives**

### **1.4.1 Aims**

The aim of the study was to quantify and qualify fats, oil and grease, appropriate chemical species, and microbes from UL food-court wastewater (effluent streams).

### **1.4.2 Objectives**

The objectives of the study were to:

- i. carry out physical, chemical, and microbial analysis from wastewater,
- ii. use ICP, UV-spectroscopy for elemental analysis and Ion chromatography for cations and anions analysis.
- iii. design extraction methods for oil, fats, and grease from wastewater,
- iv. compute the amount of FOG from the extracts,
- v. separate the extracts of FOG using distillation,
- vi. characterise the FOG using the FTIR instrument.

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## **CHAPTER 2**

### **LITERATURE REVIEW**

#### **2.1 Introduction**

Water is a scarce commodity in various nations around the globe; however, it is an essential and very important resource. Serious water contamination and lack of water rehabilitation assets are of significant difficulties these days (Enoh and Christopher, 2015). South Africa is the world's 30<sup>th</sup> driest nation with a normal annual precipitation of 495 mm contrasted to the world normal precipitation of 1033 mm for every annum. South Africa is supposed to be a water-scarce nation with evaporation much higher than precipitation, so there is a need to consider the use of researched and scientific water management systems and protocol (Hedden and Cilliers, 2014).

Wastewater is water after it has been used in a variety of applications usually leaching, flushing, or washing away wastes from locations those wastes were generated (Fillaudeau *et al.*, 2006). It includes substances such as human waste, food scraps, oils, soaps, and chemicals, some of this organic content is dissolved into the water and some exist as separate particles. The portion of organic material that does not dissolve but remains suspended in the water is known as suspended solids (Woodard, 2001). Wastewater is treated to remove as much organic material as possible. The extreme bad aroma emanation from these wastewaters makes residential areas intolerable.

In spite of their technological demand, food preparation facilities are among probably the biggest clean water consumers in regions. Food production and processing produce wastewater for the most part with high natural substance alongside raised convergences of FOG. Studies concluded that per capita FOG production is growing in developing countries, with each person generating 50 kg/annum as of 2015, while in non-developing countries the per capita production was 20 kg/annum (European, 2015). FOG has a negative impact on treatment systems by clogging the pipelines and making further procedures for treating wastewater very difficult (Xia *et al.*, 2013).

Off-lately numerous individuals are alert about the significance of arranging waste. On account of food squander, the green receptacle, 'the trash and down the drain system' are some of the removal techniques and the last incorporate the sinks, food

processors and toilets. Wastewater frameworks are not unequivocally intended to deal with food squander, be that as it may, so these may cause critical issues after some time. Most food and food ingredient by-products contain FOG, the main chemical constituents of which are free fatty acids (FFAs). Cooking oil, meat fats, gravy, margarine, food scraps, sauces, butter, deep fried food, and cheese are some examples of foods rich in FOG (Husain *et al.*, 2014).

When FOG enters the pipes, it chills off and gathers until a core is made that is fixed to the sewer walls. With the steady flow of wastewater, varying debris, dirt and more FOG aggregates around the preliminary centre until they form a solid layer that covers drain lines and eventually blocks the sewers (He *et al.*, 2013). It may have different properties, depending on which of the two general types of FFAs (saturated or unsaturated) is most abundant in a FOG deposit. Deposits made from saturated FFAs are thick and less viscous than those made under laboratory conditions with unsaturated FFA (He *et al.*, 2013). Previous research has also shown that saturated FFAs (palmitic acid) are the most frequently found in sewage deposits (Benecke *et al.*, 2017; Keener *et al.*, 2008; Williams *et al.*, 2012). Such results suggest that deposits with high concentrations of saturated FFA may be harder to remove from walls of sewers.

Multi-site FOG waste can be aggregated with other non-flushable waste such as wet wipes and sanitary items (tampons, cotton, and buds) in the sewer to produce what is often referred to as 'fatbergs' (coined by Thames Water, UK). Reports of fatbergs in London have been ubiquitous in recent years, with a 10 ton fatberg in 2015 and an example of 15 tonnes in 2013 (Bufe, 2013). The term fatberg entered the Oxford online dictionary in 2015 and is well defined as 'a very large form of solid waste in a sewer system consisting of congealed fats and toilet-flushed personal hygiene products' (Oxford, 2015).

The problems caused by fatberg through FOG deposits can range from local property level flooding with sewage to city-wide problems caused by a complete blockage and overflow of sanitary sewer. These problems require road closures for mechanical sewage repairs, and can potentially release high concentrations of bacteria, pollutants and solids into water bodies that pose a risk to public health and the environment (He *et al.*, 2013). It has been estimated that approximately 24,750 events per year in the

United Kingdom are the result of line blockages (Arthur *et al.*, 2008), of which 50 to 75% are caused by FOG deposits (Keener *et al.*, 2008).

Fatberg may be a new term, but FOG waste deposit problems in the sewer system are not recent. FOG waste was deemed a sewer system concern as far back as 1944, when it was discussed at the New York conference on grease removal in relation to grease problems in sewer maintenance (Cohn, 1944; Dawson and Kalinske, 1944). There was then no coherent solution decided, this remains the case. Fatberg's coverage in London and the UK is not a localised issue. Without a proactive method, the FOG deposit epidemic and the negative effects attributed to it will only increase internationally due to growth in population and food service outlets (FSOs) and the tension of these places on urban sewer systems that were not designed for that level of input.

Within any sewer system, some locations are more prone to blockage by FOG than others. A number of factors may contribute to why these locations have become blockage 'hot spots' (Williams *et al.*, 2019). These include: the location of the sewer in relation to residential, commercial, and industrial areas; characteristics of the individual sewer pipe (e.g., diameter, material, joins); characteristics of the sewer network (bends, sags, number of inflows, effluent volume); and existing maintenance and cleaning programmes (Shaffer, 2018).

Appropriate waste management is recognised as a necessity for sustainable development (Papargyropoulou *et al.*, 2014). The waste hierarchy (Rosu, 2016) must be used for effective control of FOG. The waste hierarchy refers to prevention as the preferred method when dealing with waste, but the next option are the 3Rs (reduction, reuse, or recycling) when this is not feasible. Sustainable resource management is based on the ideal of "waste" being a resource, potentially creating a circular economy and a sustainable urban environment around a stream of renewable waste.

One of the most effective methods of reducing the detrimental effects of FOG waste is through stakeholders' awareness-raising and education initiatives. Many of these measures include encouraging practical activities that might prevent large quantities of FOG waste from entering drains. Such procedures vary from dry cleaning of dishes before washing operations to allow waste oil to cool and dispose it with general waste. These practices do not prevent the residual FOG from entering the sewer in the

washing operation wastewater, so further preventive measures are needed, especially in food service outlets (FSOs), which are one of FOG's major contributors to urban-centre sewer systems (Curran, 2015).

Metro Vancouver estimates that the region is spending R38.15 million on repairing residential, commercial, and even industrial damage in the wastewater system caused by FOG (Metro Vancouver 2018a, 2018b). That is why they have developed various strategies to address this issue. In order to address FOG discharges from the food services industry, Metro Vancouver has a Food Division Grease Interceptor (GI) By-law and Food Division sectors without GI, the 'Wipe It, Green Bin It' initiative launched in September 2017, aimed at raising awareness among the region's residents and food business sectors about the adverse effects of FOG on wastewater systems and regional food companies on the detrimental effects of FOG on drainage systems and how to properly dispose of such waste (Vancouver, 2018).

Similarly, the Metro Vancouver 'Love Food, Hate Waste' initiative, inspired by the United Kingdom's effective campaign of the same name, "provides simple steps for people and food production industries to use more of the food they buy" (Love Food, Hate Waste, 2017). Metro Vancouver conducted a Food Waste Survey from November 20<sup>th</sup> to November 26<sup>th</sup>, 2014 as part of this program. This research provided food diaries for residents where they recorded the "food being disposed of and why it is discarded rather than eaten, how it was disposed of, and current attitudes toward avoidable food" (Metro Vancouver, 2014).

## **2.2 Definition of FOG**

FOG is the cooking by-product (also known as brown grease). FOG typically includes food scraps, meat fats, cooking oil, margarine, sauces, gravy, dressings, deep-fried food, milk, and butter. Based on the carbon chain concentration, FOG may be solid or a viscous liquid (Lewkowltsch, 1993). Oils and fats are a class of fatty acids, triacylglycerols, and lipid-soluble hydrocarbons consisting of small but important components of FOG (Voet, 2012).

## **2.3 Physical properties of FOG**

FOG can occur as liquid or solid and has a greasy texture. FOG is colourless, odourless, and tasteless in its purest state. In addition, FOG is insoluble in water but soluble in organic solvents such as hexane, chloroform, and ether (Sincero and

Sincero, 2002). FOG has a density not as much as water (specific gravity < 1) and therefore drifts on the surface of the water. Nevertheless, in the presence of soap or other emulsifying agents, FOG can form emulsions with aqueous media (Madan, 2013; Patrick, 2013). Generally, FOG has high viscosity, which varies according to the composition of fatty acids and the presence of double bonds. Due to the more loosely packed structure, the more double bonds present in the carbon chain, the lower the viscosity of FOG (Firestone, 2006). The presence of large amounts of FFAs in FOG, usually produced by oils ' hydrolysis and oxidation reactions during deep food frying, results in a characteristically low FOG pH (Widlad, 1999; Sharoba, 2012).

#### **2.4 Sources of FOG in the sewer system**

FOG is frequently produced in residential and food service establishments. In addition, FOG components are introduced into the sewer system by direct dumping into the sewer or by leakage from usually restaurant-installed grease traps (GTs). The GTs (interceptors) are intended to trap most of the FOG in the effluent of the restaurant and separate it from the sewage before entering the sewer pipe (Aziz *et al.*, 2010). Nevertheless, the GT's performance is strongly dependent on the level of its maintenance (Wong, 2007). In addition, if a high-temperature dishwasher or water is used for dishwashing, FOG will dissolve and emulsify within the wastewater process, and thus outflow the GT. Upon subsequent discharge into the sewer pipe, FOG can congeal and form particles depositing on the pipe surface thus obstructing the flow of wastewater (Xia He, 2013). According to (Stoll and Gupta, 1997) FOG concentrations in wastewater from Thailand's Asian restaurants ranged from (730-100) mg/L, this high amount of FOG comes from fast-food restaurant wastes.

#### **2.5 Characteristics of wastewater from food processing facilities**

Because of the food preparing advancements utilized, the physico-chemical attributes of food handling wastewater shift (Owens *et al.*, 2000); yet they all have comparative issues, for instance; high Biological Oxygen Demand (BOD), expanded Total Suspended Solid (TSS) content, wealthy in protein and absolute nitrogen ( $N_{tot}$ ), and

high FOG concentration. FOG production in wastewater originates from the food business ventures (Table 2.1)

Table 2.1: Wastewater chemical composition of restaurant wastewater effluent in comparison to municipal wastewater parameters (Klaucans and Sams, 2018).

Parameters	Restaurant wastewaters (mg/L)	Typical municipal wastewaters (mg/L)
Total COD	1250-4500	210-740
Total BOD	820-300	150-350
Total Suspended Solids (TSS)	220-2700	120-450
FOG	140-4100	-

## 2.6 Wastewater-associated pathogenic microorganisms

Microbiological water quality is a significant worry among specialists worried about drinking, recreational and aquaculture waters (Whitman and Nevers, 2004) where microbiological contamination (Ashbolt, 2004) can represent a genuine wellbeing danger and lead to extreme sickness and even demise: as indicated by the World Health Organization (WHO), waterborne maladies execute around 2,2 million individuals consistently. In various nations there are different legislative guidelines which force quality norms for water applications. *E. coli* is generally viewed as a solid pointer and tracer of ongoing microbial and faecal sully (Edberg *et al.*, 2000), while different pathogens, for example, all out coliforms, faecal coliforms as well as *Enterococci*, may require observing relying upon the nation and application.

There is an assortment of microbiological verdure in wastewater which contains bacterial, protozoan, and viral pathogens (Ottoson *et al.*, 2006a). Wastewater has for some time been viewed as a wellspring of microbial pathogens, given that sickness and disease are endemic in the populace whenever. Since waterborne cholera transmission was first announced in 1854 (Snow, 2005), techniques for shielding general wellbeing from defilement of drinking water have been set up (Bitton, 2005). Given these methodologies, waterborne ailments keep on happening, of which particularly in creating nations are found in wastewater (Reynolds *et al.*, 2008; Lee and Schwab, 2005).



## 2.7 Bacterial pathogens

Numerous pathogenic microorganisms are present in human excrement, bringing about high centralizations of microbes in sewage-sullied waters. During the twentieth century, the progression of wastewater treatment innovations was credited to the decrease in created world bacterial-related waterborne infection (Leclerc *et al.*, 2000). Confinements in wastewater treatment forms, be that as it may, may prompt pathogenic microbes being transmitted through recreational use or utilization of tainted water.

The numbers and range of microbes found in wastewater are critical contemplations. The range of bacterial microbes is significant in light of the fact that it directs the idea of the danger presented by the wastewater. Then again, the quantities of microbes have an immediate bearing on the danger those microorganisms present and the size of the assignment of lessening that hazard. A knowledge of the range of pathogens also tells us something of the ecology of the organism being analysed, in (table 2.2) below it shows the bacterial pathogen present in municipal water (Bitton, 2005).

Table 2.2: Bacterial pathogens present in municipal wastewater (Bitton, 2005).

Genus	Organism	Possible symptoms of illness	Implicated in water or food borne illness
<i>Escherichia</i>	<i>E. coli</i> VTEC Serogroups	Abdominal cramping, severe bloody diarrhoea, sometimes leading to haemolytic uremic syndrome, renal failure and death	Both, mainly foodborne

<i>Shigella</i>	Various species	Causes “shigellosis” or bacillary dysentery. Fever, abdominal cramps, chills, vomiting, and diarrhoea containing blood and mucus	Both, mainly foodborne
<i>Salmonella</i>	<i>S. enterica</i> (various serogroups)	Stomach pain, diarrhoea, nausea, chills, fever, and headache	Both, mainly foodborne

## 2.8. Adapted Analytical methods for sample analysis

### 2.8.1 Sampling

Sampling of water is an essential part of the study and can be done manually or automatically. There are governmental standard methods and guidelines (EPA, 2016) for manual sampling that require care in collecting without contamination and maintaining validity of the sample. The representativeness of control may be influenced by various issues. Variations in sampling time, depth and sampling techniques can affect the quality of the sample (Nevers *et al.*, 2013).

### 2.8.2 Physico-chemical parameters

Testing the wastewater before it discharges into the rivers, lakes and dams is very necessary and important. Wastewater shall be measured using different physico-chemical parameters. The selection of parameters for wastewater testing depends solely on the nature of the analysis and the degree to which its quality and quantity are required. Wastewater contains various types of floating, dissolved, suspended and microbiological impurities as well as bacteriological ones (Aniyikaiye *et al.*, 2019). Some physical tests should be carried out to test its physical appearance, such as temperature, colour, odour, pH, turbidity, total dissolved solids (TDS), etc., whereas

chemical tests should be carried out for its biochemical oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), alkalinity, hardness and other characters.

### **2.8.2.1 pH**

pH is known as the H<sup>+</sup> ion activity measure and the hydrogen presence indicator in water (DWAF, 1996). The pH tests the level of acidity in water, the lack of acidity or alkalinity by comparable quantities of acids and water bases. If both are present in equal proportion (as in pure water), the pH shall be specified as neutral or 7. If more acids are present than bases, the water is acidic, and the pH will be below 7. When bases dominate, the water will be alkaline or basic, and pH above 7. Water with a pH of less than 4.8 or more than 9 may be harmful to the aquatic environment as the pH is a reliable measure of chemical balance in water. High (>9) or low (<5) pH will have an unpleasant effect on the availability of certain chemicals or nutrients in the water to be used by plants (Powell *et al.*, 2008; Jensen, 2010). Instability of water pH disturbs the solubility of chemicals that may also disturb the availability of toxic and nutritious chemicals to aquatic organisms. Most of the metals become more water-soluble and more toxic with an increase in acidity (Mosley *et al.*, 2004). The pH may also have an impact on wastewater treatment plant efficiency. According to (Hodgson and Larmee's, 1998) study indicated that an increase in pH of which is above 10.7 of the final could also lead to a decrease in coliforms in the final effluent. A higher pH also allows phosphates to precipitate the metal ions in wastewater, such as magnesium, calcium, and iron, causing a drop in phosphorus concentrations (Powell *et al.*, 2008).

### **2.8.2.2 Electrical Conductivity**

Conductivity is a parameter that cannot be evaluated in a complex way and can be easily measured using a continuous measuring device. This parameter is a general water quality measure, especially a function of the amount of salt dissolved. Conductivity can be used to track wastewater treatment processes that cause changes in the total salt concentration and consequently change its conductivity. Analysing samples in the laboratory will determine the amount of salt in the wastewater. However, the analysed result will be available with a delay using the laboratory which makes it less useful for controlling the process of treatment. In the process, conductivity can be measured straight through which values can be collected directly and can therefore be used to monitor and control the process. Electrodes or

computerized analytical devices can conduct chemical analysis on-line. Nevertheless, the cost of this is high, which can make measuring on-line conductivity a fascinating alternative. Most municipalities include industries and similar companies that discharge wastewater to the conductivity of the sewage net measure and are not allowed to exceed 500 mS/m (Levlin, 2010). A very high conductivity leads to problems such as corrosion in the sewage pipe network. Otherwise, conductivity measurement is not so much used in wastewater treatment.

### **2.8.2.3 Turbidity**

Turbidity is a measurement used to denote the consistency of waste deposited into water in relation to colloidal and residual suspended matter, it is a measure of water's light-transmitting properties. The calculation is based on a comparison of the light intensity emitted by a sample as compared with the light dispersed under the same conditions by a reference suspension. The colloidal matter disperses or absorbs light, thus preventing its transmission (Metcalf and Eddy, 1991). The cloudiness or haziness of a fluid is, by other means, caused by suspended solids that are normally invisible to the naked eye. The turbidity measure is an important test when trying to determine the water quality. It is an aggregate optical property of water and does not identify individual substances, it is simply saying that there is something.

### **2.8.2.4 Total Dissolved Solids**

Total dissolved solids (TDS) refer to inorganic salts (mainly potassium, sodium, calcium, magnesium, bicarbonates, sulphates, and chlorides) and small amounts of organic matter found in water solution from natural sources, sewage, urban and agricultural run-offs, and industrial wastewater (Elamassi, 2009). Majed (2006) emphasized that wastewater TDS increases due to evaporation while going over the treatment process. According to (Nadia, 2006), the discharge of wastewater effluents with a huge amount of TDS level (above 470 mg/L) will adversely affect aquatic life, making the receiving water incompetent for drinking and domestic purposes as well as crop yields.

### **2.8.2.5 Salinity**

Saline contaminated water originates from many sources such as seawater, groundwater, concentrate from decantation plants, effluent from sewage treatment plants, brine from natural salt lakes or freshwater effluent storage basins, brine from

salt mining operations, agricultural and irrigation effluent schemes, pulp and paper effluent, food It also comes from fertilizers, additives, paint, ink, pharmaceuticals, oil and gas produced water, mining and mineral processing effluents, pickling processes, meat packing and dyestuffs, pesticides, herbicides, polyhydric compounds, organic peroxides, and pharmaceutical products (Woolard and Irvine, 1995).

#### **2.8.2.6 Temperature**

Temperature is essentially an important factor for its effects on the chemical and biological water reaction. An increase in water temperature speeds up chemical reactions, decreases gas solubility, increases taste, odour, and organism metabolic activity (Saxena, 1989). Freshwater temperatures normally vary from (0-35) °C depending on the resource, depth, and season. High water temperature stimulates the development of microorganisms and results in the acceleration of chemical reaction in water (WHO, 1993).

#### **2.8.2.7 Biological oxygen demand**

Biochemical oxygen demand (BOD) refers to the amount of dissolved oxygen in the aquatic environment used to break down organic materials over a defined time frame in a given water sample at a certain temperature (Chapman, 1996). This measures the amount of organic compounds in water and is used as an indicator of wastewater treatment plants ' performance. According to (Suthar *et al.*, 2010), the presence of BOD in water indicates that in the aquatic environment there is an organic contaminant which unpleasantly affects the water quality and biodiversity of the river system. The more organic materials released into the aquatic environment, the greater the need for oxygen to break them apart. Low levels of aquatic oxygen (below 92% saturation) make aquatic species more susceptible to disease and hamper swimming. According to (Majed, 2006) wastewater effluent BOD and influents rise in the winter months as opposed to the summer months due to the increased use of water in the summer season compared with the winter season. (Majed, 2006) further claimed that while the BOD values in the winter seasons are rising, the biological loads remain constant, leading to dilution in the powerful BOD.

#### **2.8.2.8 Chemical oxygen demand**

Chemical oxygen demand (COD) is used as an oxygen-equivalent indicator of a sample's organic content that is prone to oxidation by a strong chemical oxidant

(Standard Methods, 1995). This test allows wastewater to be measured in terms of the total quantity of oxygen available to oxidize to carbon dioxide and water. COD indicates the quantity of oxygen required to oxidize all soluble and organic particulate matter in water under specific conditions. All organic compounds can be converted into oil, water, and ammonia. Determining water characteristics is a significant parameter. The value of COD depends on the oxidizing agent, the pH, the temperature, and the time period. It is expressed as parts of oxygen per million (ppm) or milligrams per litre (mg/L) (Patil *et al.*, 2012).

#### **2.8.2.9. Ammonia**

The term ammonia includes the non-ionized ( $\text{NH}_3$ ) and ( $\text{NH}_4^+$ ) ionized species. Ammonia in the atmosphere has its roots in microbial, agricultural, and industrial processes as well as chloramine disinfection. Natural groundwater and surface water levels are usually below 0.2 mg/L, and soil anaerobic waters may contain up to 3 mg/L. Intensive farm animal rearing can also lead to much higher surface-water levels. Contamination with ammonia can also grow from linings of cement mortar pipes. In water, ammonia is an indicator of possible contamination of bacteria, sewage, and animal waste. Ammonia is a key component of mammalian metabolism. Environmental exposure is insignificant when compared to the endogenous ammonia synthesis. Toxicological effects are only found at concentrations of approximately 200 mg/kg body weight. Ammonia in drinking water is not of immediate significance to the health and therefore no guideline importance based on health is suggested. Ammonia, however, can compromise the efficiency of disinfection, result in nitrite formation in distribution systems, cause filter failure to remove manganese, and cause problems with taste and odour (WHO, 2003).

#### **2.8.2.9. Hardness**

Hardness is a physico-chemical property of water and is commonly a calcium and magnesium ion measurement in water. Zinc, iron, strontium, aluminium, and manganese can also contribute to water hardness; however, they usually occur at very low concentrations (NRC, 1974). Such ions enter a body of water by leaching from rock and soil minerals. Common mineral calcium is granite (calcium carbonate), and chalk (calcium sulphate). A common mineral of magnesium is dolomite which contains calcium as well (Gumashta *et al.*, 2012). Initially, water hardness was understood to

be a measure of the water's capacity to precipitate soap, which in practice is the summation of concentrations of all polyvalent cations present in water (Ca, Mg, Sr, Ba, Fe, Al, Mn, etc.) later it was generally accepted that hardness is defined as the summation of concentrations of Ca and Mg, calculated by the EDTA titrimetric method and express. The type of anion found in these salts differentiates between the two hardness types which are hardness of carbonate and non-carbonate.

Carbonate ( $\text{CO}_3^{2-}$ ) hardness is sometimes referred to as temporary hardness because boiling water will kill it. By heating the bath, non-carbonate hardness cannot be broken, so it is also known as permanent hardness. Ultimately, differentiating between the two kinds of hardness is important because the method of removal varies for the two. Complete hardness consists of both temporary and permanent hardness caused by calcium and magnesium, which characterizes water as soft or hard and very hard (Sengupta, 2013). From a technical point of view, it has proposed many different water hardness scales (e.g., very soft, soft–medium, hard, very hard). Expectedly, from the technological and safety points of view, both extreme degrees (i.e., very soft and very hard) are calculated as unacceptable, but the optimal amounts of Ca and Mg water are not easy to determine since the health criteria may not be compatible with the technical ones (Kozisek, 2014).

Soft: (0-60) mg/L

Medium hard: (60-120 )mg/L

Hard: (120-180) mg/L

Very hard: > 180 mg/L.

### **2.8.3 Chemical parameters**

#### **2.8.3.1 Principle of Inductively coupled plasma optical emission spectroscopy**

Inductively coupled plasma optical emission spectroscopy (ICP-OES) is one of the most powerful and popular analytical tools for the determination of trace elements in a myriad of sample types. The technique is based upon the spontaneous emission of photons from atoms and ions that have been excited in a RF discharge. Liquid and gas samples may be injected directly into the instrument, while solid samples require extraction or acid digestion so that the analytes will be present in a solution. The sample solution is converted to an aerosol and directed into the central channel of the

plasma. At its core the ICP sustains a temperature of approximately 10000 K, so the aerosol is quickly vaporized. Analyte elements are liberated as free atoms in the gaseous state. Further collisional excitation within the plasma imparts additional energy to the atoms, promoting them to excited states. Sufficient energy is often available to convert the atoms to ions and subsequently promote the ions to excited states. Both the atomic and ionic excited state species may then relax to the ground state via the emission of a photon. These photons have characteristic energies that are determined by the quantized energy level structure for the atoms or ions. Thus, the wavelength of the photons can be used to identify the elements from which they originated (Naumann and Meyers, 2000). The total number of photons is directly proportional to the concentration of the originating element in the sample. The instrumentation associated with an ICP-OES system is relatively simple. A portion of the photons emitted by the ICP is collected with a lens or a concave mirror. This focusing optic forms an image of the ICP on the entrance aperture of a wavelength selection device such as a monochromator. The particular wavelength exiting the monochromator is converted to an electrical signal by a photodetector. The signal is amplified and processed by the detector electronics, then displayed and stored by a personal computer.

### **2.8.3.2 Aluminium**

Aluminium (Al) is the most abundant metallic element, and it constitutes about 8% of the earth's crust. Al salts are commonly used in water treatment as coagulants to decrease organic matter, colour, turbidity, and microorganism levels. Such use may lead to increased concentrations of Al in finished water, where residual concentrations are high, undesirable colour and turbidity may arise (Meija, 2013). Concentrations of Al at which such problems may occur are extremely dependent on a number of water quality parameters and operational factors at the water treatment plant. Al intake from foods, mainly those containing Al compounds used as food additives, represents the major route of Al exposure for the general public. The contribution of drinking water to the total oral exposure of Al is usually less than 5% of the total intake. In humans, Al and its compounds seem to be poorly absorbed, although the rate and extent of absorption have not been sufficiently studied for all sectors of the population (WHO, 2009). The degree of Al absorption depends on a number of parameters, such as the Al salt administered, pH (for Al speciation and solubility), bioavailability and dietary



factors. These parameters must be taken into consideration through tissue dosimetry and response assessment. The use of presently available animal studies to develop a guideline value for Al is not appropriate because of these specific toxicokinetic/toxicodynamic considerations. There is little indication that orally ingested Al is acutely toxic to humans regardless of the widespread occurrence of the element in foods, drinking water and many antacid preparations. It has been hypothesized that Al exposure is a risk factor for the development or acceleration of onset of Alzheimer disease (AD) in humans (WHO, 2003).

#### **2.8.3.3 Zinc**

Zinc (Zn) is a crucial trace element found in virtually all food and potable water in the form of salts or organic complexes. The diet is normally the principal source of zn, although levels of zn in surface water and groundwater normally do not exceed (0.01 and 0.05) mg/L respectively, concentrations in tap water can be much higher as a result of dissolution of zinc from pipelines. In 1982, JECFA proposed a PMTDI for zn of 1 mg/kg of body weight and the daily requirement for adult men is (15–20) mg per day (Musa *et al.*, 2013). It was considered that, taking into the account recent studies on humans, the derivation of a guideline value is not required at this time. However, drinking water containing zn at levels above 3 mg/L may not be acceptable to consumers.

Zn is also important to humans and plants but can be harmful to the body in very high concentrations (Abagale *et al.*, 2013). Zn can hinder algal photosynthesis and prolonged exposure to sub-lethal concentrations of zn in fish which causes liver necrosis and oedema. Zn comes from corrosion and leaching of plumbing, water-proofing products (zn-formate, zn-oxide), anti-pest products (zn-arsenate - in insecticides, zn-dithioamine as fungicide, rat poison, rabbit and deer repellents, zn-fluorosilicate as anti-moth agent), paints and pigments (zn-oxide, zn-carbonate, zn-sulphide), so most of solubility of zinc found in wastewater is due to its presents in plumbing by corrosion and leaching and other used as anti-pest products can have effect (DWAF, 1996).

#### **2.8.3.4 Sodium**

Sodium (Na) salts (e.g., sodium chloride) are found in almost all food (the main source of daily exposure) and drinking water. Even though concentrations of Na in potable

water are typically less than 20 mg/L, they can significantly exceed this in some countries. The levels of Na salts in air are usually low in relation to those in food or water. It should be distinguished that some water softeners can add significantly to the Na content of drinking water, no firm conclusions can be drawn regarding the possible association between Na in drinking water and the occurrence of hypertension. Therefore, no health based guideline value is proposed but however, concentrations in excess of 200 mg/L may give rise to unacceptable taste (Manoj and Padhy, 2015).

#### **2.8.3.5 Manganese**

Manganese (Mn) is one of the most abundant metals in the earth's crust, typically occurring with iron. It is used mainly in the manufacture of iron and steel alloys, as an oxidant for cleaning, bleaching and disinfection as potassium permanganate and as an ingredient in various products. More lately, it has been used in an organic compound as an octane enhancer in petrol in North America (Howe *et al.*, 2004). Mn greensands are used in some locations for potable water treatment and is a vital element for humans and other animals and occurs naturally in various food sources. The most significant oxidative states for the environment and biology are  $Mn^{2+}$ ,  $Mn^{4+}$  and  $Mn^{7+}$ . Mn is naturally occurring in several surface water and groundwater sources, mostly in anaerobic or low oxidation conditions, and this is the most important source for drinking water. The greatest exposure to manganese is generally from food (WHO, 2003).

#### **2.8.3.6 Lead**

Lead (Pb) is used primarily in the production of lead-acid batteries, solder, and alloys. The organo-lead compounds tetraethyl and tetramethyl lead have also been used broadly as antiknock and lubricating agents in petrol, even though their use for these purposes in many countries is being phased out. Owing to the declining use of lead containing additives in petrol and of lead containing solder in the food processing industry, concentrations in air and food are declining, and consumption from drinking water constitutes a greater amount of total intake (Abagale *et al.*, 2013). Pb is hardly present in tap water as a result of its dissolution from natural sources, rather, its presence is mostly from household plumbing systems consisting of lead in pipes, solder, fittings, or the service connections to homes. The quantity of lead dissolved from the plumbing system depends on several factors such as pH, temperature, water

hardness and standing time of the water, with soft, acidic water being the most plumbosolvency (WHO, 2003).

Pb can have harmful effects in humans and aquatic life. In humans it can accumulate in bones and teeth, and although it does not harm the bones and teeth, they are reservoirs for realising the lead into the bloodstream where it travels and reaches the brain. At more than 500 ppb Pb inhibits enzymes involved in algal photosynthesis. At concentrations exceeding 100 ppb lead affects gill function in fish (Frances, 2008). Pb also has an effect on the central nervous system, and haemoglobin synthesis, and has been known to cause kidney damage (Baysal *et al.*, 2013; Kiran *et al.*, 2015).

### **2.8.3.7 Iron**

Iron (Fe) is one of the most abundant metals in the earth's crust, and it is found in natural fresh waters at levels ranging from (0.5-50) mg/L. Fe may also be present in drinking water as a result of the use of iron coagulants or the corrosion of steel and cast Fe pipes during water dispersal. Fe is an essential element in human nutrition and estimates of the minimum daily requirement for iron depend on age, sex, physiological status, and iron bioavailability and range from about 10 to 50 mg per day. As a safeguard against storage in the body of excessive Fe, in 1983 JECFA established a PMTDI of 0.8 mg/kg of body weight, which applies to iron from all sources except for iron oxides used as colouring mediators and iron supplements taken during pregnancy and lactation or for specific clinical requirements. An allocation of 10% of this PMTDI to drinking water gives a value of about 2 mg/L, which does not present a threat to health. The taste and appearance of drinking water will generally be affected below this level (Yassen *et al.*, 2016).

### **2.8.3.8 Copper**

Copper (Cu) is both an essential nutrient and a drinking water contaminant and it has many commercial uses, it is used to make pipes, valves and fittings and is found in alloys and coatings. Copper sulfate pentahydrate is occasionally added to the surface water for the control of algae. Cu concentrations in drinking water differ widely, with the primary source most often being the corrosion of interior Cu plumbing. Levels in running or entirely flushed water tend to be low, while those in standing or partially flushed water samples are more variable and can be significantly higher (frequently > 1 mg/L) (IPCS, 1998). Cu concentrations in treated water often increase during

dispersal, especially in systems with an acidic pH or high carbonate waters with a basic pH. Food and water are the main sources of Cu exposure in developed countries. The use of standing or partially flushed water from a distribution system that include copper pipes or fittings can significantly increase total daily copper exposure, particularly for infants fed formula reconstituted with tap water (Kim *et al.*, 2013).

The toxicity of Cu is dependent on the local water quality, it increases when it is present in combination with other metals and when dissolved oxygen and water hardness decreases. It also decreases when alkalinity increases and in the presence of chelating agents such as amino acids and suspended solids. Cu toxicity also decreases in the presence of zinc, sulphate, calcium, or magnesium (DWAF, 1996). Cu can become toxic to the larval stages of marine invertebrates in concentrations above those required as a micronutrient, and also accumulation of copper over a long time in soil can reduce food quality and quantity (Abagale *et al.*, 2013), dissolved Cu can also cause harm to salmon in aquatic life and may also be toxic to phytoplankton (Smith and Shilley, 2009). Toxicity effects of Cu are seen at concentrations above 0.14 mg/L (Frances, 2009).

#### **2.8.3.9 Chromium**

Chromium (Cr) is widely distributed in the earth's crust. In general, food appears to be the major source of intake (Sharma *et al.*, 2008). Cr can occur in a number of oxidation states, but the two states that are of biological relevance are trivalent chromium (III) and hexavalent chromium (VI). These two states are very different in terms of their toxicological and environmental properties, and the trivalent Cr is generally less toxic than the hexavalent Cr (Baysal *et al.*, 2013). Cr exerts a toxic effect at different concentrations in different groups of aquatic organisms.

Cr in its hexavalent form can have a harmful effect on human health (Abagale *et al.*, 2013), and has been shown to have a number of adverse effects on humans such as causing irritations and cancer. Cr also has effects on fish by making them more susceptible to infections, and it affects reproduction of the water flea *Daphnia* (Baysal *et al.*, 2013). At 62 ppb Cr inhibits growth in algae and at 16ppb inhibits growth in Chinook salmon. Toxicity of Cr is also dependent on pH, temperature, and salinity. As pH and salinity decreases and temperature increases, toxicity of chromium also

increases. Additionally, chromium is more toxic in soft water than in hard waters (Frances, 2008).

## **2.8.4 Anions**

### **2.8.4.1 Ion Chromatography (IC)**

Ion chromatography involves the collection of analytical liquid chromatographic techniques used to separate and determine inorganic cations, anions, and low molecular weight water-soluble organic acids and bases. The core of this technique lies by means of the use of an ionic liquid (designated the eluent or mobile phase), which passes through a solid stationary phase (uniform small diameter inorganic or polymeric particles packed into a cylindrical column) before reaching a flow-through detector. The stationary phase particles contain fixed ions and exchangeable counterions, which are displaced by eluent ions and sample ions as they pass through the column. A discrete amount of sample containing the analytes of interest is applied to the top of the column through an injector, whilst the eluent is continuously pumped through the column. Dependent upon the ion exchange groups attached to the column particles, the ionic strength of the eluent and the affinity of the sample ions for the fixed ion exchange sites (the selectivity coefficient), the sample analytes pass through the column at different rates, thereby entering the detector cell at different times and resulting in separation (Small *et al.*, 1975).

### **2.8.4.2 Fluoride**

Fluorine (F) is a common element that is widely distributed in the earth's crust and exists in the form of fluorides in a number of minerals, such as fluorspar, cryolite and fluorapatite. Traces of fluorides are present in many waters, with higher concentrations often associated with underground sources. In areas rich in fluoride-containing minerals, well water may contain up to about 10 mg/L of fluoride, although much higher concentrations can be found. High F concentrations can be found in many parts of the world, particularly in parts of India, China, Central Africa, and South America, but high concentrations can be encountered locally in most parts of the world (Fawell, 2006).

Virtually all foodstuffs contain at least traces of fluorine. All vegetation contains some F, which is absorbed from soil and water. Tea in particular can contain high F concentrations, and levels in dry tea are on average 100 mg/kg. The F is widely used in dental preparations to combat dental caries, particularly in areas of high sugar

intake. These can be in the form of tablets, mouthwashes, toothpaste, varnishes, and gels for local application. In some countries, fluoride may also be added to table salt or drinking-water in order to provide protection against dental caries (WHO, 2019). The amounts added to drinking-water are such that final concentrations are between (0.5 and 1) mg/L. The F in final water is always present as fluoride ions, whether from natural sources or from artificial fluoridation. Total daily F exposure can vary markedly from one region to another. This will depend on the concentration of fluoride in drinking-water and the amount drunk, levels in foodstuffs and the use of fluoridated dental preparations (US NRC, 2006). In addition, F exposure in some areas is considerably higher as a consequence of a range of practices, including the consumption of brick tea and the cooking and drying of food with high-fluoride coal (Ruma *et al.*, 2014).

#### **2.8.4.3 Chloride**

Chloride (Cl<sup>-</sup>) in drinking water comes from natural sources, sewage systems, industrial effluents, urban runoff consisting of de-icing salt and saline intrusion. The main source of human exposure to Cl<sup>-</sup> is the addition of salt in the food and the intake from this source is usually seriously in excess of that from drinking water. Excessive chloride concentrations increase rates of corrosion of metals in the distribution systems, depending on the alkalinity of the water and this can lead to increased concentrations of metals in the supply. No health-based guideline value is anticipated for chloride in drinking water. However, chloride concentrations in excess of about 250 mg/L can give rise to measurable taste in water (WHO, 2003).

#### **2.8.4.4 Bromide**

Bromide (Br<sup>-</sup>) is the bromine element anion which is a member of the common halogen element sequence comprising fluorine, chlorine, bromine, and iodine. Both elements have similarities in chemistry but also significant differences. When accepting an electron, they are the oxidizing agents and all form anions. In the periodic table the elements below the fluorine also form various oxyanions. Bromine atomic weight is 79.909. Naturally occurring bromine consists of <sup>79</sup>Br 50.57% and <sup>81</sup>Br 49.43%. Bromine is a dense, mobile, dark liquid that freezes at -7°C and boils at 58°C at room temperature (Cotton *et al.*, 1988). Br<sup>-</sup> is commonly found as salts containing sodium, potassium, and other cations, which are usually very water soluble. It also forms the oxyacids of the strong acid, hydrobromic acid (HBr) and the weaker hypo-bromic

(HOBr), bromous (HBrO<sub>2</sub>) and bromic (HBrO<sub>3</sub>). The basics of OBr are stable at 0°C, but quickly disproportionate to Br<sup>-</sup> and BrO<sub>3</sub><sup>-</sup> at temperatures above and below 50°C (Cotton *et al.*, 1988).

#### **2.8.4.5 Sulfate**

Sulfate (SO<sub>4</sub><sup>2-</sup>) occurs naturally in numerous minerals and are used commercially mostly in the chemical industry. They are discharged into water in industrial wastes and over atmospheric deposition; however, the highest levels generally occur in ground water and are from natural sources. In general, the average daily intake of sulfate from drinking water, air and food is approximately 500 mg, with food being the major source. However, in areas with drinking water supplies consisting of high levels of SO<sub>4</sub><sup>2-</sup>, drinking water may constitute the major source of intake. The existing data do not identify a level of SO<sub>4</sub><sup>2-</sup> in drinking water that is possible to cause adverse human health effects. The data from a liquid diet piglet study and from tap water studies with human volunteers specify a laxative effect at concentrations of (1000–1200) mg/L but no increase in diarrhoea, dehydration, or weight loss. No health-based guideline is proposed for sulfate. However, because of the gastrointestinal effects resulting from consumption of drinking water containing high SO<sub>4</sub><sup>2-</sup> levels, it is suggested that health authorities be notified of sources of drinking water that comprise of SO<sub>4</sub><sup>2-</sup> concentrations in excess of 500 mg/L. The presence of SO<sub>4</sub><sup>2-</sup> in drinking water may also cause noticeable taste and may contribute to the corrosion of distribution systems (Williams *et al.*, 2015).

#### **2.8.5 Microbial analysis**

The microbial properties of wastewater are determined by the type and number of microorganisms present in the water. A variety of microorganisms can be present even in very good quality domestic waters. Most of these microorganisms are harmless but if the water is polluted pathogens may be present, pathogens are disease causing microorganisms such as those causing cholera, gastro-enteritis, and hepatitis. It is difficult to determine the presence of all the different pathogenic organisms and therefore the presence of certain indicator organisms is used to give an indication of the possible presence of pathogens. Indicator organisms are specific types of microorganisms which are present in very large numbers in the intestines of people and warm blooded animals. They are easy to detect and the presence of these

organisms in water serves as an indication of pollution of water by human wastes. The most commonly used indicator organisms are total coliform and faecal coliforms.

#### **2.8.5.1 Total coliform bacteria**

Total coliform bacteria consist of a wide range of aerobic and facultatively anaerobic, Gram-negative, non-spore-forming bacilli capable of growing in the presence of *Escherichia coli* (*E. coli*) pathogenic strains comparatively high concentrations of bile salts with the fermentation of lactose and production of acid or aldehyde within 24 hours at a temperature of (35–37) °C. *E. coli* and thermotolerant coliforms are a subset of the total coliform group that can ferment lactose at higher temperatures. As part of lactose fermentation, total coliforms produce the enzyme  $\beta$ -galactosidase. Traditionally, coliform bacteria were regarded as belonging to the genera *Escherichia*, *Citrobacter*, *Klebsiella* and *Enterobacter*, but the group is more heterogeneous and includes a wider range of genera, such as *Serratia* and *Hafnia*. The total coliform group includes both faecal and environmental species (Sueiro *et al.*, 2001).

They include organisms that can survive and grow in water. Hence, they are not useful as an indicator of faecal pathogens, but they can be used to assess the cleanliness and integrity of distribution systems and the potential presence of biofilms. It has been proposed that total coliforms could be used as a decontamination indicator. However, the test for total coliforms is far slower and less reliable than direct measurement of contaminants residual. In addition, total coliforms are far more sensitive to decontamination than are enteric viruses and protozoa. HPC measurements detect a wider range of microorganisms and are generally considered a better indicator of distribution system integrity and cleanliness (Snozzi, 2001).

Total coliform bacteria (excluding *E. coli*) occur in both sewage and natural water, some of these bacteria are excreted in the faeces of humans and animals, but many coliforms are heterotrophic and are able to multiply in water and soil environments. They can also survive and grow in water distribution systems, particularly in the presence of biofilms (Grabow, 1996).

#### **2.8.5.2 *Escherichia coli***

*E. coli* is present in massive numbers in the normal intestinal flora of human beings and animals, where it generally causes no damage. Nevertheless, in other parts of the body *E. coli* can cause serious diseases such as urinary tract infections (UTI),



bacteraemia and meningitis. An inadequate number of enteropathogenic strains can cause acute diarrhoea, several classes of enteropathogenic *E. coli* have been identified on the basis of different virulence aspects, including enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and prolixly enthusiast *E. coli* (DAEC). More is known about the first four classes named; the pathogenicity and prevalence of EAEC and DAEC strains are less well reputable (Nataro and Kaper, 1998).

Enteropathogenic *E. coli* are enteric organisms and humans are the major reservoir, particularly of strains such as EPEC, ETEC and EIEC. Livestock such as cattle and sheep and, to a minor extent goats, pigs and chickens are a major source of EHEC strains. The latter have also been linked with raw vegetables such as bean sprouts. The pathogens have been detected in a variety of water environments. Infection is related with person to person transmission, contact with animals, food, and consumption of contaminated water (O'Connor, 2002). Person to person transmissions is mainly dominant in communities where there is close contact between individuals, such as nursing homes and day care centres.

The US Environmental Protection Agency (EPA) recommended that *E. coli* is a better indicator of faecal pollution than faecal coliform for purposes of evaluating ambient freshwater quality (Dulfour and Ballentine, 1986). The presence of *E. coli* in water indicates that the water was contaminated by faecal material of humans or other warm-blooded animals, and also indicates the potential for the presence of pathogenic organisms. The source of *E. coli* contamination in surface water includes municipal waste-water discharges, septic leachate, agricultural or storm runoff, wildlife populations, or nonpoint sources of human and animal waste (Dulfour, 1977) .

#### **2.8.5.3 Pseudomonas aeruginosa**

*Pseudomonas aeruginosa* (*P. aeruginosa*) is a member of the family *Pseudomonadaceae* and it is a polarly flagellated, aerobic, Gram-negative rod bacteria. When the bacteria are grown in suitable media, it produces the non-fluorescent bluish pigment of pyocyanin. Many strains also produce the fluorescent green pigment of pyoverdin. *P. aeruginosa*, like other fluorescent *pseudomonads*,

produces catalase, oxidase and ammonia from arginine and can also grow on citrate as the sole source of carbon (Bartram *et al.*, 2003).

*P. aeruginosa* can cause a variety of infections but hardly causes serious illness in healthy individuals lacking some predisposing factor. It mainly colonizes damaged sites such as burns, surgical wounds, the respiratory tract of people with underlying disease and physically damaged eyes. From these sites, it may attack the body, causing destructive lesions or septicaemia and meningitis. Cystic fibrosis and immunocompromised patients are disposed to colonization with *P. aeruginosa*, which may lead to serious progressive pulmonary infections. Water related folliculitis and ear infections are related to warm, moist environments such as swimming pools and spas. Many strains are resistant to a series of antimicrobial agents, which can increase the significance of the organism in hospital settings (De Victorica *et al.*, 2001).

*P. aeruginosa* is a communal environmental organism and can be found in faeces, soil, water, and sewage systems. It can multiply in water environments and also on the surface of appropriate organic materials in contact with water. *P. aeruginosa* is a recognized cause of hospital acquired infections with potentially serious complications. The bacteria can be isolated from a range of moist environments such as sinks, water baths, hot water systems, showers, and spa pools. The main way of infection is by exposure of susceptible tissue, notably wounds and mucous membranes to contaminated water or contamination of surgical instruments. Cleaning of contact lenses with contaminated water can cause a form of keratitis and ingestion of drinking water is not an important source of infection (Hardalo and Edberg, 1997).

#### **2.8.5.4 Salmonella**

*Salmonella spp.* belong to the family Enterobacteriaceae, they are motile Gram negative bacilli that do not ferment lactose, but most produce hydrogen sulfide or gas from carbohydrates fermentation. Initially, they were grouped into more than 2000 species (serotypes) according to their somatic (O) and flagellar (H) antigens (Kauffmann-White classification). There has been much argument about nomenclature and taxonomy of *Salmonella*, but it is now considered that there are in fact two species (*Salmonella enterica* and *Salmonella bongori*). Other previously named species, including *S. typhi* and *S. paratyphi*, are considered to be serovars (Tindall, 2005).

*Salmonella* infections typically cause four clinical manifestations: gastroenteritis (ranging from mild to fulminant diarrhoea, nausea, and vomiting), bacteraemia or septicaemia (high spiking fever with positive blood cultures), typhoid fever/enteric fever (sustained fever with or without diarrhoea) and a carrier state in persons with previous infections. With regard to enteric illness, *Salmonella spp.* can be divided into two fairly distinct groups: the typhoidal species/serovars (*S. typhi* and *S. paratyphi*) and the remaining non-typhoidal species/serovars. Symptoms of non-typhoidal gastroenteritis appear from (6-72) hours after ingestion of contaminated food or water. Diarrhoea lasts (3–5) days and is accompanied by fever and abdominal pain. Usually, the disease is self-limiting. The incubation period for typhoid fever can be (1–14) days but is usually (3–5) days. Typhoid fever is a more severe illness and can be fatal. Although typhoid is uncommon in areas with good sanitary systems, it is still prevalent elsewhere, and there are many millions of cases each year (Angulo *et al.*, 1997).

*Salmonella spp.* are extensively distributed in the environment, but some species or serovars show host specificity. Markedly, *S. typhi* and usually *S. paratyphi* are restricted to humans, although livestock can occasionally be a source of *S. paratyphi*. A huge number of serovars including *S. typhimurium* and *S. enteritidis* infect humans and also a wide range of animals including poultry, cows, pigs, sheep, birds and even reptiles. The pathogens typically enter water systems through faecal contamination from sewage system discharges, livestock, and wild animals. Contaminants have been detected in a wide variety of food and milk (Escartin *et al.*, 2002).

*Salmonella* is spread by the faecal oral route and infections with non-typhoidal serovars are primarily associated with person to person contact, the consumption of a variety of contaminated foods and exposure to animals. Infection by typhoid species is related with the consumption of contaminated water or food and with direct person to person spread being uncommon (Koplan *et al.*, 1978).

The biochemical identification of foodborne and clinical *Salmonella* isolates is generally coupled to serological confirmation, a complex and labour-intensive technique involving the agglutination of bacterial surface antigens with *Salmonella*-specific antibodies. National epidemiologic registries continue to underscore the importance of *Salmonella spp.* as a leading cause of foodborne bacterial illnesses in humans, where reported incidents of foodborne salmonellosis tend to dwarf those

associated with other foodborne bacterial pathogens. The antibiotic resistance genes currently present in bacterial pathogens may originate from antibiotic-producing bacteria present in soil (D'Aoust and Maurer, 2007).

#### **2.8.5.5 *Shigella***

*Shigella spp.* are Gram-negative, non-spore forming, non-motile, rod-like members of the family Enterobacteriaceae, which grows in the presence or absence of oxygen. Members of the genus have a complex antigenic form and classification is based on their somatic (O) antigens, many of which are shared with other enteric bacilli, including *E. coli*. There are four species: *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*.

*Shigella spp.* can cause serious intestinal diseases including bacillary dysentery. Over 2 million infections occur per annum, resulting in about 600 000 deaths, mostly in developing countries. Most cases of *Shigella* infections occur in children under the age of 10 years. The incubation period for shigellosis is usually (24-72) hours. Ingestion of as few as 10 to 100 organisms may lead to infection, which is significantly less than the infective dose of most other enteric bacteria, abdominal cramps, fever, and watery diarrhoea occurs early in the disease. All species can produce severe disease, but illness due to *S. sonnei* is typically relatively mild and self-limiting. In the case of *S. dysenteriae*, clinical manifestations may proceed to an ulceration process with bloody diarrhoea and high concentrations of neutrophils in the stool. The production of Shiga toxin by the pathogen plays an important role in this outcome and *Shigella spp.* appear to be better adapted to cause human disease than most other enteric bacterial pathogens (Alamanos *et al.*, 2000).

Humans and other higher primates seem to be the only natural hosts for the *shigella*, the bacteria remain localized in the intestinal epithelial cells of their hosts. Epidemics of shigellosis occur in crowded communities and where hygiene processes are poor. Many cases of shigellosis are associated with day care centres, prisons, and psychiatric institutions. Military field groups and travellers to areas with poor sanitation are also disposed to infection. *Shigella spp.* are enteric pathogens predominantly transmitted by the faecal oral route through person to person contact, contaminated food, and water. Flies have also been recognized as a transmission vector from contaminated faecal waste (Pegram *et al.*, 1998).

### **2.8.5.6 *Staphylococcus aureus***

*Staphylococcus aureus* is an aerobic or anaerobic, non-motile, non-spore-forming, catalase, and coagulase positive, Gram-positive coccus, usually arranged in a grape-like irregular cluster. The genus *Staphylococcus* contains at least 15 different species, apart from *S. aureus*, the species *S. epidermidis* and *S. saprophyticus* are also associated with disease in humans. Although *S. aureus* is a common member of the human microflora, it can produce disease by two different mechanisms, of which one is based on the ability of the organisms to multiply and spread widely in tissues and the other is based on the ability of the organisms to produce extracellular enzymes and toxins.

Infections based on the multiplication of the organisms are a substantial problem in hospitals and other health-care facilities. Multiplication in tissues can result in manifestations such as boils, skin sepsis, post-operative wound infections, enteric infections, and pneumonia. The beginning of clinical symptoms for these infections is relatively long, usually several days. Gastrointestinal disease (enterocolitis or food poisoning) is caused by a heat stable staphylococcal enterotoxin and characterized by projectile vomiting, diarrhoea, fever, abdominal cramps, electrolyte imbalance and loss of fluids. Beginning of disease in this case has a characteristic short incubation period of (1-8) hours, and the same applies to the toxic shock syndrome caused by toxic shock syndrome toxin-1 (Antai, 1987).

*S. aureus* is relatively widespread in the environment but is found mainly on the skin and mucous membranes of animals. The organism is a member of the normal microbial flora of the human skin and is found in the nasopharynx of (20-30) % of adults at any one time. *Staphylococci* are occasionally detected in the gastrointestinal tract and can be detected in sewage systems. *S. aureus* can be released by human contact into water environments such as swimming pools, spa pools and other recreational waters and it has also been detected in drinking-water supplies.

Hand contact is by far the most communal route of transmission. Inadequate hygiene can lead to contamination of food and foods such as ham, poultry, potatoes, and egg salads kept at room or higher temperatures offer an ideal environment for the multiplication of *S. aureus* and the release of toxins. The ingestion of foods containing

*S. aureus* toxins can lead to enterotoxin food poisoning within a few hours (LeChevallier, 1980).

#### **2.8.5.7 Enteric viruses**

The viruses mentioned here are a combined group of those that infect the human gastrointestinal tract and are mostly transmitted by the faecal oral route. Well known members of this group include the enteroviruses, astroviruses, enteric adenoviruses, orthoreoviruses, rotaviruses, caliciviruses, hepatitis A and E viruses. The enteric viruses cover an extensive spectrum of viruses, members of which are a main cause of morbidity and mortality worldwide. Members of the group of enteric viruses vary with respect to structure, composition, nucleic acid, and morphology. There are also variances in the numbers and frequency of excretion, survival in the environment and resistance to water treatment processes. Enteric viruses have strong capsids, that allow them to survive through unfavourable conditions in the environment as well as allowing passage through the acidic and proteolytic conditions in the stomach on their way to the duodenum, where they infect susceptible epithelial cells (Ashbolt *et al.*, 2001).

Enteric viruses are excreted by people worldwide at a frequency and in numbers that result in large numbers of these viruses being universally present in substantial numbers in wastewater. However, the prevalence of individual members may differ to a huge extent as a result of variations in rates of infection and excretion and much higher numbers would be present during outbreaks (Grabow *et al.*, 2001).

#### **2.8.5.8 *Bacillus cereus***

*Bacillus cereus* is a Gram-positive aerobic or facultative anaerobic spore-forming rod. It is a cause of food poisoning, which is frequently associated with the consumption of rice-based dishes. The organism produces an emetic or diarrheal syndrome induced by an emetic toxin and enterotoxin, respectively. Other toxins are produced during growth, including phospholipases, proteases, and hemolysins, one of which, cereolysin, is a thiol-activated hemolysin. These toxins may contribute to the pathogenicity of *B. cereus* in non-gastrointestinal disease. *B. cereus* isolated from clinical material other than faeces or vomitus was commonly dismissed as a contaminant, but increasingly it is being recognized as a species with pathogenic potential (Drobniewski, 1993). It is now recognized as an infrequent cause of serious

non-gastrointestinal infection, particularly in drug addicts, the immunosuppressed, neonates, and postsurgical patients, especially when prosthetic implants such as ventricular shunts are inserted. Ocular infections are the commonest types of severe infection, including endophthalmitis, panophthalmitis, and keratitis, usually with the characteristic formation of corneal ring abscesses.

## **2.8.6 Extraction method**

### **2.8.6.1 Liquid-Liquid Extraction**

Liquid-liquid extraction (LLE) is a separation technique for a wide series of applications in chemical process industries. The most commonly used method of LLE is performed using a separatory funnel (Koch and Shivelier, 2015) and it is frequently used for extraction of water samples (Nikolaou *et al.*, 2009). This extraction separates components according to their relative solubilities in two immiscible liquids. When the liquids are immiscible, this commonly means that they will form two layers when they are together, similar to oil and water (Yates *et al.*, 2013). Some of the compounds are more soluble in the organic layer, which is often FOG while some compounds are more soluble in the aqueous layer (water) (Nikolaou *et al.*, 2009; Xia *et al.*, 2013).

In the LLE unit, a liquid stream consisting of the components that must be recovered (solute) is added into an extractor where it is in contact with a solvent. The two liquids should be immiscible or slightly miscible (Koch and Shivelier, 2015). These conditions will then allow the solutions to form an emulsion, with one liquid dispersed as droplets in the other. Mass transfer will occur between the dispersed phase and the surrounding liquid which is the continuous phase (Nguyen *et al.*, 2004). In order for the two liquids to be eventually separated, they must have different densities. The droplets then accumulate below or above the continuous phase, and this will depend on the liquid's relative densities (Leon *et al.*, 2014; Chen *et al.*, 2016).

The types of solvents used in LLE are chosen to obtain the maximum transfer of the solute from the carrier to the solvent (Tavakoli *et al.*, 2008; Chen *et al.*, 2016). They must not be completely miscible with the carrier liquid and must have a high affinity for the solute molecules. Generally, an ideal solvent for LLE will normally have the following properties: high boiling point, low viscosity, high resistance to thermal degradation, density difference and high solubility for the solute and low solubility for the carrier liquid (Andersson *et al.*, 2002).

FOGs are insoluble in water but soluble in organic solvents such as chlorinated fluorocarbons and alcohols, such that recent measurement methods incorporate a solvent extraction step (US, 1999). In the United Kingdom, the standard procedure involves LLE with 1,1,2-trichloro-1,2,2-trifluoroethane ( $C_2Cl_3F_3$ ), which due to environmental concerns has been substituted with more proper options such as carbon tetrachloride ( $CCl_4$ ) and n-hexane ( $C_6H_{14}$ ). As each solvent extract different materials, it is common practice to refer to the solvent used when expressing a concentration, for example hexane extractable material (HEM). Quantification of the FOG content in FSEs wastewater is usually based on gravimetric measurement of residuals post-solvent evaporation, with reported alternatives of utilising infrared spectroscopy or gas chromatography. The current solvents intensify an issue with incomplete phase separation, demanding remedial actions if an emulsion persists such as increased tension (Ducoste *et al.*, 2009) or solid phase extraction (SPE) (Barton, 2012). The latter includes use of a hydrophobic matrix that holds all non-aqueous components as the sample passes through the material. The reserved oils are back eluted with an appropriate solvent (i.e., n-hexane) and the post-evaporated residual weighed (Martha, 1995). A benefit of such approaches is the ability to pre-concentrate, enabling more accurate measurement of low concentrations of FOG.

### **2.8.7 Fourier Transform Infrared spectrophotometer**

IR spectroscopy is one of the vibrational spectroscopies intensively used to classify all aspects of edible oils (Yang and Irudayaraj, 2000). IR spectroscopy can be described as the interaction between electromagnetic radiation in the IR region by scattering, reflecting, absorbing, or transmitting it to analysed substances (e.g., edible oils) as a function of wavenumbers (Sun, 2009). Frequencies or wavelengths are recorded in an IR spectrum at which samples absorb IR radiation and its corresponding intensities (either transmittance or absorbance) (Yueh *et al.*, 2000).

IR spectroscopy can be considered as an ideal technique for the analysis of edible oils as this technique is fast, sample-friendly, non-destructive, and non-invasive, which means that the samples analyzed with IR spectroscopy can be analyzed using different instruments (Bunaciu *et al.*, 2015; Prazeres *et al.*, 2015). Today, because of its ability to classify the structure of various molecular species without destroying them, a number of researchers use IR spectroscopy. For a given sample, IR spectroscopy produces a "chemical fingerprint" which can be used to classify, define, evaluate, and



monitor the FOG parameters qualitatively and quantitatively (Guillén and Cabo, 1997; Rohman and Man, 2012).

Spectra IR can be obtained using a dispersive and Fourier transformed IR (FTIR) spectrophotometer. The dispersive instrument has been scarcely used in edible oils due to the difficulties in sample handling technique and is not equipped with proper spectral acquisition and processing systems in order to give the valuable information for quantitative analysis (Guillen and Cabo, 1997) as a consequence, today, the FTIR spectrophotometer has replaced dispersive IR spectrometer (van de Voort *et al.*, 2008). In addition, the FTIR spectrophotometer can be equipped with the advanced chemometrics software which is able to handle the calibration development (Bendini *et al.*, 2007).

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## CHAPTER 3: EXPERIMENTAL

### 3.1 Materials and method

#### 3.1.1 Chemicals, solvents, and instruments

#### 3.1.2 Sampling

Sampling of water is an essential part of the study and can be done manually or automatically. There are governmental standard methods and guidelines (EPA, 2016) for manual sampling that require care in collecting without contamination and maintaining validity of the sample. The representativeness of control may be influenced by various issues. Variations in sampling time, depth and sampling techniques can affect the quality of the sample (Nevers *et al.*, 2013).

By definition sampling refers to the collection of a sample whose composition is a true representative of the bulk material. Six samples were collected from two different sites at University of Limpopo Mankweng business centre, three samples for each site. The samples were obtained by scooping water from basin and pots with sterilized sampling bottles, the samples were carried back to the laboratory and offline analysis was carried out (Figure 3.1).

#### 3.1.3. Analysis scheme using analytical techniques

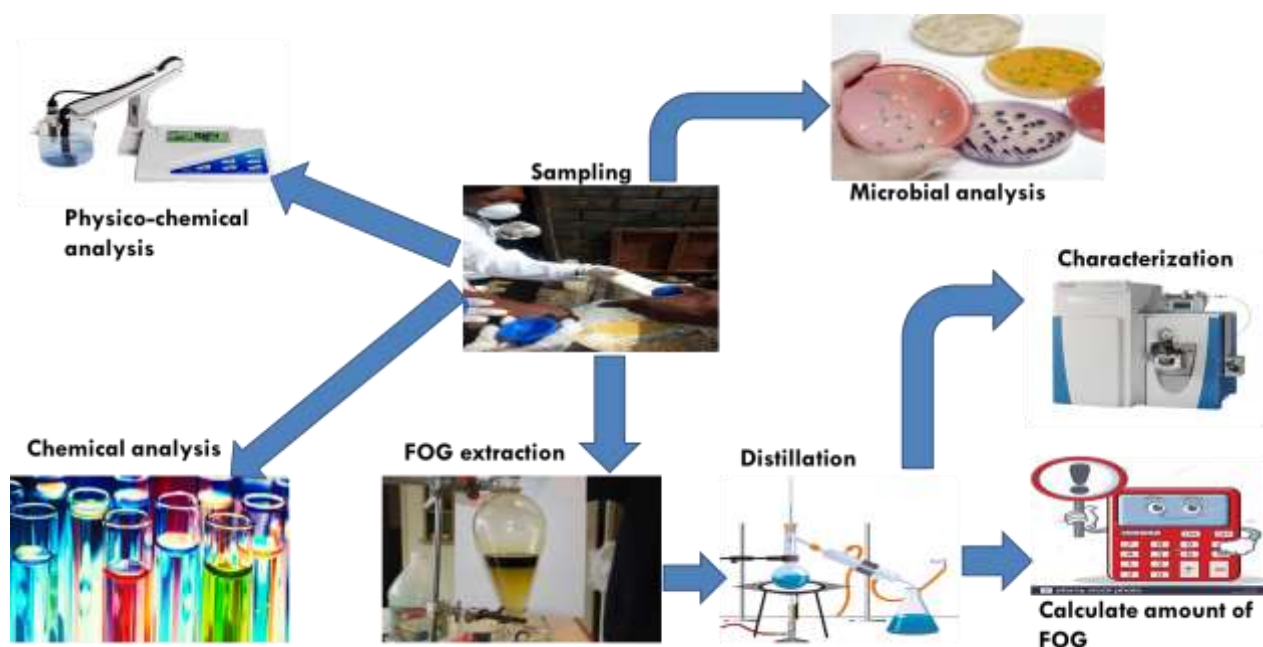


Figure 3.1: Schematic diagram of the analytical procedure that are followed from sampling to analysis.

### 3.1.3.1 Physicochemical analysis

Physicochemical analysis is a method of investigating physicochemical systems that makes possible a determination of the nature of the interactions between the components of a system through a study of the relations between the system's physical properties and composition. The principles of the physicochemical analysis were established in the late 19<sup>th</sup> century (Simpì *et al.*, 2011). It involves the measurement of various physical properties of systems, most often phase transition temperatures and other thermal properties like (thermal conductivity, heat capacity and thermal expansion) and for electrical properties (conductivity, dielectric permittivity). Also measured are density, viscosity, and hardness.

Physicochemical analysis is based on the phase rule and on the principles of continuity and correspondence which were introduced (Kauffman, 1982). The continuity principle holds that during continuous changes in the parameters of the state of the properties of a system also undergo continuous change ( provided that the numbers of phases in the system remains constant) when the numbers of the phases change, certain properties change abruptly (continuity being broken). The (Figure 3.2) shows pH Model 1120 (Metler Toledo.) that is intended to promptly withstand the afflictions of convenient analysis, while dependably estimating pH and conductivity in a basic and expert way. This item permits the synchronous readout of pH, conductivity and temperature utilizing a custom backlit liquid crystal display. Calibration errors are clearly indicated, together with the parameter in error.

For turbidity measurements which is a qualitative parameter for water, however its discernibility to an essential standard permits the estimation to be applied as a quantitative estimation. At the point when utilized as a quantitative estimation, turbidity is commonly revealed conventionally in Nephelometric Turbidity Units (NTU's). The essential norm for this boundary is a polymer compound known as formazin and this standard gives the recognizable way to any remaining turbidity principles and is utilized to align a wide range of turbidimeters (Sadar, 2002). In (Figure 3.3) it shows a Turbidimeter instrument that was used for analysis.

Inductively coupled plasma optical emission spectroscopy (ICP-OES) has become the main innovation for routine analysis of liquid samples just as materials that can be handily transformed into a liquid form by disintegration or absorption. Its source lies in

purported spectrosopes and spectrographs that permit visual assessment of otherworldly lines on a film, which required a lot of involvement and time to get solid outcomes (Falkner *et al.*, 1995). The (Figure 3.5) shows an ICP-OES instrument used in the elemental analysis.

#### **3.1.3.1.1 pH**

In this study, an electronic pH meter 1120 (Metler Toledo.) was used for measuring pH of the sample (wastewater). The instrument was calibrated with Metler Toledo buffer solutions pH 2.0, 4.01, 7.0 and 9.21. The prepared test solution was put inside the beaker for measurement. The electrode probe was inserted in the test solution to give the pH value (Alwan, 2008). The measurement was repeated 3 times, and an average value calculated.

#### **3.1.3.1.2 Turbidity**

Turbidity was measured by using the TU5200 turbidimeter. A sample of wastewater was placed on the turbidimeter and the results were expressed as nephelometric turbidity units (NTU), the measurement was repeated three times and the average value was calculated.

#### **3.1.3.1.3 Total dissolved solids**

Total dissolved solids (TDS) in the wastewater sample were quantified by gravimetric method. A clean Petri dish was subjected to a temperature of 100 °C in an oven, cooled in a desiccator and then weighed to constant weight. The collected wastewater sample was filtered into a clean conical flask using a pre-weighed filter paper. A known volume of the filtrate was poured into the petri-dish and heated in an oven at temperature 180 °C. The obtained residue was then cooled in the desiccator and weighed to a constant weight. The TDS is calculated with the formula below:

$$\text{Total Dissolved Solids (mg/L)} = \frac{(A-B) \times 1000}{\text{Volume of sample (mL)}}$$

where A = weight of dried residue + evaporating dish (mg); B = weight of the evaporating dish (mg).

#### **3.1.3.1.4 Conductivity**

The electronic pH meter consists of two electrodes in which the other electrode is responsible for measuring conductivity, the wastewater sample was placed inside a beaker for measurement and the electrode probe was inserted in the sample. The

measurement was repeated three times and the average value will be calculated, and the results will be expressed as  $\mu\text{S}$ .

### 3.1.3.1.5 Temperature

The temperature was determined with a centigrade thermometer capable of reading from (0 to 110)  $^{\circ}\text{C}$ . The sample was poured into a beaker and the thermometer was dipped in the wastewater sample, the measurements were repeated three times and the average value was calculated, the results were expressed as  $^{\circ}\text{C}$ .

### 3.1.3.1.6 Salinity



Figure 3.2: A pH and conductivity meter 1120 (Metler Toledo) used to measure pH, conductivity, salinity, and temperature.

An electrode capable for measuring conductivity was also used for salinity content in the wastewater sample, the measurements were done by changing the mode reading from the instrument, the results were expressed as ppm.





Figure 3.3: A TU5200 turbidimeter used in the analysis of turbidity measured in NTU as the units.

#### **3.1.3.1.7 Chemical oxygen demand**

The wastewater samples were mixed thoroughly by shaking before use. The COD vial of a concentration of (20 to 1500) mg/L were used since the samples had a bad colour. The COD vials were labelled according to the sample code and the blank was included. For blank 2 mL of deionized water was pipet into the COD vial and the lid was replaced and inverted the vial to mix the content, the same procedure was done for the samples. After adding of the samples, the filled COD vials were added into the reactor which is ideally in a fume cabinet. The reactor was set to a temperature of 150 °C and a time of 2 hours, after 2 hours the vials were cooled. The photometer (DR6000) was used to read the COD level in the samples at a wavelength of 620 nm, a blank was first placed to zero the instrument and then followed by the sample to give a reading of COD in mg/L.



Figure 3.4: COD vials from the concentration of (0-1500) mg/L in ascending order.

#### 3.1.3.1.8 Biochemical oxygen demand

The BOD level is determined by comparing the DO level of a water sample taken immediately with the DO level of a water sample that was incubated at 20 °C temperature for 5 days (Baker *et al.*, 1999). The principal difference between the two Levels Represents the amount of oxygen required for the decomposition of organic materials.

$$\text{Hence BOD}_5 (20\text{ }^\circ\text{C}) = [\text{DO}]_{\text{initial}} - [\text{DO}]_{\text{final}}$$

The sample was poured in bottles with specific volume then measuring immediately the quantity of dissolved oxygen by using an Oximeter. After that, incubate them at 20 °C for five days in a darkened environment.

#### 3.1.3.1.9 Calcium hardness

##### Method

A 50 mL sample was measured into 125 mL Erlenmeyer flask and 2 mL of the 1N hydroxide solution was added, or the volume that was sufficient to produce a pH of 12-13 in the 50 mL sample. About (0.1-0.2) g of Calver II calcium indicator was added, and the sample was titrated slowly with the EDTA disodium salt solution (0.01 M) until the colour changes from red to blue. For a 1 mL of titrant (0.0100 M EDTA) was

equivalent to exactly 400.8  $\mu\text{g Ca}^{++}$ . Sample size, dilution and ml of the titrant used to reach the end point were recorded.

### Calculation

Calcium as  $\text{Ca}^{++}$ :

$$\text{mg Ca}^{++}/\text{L} = \frac{(A - B) \times D \times 400.8}{\text{mL sample}}$$

Calcium hardness as  $\text{CaCO}_3$ :

$$\text{Mg CaCO}_3/\text{L} = \frac{(A-B) \times D \times 1000}{\text{mL sample}}$$

Where

A= mL of titrant used for the sample

B= mL of titrant used for the blank

C= mg mL of titrant used for standard

D= mg  $\text{CaCO}_3$  equivalent to 1.00 mL EDTA titrant

### 3.1.3.2 Chemical analysis

#### 3.1.3.2.1 Method for elemental analysis using ICP-OES

The samples were first filtered through the 0.45  $\mu\text{m}$  filters and a drop of nitric acid was added in the samples in order to keep the metals in solution. After filtration the standard was prepared in order to calibrate the instrument and then when the instrument was ready for analysis, the solution to be analysed was conducted by a peristaltic pump through a nebulizer into a spray chamber. The produced aerosol was led into an argon plasma. Plasma is the fourth state of matter next to the solid, liquid, and gaseous state. In the ICP-OES the plasma is generated at the end of a quartz torch by a cooled induction coil through which a high frequency alternating current flows. As a consequence, an alternate magnetic field was induced which accelerated electrons into a circular trajectory. Due to collision between the argon atom and the electrons ionization occurring, giving rise to a stable plasma. The plasma was extremely hot, 6000-7000 K. In the induction zone it can even reach 10000 K. In the torch de-solvation, atomization and ionizations of the sample took place. Due to the thermic energy taken up by the electrons, they reach a higher "excited" state. When the electrons drop back to ground level energy is liberated as light (photons). Each element has its own characteristic emission spectrum that is measured with a

spectrometer. The light intensity on the wavelength was measured and with the calibration calculated into a concentration.



Figure 3.5: A Sequential Type ICP-OES SPS3000 instrument used for elemental analysis.

### 3.1.3.2.2 Inorganic (Anions analysis)

The sample preparation procedure is essential before the ion chromatography (IC) analysis so as to protect the analytical columns and obtain consistent and repeatable results (Smith, 2003). A sample that is not appropriately prepared can cause increased column back pressures and change their performances. The most important reasons why sample preparation in IC is necessary include the following aspects: analytes concentrations are too high or too low, analytes concentrations differ primarily and the presence of compounds that may cause interference by peak overlapping (Saubert *et al.*, 2004). Usually, the choice of the sample preparation method depends on the physical state of the sample and the sample matrices, as well as availability of the apparatus configurations (Slingsby and Kiser, 2001). The typical sample preparation methods used for IC analysis include the following: filtration, dilution, pH adjustment, liquid–liquid extraction, solid-phase extraction, distillation, and membrane separations (Michalski, 2016). The wastewater samples for the IC analysis should be collected in plastic containers made of polytetrafluoroethylene (PTFE), polypropylene (PP), polystyrene (PS), or high-density polyethylene (HDPE), because glass containers can cause ionic contamination when performing trace analysis. Most wastewater samples

often require only dilution and filtration to bring analytes of interest into the working range of the method. For filtration, membrane filters with a pore size of 0.45  $\mu\text{m}$  are strongly recommended, while for biologically active samples, sterile filters with a pore size of 0.2  $\mu\text{m}$  should be used.

The filtration method was considered for this samples, whereby wastewater samples were first filtered using 0.45  $\mu\text{m}$  filters and after filtration the samples were analyzed using Ion Chromatography (IC) to detect anion such as fluoride, chloride, bromide, sulphate, and ammonia. Before analysis, eluent composition of 5.0 Mm  $\text{Na}_2\text{CO}_3$  and 10.0 mM  $\text{NaHCO}_3$  were prepared in 2 L ultrapure water, the preparation was done by filling the 2 L volumetric flask with approximately 1.5 L of ultrapure water. An amount of 1.06 g of sodium carbonate and 1.68 g of sodium hydrogen carbonate were weighed and transferred to the volumetric flask containing the ultrapure water and it was filled to the mark by ultrapure water. The eluent was filtered and degassed using a vacuum pump. Approximately 20 mL aliquot of the fresh eluent was added to the IC eluent bottle, and the bottle was swirled with aliquot of the fresh eluent and discarded the contents, and the eluent bottle was filled with freshly prepared eluent.

Before the IC was started to equilibrate, the eluent lines were purged to remove the old eluent and any potential air bubbles. MSM 0.1 M phosphoric acid solution was prepared by adding approximately 500ml of ultrapure water to 1 L volumetric flask, followed by adding 6.84 mL of 85%  $\text{H}_3\text{PO}_4$  to the 500ml ultrapure water in the volumetric flask. The volumetric flask was filled to the mark with ultrapure water and the MSM regenerant bottle was filled with freshly prepared regenerant solution. The purging of the instrument was done using the prepared solution and after purging the system was started by ensuring that the pressure is between 12-15, conductivity is <40 and flow rate is 1.0.



Figure 3.6: A Metrohm 850 Ion Chromatography System used for anions analysis.

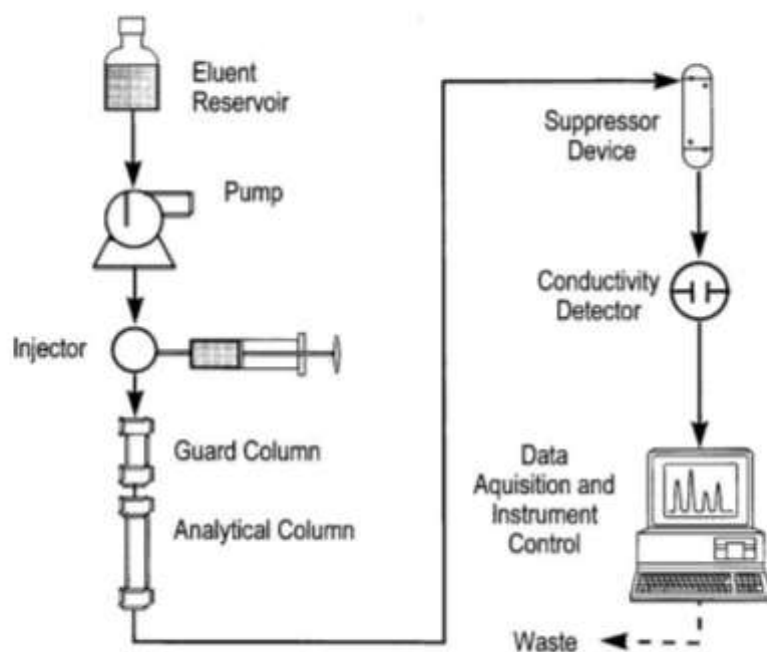


Figure 3.7: Schematic diagram of (IC)

### 3.1.3.2.3 Ammonia analysis

Firstly, the instrument was switched on and the program of 380 N Ammonia Ness was selected, after the samples and blank were prepared using the following procedure: 25 mL mixing graduated cylinder was filled to the mark with the sample, and another 25 mL cylinder was filled to the mark deionized water. About three drops of mineral stabilizer was added to each cylinder, a stopper was inserted and inverted several times to mix, followed by adding three drops of polyvinyl alcohol dispersing agent to each cylinder and a stopper was inserted and inverted several times to mix. A Nessler reagent of 1mL was pipetted into each cylinder and after it was inverted several times to mix, and 10ml of each sample was used for the analysis along with 10 mL of blank.



Figure 3.8: A DR 6000 instrument used for ammonia analysis along with the prepared solutions on the right.

### 3.1.3.2.4 Extraction of FOG from wastewater.

The fats, oil and grease in the collected sample was extracted using n-hexane. A 120 mL of the sample was transferred to a separatory funnel, received 30 mL of n-hexane, mixed vigorously, and allowed to settle to let the organic and aqueous phases separate. The lower layer (aqueous layer) was drained into the original sample container and the hexane layer remains in the separatory funnel. The hexane extract was drained through a funnel containing anhydrous sodium sulphate to remove any excess water from the extract and was collected in a pre-weighed container (flask or pan) to evaporate the hexane. The original sample went through the process two

more times to remove all fats, oil and grease compounds for the analysis. Once the three extract portions were collected, the solvent was evaporated, and the pre-weighed container was weighed to constant weight. The difference in mass is the amount of oil and grease in the collected sample and then FOG was characterized using analytical techniques such as Infra-Red (IR) spectroscopy to determine the specific bond lengths that are contained in the compound. The (Figure 3.9) below shows the separating funnel with wastewater containing FOG

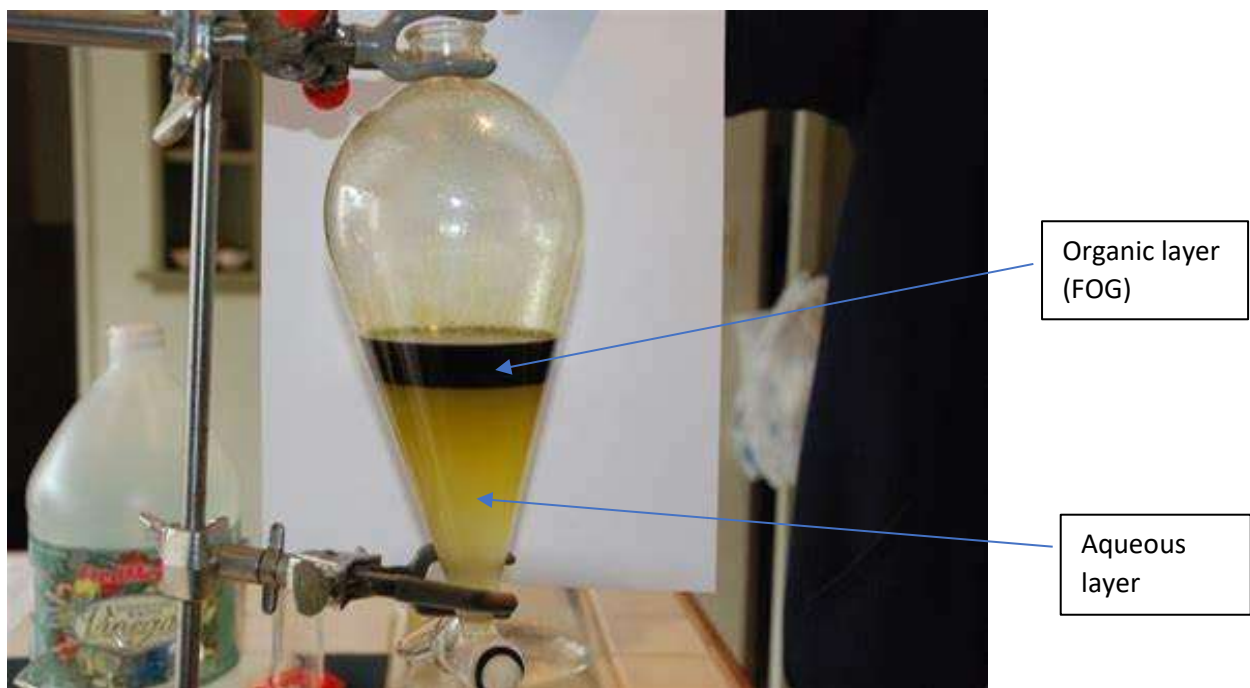


Figure 3.9: A separating funnel showing separation of wastewater with FOG whereby the top layer is the organic and the bottom layer is the aqueous layer.

### 3.1.3.3 Microbial Analysis

#### 3.1.3.3.1 Colilert test method

For *E. coli* and total coliform, a colilert test method (colilert-18 98-08876-00) was used whereby one packet of colilert powder was added to the 100 mL water sample in a sterile, non-fluorescing vessel, then vessel was capped and shaken to dissolve the powder, the sample was transferred into a quantity tray and sealed. Incubation was done at 36 °C between (18-24) hours. The results were read and compared against the comparator. If there is no yellow colour, the test is negative and if the sample had a yellow colour equal to or greater than the comparator, the presence of total coliforms was confirmed. If there is yellow colour, blue fluorescence was checked by placing a



6 W, 365 nm UV light within 5 inches of the sample. If blue fluorescence is greater or equal to the fluorescence of the comparator the presence of *E. coli* will be confirmed.



Figure 3.10: A colilert test method used for total coliform and *E.coli* detection.

#### **3.1.3.3.2 For other microbial analysis using the TEMPO instrument**

The water was kept in the fridge after reception. About 10 ml of the water was poured into 100 mL of buffered peptone water (primary diluent), then the mixture (1 mL) was then poured into 3 mL of the media which was hydrated with sterile distilled water (secondary diluent). The TEMPO cards were placed in the media and were placed in the TEMPO machine. The liquid mixture was transferred from the bottles into the cards. The cards were incubated at the 37 °C for 22 hours for *B. cereus* & *enterics* for 24 hours for *E. coli*, *Staphylococcus* and Total coliforms and 48hrs for aerobic count. After incubation the cards were read as CFU/mL

#### **3.1.3.3.3 Spread plate technique**

Spread plate technique was used for the analysis of *Pseudomonas*, *Shigella* and *salmonella*. *Pseudomonas* was grown on *pseudomonas* agar and incubated at 37 °C for 24 hours, *Shigella* and *salmonella* grown on XLD agar which was incubated at 37 °C for 24 hours.

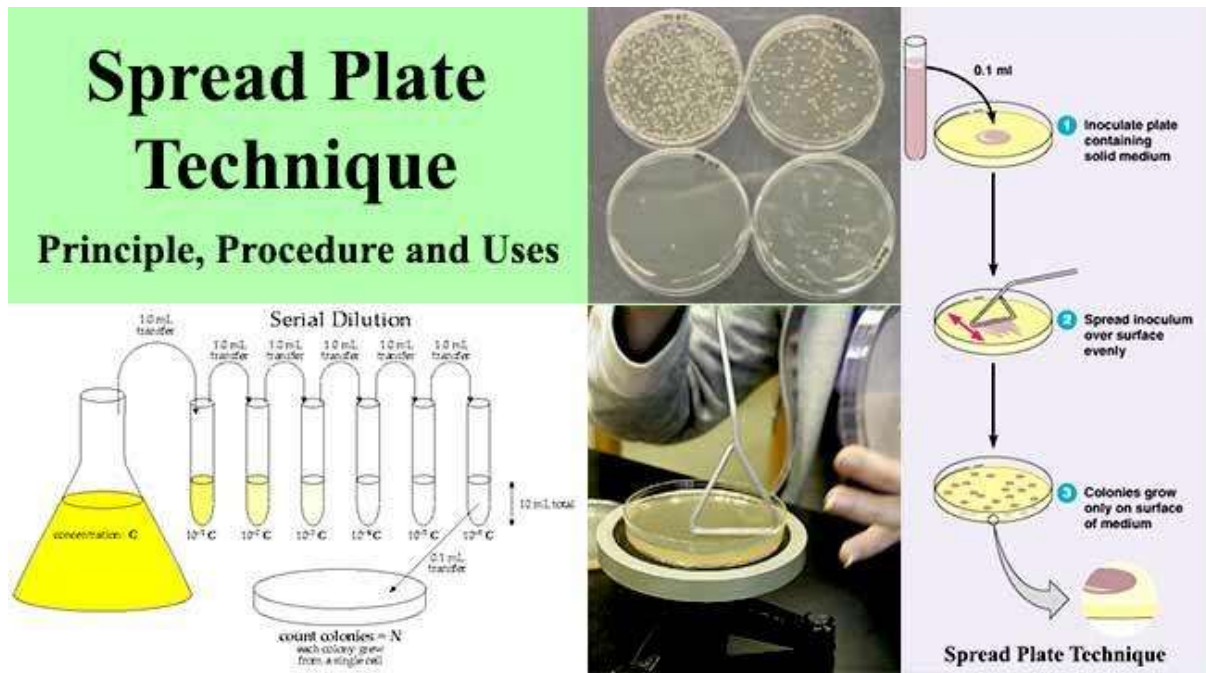


Figure 3.11: Schematic diagram for spread plate technique used in microbial enumeration (Mackie, 1996).

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## CHAPTER 4: RESULTS AND DISCUSSION

### 4.1 Physico-chemical parameters (Summer results)

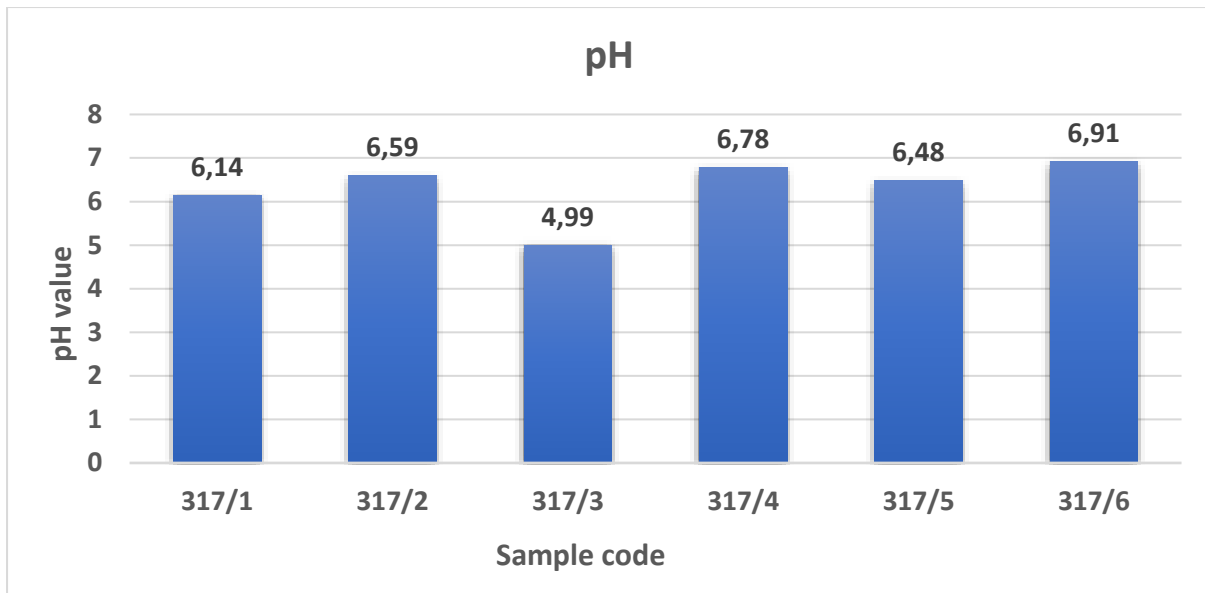
Water does contain different types of floating, dissolved, suspended and microbiological as well as bacteriological impurities. Selection of parameters for water testing solely depends upon the purpose for which the water is used for and this will give guidance about the degree of the water quality and purity needed. The analysis was done in both summer and winter in-order to compare the results. Some physical tests such as temperature, colour, odour, pH, turbidity, TDS etc. and chemical tests such as BOD, COD, DO, alkalinity, and water hardness were performed as shown in (Table 4.1 and Table 4.2), respectively. The water was tested for the presence of trace metal and heavy metal in order to obtain the quality and purity of water. In this study ICP-OES was used for testing the presence of heavy metal because of its capability for efficient and reproducible vaporization, atomization, excitation, and ionization for a wide range of elements in various sample matrices as compared to other excitation analytical instruments. The results from (Table 4.3 to 4.10) represent the amount/concentration of the anions found in the wastewater samples.

The results in both Table 4.4 and 4.11 are for elemental analysis. It is obvious that drinking water should pass these entire tests and it should contain the required amount of mineral level. Ion chromatography was also used to detect anions from the wastewater, because it is one of the most efficient methods used for separation of charged molecules.

**4.1.1 This section summarises and discusses the concentrations of some of the physicochemical tests in water samples from University of Limpopo gate 2 obtained using different analytical techniques. The results including mean concentration and standard deviation of all samples are given in tables. It also includes methods that can be used in-order to limit the deposition of FOG in wastewater at a higher rate.**

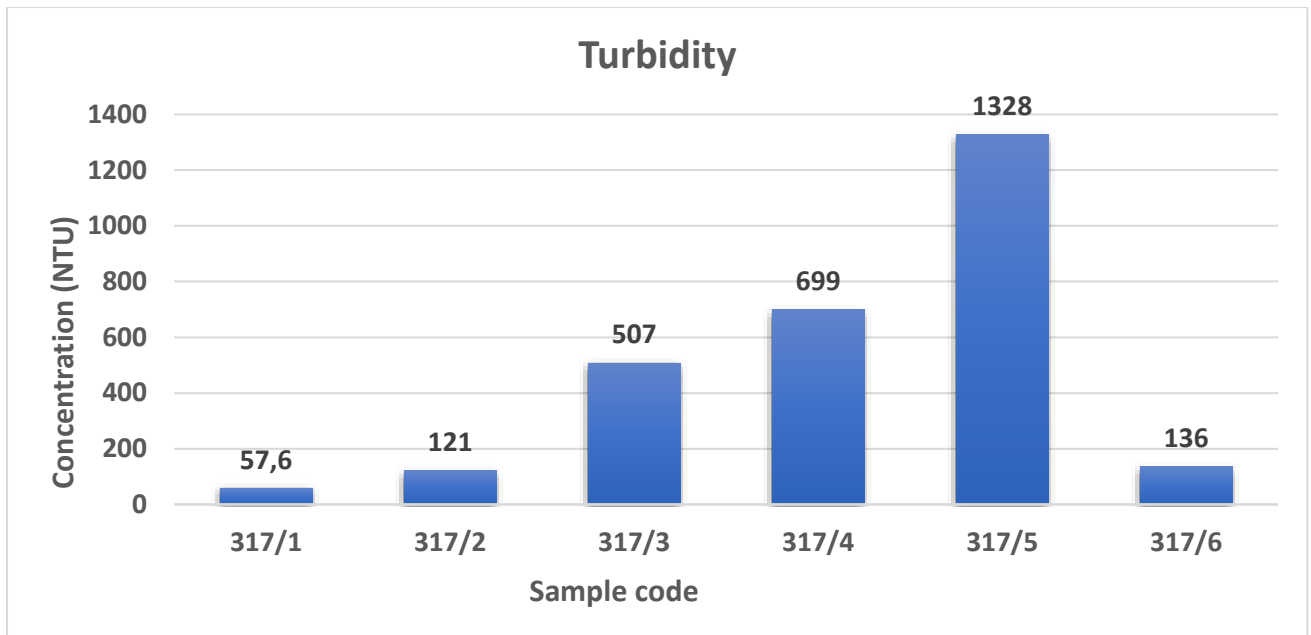
Table 4.1: Results for physico-chemical properties from the wastewater.

Parameter	317/1	317/2	317/3	317/4	317/5	317/6	SANS241
	Basin	Bucket	Pots	Pots	Bucket	Basin	Limit
<b>pH</b>	6.13±0.04	6.59±0.03	4.99±0.06	6.78±0.04	6.48±0.05	6.91±0.03	5-9.7
<b>TDS (mg/L)</b>	302.5±1	606.5±1	1835±1	2155±2	4043±2	894±3	≤2000
<b>Turbidity (NTU)</b>	57.6±0,7	121±0.9	507±0.5	699±1	1328±1	136±2	≤500
<b>Salinity (mg/L)</b>	226±1	501±3	1793±2	1942±1	3390±2	731±1	-
<b>Conductivity (µS/m)</b>	1465±2	1933±1	2790±3	3350±2	6223±3	1409±2	≤170
<b>Temperature (°C)</b>	27±0.1	28±0	28±0	28±0	27±0.1	28±0	≤40
<b>Calcium hardness (mg/L)</b>	17.17±0.02	85.47±0.6	17.03±0,428	55.72±1	68.20±1	402.6±0.5	-



**Figure 4.1: The pH of different samples of water.**

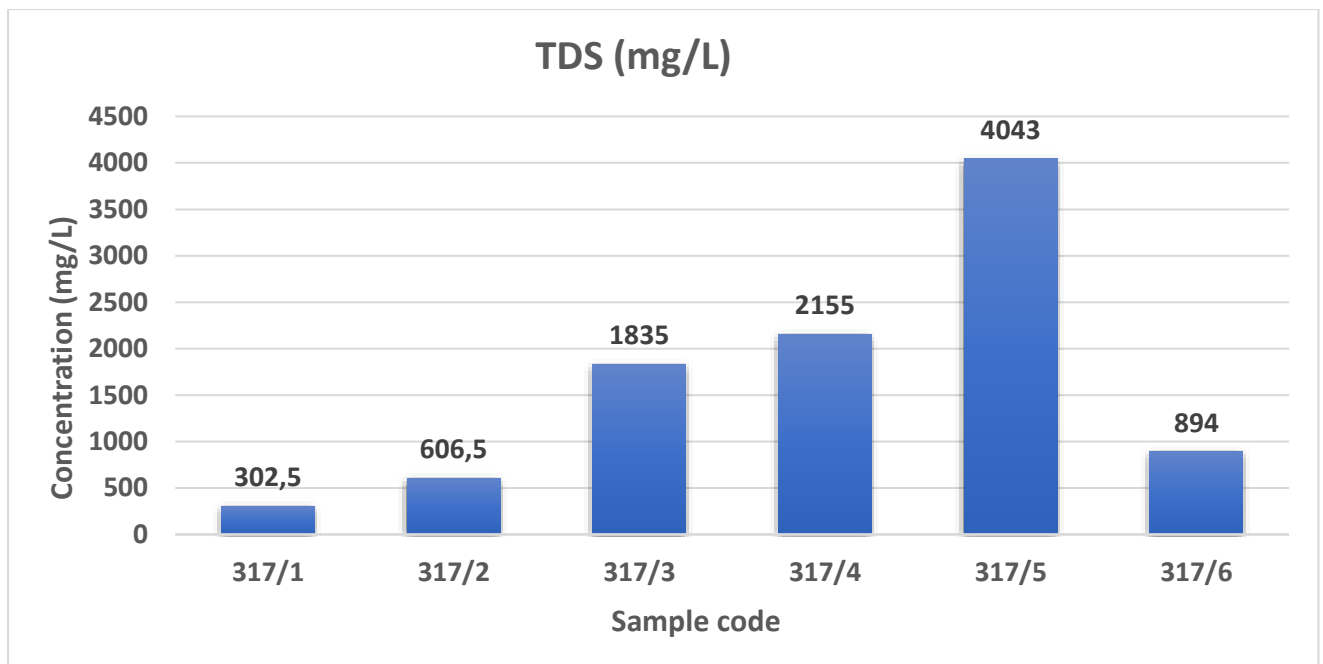
The pH is the negative log of the concentration of hydronium ions ( $\text{pH} = -\log[\text{H}^+]$ ). It indicates the level of acidity or basicity of water samples. The concentration of hydrogen ions is influenced by biological activities such as fermentation, BOD and COD. Besides chemical substances, the presence of organic matter in large quantities may decrease the pH value due to  $\text{CO}_2$  and  $\text{SO}_2$  releases. Change in pH value of wastewater can affect the biological reaction rate and the survival of different microorganisms (Hafemeister, 2014). The presence or absence of specific ionic species (hydronium ions) can have the wastewater's direct relation to pH. A similar study conducted on the determination of physicochemical parameters in food wastewater showed that the pH did not fall within the specified range due to change in values of Carbon dioxide ( $\text{CO}_2$ ), carbonate and bicarbonate in water (Ma *et al.*, 2020). The pH value of all samples falls within the normal limits of (5-9.7) as presented in (Figure 4.1) in the present investigation. Then sample 3 showed that it was starting to ferment because the pH (4.99) in sample (317/3) was reducing due to formation of lactic acid through fermentation which reduces the pH value of the wastewater because of the presence of high organic load (food scraps). The rise in pH of the sample implies that if the wastewater contains too much organic load and is left for some hours, it will ferment due to high temperatures from solar energy. The higher the pH value in wastewater, the higher the rate of corrosion because some elements' solubility depends on the acidity of the solution in-order to be secreted.



**Figure 4.2: Results for turbidity measurement reported in NTU as units.**

Turbidity is the measure of relative clarity of a liquid caused by particles suspended or dissolved in water that scatter light making the water appear cloudy or murky. Particulate matter can include sediment especially clay and silt, fine organic and inorganic matter, soluble coloured organic compounds, algae, and other microscopic organisms. Turbidity is affected by several factors in water: presence of dissolved and suspended solids, size and shape of particles and composition of the particles (Biswas, 2015). Water quality measurements that can help in the characterization of turbidity include total suspended solids, volatile suspended solids, total dissolved solids, suspended sediment concentration, chlorophyll a, and particle size analysis. From the food court at gate2 University of Limpopo, food scraps, steel wool, pot scourers and FOG is the primary contributor for turbidity, and this resulted in high turbidity for sample 317/1-317/5 for which they are above 500 NTUs which is the standard limit. High turbidity from these samples significantly reduces the aesthetic quality of water, having a harmful impact on recreation and tourism. It can increase the cost of water treatment for drinking and food processing.

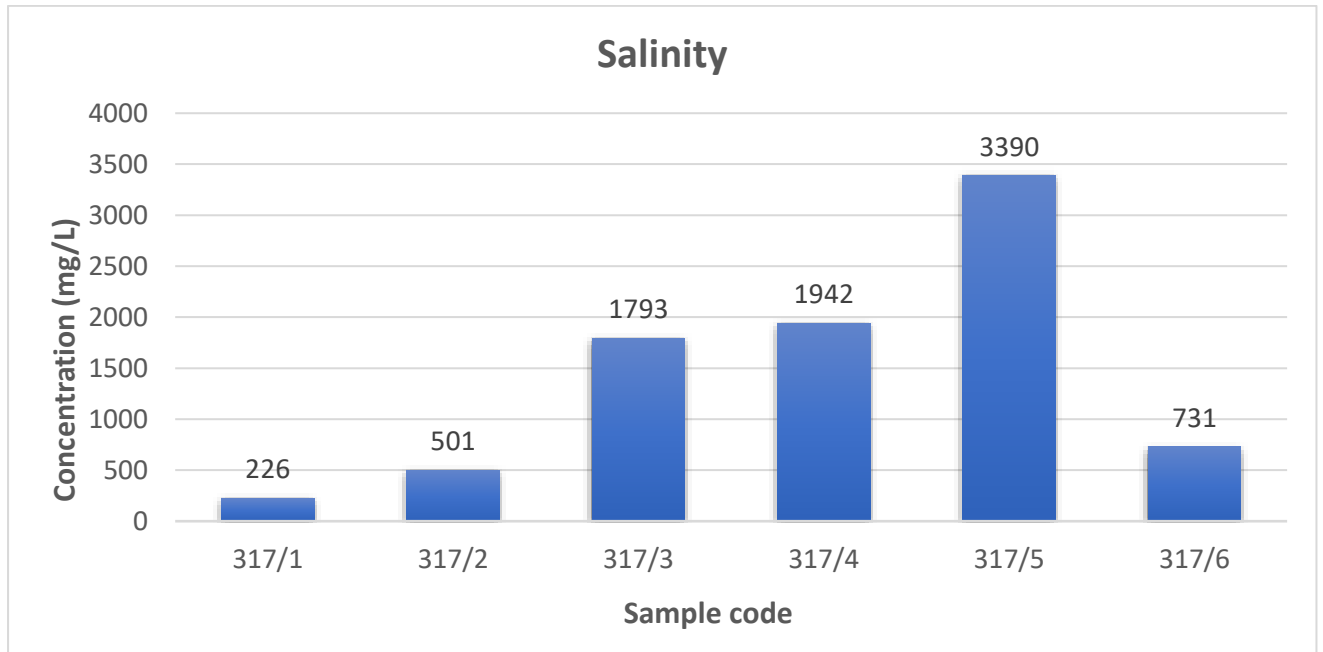




**Figure 4.3: Results for concentration of TDS measured in mg/L.**

According to (Wetzel, 2001), turbidity as from TDS refers to fragments of organic and inorganic particles in the water. It may incorporate anything floating or drifting within the water, from food scraps, fats and oil, even natural particles from breaking down materials can contribute to the TDS concentration. When organic materials decompose, some of the decomposed material may be tiny but still be detected as the dissolved solids. Even tiny chemical particles are considered as part of dissolved solids in the water (National Geographic Society). Cerco *et al.* (2003) points that TDS affects water clarity. The more solids found in the water, the more turbid the water is. TDS can be predicted using turbidity as an indicator parameter (Hannouche *et al.*, 2011). It is also a measure of the combined dissolved content of all inorganic and organic matter contained in a sample. Generally speaking, the operational concept is that the solids must be small enough to withstand filtration through a 0.002 mm (nominal size or smaller) pores filter, so the solids from the samples were too big to survive filtration, resulting in high TDS detection on 317/4 and 5. The TDS was not above the limit for sample 317/1,2,3 and 6 because the water contained small amounts of both inorganic and organic load. The dissolved content in the water includes a large number of parameters from food scraps, natural particles and even FOG. Other studies have shown that food wastewater from the major cooking facilities can be classified as strong wastewater and cannot be discharged into the stream because

their concentrations were above the required limit and they ranged from (250-400) mg/L (Akan *et al.*, 2010) and checking the results from the current study the concentrations are way above the results reported by other authors, meaning the wastewater contamination is too much.

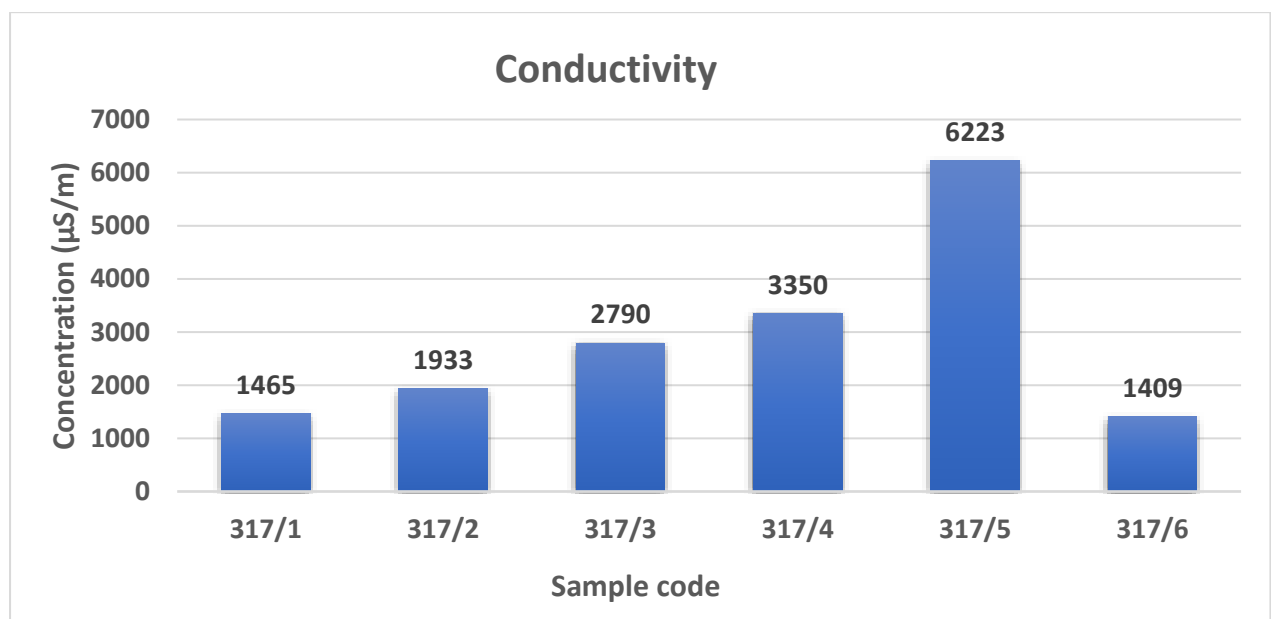


**Figure 4.4: Graphical concentration of salinity measured in mg/L.**

Salinity (salt) in water is a measure of dissolved mineral salts such as calcium, magnesium, potassium, sodium, sulfate, and chloride. All water supplies naturally contain some salt, but agricultural, industrial, residential and food preparation facilities water users often add more salt to the water they use. Domestic use of water softeners, detergents, cleaning products, liquid fabric softeners, soaps, and shampoos all add salt to the wastewater, same applies to food preparation facilities. The salinity in the wastewater was tested using a probe for conductivity measurement (1120 Metler Toledo) which measures salinity, TDS, and conductivity, the probe was immersed in the wastewater and salinity concentration was recorded in mg/L, although there is no standard salinity limit from SANS241, its detection is from the salt they used during the preparation of the food and cleaning detergents they use.

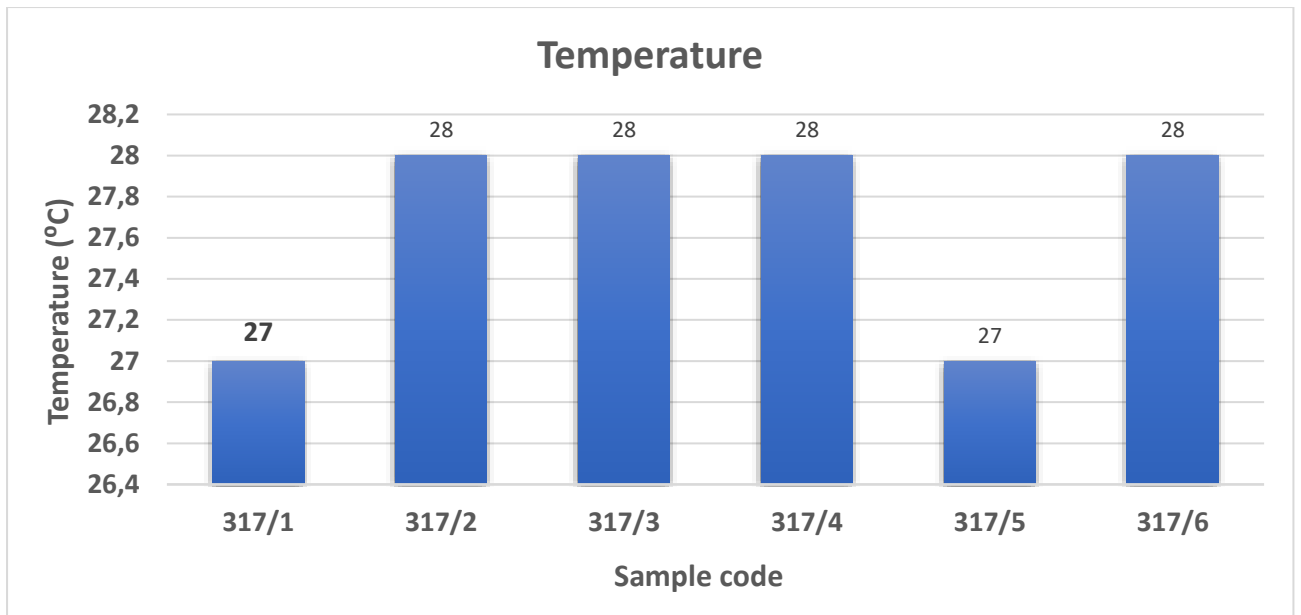
Too much salt in wastewater can affect sensitive ecosystems and degrade the quality of water supply for drinking, farming, industry, and recreation (Singh *et al.*, 2012).

Unfortunately, high levels of salt in wastewater may result in regulatory mandated treatment improvements. Installing costly systems at the treatment plant to remove excess salt would result in significantly increased rates for residential and commercial users, therefore, it is important for all of us to take simple measures to decrease the salinity in wastewater, because this measures will help to extend the life of water pipes, appliances, equipment and also reserve water quality for potential re-use (high salinity levels limits water resource management options and the ability to use reclaimed water in the future). This extra treatment will escalate the cost for water supply.



**Figure 4.5: Conductivity measurement reported in µS/m.**

Electrical conductivity is a numerical measure of the electricity carrying capacity of an aqueous solution (µS/m). It is an important tool for accessing the purity of water, all the samples had high conductivity detection. It is understood that steel wool is relatively conductive like all metals, since well the samples are from food preparation facilities and they use steel wool mostly when they wash their dishes, and its presence makes the samples conductive. The source of conductivity may be an abundance of dissolved salts due to addition of table salt in food materials, actual salt present in pure water, and other mineral discharges (Acharya *et al.*, 2008) and also electrical conductivity increases at higher temperature, the analyses were conducted during hot season, temperature could have also played a major role (Hamid *et al.*, 2013). Conductivity also gives an indication of the amount of inorganic materials in the water including calcium, nitrogen, bicarbonate, and others.



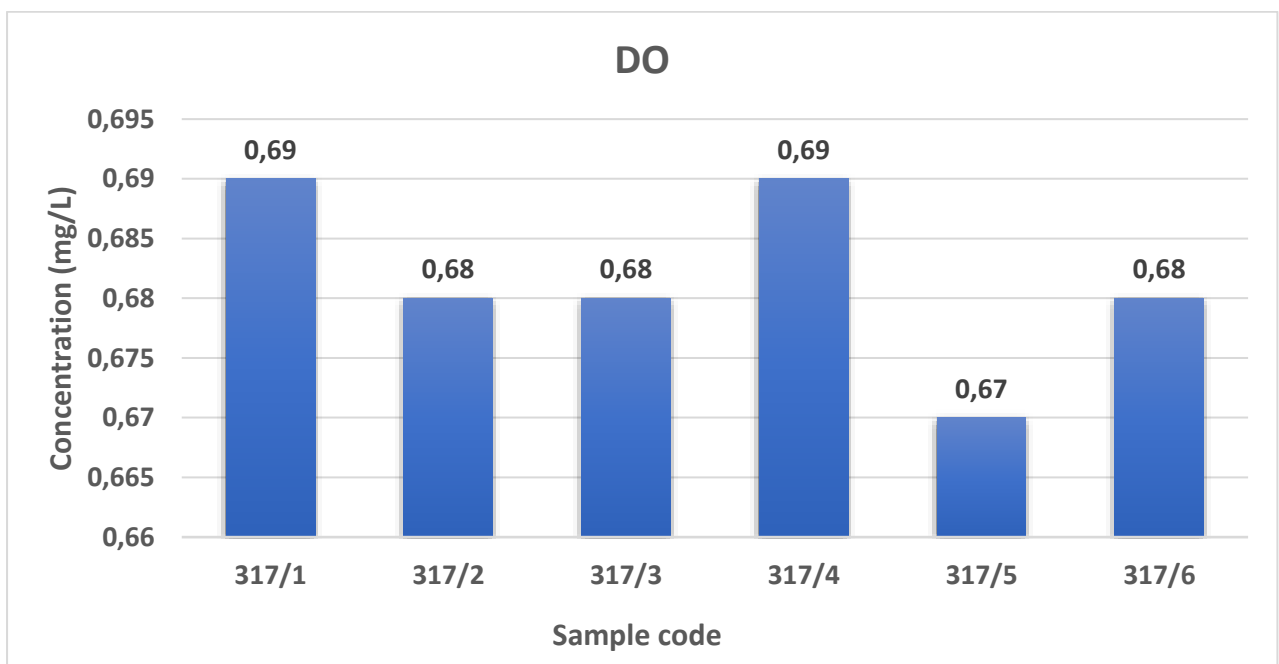
**Figure 4.6: Graphical representation of temperature from the samples reported in °C.**

Temperature is important for its impact on certain chemical and biological reactions occurring in water and aquatic media-inhabiting species and will rely on seasons and sampling time. In the present investigation the sample temperature was between (27-28) °C and falls within the range of which is as much as 40 °C, but due to clear atmosphere and great solar radiation the water temperature is above room temperature. Results obtained from the study (Ma *et al.*, 2012) showed that the temperature ranged from (31.15-34.30) °C, and he stated that “release of high-temperature wastewater into water bodies may speed up some reactions in the water body. It will also reduce solubility of oxygen and amplified odour due to anaerobic reaction (less oxygen)”. According to the results obtained the temperature is not too high from the room temperature hence the solubility of oxygen in the water is high.

**4.1.2 The table and the figures below include the results of oxygen demand found in the samples using different analytical methods. The results obtained were discussed under each figure from 4.7 to 4.9.**

Table 4.2: Results for COD, BOD, and DO using DR 6000 and Portable Dissolved Oxygen meter-H19146-Hanna instrument.

Parameter (mg/L)	317/1	317/2	317/3	317/4	317/5	317/6	SANS241 Limit (mg/L)
	Basin	Bucket	Pots	Pots	Bucket	Basin	
<b>DO</b>	0.69±0.006	0.68±0	0.68±0.006	0.69±0	0.67±0.02	0.68±0	-
<b>BOD<sub>5</sub></b>	2124.31±0. 6	3222.32±2	1189.32±0. 5	1201.31±0. 5	1984.33±0. 1	1101.32±0. 03	50
<b>COD</b>	1853±0	4709±0	7196±0	2136±0	4685±0	1784±0	75

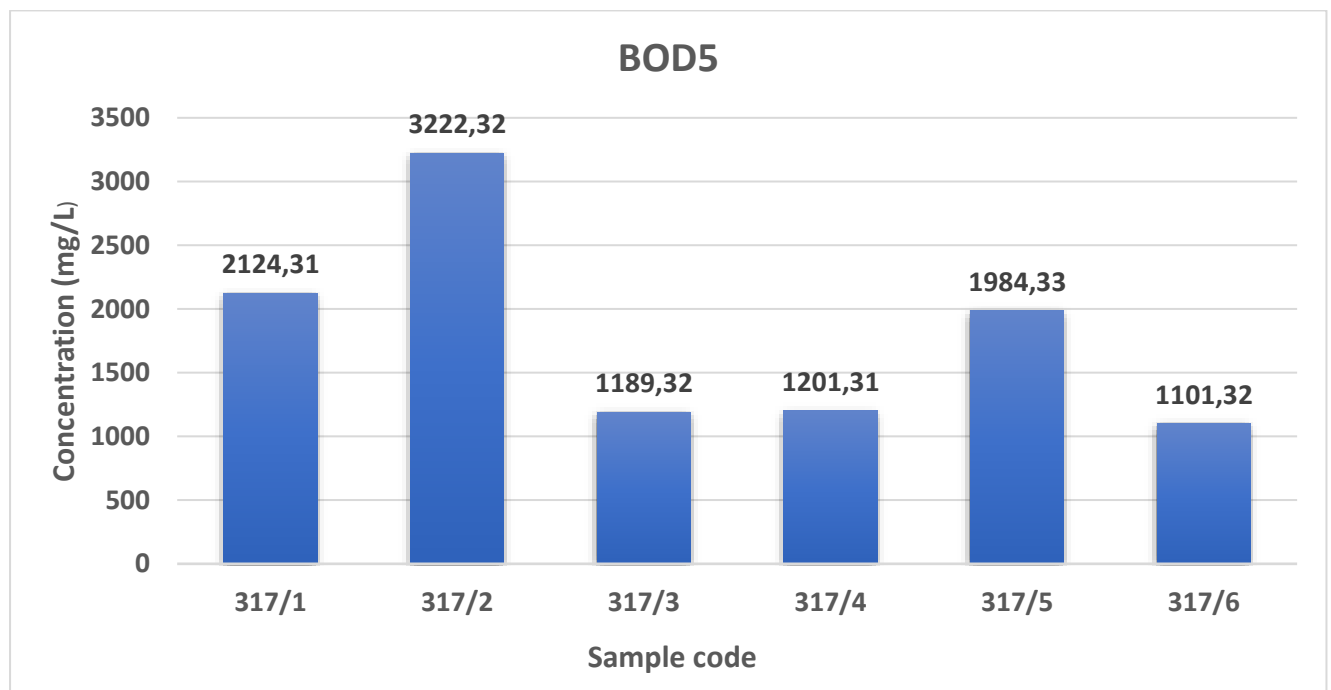


**Figure 4.7: Graphical representation of DO concentration measured in mg/L as units.**

Dissolved oxygen is essential for aquatic life's survival and thus serves as an important indicator of ecosystem condition, its water levels are partially dependent on the

chemical, physical, and biochemical activities that occur in the water. The concentrations of DO depend directly on the generation of oxygen through photosynthesis and ingestion by living organisms, especially bacteria. Therefore, DO among others is affected by water temperature, water movement, and salinity. Oxygen has limited water solubility directly associated with atmospheric pressure and is inversely correlated with water temperature and salinity and DO is greatly influenced by the BOD level in water. The higher the BOD concentration, the greater the extent of oxygen depletion in the water bodies. This results in the reduction of oxygen available for higher forms of aquatic life which consequently leads to the death of aquatic organisms (Bhateria and Jain, 2016).

The wastewater coming from food preparation facilities mostly has organic materials that are decomposed by microorganisms and uses oxygen to carry out their activities and this increases the amount of oxygen (Sahu *et al.*, 2007). From the results obtained, the sample concentrations ranged from (0.67-0.69) mg/L and it was small because the organic matter was not yet decomposed. The sample was analysed at the site of sampling, so the microorganism did not have enough time to degrade the organic material available in the wastewater.

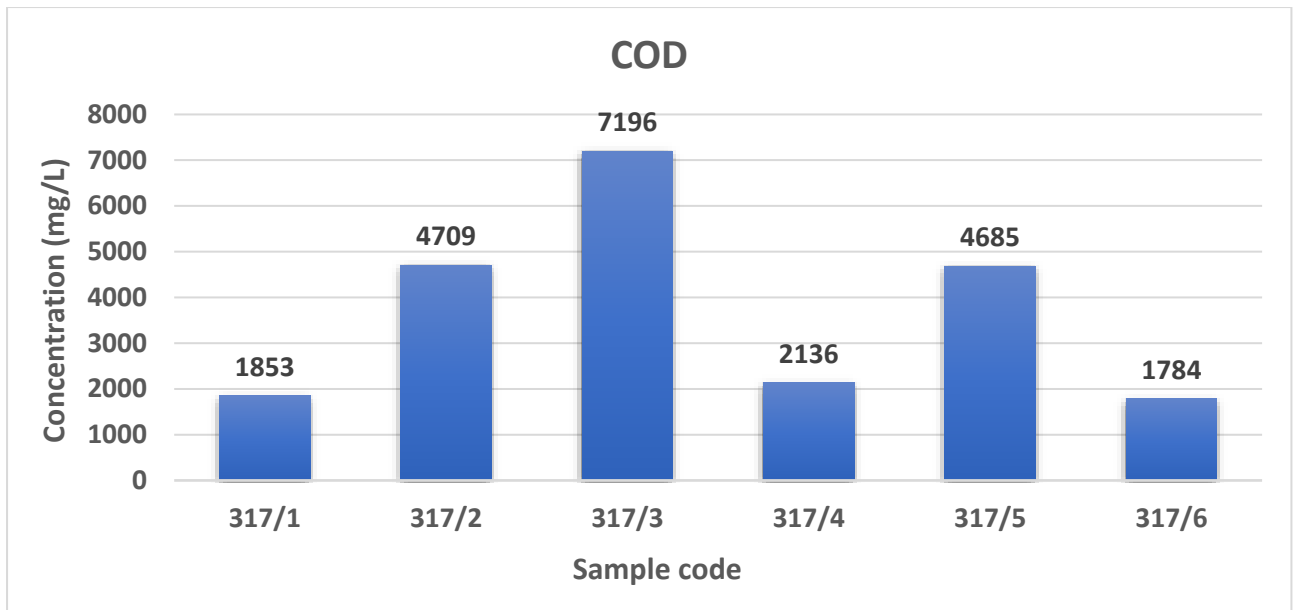


**Figure 4.8: A bar graph of BOD<sub>5</sub> concentration from the samples measured in mg/L.**

Five day BOD test, also called BOD<sub>5</sub>, is the most important parameter for water quality. This is the calculation of the oxygen required to break down organic matter in a water sample by bacteria and other microorganisms. Microorganisms (such as bacteria and fungi) feed on this decaying content as organic matter decomposes and gradually the matter is oxidized. BOD measures the amount of oxygen used by microorganisms when the organic matter in the sample is decomposed. The greater the microorganism activity, the more oxygen they will be using and the higher the BOD level calculation (Howe *et al.*, 2012).

This is an indirect measure of the amount of organic matter in the sample and provides an indicator of how much dissolved oxygen might be removed from water as the organic matter decays. BOD has a strong effect on the amount of oxygen dissolved in rivers and streams. The sooner the supply of oxygen is reduced, the greater the BOD. This means less food for other aquatic life, such as insects and fish, is available. For example, a high BOD measure affects the health of streams in the same way as low dissolved oxygen: aquatic life is overwhelmed, suffocated, and dies. The few species, including carp and sewage worms, that can live with less oxygen, would increase in number (Lack, 2001).

As mentioned from the DO, microorganisms use oxygen to carry out their activities (degrading organic matter) from the wastewater. The sudden increase in BOD<sub>5</sub> shows that the microorganisms had enough time to degrade the waste. In wastewater from food preparation services, these organic matters are mainly derived from food scraps (meat, sauce, pap and related species) introduced by washing dishes into the water. From the analyzed samples, after five days since the organic content was high, all the samples contained elevated BOD<sub>5</sub> levels at the average between (1189.32 to 3222.32) mg/L which is above the required standard limit of 50 mg/L and this is because the amount of dissolved oxygen (DO) to degrade the matter increased daily.



**Figure 4.9: COD concentration from the samples reported in mg/L.**

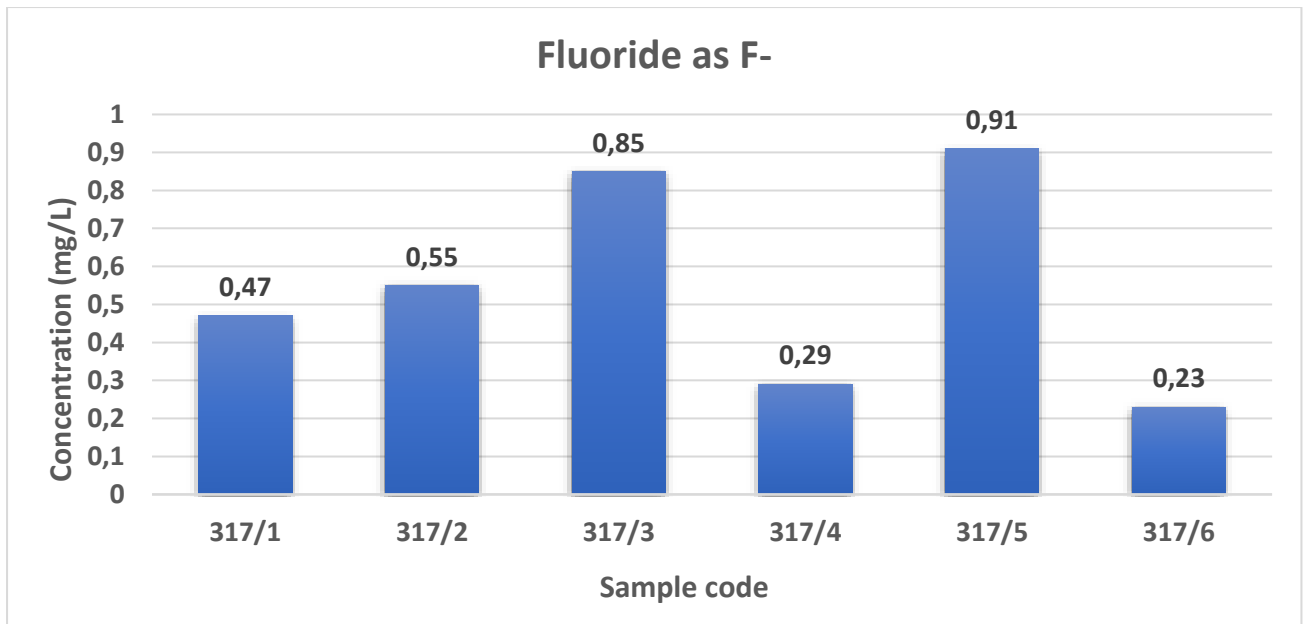
COD determines the quantity of oxygen required for chemical oxidation of organic matter (pap, meat, gravy, etc.) produced from cooking using a potent chemical oxidant. The COD is a test which is used to measure household waste pollution. The waste is measured in terms of the balance of oxygen required to produce carbon dioxide (CO<sub>2</sub>) and water for oxidation of organic matter. It's a fact that under acidic conditions all organic compounds with a few exclusions may be oxidizing agents. The COD test is useful for signalling harmful environments and the presence of resistant biologic substances (Simpi *et al.*, 2011) . In the present study the COD value for all the samples was obtained to be above the standard limit of 75 mg/L because more oxygen was required to degrade the high organic load present in the samples.



**4.1.3 In-order to determine the anions concentration in water, Ion Chromatography instrument is used. The (Table 4.3) below shows the concentrations of different anions and ammonia found in the wastewater samples followed by the figures from 4.10-4.13 which is the discussion of each concentration.**

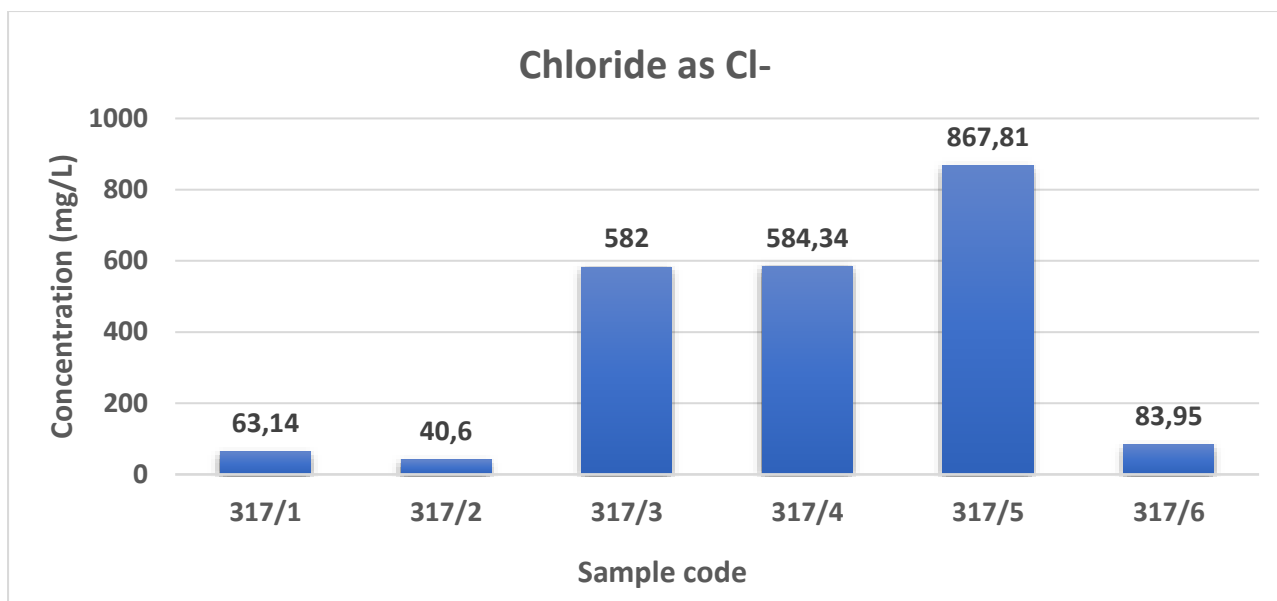
Table 4.3: Results for anions analysis using IC.

Parameter (mg/L)	317/1	317/2	317/3	317/4	317/5	317/6	SANS241 limit (mg/L)
	Basin	Bucket	Pots	Pots	Bucket	Basin	
<b>Fluoride as F<sup>-</sup></b>	0.47±0.0 01	0.55±0	0.85±0.005	0.29±0	0.91±0.000	0.23±0	≤1,5
<b>Chloride as Cl<sup>-</sup></b>	63.14±0	40.60±0.00 1	582±0.03	584.34±0.0 2	867.81±0	83.95±0	≤300
<b>Bromide as Br<sup>-</sup></b>	468.63± 0.003	446.04±0.0 05	464.18±0.0 1	505.01±0	631.83±0	451.41±0	-
<b>Sulfate as SO<sub>4</sub><sup>-</sup></b>	31.44±0. 01	30.94±0	62.30±0.00 1	49.93±0	83.43±0	30.09±0	≤500
<b>Ammonia NH<sub>3</sub>-N</b>	1853±0	4709±0.03	7196±0.05	2136±0.008	4685±0.07	1784±0	≤1.5



**Figure 4.10: Fluoride as an anion measured in mg/L.**

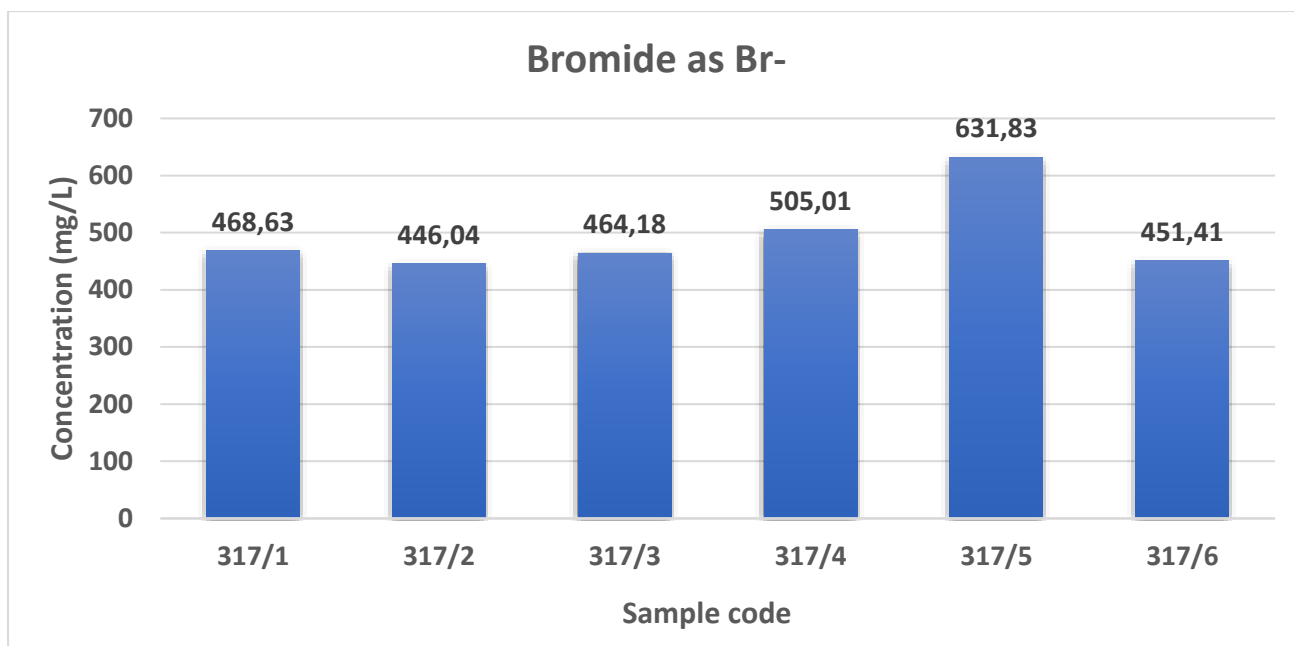
Many foodstuffs are known to contain traces of fluorine. All vegetation includes fluoride and from FSEs they serve vegetables on their menu, its fluoride ion concentrations can be added into the wastewater through the washing process. In the final drinking water fluoride ions are also present at a concentration of (0.5-1) mg/L. The concentration of fluoride ions in the wastewater was found to be within the range of (0.23-0.85) mg/L, since the concentration was not higher than 1 mg/L, it suggests that its existence is from the natural water or from the vegetables in low concentration.



**Figure 4.11: Chloride ion concentration measured in mg/L.**

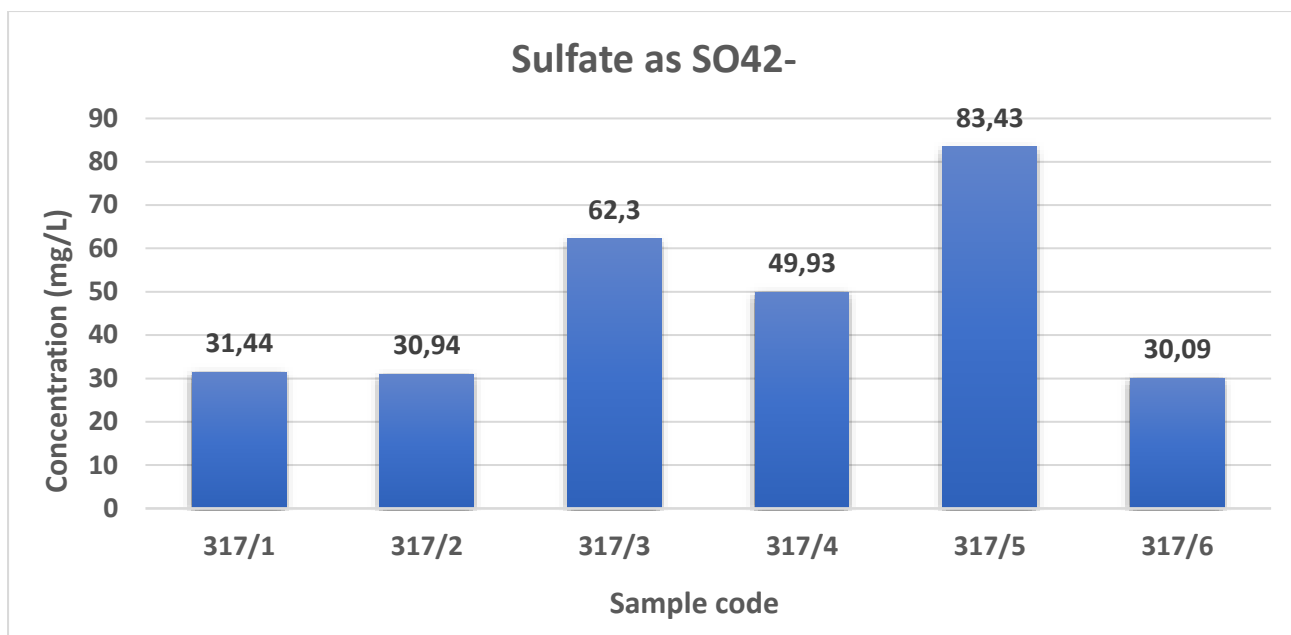
Chloride is a common inorganic anion present in water. It usually occurs in the form of chelates. Generally, a major amount of chlorides comes from use of chemical fertilizers, human urine, faeces, animal excretory material and NaCl. Contaminations with high chloride ion concentrations in water can cause damage to metal pipes and concrete structures (de Almeida *et al.*, 2015). Due to the human diet and the composition of cleaning products like soap, it is common to find high concentrations of chloride and sodium in domestic wastewater. Chloride tends to stay constant through conventional effluent processing, but it is known that ion exchange and reverse osmosis may decrease it.

Water containing residual chlorine exceeding 10 mg/L will damage agricultural crops (Emongor and Ramolemana, 2004) like water reuse with high sodium concentrations, problems with crop handling can arise (Powel, 1987). Studies also show that chloride has harmful effects on soil, contributing to plant toxic effects (Rodrigues *et al.*, 2010; Rodrigues *et al.*, 2012) and for safety reasons, chloride in wastewater should not exceed 350 mg/L as directed by WHO Standard (WHO, 2006). The three samples ranging from 317/3 to 317/5 were high in chloride concentration due to sodium chloride dissolution from the salt used in cooking to form ions such as sodium and chloride ion or it can be occurring naturally as its commonly found in water and it can also be excreted from the faeces and animal excretory material from the chicken intestines and tripe.



**Figure 4.12: Bromide ion concentration from the samples measured in mg/L.**

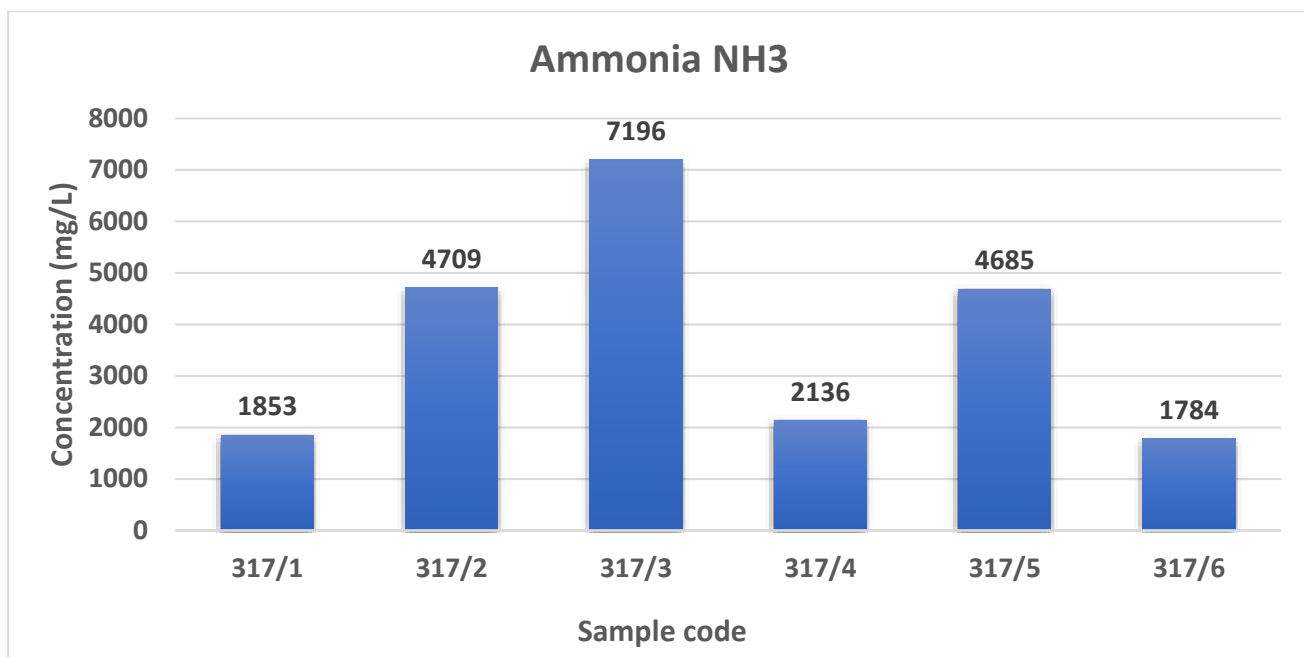
Methyl bromide (MBr) is mainly used as a fumigant in soil for controlling fungi nematodes, and weeds; in-space fumigation of food resources (e.g. grains, vegetables); and in storage facilities such as pest and rodent control mills. In general, favorable effects of MBr as a soil sterilant on the growth of crops can be attributed to the alleviation of injuries caused by soil-borne diseases and nematodes in continuous cropping. In another research, authors (Masui *et al.*, 1978) found that the germination of tomato and spinach seeds are very sensitive to MBr and most farmers if not all use MBr as their fumigant. So, at the food preparation facility, vegetables like tomatoes and spinach are used to make salads as one of the side dishes. These veggies are washed first before use, so through the washing process bromide was introduced in wastewater.



**Figure 4.13: Sulfate ion measured in mg/L from the wastewater samples.**

As the major anion in hard water reservoirs, sulfate is second to bicarbonate. Sulfates ( $\text{SO}_4^{2-}$ ) may occur naturally or result from municipal or industrial discharges. Sulfate minerals can cause scale build-up similar to other minerals in water pipes and may be associated with a bitter taste in water that can have a laxative effect on humans and young animals. Elevated levels of sulfate in combination with chlorine bleach can make it difficult to clean clothes. The sulfur-oxidizing bacteria have similar effects to those of iron bacteria. They transform sulfide into sulfate, producing a dark slime that can clog plumbing and stain clothing (Patil *et al.*, 2012). A problem with sulfur-oxidizing bacteria may indicate blackening of water or dark slime coating inside toilet tanks. Bacteria that oxidise sulfur are less common than bacteria that reduce sulfur.

The primary producers of large quantities of hydrogen sulfide are sulfur-reducing bacteria which use sulfur as an energy source. These bacteria are chemically converting natural sulfates to hydrogen sulfide in water. Sulfur-reducing bacteria live in environments that lack oxygen, such as deep wells, plumbing systems, water softeners, and water heaters. Such bacteria typically thrive along a water distribution system's hot water side. If organic load (pap, gravy, meat) concentrations are high in wastewater, sulfate acts as an acceptor to remove oxygen for the production of hydrogen sulfide (Tüfekci *et al.*, 2007). The analytical samples had a high organic load concentration and therefore sulfate was detected as an acceptor to eliminate oxygen from the samples.



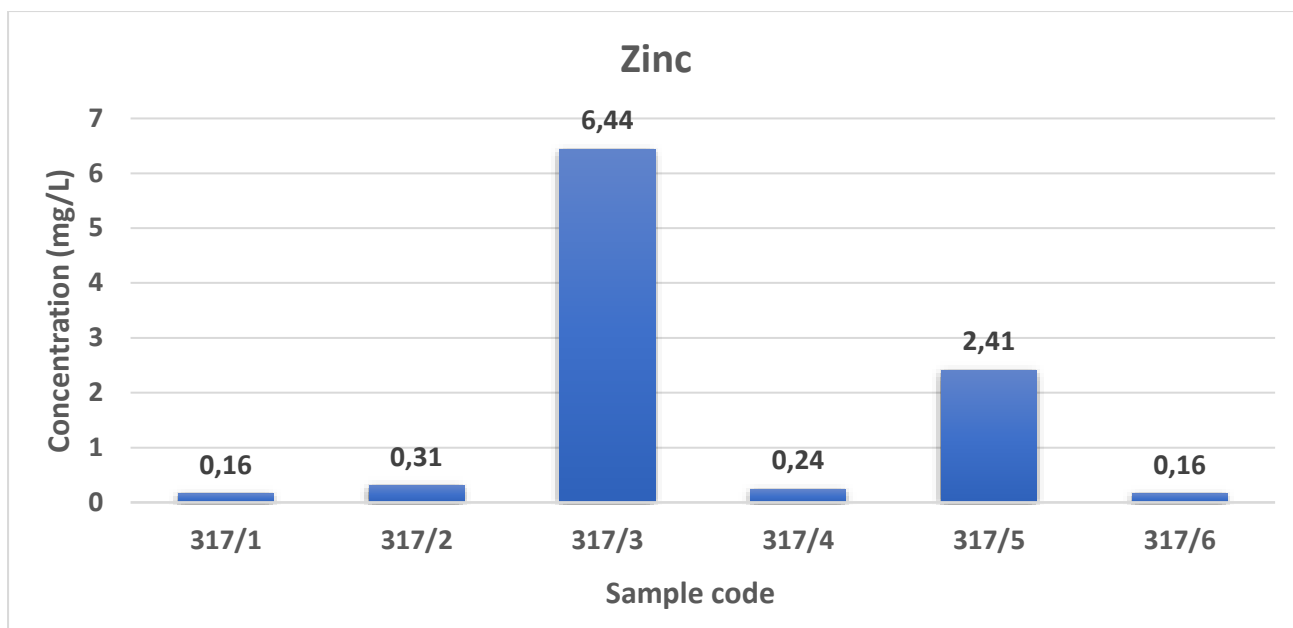
**Figure 4.14: Ammonia concentration measured in mg/L.**

The high intensity of some ions can affect plants, animals, and humans; the high concentration of ammonium ions can cause noticeable foliar injury. Additionally, a significant increase in nitrogen in organic tissue may occur when plant assimilation of this ion is high. In addition to changing the biomass production, the plant's excessive absorption of ammonia and ammonium ions also modifies the susceptibility to drought and frost resistance (Krupa, 2003). In addition, when converted to nitrate, high ammonium ion concentrations in water bodies can be toxic to aquatic organisms and ultimately to humans (Dong and Reddy, 2012). The ammonia concentrations from the samples were found to be high, the main reason could be that ammonia has long been used as a cleaning agent, widely used in houses, restaurants, and other public places. The drug is perfectly safe to use with no precautions, is used in a variety of cleaning products and is used in homes even in the form of pure or scented ammonia, and the use of detergents to wash dishes has resulted in high levels of ammonia.

**4.5 Below is the elemental analysis of the wastewater in-order to get the elemental composition of each sample. The figure 4.15-4.21 discusses the concentrations found in the water.**

Table 4.4: Results for elemental analysis using ICP-OES as an analytical instrument.

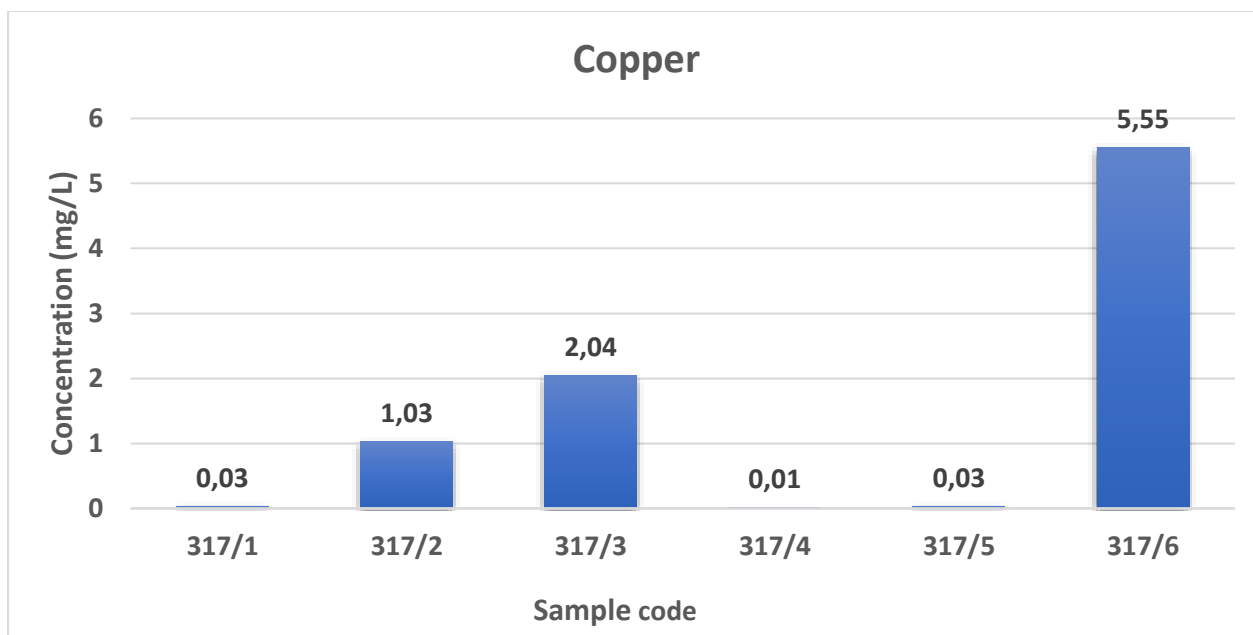
Parameter (mg/L)	317/1	317/2	317/3	317/4	317/5	317/6	SANS241
	Basin	Bucket	Pots	Pots	Bucket	Basin	Limit (mg/L)
<b>Potassium</b>	36.16±0.1	42.60±0.02	80.86±0.1	139.92±0.4	282.66±0.1	67.88±0.1	≤50
<b>Calcium</b>	4.84±0.01	26.69±0.003	57.02±0.01	10.77±0.005	13.98±0.01	9.10±0.001	≤150
<b>Magnesium</b>	1.23±0.01	4.55±0.01	8.37±0.01	6.99±0.01	8.07±0.02	4.25±0.01	≤70
<b>Sodium</b>	58.14±0.02	143.38±0.02	359.07±0.01	399.80±0.02	606.27±0.03	172.72±0.03	≤200
<b>Zinc</b>	0.16±0.01	0.31±0.01	6.44±0.01	0.24±0.01	2.41±0.001	0.16±0.001	≤5
<b>Lead</b>	0.02±0	ND	ND	0.01±0	ND	ND	≤0.01
<b>Manganese</b>	0.02±0	0.02±0	0.53±0.002	0.02±0	ND	ND	≤0.4
<b>Vanadium</b>	0.01±0	ND	ND	ND	ND	ND	≤0.2
<b>Aluminium</b>	0.14±0.01	0.25±0.006	0.15±0.01	0.18±0.02	0.24±0.008	0.12±0.06	≤0.3
<b>Chromium</b>	ND	ND	ND	ND	ND	ND	≤0.05
<b>Copper</b>	0.03±0.02	1.03±0.01	2.04±0.03	0.01±0	0.03±0.003	5.55±0.1	≤2
<b>Iron</b>	0.31±0.05	0.92±0.01	4.08±0.2	2.33±0.05	0.48±0.004	0.16±0.007	≤2



**Figure 4.15: Zinc concentration reported in mg/L.**

In the environment zinc occurs in an inorganic form, with complexes and components dissolved or as insoluble. It has no known adverse physiological effects on humans. It is an important and really helpful component of human nutrients. This combines primarily with suspended materials in an aquatic environment before settling in the sediment (Sharma *et al.*, 2007). While zinc levels in surface water and groundwater typically do not exceed (0.01 and 0.05) mg/L respectively, tap water concentrations can be much higher as an end result of zinc dissolution from pipelines. Zinc solubility depends on Wastewater temperature and pH. When the pH is normally neutral, zinc will become insoluble in water and its solubility will rise as quickly as the water acidity increases. Solubility increases, too, above pH 11. Zinc dissolves as  $ZnOH^+$  (aq), or  $Zn^{2+}$  (aq) in water (Gupta *et al.*, 2017). The solubility of anionic  $ZnCO_3$  is 0.21 g/L. From the results obtained in sample 317/3, its zinc concentration was above the limit of 5 mg/L due to the presence of the wastewater's acidic portion sample.



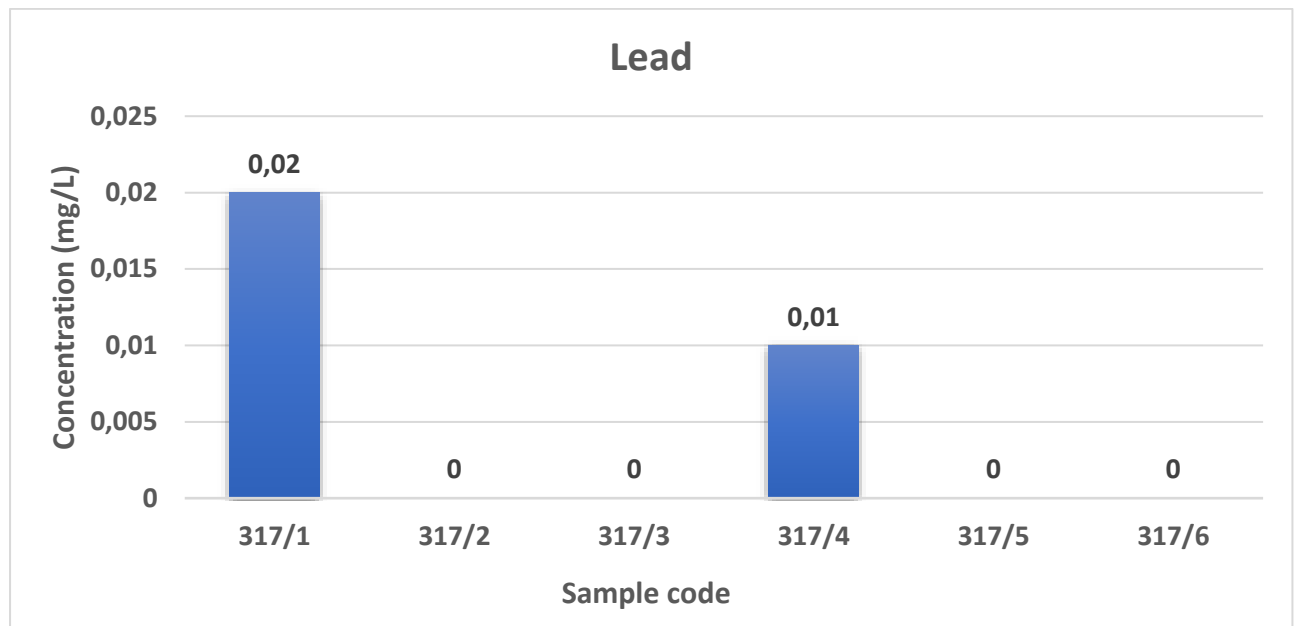


**Figure 4.16: Copper concentration from samples measured in mg/L.**

In the minerals, copper is found primarily as sulphide, oxide, or carbonate. Copper reaches the water system by mineral degradation, industrial effluents due to its use as an algicide and insecticide, and by corrosion of water distribution pipes in copper alloys. It may occur in a simple ionic form or in one of several groups, such as cyanides, chlorides, ammonia, or organic ligand complexes. Copper tests are important because dissolved copper salts are poisonous to some biota even in low concentration (Eriksen *et al.*, 1989). If your water is acidic, copper can enter water either directly by contaminating the well water or by corrosion of the copper pipes. Problems of corrosion of copper and copper alloys in plumbing systems occur when the protective copper corrosion product layers either do not properly form in the first place or become disrupted as time passes-by (King *et al.*, 1995).

The two samples with high detection of copper 317/3 and 317/6, the wastewater in sample 3 is from the pot and it contained steel wool of which the food preparation facility uses vinegar to make some of their dishes including salads and pap, even if vinegar was found in low concentration its reaction with steel wool results in corrosion in which elements such as copper are released. The one for sample 6 which was sampled from the basin at the back from the pipe-line, corrosion of copper from the pipe could have occurred due to the sticking of FOG on the pipe-lines which leads to corrosion. The presence of copper in wastewater can have a negative impact on the environment because it can interrupt the activity in soils, as it negatively influences the

activity of microorganisms and earthworms. The decomposition of organic matter may seriously slow down because of its presence.



**Figure 4.17: Lead concentration measured in mg/L.**

Lead waterworks have been used extensively in former days, and these may still occur in ancient buildings in addition. Lead from pipes can dissolve moderately in the water that flows through. Furthermore, lead can bind to carbonate, thereby reducing the amount of lead dissolve in hard water. A coating of unlikely ever soluble alkaline lead carbonate is formed inside the pipes. This film acts as a protective coating for the pipes underlying lead (Cornwell *et al.*, 2016). The 317/1 sample in which its concentration was above the limit indicates that when sampling was performed, there was a flow of lead from the pipe as the wastewater was collected from the outside pipe from the basin. The lead in the wastewater is not in higher concentration, so if it ends up on the environment for example the water can be used for irrigation because plants can take up to a concentration 500 ppm from the soil. So, the slight increase of lead in the wastewater cannot have a huge impact on the ecosystem because it is not in higher concentration.

Lead is also available in tap water somewhat because of its disintegration from common sources, yet basically from household plumbing frameworks in which the pipes, patch, fittings, or services connected to homes contain lead. Polyvinyl chloride (PVC) pipes likewise contain lead exacerbates that can be filtered from them and

result in high lead concentration in drinking-water. The amount of lead dissolved from the plumbing system relies upon a few parameters, including the nearness of chloride and dissolved oxygen, pH, temperature, water softness and standing time of the water, soft, acidic water being the most plumbosolvency (Davidson and Rabinowitz, 1992 ). Despite the fact that lead can be filtered from lead channelling uncertainty, apparently the draining of lead from bound joints and metal taps diminishes with time. Welded associations in late constructed homes fitted with copper funnelling can deliver enough lead (210–390) µg/L to cause inebriation in children (Pocock *et al.*, 1983).

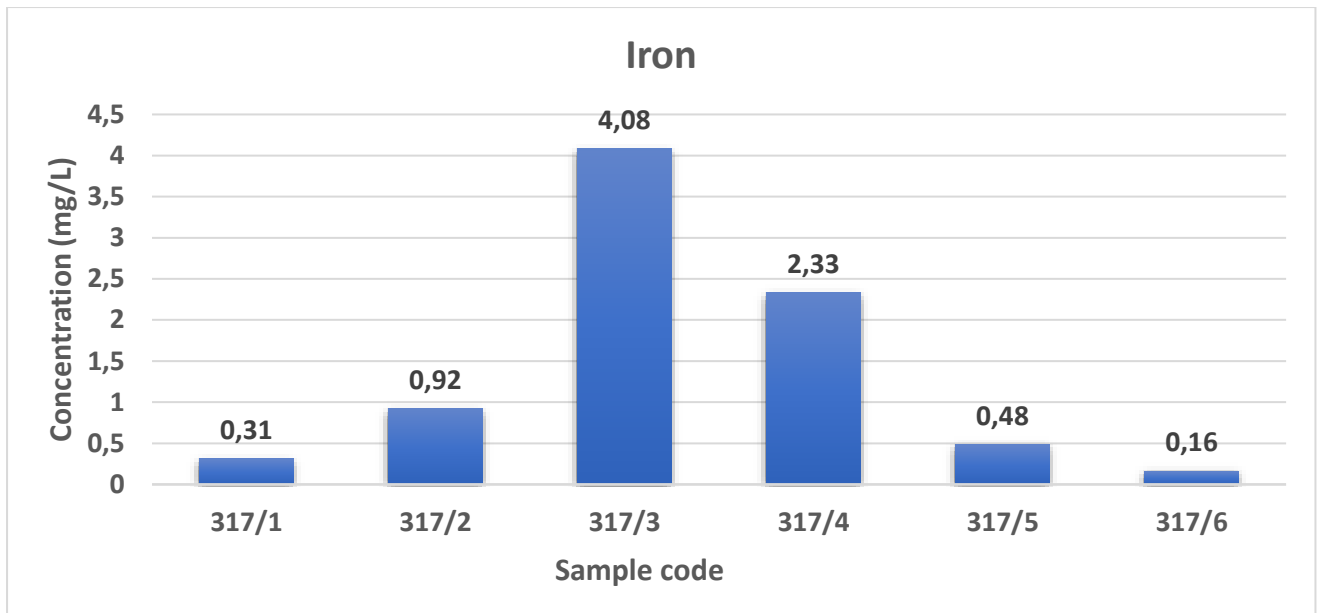
The degree of lead in drinking-water might be decreased by consumption control estimates, for example, the expansion of lime and the change of the pH in the appropriation framework from <7 to 8–9 (Lanphear *et al.*, 1998). Lead can likewise be delivered from chipping lead carbonate stores on lead pipe and from iron residue from old-aroused pipes that has amassed lead from lead sources, for example, plumbing and services connection, in any event, when the water is no longer plumbosolvency. The presence of lead in the water can lead to lead poisoning by ingesting it or breathing in dust that contains lead because you can smell and taste lead, but it is not visible to the naked eye (Davidson and Rabinowitz, 1992 ).

Introduction to lead can have a wide scope of impacts on a youngster's turn of events and conduct. Blood lead levels under 10 micrograms for every decilitre (µg/dL) are related with expanded conduct impacts, postponed pubescence, and diminishes in hearing, intellectual execution, and postnatal development or tallness. A portion of these wellbeing impacts are found even at low blood lead levels under 5 µg/dL, including lower Intelligence level scores, diminished scholarly accomplishment, and increments in both social issues and attention related practices (Asaolu and Olaofe, 2005). There is a wide scope of lead associated conduct impacts in the territory of consideration. Consideration shortage hyperactivity issue (ADHD) is one model on the more extreme finish of the range.

Lead exposure has also been connected to various wellbeing impacts in grown-ups. When in doubt, the more lead you have in your body, the more probable it is you'll have medical issues. High blood lead levels more prominent than 15 µg/dL are related with cardiovascular impacts, nerve issues, diminished kidney work, and ripeness issues, including postponed origination and antagonistic consequences for sperm and

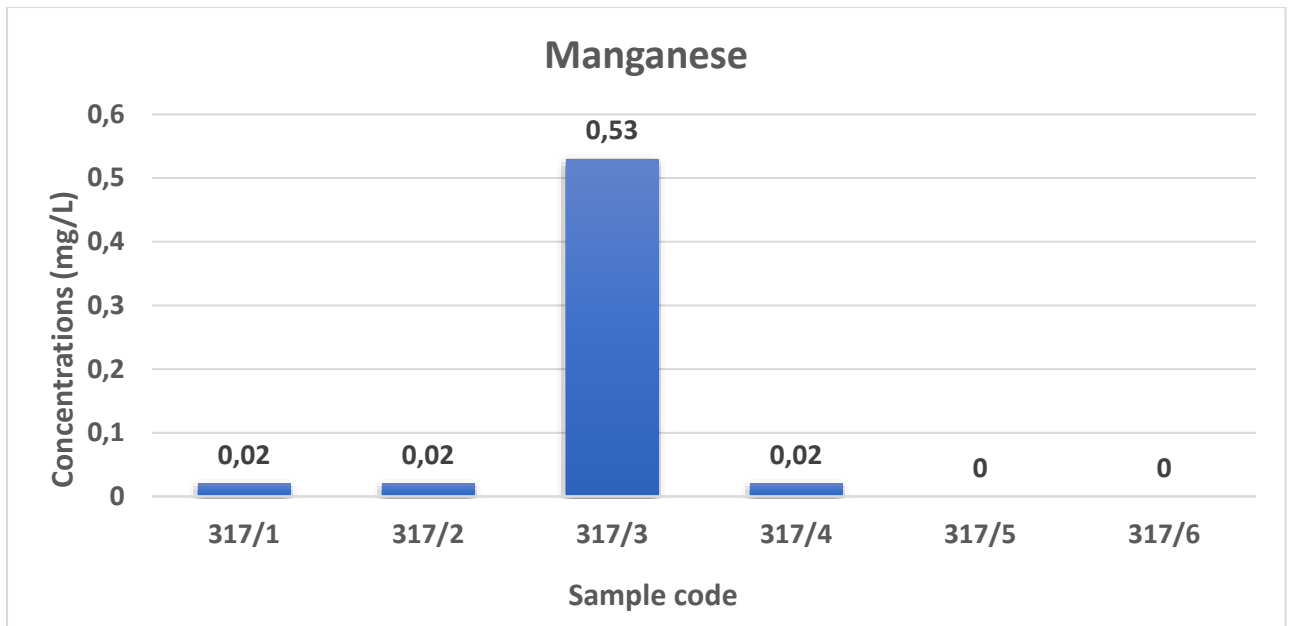
semen, for example, lower sperm counts and motility. Blood lead levels below 10 µg/dL are related with diminished kidney capacity and increments in blood weight, hypertension, and incidence of essential tremor, a degenerative issue of the focal sensory system whose most conspicuous element is tremor of the arms or hands during intentional developments, for example, eating and composing. There is moreover proof indicating that grown-ups who have low levels of introduction to lead under 5 µg/dL may have diminished kidney work (Djukić *et al.*, 2013).

Pregnant women should be especially cautious around lead. Maternal blood lead levels under 5 µg/dL are related to decreased foetal development. Since the impacts of lead are distinctive for everybody, more exploration should be never really comprehending the wellbeing impacts. A recent report, upheld by National Institute of Environmental Health Sciences (NIEHS), additionally demonstrated that lifetime lead presentation may build the danger of creating cataracts, a blurring of the eye focal point bringing about fractional loss of vision, which can be normal in more seasoned individuals. Most grown-ups with raised blood lead levels are presented to lead at work (Shavandi *et al.*, 2012). Those in occupations identified with mining, ironwork or welding, development, redesign and rebuilding exercises, smelters, terminating ranges, the assembling and removal of vehicle batteries, car radiator fix, metal shop work, and the production of stoneware or recoloured glass are especially in danger for lead presentation.



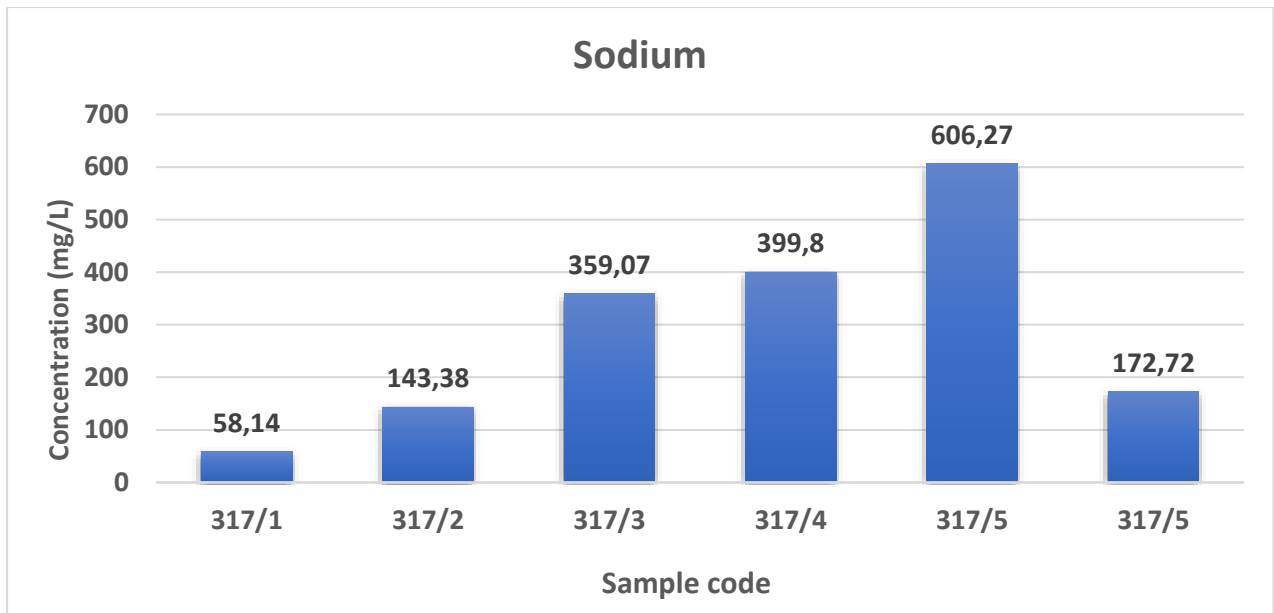
**Figure 4.18: Iron concentration measured in mg/L as units.**

Rust, in any other situation known as iron oxide, is the end result of a chemical reaction between iron and oxygen. Because time-honoured steel wool and steel is mostly made of iron, steel wool is vulnerable to rust unless it has a rust proof coating on it. The authentic rust-creating reaction occurs when two iron atoms combine in water with three oxygen atoms, the oxygen binds to the metal, and a new compound is produced. While air contains plenty of oxygen, if it just sits on a table, steel wool wouldn't rust (De Anil, 2003). To produce rust, it will certainly take hydrogen hydroxide, in any other case known to be H<sub>2</sub>O or water. When steel wool is damp, the water flows into the tiny gaps of the metal. Water acts as an electrolyte that allows the oxygen electrons to gravitate toward the iron. The water's hydrogen bond serves as an acid that combines its corrosive properties with rust. Since sodium fastens corrosion, saltwater is even more detrimental to rusting metals (Sharma and Kansal, 2011). Wastewater from samples 317/3 and 317/4 contains high levels of iron, which is from steel wool because the food preparation facilities use steel wool mostly as a cleaning essential and salt is used as part of the diet in the cooking process. The steel wool found in the pot had a chemical reaction with water and lead rust and iron oxide were formed, in which the iron was detected as an element in the water.



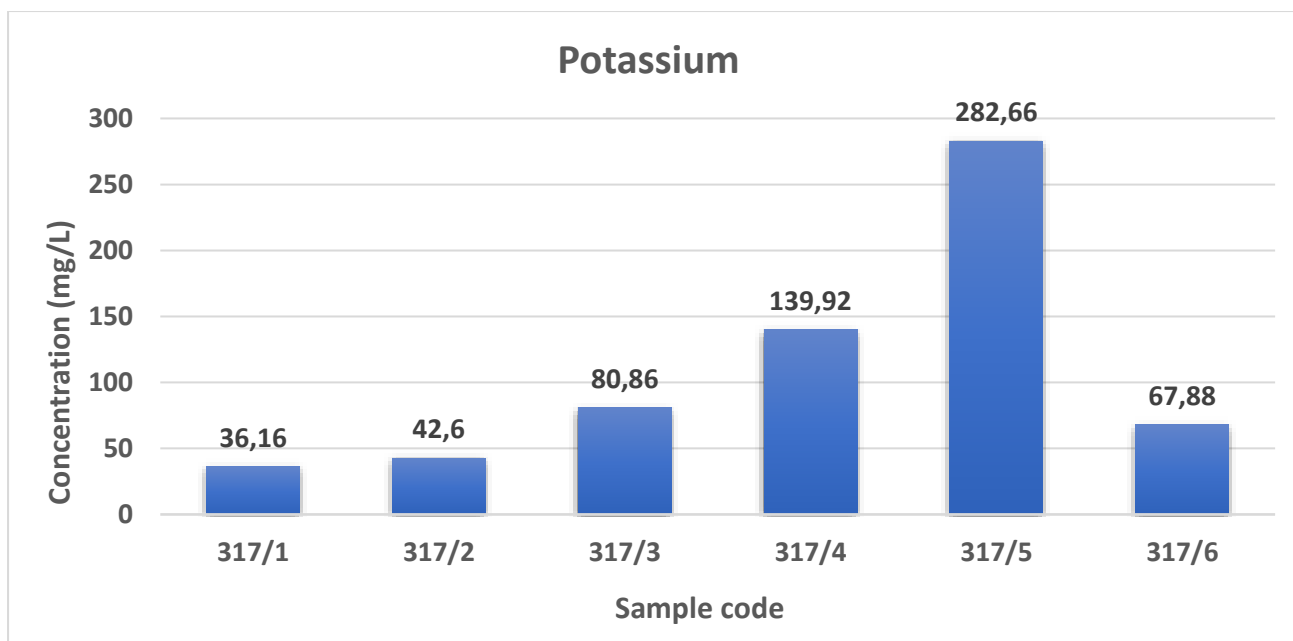
**Figure 4.19: Manganese concentration measured in mg/L.**

Metallic manganese (ferromanganese) is primarily used in steel production along with solid iron and super alloys to increase stiffness, rigidity and strength. For iron alloys, this makes manganese a matter because it is used to enhance iron toughness, hardness, and strength. Concentrations of dissolved manganese in natural waters that are essentially free of anthropogenic inputs can range from (0.1 to >10) mg/L. However, dissolved manganese concentrations in natural surface waters rarely exceed 1 mg/L and are usually less than 0.2 mg/L. The appearance of water can indicate the presence of iron and manganese. For example, reddish-brown (iron) or black (manganese) particles may be visible when water is drawn from the tap. These particles of iron and/or manganese may come from corroded pipes or from the water supply itself. The particles form because oxygen in the plumbing system is oxidizing and precipitating the iron and manganese (McFarland *et al.*, 1998). As a result, concentrations of manganese in wastewater increased with the increased concentration of iron from the sample. If iron is present in water, then manganese will also be present in wastewater.



**Figure 4.20: Sodium concentration from the samples measured in mg/L.**

Sodium is a mineral that occurs naturally in food or is added during the production process or both. It is naturally present in foods such as celery, beets and milk. Packaged and processed foods can be major culprits for sodium. Most of the salt we eat comes from processed foods such as canned soups, lunch meats and frozen dinners. Sodium is an essential mineral in our diet and is commonly found in sodium chloride (salt) form (Seldin and Giebisch, 1990). Salt has no scent and it quickly dissolves in water and gives a "salty" taste to water at levels greater than 180 mg/L. From the results obtained the detection of sodium concentration above the limit is due to the salt used in food preparation establishments. Sodium is attributed to water hazard class 2; in other words, it is a risk when present in water. Sodium chloride however is not a risk and is attributed to water hazard class 1. The sodium presence in the wastewater is the dissociation of NaCl, and its presence in the wastewater does not have an effect on the environment (WHO, 2012).



**Figure 4.21: Potassium content measured in mg/L from the samples.**

Potassium is an essential element and is present in all animal and plant tissues. The primary source of potassium for the general population is the diet, as potassium is found in all foods, particularly vegetables and fruits. Some food additives are also potassium salts (e.g., potassium iodide). Some individuals require potassium supplements, which are given under medical supervision; others take potassium supplements without supervision, although this is not recommended (Powell *et al.*, 1987).

Potassium permanganate may be used in the drinking-water treatment process. Resulting levels of potassium in water are relatively low compared with levels resulting from the use of water softeners using potassium chloride. Where potassium permanganate is used in water treatment, concentrations of added potassium can be up to a maximum of 10 mg/L, but normally concentrations would be less than this (Perazella, 2000).

Although concentrations of potassium normally found in drinking water are generally low and do not pose health concerns, the high solubility of potassium chloride and its use in treatment devices such as water softeners can lead to significantly increased exposure. From the results obtained the potassium in the wastewater cannot be attributed to the fact that potassium is found in drinking water.



The presence of potassium in sample 317/3 to 317/6 is above the maximum permissible limit of 50 mg/L of which the concentrations are (80.86, 139.92, 282.66 and 67.88) mg/L. As mentioned above that potassium levels are found in food such as fruits and vegetables. In the food preparation facilities vegetables is one of their side dishes in their menu, so during the washing process the pigmentation of some veggies is introduced in the water and that is how the potassium was introduced.

Since the high level of potassium is in wastewater, it does not have much effect on the environment because it plays a central role in plant growth and it often limits it, but at the treatment plant the high level of potassium can be reduced by using reverse osmosis in-order to avoid the high level of it to be found in tap water (Skoog *et al.*, 1992).

**4.6 in-order to know the characteristics of a specific oil, FTIR analysis is done to check the different absorption peaks found in the oil.**

Amount of FOG found in wastewater

$$\frac{Volume2}{Volume1} \times 100\%$$

$$\frac{120ml}{250ml} \times 100\%$$

$$= 48\%$$

NB: Volume 1 – the amount of wastewater plus FOG

Volume 2 – the amount of extracted FOG

**Waste Cooking Oil (WCO)**

In a modern society, oil is commonly used for the preparation of food. In today's world, oil frying method is widely used due to the contribution of good taste, attractive colour and better presentation of the food. As this method becomes increasingly popular, accumulation of waste generated from cooking oil also increases (Kulkarni and Dalai, 2005).

Improper waste management of cooking oil leads to discharge of WCO to the environment and this will cause environmental pollution particularly land and water pollution. In water, an oil layer covers the surface and prevents the dissolution of oxygen, thus causing a mass extinction of marine biota. Besides, the mixture of oil and

water increases the chemical oxygen demand (COD) of water and causes it to be poisonous due to the presence of oil degradation by-products. Carcinogenic compounds are absorbed by the sea creatures and returned to humans through the food chain (Kulkarni and Dalai, 2006; Jafari, 2010; Marjadi and Dharaiya, 2010).

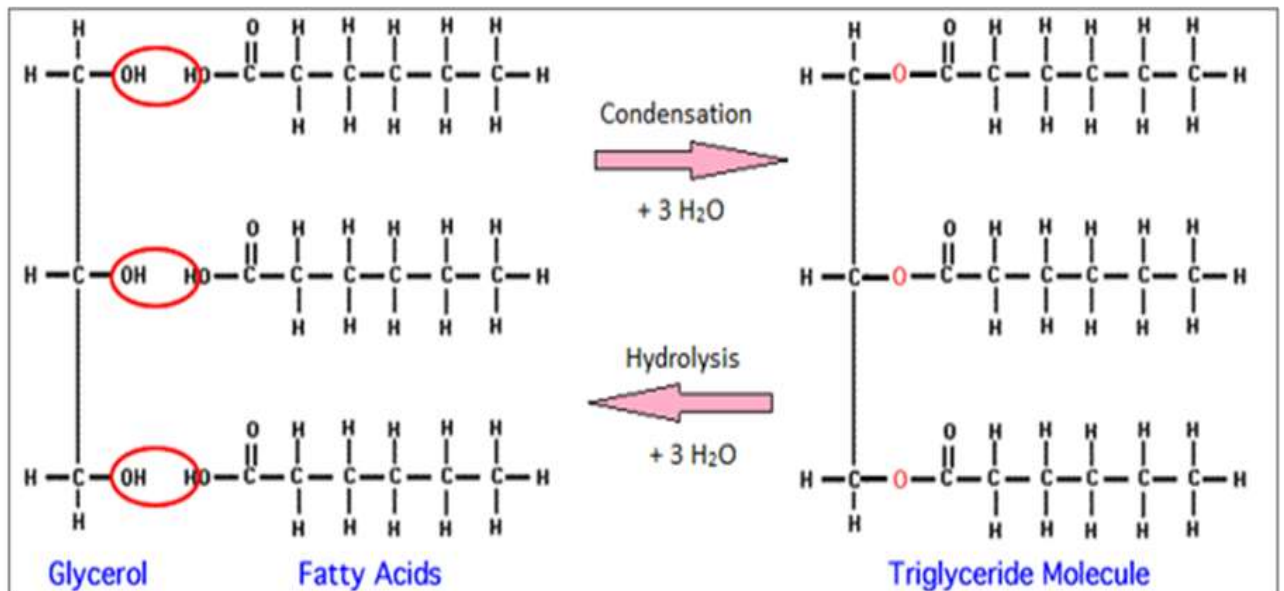
In addition, the repeated usage of WCO is hazardous to consumers because the fried oil forms toxic compounds such as peroxides, aldehyde, acrolein and polymer through several reactions such as thermolytic, hydrolysis, and oxidation (Kulkarni and Dalai, 2006). Thermolytic reaction occurs when there is no oxygen at high temperatures above 180 °C. This reaction forms by products of alkanes, alkenes, lower fatty acids, symmetric ketones, oxopropyl esters, CO and CO<sub>2</sub> which are produced from non-oxidative decomposition of saturated fatty acids (Nawar, 1984; Kulkarni and Dalai, 2006). Meanwhile, unsaturated compounds such as dehydromers, saturated dimers, and polycyclic are produced through thermolytic reaction (Nawar, 1984).

Hydrolytic reaction occurs in conditions of water presence from the food entering the oil and the water molecules cause hydrolysis of triglycerides into free fatty acids (FFA), glycerol, monoglycerides and diglycerides (Mittelbach and Enzelsberger, 1999). The product of hydrolysis can be measured from the presence of monoglycerides and diglycerides in the oil (Guesta *et al.*, 1993). Generally, polar content is used as a parameter to measure the quality of oil. Fresh cooking oil has lower polar content within the range of 0.4-6.4 mg/100 g, while after 40 sets of frying process the polar fraction increases >25% (Bastida and Muniz, 2001).

### **Fats and oils as triglycerides**

Vegetable oil, fats, and grease are chemically classified as molecules of triglycerides and are commonly used for cooking. (Maher and Bressler, 2007). Repetitive use of cooking oil under high temperatures induces deleterious changes in oil's physical and chemical properties through the hydrolysis, oxidation, and polymerization process. Reactions that occur in repeated oil cooking depend on the original oil content, type of food material, heating conditions, time, moisture, and oxygen concentration. High temperature and number of repeats reduce the quality of the oil's flavour and produce volatile or non-volatile complexes. Most of the volatile components evaporate and produce smoke which in working condition generates anoxxygenic environment due to Diels–Alder and Amadori rearrangements. The chemistry of non-volatile compounds

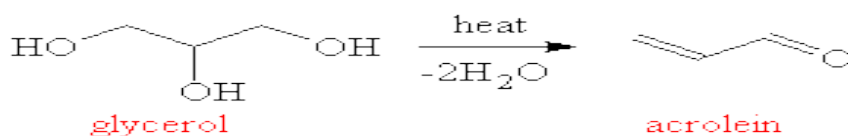
is a complex and poorly understood mechanism (Martins *et al.* 2000). The mechanism of formation of volatile or non-volatile components due to isomerization, cyclization, thermal polymerization, hydrolysis, and oxido-reduction cycle is therefore not predictable.



**Figure 4.22: A mechanism showing how triglycerides are formed (Martins *et al.*, 2000).**

#### Formation of acrolein (2-propenal)

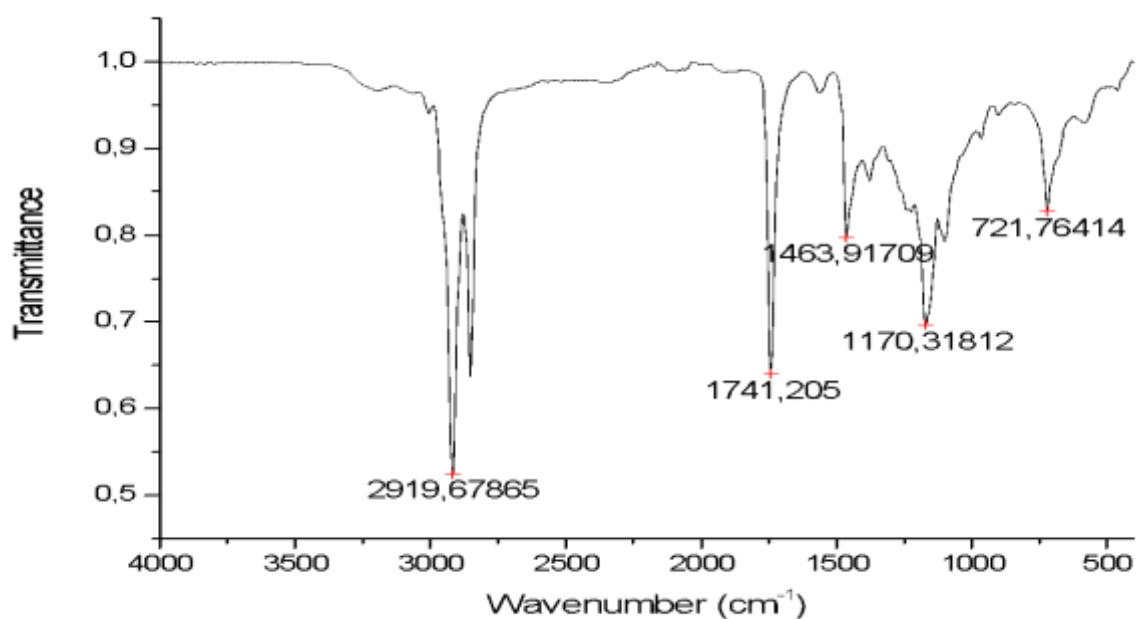
The smoke point is the temperature at which oil continuously starts to smoke and can be perceived as bluish smoke. This smoke indicates the chemical breakdown of glycerol and free-fatty acids (FFA) in the fat (Fujisaki, 2002). The glycerol is then further broken down to an acrolein (2-propenal), which is one of the bluish smoke's major constituents. This point depends heavily on the content of the free-fatty acids and partial glycerides to a lesser degree. The influence of degree of unsaturation is minimal but chain length has a significant effect; oils containing short chain fatty acids (e.g., lauric acid) have lower smoke point than oils with predominantly longer chain fatty acids. A general rule is that the higher the smoke point, the better a fat is for frying; deep-frying fats with a smoke point below 200 °C are not suitable (Zhu, 2001).



**Figure 4.23: A mechanism showing degradation of fats and oil to glycerol then production of acrolein at high temperature (Erickson, 2015).**

### FTIR Spectra

The (Figure 4.24) shows the infrared spectrum of FOG obtained using the FTIR-Spectrophotometer (name) in the region between (500-400)  $\text{cm}^{-1}$ . The prominent bands observed are indicated in the figure. Table 4.5 lists the vibrational modes of FOG that were observed after running the sample.



**Figure 4.24: An IR spectrum of the extracted FOG in summer season.**

Table 4.5 : The wavenumbers along with their functional groups from the FTIR.

Wave number (cm <sup>-1</sup> )	Functional group
2919.67865	CH <sub>2</sub> stretch
1741.205	C=O stretch
1463.91709	CH <sub>2</sub> and CH <sub>3</sub> bending
1170.31812	C-O stretch
721.76414	C=C bend

Absorption peaks for triglyceride and acrolein exist in the extracted FOG's IR spectrum. The CH<sub>3</sub> present in triglyceride causes a CH<sub>2</sub> stretching at 2919.67865 cm<sup>-1</sup> wavenumber. Due to the carbonyl group present in the triglyceride, a C=O stretch is observed at a wavenumber of 1741.205 cm<sup>-1</sup>. A bending of CH<sub>2</sub> and CH<sub>3</sub> is observed at a wavenumber of 1463.91709 cm<sup>-1</sup>, which is from the triglyceride molecule. The triglyceride bond between glycerol and fatty acids is formed by the C-O bond and the stretch is observed at 1170.31812 cm<sup>-1</sup>. At 721.76414 cm<sup>-1</sup> wavenumber there is a C=C bend observed which is present in the acrolein molecule.

From the results obtained in the FTIR analysis it is confirmed that the presence of an acrolein molecule in the Used Cooking Oil (UCO) is observed. Indeed, nobody has ever focused on what this fat and oil does to our condition. UCO is perhaps the greatest irritation of advanced civilization and a main consideration in debasement of condition, especially in the Mankweng area. Notwithstanding, that doesn't mean the provincial arrangement isn't influenced by it. It gets influenced, yet the outcomes are all the more unmistakably obvious in the Mankweng arrangement as they are all the more thickly populated (by university students) and have a ton of business foundations occupied with the food and drinks part. Enormous scope business utilization of cooking oil by the bites fabricating industry and the food and refreshments associations, for example, lodgings, cafés, food providers, street side restaurants and even the road sellers bring about the creation of many huge amounts of UCO with no arranged and logical technique for removal.

It is amusing that while our mouth waters on seeing those jalebis, samosas, potato chips or even chicken candies sizzling in the fryer, we infrequently think where they

will dump the leftover fat and oil toward the day's end. Is it going to be re-utilized or discarded in the sewer or drain? The two different ways it will hurt a person or thing. While utilization of UCO isn't prescribed for its inclination to change over into trans-fat after each rehashed use; its unabated unloading and removal into the drains or sewer has expected hurtful impacts on the environment. UCO gags and obstructs the municipal sewers as well as definitely diminishes the productivity of the Wastewater Treatment Plants.

Moreover, when this used oil ultimately ends up reaching the natural water reserves, it severely harms aquatic and marine life. It has been found to murder the fish, winged creatures, plants, and other amphibian life structures by covering them with sleek layers, truly diminishing their capacity to inhale, in this manner, gagging them to death. It is additionally a main consideration in dirtying the water bodies because of its famous ability to raise the Biochemical Oxygen Demand (BOD). With the regularly expanding mindfulness among the overall masses about the negative effects of re-utilizing cooked oil on human wellbeing, increasingly more of the UCO is getting disposed of and dumped into the environment after every last one of those luxurious galas is finished (AOCS, 1998).

It is very much imperative to care for our own health, but we do need to take care of the health of our environment, too. Unfortunately, not much thought has been dedicated towards this as far as disposal of UCO is concerned. The Food Safety and Standards Authority of India (FSSAI) has already issued a guidance note in 2018 outlining the Standard Operating Procedure (SOP) on handling and disposal of UCO. These SOPs unmistakably notice that the UCO ought not be arranged off into the city sewers or drains. Or maybe, it ought to be disposed of in an earth cordial way, ideally by giving it to the approved UCO aggregators/gatherers appropriately enrolled with approved offices, for example, State Biodiesel Board or Biodiesel Relationship of India (Canakci, 2007).

At household level, the FSSAI recommends the mixing of UCO with some absorbent material like saw dust, sand, used cloth, towel, waste-paper etc. to contain any spillage and then dispose of it off in dustbins. In any case, UCO should not be disposed of in the drains. Besides, the dumping of any environmentally hazardous material into the water bodies is a punishable offence under **The Water (Prevention and Control of**

**Pollution) Act, 1974.** Be that as it may, truly, we are certainly ailing in an exhaustive methodology, fusing all the potential stakeholders, on the utilization and removal of UCO (Jain, 1984).

UCO isn't a completely futile thing and discarding it with no thought will be rash. It has a ton of commercial value owing principally to its capacity to be reused into valuable commercial products. Next to its utilization in the production of cleansers, it tends to be handily separated into glycerine and biodiesel utilizing a straightforward synthetic cycle called 'transesterification'. Cooking oils are basically 'fatty oils or esters' that respond with an alcohol, for example, ethanol or methanol within the sight of an impetus (acid or base) to produce biofuel and glycerol, that is extremely fundamental science (Tarmizi *et al.*, 2013). Some way or another, we have not had the option to tap this high school information on an industrially practical scale.

One of the contributing components is the non-accessibility of fats and oils as crude material for biofuel or biodiesel production because most of it, especially from the disorderly food and drinks segment and individual family units goes into the drains. The little food and drink associations in the composed segment in some cases want to sell it either to the soap producers or to the sloppy food-focuses that reuse it for frying and cooking. This is essentially in light of the fact that these end users are prepared to pay some additional money when contrasted with biofuel makers. Likewise, only one out of every odd state has a State Biodiesel Board or even a Biodiesel Plant (Maddikeri *et al.*, 2012).

While the administration is en route to investigate new probabilities, the individual stakeholders can contribute their smidgen in killing the annoyance of UCO. Most importantly, they should make it a propensity to dishearten the utilization of oils, particularly for profound broiling and so forth and ought to embrace more beneficial food alternatives like prepared, steamed or even air seared indulgences. This will profit them more than the earth which for this situation, will be a true recipient. Next, they should vow not to arrange off UCO, created from their individual family units or even from the food readiness offices in the kitchen sink, drains or sewer. Instead, use an old newspaper to soak it and dispose it in the bin (Selvi *et al.*, 2013).

We can likewise contribute by teaching the small food preparation facilities around Mankweng and refreshment associations, particularly the ones working in chaotic

segments, to search for alternatives to offer any lingering oil to a recycler, however it would not be simple for them to discover one yet. The nearby local municipality can be included to arrange assortment places for UCO and the other strategy for scrapping the UCO in the containers to be gathered for reusing can be considered as opposed to tossing it down the drain.

#### 4.1.2 Enumeration of microorganisms

Microorganisms in water are, in general, not present individually but as a clump or in association with particular matter. Since the wastewater contains different types of pathogens, the approach that was adopted was to analyse for indicator organisms that inhabit the gut in large numbers and are excreted in faeces, because the presence of these indicator organisms in water is evidence of faecal contamination and therefore of a risk that pathogen is present. Microorganisms that are responsible for degrading FOG in wastewater were also taken into consideration. The tables below which are (Table 4.6-4.8 )and again with results in winter which are (Table 4.13-4.15) represent the concentration of the selected microorganisms which were analysed in the wastewater.

Table 4.6: Results for enumeration of microorganisms using a colilert test method.

<b>Sample code</b>	<b>Total coliforms</b>	<b>E. coli</b>	<b>MPN</b>
<b>317/1 (Basin )</b>	51	51	> 200.5
<b>317/2 (Bucket)</b>	51	51	>200.5
<b>317/3 (Pots)</b>	51	51	>200.5
<b>317/4 (Pots)</b>	51	51	>200.5
<b>317/5 (Bucket)</b>	51	50	>200.5 & 200.5
<b>317/6 (Basin)</b>	51	49	>200.5 & 165.2

NB: MPN: Most probable number



Table 4.7: Results for enumeration of microorganisms using Tempo method.

Sample code	<i>E. coli</i>	Total enterics	<i>S. aureus</i>	Total coliforms	<i>B. cereus</i>	Aerobic count
317/1 (Basin)	10	6.6 x10 <sup>3</sup>	0	9.1x10 <sup>3</sup>	1.2x10 <sup>2</sup>	>4.9x10 <sup>5</sup>
317/2 (Pots )	1.7x10 <sup>2</sup>	4.9x10 <sup>4</sup>	0	5.0x10 <sup>2</sup>	6.0x10 <sup>2</sup>	>4.9x10 <sup>5</sup>
317/3 (Basin)	0	3.1x10 <sup>2</sup>	0	1.2x10 <sup>2</sup>	1.3x10 <sup>3</sup>	>4.9x10 <sup>5</sup>
317/4 (Pots)	21	2.5x10 <sup>3</sup>	0	2.5x10 <sup>2</sup>	3.1x10 <sup>2</sup>	>4.9x10 <sup>5</sup>

NB: The unit of the enumerated samples is in CFU/mL.

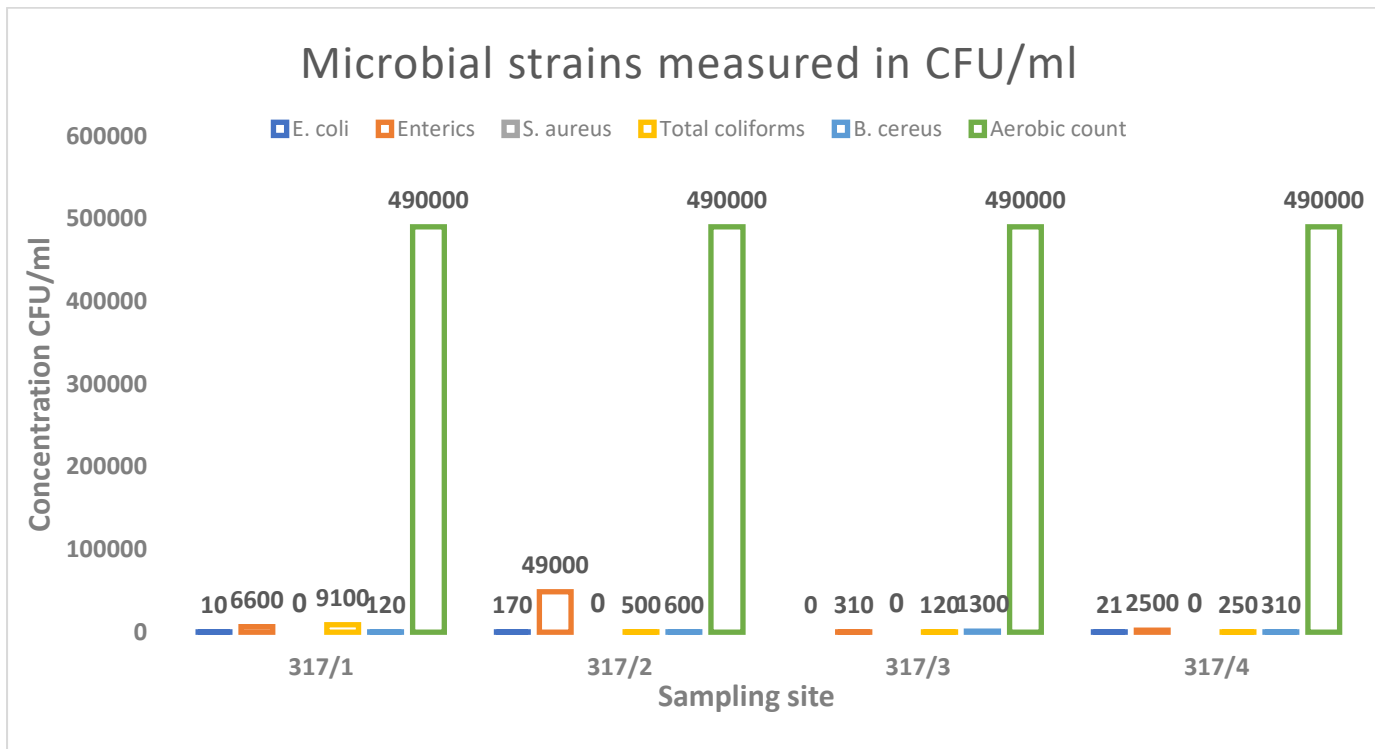


Figure 4.25: A graph showing microbial strains from the wastewater samples in concentration of CFU/mL.

Total coliform bacteria are present in the environment and faeces of all warm-blooded animals and humans. Total coliform bacteria are unlikely to cause illness, however, their presence in water indicates that disease-causing organisms (pathogens) could be in the water system (Mulamattathil *et al.*, 2015). Most pathogens that can contaminate water supplies come from the faeces of humans or animals. Total coliforms are a group of bacteria made up of primarily 18 different bacteria and are not

harmful in most cases, but if they are present in water supply it is an indicator that other harmful and disease causing bacteria may have also entered the water supply (Sobsey *et al.*, 2006). The presence of coliform bacteria in water does not guarantee that the water will cause an illness. Rather, their presence indicates that a contamination pathway exists between a source of bacteria (surface water, septic system, animal waste, etc.) and the water supply. Disease-causing bacteria may use this pathway to enter the water supply. The concentration of total coliforms in the wastewater samples ranged from (120-9100) CFU/mL and this was an indication that possibility of pathogenic microorganisms to be present in the wastewater was high as referenced in (Table 4.6).

According to several authors, faecal contamination from human and animal waste to aquatic environments is a threat to human health from exposure to pathogenic microorganisms (van Asperen Medema *et al.*, 1998). *E. Coli* is most frequently found on a small number of cattle farms where the bacteria can reside in stable cattle intestines. Millions of germs can be released from an infected human or animal within a bowel movement. *E. Coli* (O157:H7) can be found in sources of water, such as private wells, that have been polluted with tainted human faeces or animal excreta. Waste can reach water in various ways, including sewage overflows, malfunctioning sewage systems, contaminated stormwater runoff, and agricultural runoff. Wells, especially if the wells are shallow, drilled, or bored, may be more susceptible to such pollution after flooding (Mortazavi *et al.*, 2016) . From the results obtained the *E. Coli* in the wastewater ranges from a concentration of (0-170) CFU/mL, the effluents discharged during the washing of dishes and food preparation are possible sources of pathogenic bacteria including *E. Coli*.

Total *enterics* can be found at sources of water, such as private wells. Wells become contaminated when faeces from infected humans enter the water through various ways, including sewage overflows, unworkable sewage systems, and polluted stormwater runoff. Wells may be more susceptible to such pollution after flooding, particularly if the wells are shallow, have been drilled or bored, or have been for long periods of time submerged by flood water (Ray and Ryan, 2014). The concentration obtained in the *enterics* bacteria in all the samples was found to be in the range of 310-49000 CFU/ml, because *enterics* bacteria are mostly found in polluted area along with faecal contamination, the results showed that the wastewater has faecal

contamination due to the presence of *E. Coli*, of which if it is present then *enterics* bacteria are highly to be found.

*S. aureus* is a common bacterium found by about 25% of healthy people and animals in the nose and skin. *S. aureus* is capable of producing seven different toxins and often causes food poisoning. *S. aureus* is the product of contaminated food. It has a high tolerance for salt and can grow in ham, other meats, and milk products (Bhatia and Zahoor, 2007). The toxins produced by the bacteria are also heat resistant, and they cannot be destroyed by cooking. Once the food is infected, bacteria start multiplying. Alimentary items most frequently associated with *S. aureus* are dairy and cheese. And the most frequent cause of contamination is contact with food workers carrying the bacteria (Kadariya *et al.*, 2014). The bacterium in the wastewater was not found to be active, because all the samples had 0 CFU/mL concentration of the strain. The *S. aureus* as mentioned above is a bacterium mainly found in the nose and skin and in food can be found as a poison. It is known that washing hands can kill the bacteria of *S. aureus* and the food preparation facility detergents are mostly used for washing and it hinders the bacterium from growing.

*B. cereus* is one of the common contaminants found in spoiled foods and it can certainly be serious about indigestion caused by ingestion. *B. cereus* causes two distinct forms of foodborne illnesses: a diarrheal syndrome and an emetic syndrome, both by producing distinct toxins. The emetic disease is associated with the production of a preformed heat stable toxin in food while the diarrheal disease is caused by the production of toxin in the intestine (Gladwin *et al.*, 2004). The omnipresence of this group's spores makes contamination of food products a common occurrence. However specific types of foods tend to be associated with each syndrome of the disease. Desserts, meat dishes and dairy products are most often the vehicles for transmission of the disease's diarrheal type, whereas rice is the emetic disease's main vehicle (Becker *et al.*, 1994). The concentration between (120-1300) CFU/mL for this bacterium was found in the samples of the wastewater and the *B. cereus* since is one of the common pathogens found in spoiled food, the samples taken for analysis contain spoiled food which were scrapped off during the washing process which serves as growth medium as the spoilage promotes the bacterium to grow well.

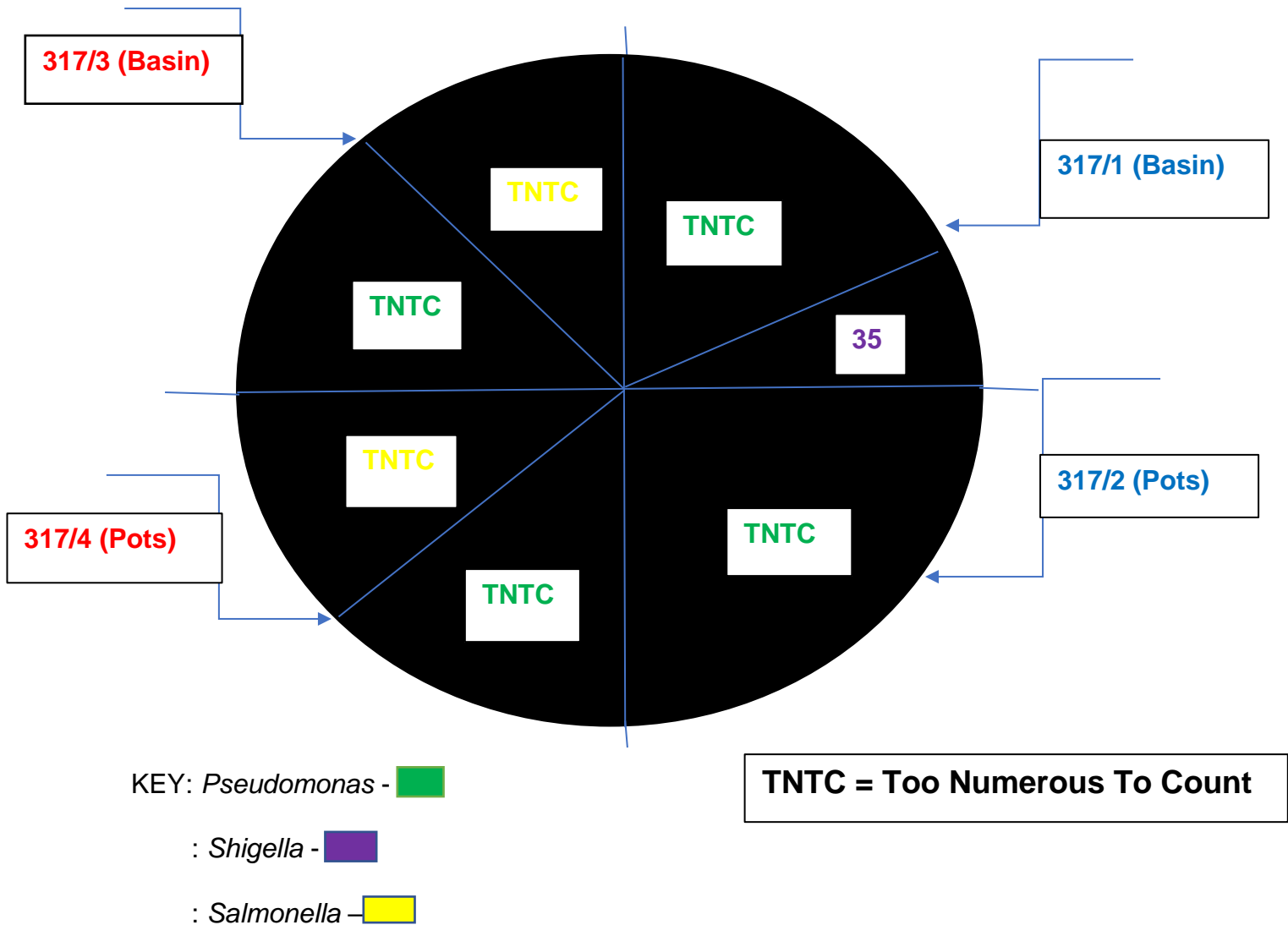
Aerobic bacteria live and increase within the presence of free oxygen. Facultative microbes consistently accomplish a vigorous state when oxygen is available. While the name "aerobic" infers breathing air, dissolved oxygen is the essential wellspring of vitality for aerobic bacteria. The digestion of aerobes is a lot higher than for anaerobes. This expansion implies that 90% less life forms are required contrasted with the anaerobic procedure, or that treatment is practiced in 90% less time (Evans, 2000). This gives various points of interest including a higher level of natural expulsion. The by-products of aerobic bacteria are carbon dioxide (CO<sub>2</sub>) and water (H<sub>2</sub>O). Aerobic bacteria live in pilgrim structures called floc and are kept in suspension by the mechanical activity used to bring oxygen into the wastewater. This mechanical activity opens the floc to the natural material while treatment happens. Following processing, a gravity clarifier isolates and settles out the floc. As a result of the mechanical idea of the high-impact absorption procedure, upkeep and administrator oversight are required (Hall *et al.*, 2005). The aerobic count from all the samples was found to be >490000 CFU/mL because in-order to degrade the organic load that was found in high concentration from the samples oxygen is required by facultative aerobes bacteria, so more oxygen was produced hence high aerobic count.

Table 4.8: Results for enumeration of microorganisms using spread plate technique.

<b>Sample code</b>	<b><i>Pseudomonas</i></b>	<b><i>Shigella</i></b>	<b><i>Salmonella</i></b>
<b>317/1 (Basin)</b>	TNTC	35	0
<b>317/2 (Pots )</b>	TNTC	-	0
<b>317/3 (Basin)</b>	TNTC	0	TNTC
<b>317/4 (Pots)</b>	TNTC	0	TNTC

Key: TNTC: too numerous to count.

The pie-chart showing results from spread plate technique



**Figure 4.26: Results from microbial analysis done using spread plate technique.**

*Shigella* finds itself in every part of the world. The bacteria can enter the water in a variety of ways, including sewage overflows, unsuitable sewage systems and contaminated stormwater runoff. Wells may be more vulnerable to such contamination after flooding, especially if the wells are shallow, have been dug or bored, or have been over long periods of time submerged by flood water (Lampel, 2009). The concentration of *shigella* from the analysis was only found at one site and in only one sample with 35 colonies. *Shigella* was not detected in other samples because the bacterium does not just grow at any environments other than sewage systems, its presence was detected due to faecal contamination from *E. coli*.

*P. aeruginosa* may not be a run of the mill *Pseudomonas* in indigenous habitats. They need a straightforward sustenance flexibly and can even fill in refined water. It can likewise fill well in a medium containing acetic acid derivation (carbon source) what's more, ammonium sulfate (nitrogen source). The ideal temperature for development is 37 °C, however *P. aeruginosa* likewise develops at temperatures as high as 42 °C . It is impervious to high groupings of salts and colours, frail cleaning agents, and numerous generally utilized anti-infection agents (Mena and Gerba, 2009). These properties of *P. aeruginosa* are basic factors for its environmental achievement, which additionally help clarify the universal idea of the living being and its unmistakable quality as a nosocomial microorganism (Wu and Li, 2015). Nonetheless, one of the factors considered in determining the general cleanliness of water distribution systems may be its existence. Its presence may contribute to degradation in bacteriological quality and is often associated with an increase in water temperature or low flow levels in the distribution system and consequent taste, odour and turbidity complaints. The colonies in *pseudomonas* were too numerous to count, because of odour and turbidity from the wastewater which contributes to high detection of *Pseudomonas* as shown in (Figure 5.5.2) It also occurs in many foodstuffs (wastewater is from food preparation facility) and may often be present in the digestive tract of humans without causing any signs of illness (Mena and Gerba, 2009). It is capable of using a large variety of compounds as nutrients, and this helps it to grow in situations where other microorganisms may be unable to survive.

*Salmonella* is found in every part of the world. Millions of germs may be released into an infected human or animal's bowel movement. *Salmonella* can be found in sources of water such as private wells that have been polluted with tainted human or animal waste. Waste can reach water in various ways, including sewage overflows, malfunctioning sewage systems, contaminated stormwater runoff, and agricultural runoff. Wells may be more susceptible to such pollution after flooding, particularly if the wells are shallow, have been drilled or bored, or have been for long periods of time submerged by flood water (Haeusler and Curtis, 2013). The wastewater from site two was more polluted than the one in site one, it had high odour, turbidity and colour was too bad and these factors can increase *Salmonella* in wastewater because its colonies were too numerous to count .

From the results above in microbial analysis it is clear that all pathogenic microorganisms are introduced by faecal coliforms and some by unhygienic processes. The FSE is in a clustered location and there is a lot of foul smell in the area, sanitary sewer overflow caused by blockages and pipes leaking which ends up being a fly breeding environment. The surrounding flies will capture bacteria from the toilet and discharge them into the FSE water systems.

Another introduction of the bacteria is through that, animals bear in their stomachs and intestines a very large number of bacteria which are excreted in their faeces. Bacteria are also present on the animals' skin, hide, fleeces and feathers, including those from direct contact with the faeces or from indirect contact with the farm's environment, vehicles for transport or lairage. The bacteria in or on animals may include those that can cause human food poisoning and identified meat hazards. Most of these bacteria do not cause disease in animal-producing meat which appears to be healthy (Lasztity, 2009). The chicken and mutton tripe sold in the FSE are bought with faeces and may contain bacteria too and this could be one of the methods of introducing all this bacterium in the wastewater.

The further processing of meat into minced meat, meat ingredients and meat products offers the opportunity for any dangerous bacteria to spread throughout the product on the surface of the carcass meat, as well as for the introduction of new bacteria from the environment, handling, and processing. In particular, bacteria will spread to the food centre, where they will be less easily destroyed when cooked. If the production process does not include a pathogen reduction step such as cooking, then there will be any bacteria present in the processed meat on the carcass meat (King, 2009). And the possibility of buying meat with bacteria is very high, because most of the FSE buy their meat from local farmers or reputable super-markets but transporting and preparation introduces these pathogens.

According to several authors, faecal contamination from human and animal waste to aquatic environments poses a serious threat to human health from exposure to pathogenic microorganisms (Asperen *et al.*, 1998). Even though the contaminated water is not used for drinking, to reuse wastewater a reliable treatment must be done to meet water with strict quality to protect public health and the environment. If proper treatment is not done at the wastewater treatment plant, these microorganisms may

find their way back into the drinking water and cause serious problems to the public health.



## 4.2 Winter results

### 4.2.1 Physico-chemical parameters

Table 4.9: Results for some parts of physico-chemical analysis using different analytical techniques.

Parameter	014/1	014/2	014/3	014/4	014/5	014/6	SANS241 Limits
	Basin	Bucket	Pots	Pots	Bucket	Basin	
<b>pH</b>	5.35±0.1	5.36±0.0 06	5.5±0.00 6	7.66±0.01	7.4±0.02	7.76±0.0 2	5-9,7
<b>TDS (mg/L)</b>	2210±6	2222±0	2210±6	3220±2	3160±2	3220±1	≤2000
<b>Turbidity (NTU)</b>	20,1±0.2	19,1±0.1	13,6±0	89±0.1	75±0	95±0.5	≤500
<b>Salinity (mg/L)</b>	1858±1	1850±2	1849±0	2460±6	2340±2	2470±6	-
<b>Conductivity (mg/L)</b>	1150±1	1120±1	1100±3	2470±0.6	2420±2	2500±3	≤170
<b>Temperature (°C)</b>	20±1	19.8±0.2	19.8±0.1	19.8±0.1	20.5±0.2	20.8±0.1	≤40
<b>Total hardness (mg/L)</b>	24.32±0. 5	123.7±0. 4	50.63±0. 2	42.01±0.0 8	27.5±0.1	173.02± 0.2	-

Table 4.10: results for anions analysis using IC as the analytical tool and ammonia.

Parameter (mg/L)	014/1	014/2	014/3	014/4	014/5	014/6	SANS241 Limits (mg/L)
	Basin	Bucket	Pots	Pots	Bucket	Basin	
<b>Fluoride</b>	ND	0.167±0	ND	0.240±0	0.237±0. 001	0.140±0	≤1,5
<b>Chloride</b>	316.18±1	304.25±1	288.44±1	93.49±2	86.79±1	92.74±0.5	≤300
<b>Bromide</b>	ND	ND	ND	ND	ND	ND	-
<b>Sulphate</b>	385.29±1	267.50±1	381.53±1	49.93±1	83.43±0. 6	30.09±0.4	≤500
<b>Ammonia</b>	5.59±0.02	5.62±0.00 1	5.42±0.02	4.51±0.0 05	4.72±0	4.71±0.03	≤1.5

Table 4.11: Results for elemental analysis using ICP-OES as an analytical instrument.

Parameter (mg/L)	014/1	014/2	014/3	014/4	014/5	014/6	SANS24 1 Limits (mg/L)
	Basin	Bucket	Pots	Pots	Bucket	Basin	
<b>Potassium</b>	195.61±1	132.48± 1	192.65±1	47.92±1	43.59±1	47,42±1	≤50
<b>Calcium</b>	21.83±0.0 4	26.13± 0.04	23.09± 0.08	6.54± 0.01	4.39±0.00 1	3.52±0.001	≤150
<b>Magnesium</b>	9.41±1	7.11±1	9.60±1	2.44±0.00 2	2,04±0.04	2.06±0.03	≤70
<b>Sodium</b>	461.90±1	380.27± 1	444.24± 1	512.03±0. 9	483.62±0. 7	513.92±2	≤200
<b>Zinc</b>	0.86±0.02	0.56±0.0 8	0.69±0.00 9	1.22±0.00 4	1.20±0.06	1.13±0.06	≤5
<b>Lead</b>	0.01±0	ND	ND	0.01±0	0.01±0	0.01±0	≤0.01
<b>Manganese</b>	0.07±0.06	0.03±0.0 09	0.08±0.01	0.04±0.1	0.03±0.00 1	0.03±0.008	≤0.4
<b>Vanadium</b>	ND	ND	ND	ND	ND	ND	≤0.2
<b>Aluminium</b>	0.22±0.00 1	0.13±0.0 3	0.03±0	0.28±0.1	0,30±0.1	0,30±0.02	≤0.3
<b>Chromium</b>	ND	ND	ND	ND	ND	ND	≤0.05
<b>Copper</b>	0.04±0	0.07±0.0 01	0.03±0.01 3	0.05±0.00 2	0.07±0.02 0	0.03±0.03	≤2
<b>Iron</b>	0.21±0.00 1	0.09±0.0 02	0.20±0.00 3	0.35±0.01	0.37±0.00 4	0.44±0	≤2

Table 4.12: Results for COD, BOD, and DO using DR 6000 and potable water meter.

Parameter	014/1	014/2	014/3	014/4	014/5	014/6	SANS241
	Basin	Bucket	Pots	Pots	Bucket	Basin	Limits (mg/L)
<b>DO (mg/L)</b>	0.89±0	0.78±0.0 3	0.78±0.00 1	0.88±0	0.69±0.00 3	0.70±0.00 8	-
<b>BOD<sub>5</sub> (mg/L)</b>	2124.31±0. 001	3222.32 ±0.008	1189.32±0 .03	1201.31±0	1984.33±0 .012	1101.32±0 .05	≤50
<b>COD (mg/L)</b>	1853±0	4709±0	7196±0	2136±0	4685±0	1784±0	≤75

#### 4.2.2 Enumeration of microorganisms

Table 4.13: Results for enumeration of microorganisms using a colilert test method.

Sample code	Total coliforms	E.coli	MPN
<b>014/1 (Basin)</b>	51	49	>200,5 & 165,2
<b>014/2 (Bucket)</b>	51	48	>200,5 & 144,5
<b>014/3 (Pots)</b>	51	44	>200,5 & 101,3
<b>014/4 (Pots)</b>	51	51	>200,5
<b>014/5 (Bucket)</b>	51	47	>200,5 & 129,8
<b>004/6 (Basin)</b>	51	51	>200,5

Key : MPN (Most Probable Number)

Table 4.14: Results for enumeration of microorganisms using Tempo method.

Sample code	<i>E. coli</i>	<i>Total enterics</i>	<i>S. aureus</i>	Total coliforms	<i>B. cereus</i>	Aerobic count
014/1 (Basin)	25	6.3 x10 <sup>3</sup>	0	7.3x10 <sup>3</sup>	2.2x10 <sup>2</sup>	>4.9x10 <sup>5</sup>
014/2 (Pots)	1.3x10 <sup>2</sup>	3.9x10 <sup>4</sup>	0	5.3x10 <sup>2</sup>	4.6x10 <sup>2</sup>	>4.9x10 <sup>5</sup>
014/3 (Basin)	32	5.1x10 <sup>2</sup>	0	1.7x10 <sup>2</sup>	1.5x10 <sup>3</sup>	>4.9x10 <sup>5</sup>
014/4 (Pots)	12	5.9x10 <sup>3</sup>	0	3.8x10 <sup>2</sup>	3.8x10 <sup>2</sup>	>4.9x10 <sup>5</sup>

NB: The unit is of the enumerated samples is in CFU/ml

Table 4.15: Results for enumeration of microorganisms using spread plate technique.

Sample code	<i>Pseudomonas</i>	<i>Shigella</i>	<i>Salmonella</i>
014/1 (Basin)	TNTC	15	TNTC
014/2 (Pots)	TNTC	0	TNTC
014/3 (Basin)	TNTC	0	0
014/4 (Pots)	TNTC	0	TNTC

Key: TNTC: too numerous to count.

Amount of FOG found in wastewater

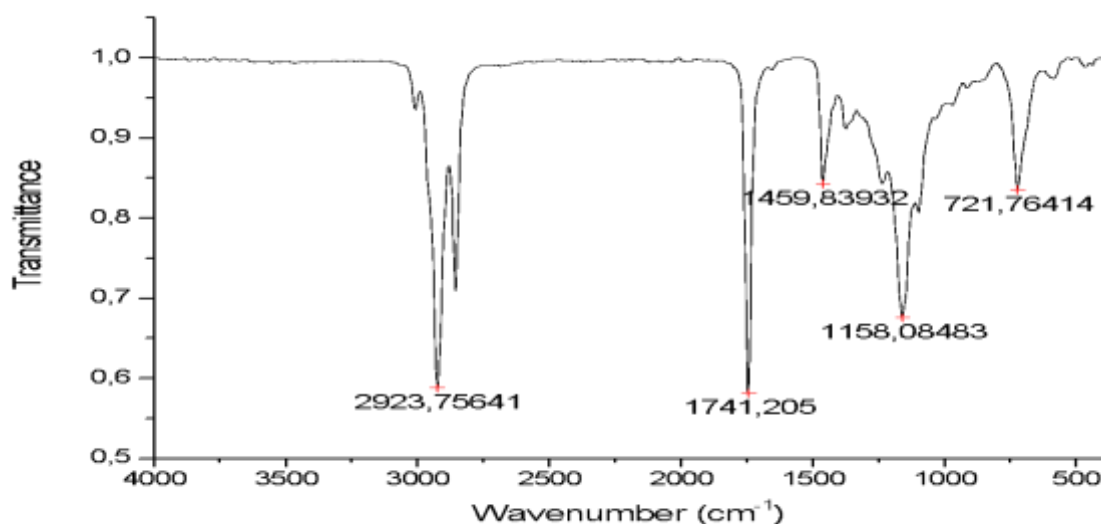
$$\frac{Volume2}{Volume1} \times 100\%$$

$$\frac{133ml}{250ml} \times 100\%$$

$$= 53.2\%$$

## FTIR Spectra

Figure 4.27 shows the infrared spectrum of FOG obtained using the FTIR-Spectrophotometer (name) in the region between 500 to 4000  $\text{cm}^{-1}$ . The sample is for the extracted FOG that was obtained in the winter season. The prominent bands observed are indicated in the figure. Table 4.16 lists the vibrational modes of FOG that were observed after running the sample.



**Figure 4.27: An IR spectrum of the extracted FOG in winter season.**

Table 4.16: Functional groups from IR along with its wavenumber at  $\text{cm}^{-1}$

Wave number ( $\text{cm}^{-1}$ )	Functional group
2923.75641	CH <sub>2</sub> stretch
1741.205	C=O stretch
1459.83932	CH <sub>2</sub> and CH <sub>3</sub> bending
1158.08483	C-O stretch
721.76414	C=C bend

From the results obtained in winter, few parameters were found to be above the standard limit, and this is due to temperature effects. Cold weather can slow down so many things, people, water and even the viscosity of some oil. During the cold season

the FOG from wastewater solidifies and when sampling was done most of it was floating on the surface of the water. The FOG when is introduced down the drain or in the sink is warm, but the moment they combine with cold water or the temperature of the water drops, most of it solidifies and they travel through the pipe-lines of which they may not travel very far through the pipes before they begin to form large, nasty conglomerations with other debris and chemicals and some of it stick to the walls of the pipes. These FOG deposits could cause future sewer backups in your home, condominium, restaurant, or other building (Tsutsui *et al.*, 2007).

Turbidity of all the samples were within the range because the wastewater samples were not too dark in order to increase its turbidity, but they contained a lot of pap and other organic matters which influenced the increase in TDS of all the samples ranging from (2210±5.774 to 3220±2.221) mg/L and this high amount of TDS has influenced an increase in both BOD<sub>5</sub> ( 1101.32±0.052 mg/L to 3222.32±0.008) mg/L and COD (1784±0.000 mg/L to 7196±0.000) mg/L by triggering the aerobic biological organisms to break down organic material present in the samples, if there is an increase in BOD in the wastewater what it does when is discharged in the rivers from sewage plants is that the increasing oxygen consumed in the decomposition process robs other aquatic organisms of the oxygen they need to live. Therefore, organisms that are more tolerant of lower dissolved oxygen levels may replace a diversity of natural water systems containing bacteria, which need oxygen (aerobic) to survive. These organisms are part of the decomposition cycle and they feed on dead algae and other dead organisms. High COD in water indicates greater levels of oxidizable organic matter and consequently, a lower amount of DO can occur. Critical DO depletion due to organic contamination can kill off aquatic life forms.

On the other hand, conductivity for all the samples was high and is due to the presence of steel wool and other related ions such as Na<sup>+</sup>, SO<sub>4</sub><sup>2-</sup>, Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, etc. in the samples of which is a good electric conductor, as mentioned from the results in summer “it is also known that water having more salt contents carries more electrical current and salt is used at the preparation facility to give taste to food and the presence of inorganic materials in the water including calcium, nitrogen, bicarbonate and others may also increase salinity”. (Hamid *et al.*, 2013). Temperature plays a significant role on pH measurements. As the temperature rises, molecular vibrations increase which results in the ability of water to ionise and form more hydrogen ions (H<sup>+</sup>). As a result, the pH

will drop. From the sample the pH values range from 5.35-7.76 (acidic), the organic and inorganic particles found in wastewater played a role in fermentation to produce acid in the wastewater.

Wastewater is a dynamic system containing both organic and inorganic compounds, dissolved compounds, and insoluble substances. Moreover, the composition of samples can change dramatically during or after the sampling (Howe *et al.*, 2012). Therefore, analyses require available, reliable, and fully-automatic methods for simultaneous determinations of several analytes. Ion chromatography offers several advantages over the classic wet methods for determinations of inorganic and organic ions in wastewater, such as: short time of analysis (10–15 min), high sensitivity and selectivity in samples with complex matrices.

The results from the anions analysis conducted which include the following: fluoride, chloride (Cl<sup>-</sup>), bromide (Br<sup>-</sup>) and sulphate (SO<sub>4</sub><sup>2-</sup>), shows that chloride had higher concentration in sample 014/1 and 014/2 with values of (316.18±1.102) mg/L and (304.25±1.327) mg/L respectively which are above the maximum permissible limit of 300 mg/L. The amount of chloride in the wastewater at the end zone can have negative effects on an ecosystem. Chloride may impact water organisms and plants by altering reproduction rates, increasing species mortality, and changing the characteristics of the entire ecosystem.

The analysis of wastewater for trace and heavy metal contamination is an important step in ensuring human and environmental health. Wastewater is regulated differently in different countries, but the goal is to minimize the pollution introduced into natural waterways. In recent years, metal production emissions have decreased in many countries due to heavy legislation, improved production, and cleaning technology. A variety of inorganic techniques can be used to measure trace elements in wastewater including flame atomic absorption spectrometry (FAAS) and graphite furnace (or electrothermal) atomic absorption spectrometry (GFAAS or ETAAS), inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). Depending upon the number of elements to be determined, expected concentration range of analytes and the number of samples to be run, in our case ICP-OES was used since it is more reliable and runs many elements at the same time (Fresenius *et al.*, 1989).



The results showed that only potassium ( $195.61 \pm 1.193$ ,  $132.48 \pm 1.012$ ,  $192.65 \pm 1.207$ ) mg/L which is above the required limit of 50 mg/L and sodium in all the samples with a range of ( $380.27 \pm 1.154$  to  $513.92 \pm 1.502$ ) mg/L of which is above the limit of 200 mg/L were detected to be above the required limit as referenced from (Table 4.10). The samples where sodium was analysed, all the samples had the highest value of which is above the required 200 mg/L SANS241 limit, the concentration range from ( $380.27$ - $513,92$ ) mg/L. Sodium is attributed water hazard class 2, in other words it is a risk when present in water. Sodium chloride however is not a risk and is attributed class 1. Since the wastewater in the study is from food preparation facilities, the presence of sodium is from dissociation of NaCl into sodium and chloride.

The introduction of microorganisms in the wastewater does not depend on the type of season. The results for winter analysis also contained some microorganisms which were also detected in summer. The FTIR spectrum for winter results in (Figure 4.2) showed the same absorption peaks of the summer one from (Figure 4.1), which confirms the presence of an acrolein.

Table 4.17: The table below shows comparison of the national and international standards limit of the concentration of the samples.

Parameter	Concentration	National standard limit	International standard limit	% rate national limit	% rate international limit	Flag	
						National	international
Ph	4.99	6-8	6-9				
Turbidity	1238 NTU	$\leq 300$ NTU	-	>100%	-	<b>RED</b>	
TDS	4043 mg/L	$\leq 200$ mg/L	-	>100%	-	<b>RED</b>	
Salinity	3390 mg/L	$\leq 2000$ mg/L	-	>100%	-	<b>RED</b>	
Conductivity	6223 $\mu$ S/m	-	$\leq 250\mu$ S/m	-	>100%	-	<b>RED</b>
Temperature	28 °C	20-35 °C	$\leq 37$ °C				
BOD5	3222.32 mg/L	$\leq 50$ mg/L	$\leq 30$ mg/L	>100%	>100%	<b>RED</b>	<b>RED</b>
COD	7196 mg/L	$\leq 100$ mg/L	-	>100%	-	<b>RED</b>	-

Fluoride	0.91 mg/L	≤1.5 mg/L	≤1.5 mg/L	61%	61%	<b>GREEN</b>	
Chloride	867.81 mg/L	≤500 mg/L	≤250 mg/L	>100%	>100%	<b>RED</b>	<b>RED</b>
Bromide	631.83 mg/L	-	-				
Sulfate	83.43 mg/L	≤500 mg/L	-	17%	-	<b>GREEN</b>	-
Ammonia	7196 mg/L	≤10 mg/L	-	>100%		<b>RED</b>	<b>RED</b>
Zinc	6.44 mg/L	≤5 mg/L	≤0.1	>100%	>100%	<b>RED</b>	<b>RED</b>
Copper	5.55 mg/L	≤1.0 mg/L	≤0.5 mg/L	>100%	>100%	<b>RED</b>	<b>RED</b>
Lead	0.02 mg/L	≤0.1 mg/L	≤0.1 mg/L	>100%	>100%	<b>RED</b>	<b>RED</b>
Iron	4.08 mg/L	≤10 mg/L	≤0.2 mg/L	41%	>100%	<b>GREEN</b>	<b>RED</b>
Manganese	0.53 mg/L	≤1.0 mg/L	≤0.05 mg/L	53%	>100%	<b>GREEN</b>	<b>RED</b>
Sodium	606.27 mg/L	-	-	-	-		
Potassium	282.66 mg/L	-	-	-	-		

**Red** → Above the maximum value

**Green** → Below the maximum value

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## **CHAPTER 5: CONCLUSION AND RECOMMENDATIONS**

### **5.1 Disadvantages of method used to prevent FOG from entering the sewer collection system**

Problems associated with FOG (lipids) discharges from restaurants have not been the concern of the municipalities in older days. The main focus was on how to manage industrial waste discharges and sewer line rehabilitation of which the presence of FOG affects them. With the increased pressure on treatment facilities, it is now time to engage capital to tackle this form of waste as it impacts very negatively on the wastewater system. Through minimizing FOG's contribution to the collection system, some projections or even more will reduce the risk of sewer line blockages and sewage back up into service laterals by as much as 50% by some estimates or even more. Solvents, caustics, and acids used may dissolve the FOG and take it out of the facility but may have harmful effects on the treatment system. These may also pose a potential danger to employees of the municipal sewer system, and the use of such chemicals for FOG control in the sewer collection system should be completely forbidden and there is little knowledge about it (Bligh and Dyer, 1997).

Many people are swearing by enzymes while others are swearing at them. Basically, an enzyme is a protein that acts on a compound and breaks it up into several smaller fragments. Enzymes are compound-specific, there are also enzymes that are present in FOG (selectivity) only to function on the compounds (WJ, 1959). Although the actual enzymatic action is quite complex, the end result is that the fatty acids are removed from the base of glycerol and this will allow the FOG to dissolve and pass forward.

Nevertheless, enzyme reactions are all reversible chemical reactions because the free fatty acids will re-join the base of glycerol and become a complete FOG with the same characteristics that it once had. While this may be helpful for the restaurant owner as the grease interceptor or trap may not need to be drained as frequently, the FOG problem will only be transferred downstream and will reappear in the drain. In fact, enzymes do not repeat themselves, they will be done in combination with the dissolved FOG and, as a result, enzymes must be regularly incorporated into the grease interceptor/trap in-order to have a continuous operation and maintenance component of the restaurant management, but the issue of FOG build-up will be directed to the local sewage network (Brooksbank *et al.*, 2007).

Again, detergents are also not an effective treatment option, they may break up FOG deposits but in the sewer collection system, the FOG may re-congeal further downstream. This can clear blockages from the drains of the restaurant but causes other issues for the lines along the road leading to the sewer system. Using bacteria to degrade/consume FOG can only be effective when using and applying the proper microorganisms through a highly developed service system. Bacteria items usually sold with "do it all" promises, generally give a false sense of safekeeping to facility managers. Most distributors of biological liquifiers, enzymes, and other related products say that their products will remove ever again the need to pump a grease trap, in-order for selling their products. Some of the products claiming to be bacterial products are essentially inactive types of bacteria that are packed into solvents including toluene, surfactants, kerosene, terpene and so on. In reality it is the solvent that dissolves the FOG, not the bacteria. This can push it out of the grease trap of the food processor, but again in the sewage collection system the same as above will re-congeal (Keener *et al.*, 2008).

## **5.2 Conclusion**

The FSEs produce too much waste (porridge, gravy, meat, baked goods, etc) of which all these wastes are introduced in wastewater, when all this organic load is introduced in the wastewater it triggers a lot of physico-chemical properties, of which at a later stage their concentrations increase causing a damage to the sewer system. From the results obtained, it clearly shows that the main problem here is FOG, since well the summer season results in which the temperatures are high and it allows the FOG to emulsify better in the wastewater, and has many elements found which are harmful to both the sewer system and the ecosystem. In hot conditions, the FOG is able to melt more easily in the wastewater, in which some of it sticks to the pipes line during its flow into the sewer system and contributes to corrosion resulting in release of metals such as iron as shown in the results in (Table 4.4) of which the concentration of iron is above the limit of 2 mg/L in sample 317/3 and 317/4 with values of ( $4.08 \pm 0.210$  and  $2.33 \pm 0.045$ ) mg/L respectively.

Although the study was based on FSEs, both residential and commercial property in the wastewater system contribute significantly to the FOG. In this regard, a successful awareness, registration, inspection, and compliance system will lead to significant reductions in the blockage and sewer overflows associated with FOG. The study

focussed on a very small percentage of the whole fast food preparation and distribution within the Mankweng region, but the results are quite alarming, the conclusion therefore indicates that we are in trouble and sitting on a catastrophically time bomb for environmental health. The combination of source control and operation and maintenance measures is required for an effective FOG management programme. While FOG is a major problem in the wastewater system, there is an opportunity to recover these materials and use them beneficially in processes that will improve both the environment and wastewater treatment efficiency encouraging recycling. Central to this is informing and enabling communities and companies to think of FOG rather than waste as a valuable commodity. The most cost-effective option for FOG recovery seems to be collection at source. In conjunction with other products, anaerobic digestion of recovered FOG and conversion to biodiesel can pose opportunities to reduce the cost of running wastewater treatment work while minimizing landfill load.

The present study has shown that the wastewater samples are significantly contaminated with faecal coliforms because the concentration of microbes from *E.coli* with an MPN >200.5 in almost all the samples, *pseudomonas* was TNC, meaning the colonies were too much to count and total coliforms at the highest concentration of  $9.1 \times 10^3$  CFU/mL which confirmed that the wastewater was contaminated with faecal coliforms. The physico-chemistry of the water reveals that the water is not suitable to be introduced down the drain due to the organic load (FOG) it contains which causes a lot of damage. The presence of the FOG in a form of acrolein in the wastewater changes the physical state of the water, for example the DO oxygen in day one was found to be around (0.67-0.69) mg/L but as the water was incubated for 5 days in order to measure the BOD<sub>5</sub>, the concentration raised with a huge number (1101.32-3222.2 mg/L) due to the influence of the waste available in the water which needed to be degraded, when comparing the concentration of BOD<sub>5</sub> to the national and international standard limit of which is  $\leq 50$  mg/L and 30 mg/L, the flag falls within the red flag which shows that its concentration is way above the standard limit. When it comes to the elemental parts, elements such lead (0.02 mg/L), zinc (6.44 mg/L) and manganese (0.53 mg/L) just to mention a few were detected to be above the limit and their rise in concentration was influenced by some of the parameter like pH and conductivity. Comparison of national and international standard limits to the following elements shows that all of them are within the red flag as shown on the table above.

The results show that the wastewater is too bad to be introduced down the drain, because they don't meet the SANS241 standard limits and even on the national and international standard limits they reach the red flag as displayed in the table above (Table 6.1). Most if not all the physicochemical parameters including TDS, turbidity, conductivity, COD, chloride, ammonia, zinc, copper, iron to mention few are above the required standard limit. When comparing the results obtained in summer and winter, during summer season more waste was produced related to dissolution of FOG in the wastewater due to temperature effect, because the FOG could emulsify better in the wastewater than in winter season where it could solidify fast and the interaction between oil and water was low (As results stated in Table 4.1,4.2, 4.8 and 4.11). Although the physicochemical properties showed a significant difference between the two seasons, the microbial part showed that the growth of microbes does not depend on the temperature, as long as there is faecal contamination in the wastewater microorganisms will grow.

Flies in an FSE present a genuine cleanliness hazard and are known to spread various illnesses including typhoid, cholera, and TB. Tragically, because of the idea of their business, restaurants will consistently need to manage a type of fly issue. Floor and sink channels are the most widely recognized and significant reproducing flies for some kinds of flies found in FSE. Drain flies, natural product flies, phorid flies are for the most part species we find normally in FSE.

Authorized exterminators approach items that are very powerful for treating favourable places for flies. While treating for flies they clean depletes completely utilizing biodegradable froth to convey the treatment in difficult to get territories. Bacterial medicines can be utilized to dispose of FOG totally clearing out fly harbourage regions in difficult to get places. But all these methods are expensive and require time and the cheap method that can be used is to wipe everywhere using detergents to avoid flies and to manage the FOG by scrapping it in the bin before washing the dishes and FOG can be recycled for production of biodiesel in the future. As per microbial aspect proper purification technique should be adopted at the treatment plan in-order to avoid the microorganisms to be introduced back into the freshwater. Exercising good habits when it comes to disposal of FOG after cooking can be all of the prevention needed to be practised at the FSE. Further studies are required in order to quantify and qualify

the specific FOG's chemical reactions that occur, whether at the restaurants, in the grease interceptor, or along the sewer pipes (He *et al.*, 2011).

### **5.3 Recommendations**

The following FOG management recommendations are based on proven strategies currently in use in other countries and are divided into two categories: knowledge, awareness, and engagement; licensing, tracking, and implementing. What is critical from this research is that pre-treatment is crucial before the waste is discharged into the sewer system.

#### **5.3.1 Education, awareness, and engagement**

It was recognized that education and awareness are central to any approach to FOG management. Using posters, stickers and FSE information packs which provide simple graphical instructions for best practice in FOG management can be useful tools to reduce FOG discharge. Initially these could be given to FSEs in problematic areas where the study was conducted to understand current conditions. Manual distribution of this material by a 'FOG manager' would also allow a partnership with the company to be formed and it can be successful in other ways as it offers a single point of contact for the business. The same visit could be used to evaluate premises using grease traps, or just to see if there are any grease traps.

Although educating individual businesses is important, teaching potential employees and employers will provide benefits over the longer term. Another effective approach could be to engage with educational providers to ensure that all catering, food handling and food management courses cover best management practices for FOG. Residential FOG sources are also of great concern. Targeted programs seem to offer the best way to raise public awareness of FOG disposal related issues. Often, a training program targeted at schools can be effective in getting messages into households and can be a useful approach. Even Radio talk shows and high school competitions about waste disposal could aid in bringing more awareness.

#### **5.3.2 Licensing, monitoring, and enforcement**

While current legislation does not require FSEs to obtain a consent to trade waste before discharging FOG waste into the public sewer, licensing can be an extremely effective measure for controlling FOG. The best approach might be to move towards some form of consent or licensing requirement for all FSEs or for all FOG dischargers.

The scientific knowledge and conclusion derived from this research can then be used for drafting of both national and local legislatures for waste disposal. This can also influence the formulation of local municipality by-laws concerning FOG control.

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## 5.4 Conference presentation

- Rapeta, M.G., Mulaudzi, V.L., and Masoko, P. Synchronised physical, chemical and microbial analysis of wastewater from University of Limpopo food-court., Faculty of Science and Agriculture (FSA) 10<sup>th</sup> Postgraduate Research Day, 19 September 2019, at The Ranch Hotel, Polokwane.
- Rapeta, M.G., Mulaudzi, V.L., and Masoko, P. Synchronised physical, chemical and microbial analysis of wastewater from University of Limpopo food-court., South African Chemical Institute Young Chemists Symposium (SACI-YCS), 31 October 2019, at Research Conference Centre, University of Venda. *Poster presentation.*

### Appendix

#### Calculating amount of FOG found in wastewater

$$\frac{Volume2}{Volume1} \times 100\%$$

$$\frac{120ml}{250ml} \times 100\%$$

= 48%

NB: Volume 1 – the amount of wastewater plus FOG

Volume 2 – the amount of extracted FOG

#### Calculation to show the standard deviation was calculated.

Standard Deviation, s: 0.034641016151377

Standard Deviation, s: 0.034641016151377

Values : 6.12, 6.12, 6.18

Count, N: 3

Sum,  $\Sigma x$ : 18.42

Mean,  $\bar{x}$ : 6.14

Variance,  $s^2$ : 0.0012

Steps

$$s = \sqrt{\frac{1}{N-1} \sum_{i=1}^N (x_i - \bar{x})^2},$$

$$s^2 = \frac{\Sigma(x_i - \bar{x})^2}{N - 1}$$

$$= \frac{(6.12 - 6.14)^2 + (6.12 - 6.14)^2 + (6.18 - 6.14)^2}{3 - 1}$$

$$= \frac{0.0024}{2}$$

$$= 0.0012$$

$$s = \sqrt{0.0012}$$

$$= 0.034641016151377$$

**COOK IT...**



**CAN IT...**



**TRASH IT!**



**Fats, Oils and Grease clog pipes.  
Trash the FOG!**

*Figure 5.1: This can be the most effective method to remove FOG from both the sewer system and in grease traps to the FSEs that have the traps.*

**Always remember, every effort, however small, counts.**