# Assessment of Bacteriological and Physical-Chemical of Drinking Water Quality in Tukarh, Libya

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**الملخص :**

تم اعداد هذا البحث لتقييم مدى نقاوة وجودة مياه الشرب المزودة للمواطنين في منطقة توكرة وضواحيها, حيث تم جمع 21 نموذج من مياه الشرب من مصادر مختلفة شملت اغلب مصادر مياه الشرب في المنطقة حيث تتنوع فيها مصادر مياه الشرب. جمعت ستة نماذج من مياه محطة التحلية من أماكن مختلفة وكذلك خمسة نماذج من خزان توكرة الرئيسي الذي يتم فيه تجميع المياه من ستة ابار ليتم ضخها للمؤسسات الحكومية في المنطقة وللمنازل اما باقي النماذج كانت تتضمن أبار عميقة تحت داخل المؤسسات (مستشفى توكرة القروي و جامعة توكرة, الشركة الرومانية), وسبعة نماذج من الابار الخاصة والأقل عمقا وحديثة الإنشاء. تم دراسة الخصائص الفيزيوكيميائية والميكروبية ومقارنتها مع المعايير الدولية لجودة مياه الشرب WHO ومعايير الجودة الليبية القياسية .

وشملت الفحوصات الميكروبية تقدير العدد الكلي للبكتيريا الهوائية Abtc, و العدد الكلي لبكتيريا القولون Tc , و بكتيريا القولون البرازية Fc, كما تم الكشف علي البكتيريا المعزولة من النماذج عند درجة حرارة 37 درجة مئوية و 45 درجة مئوية وتعريفها بالطرق البيوكيميائية , وبعضها الخر عرف بجهاز الفونكس اما الفحوصات الفيزيائية والكيميائية شملت قياس كل من درجة الحرارة والرقم الهيدروجيني والعكارة و الكلوريد المتبقي و القلوية الكلية, المواد الصلبة الذائبة , الموصلية الكهربائية, العسر الكلي و الكالسيوم والماغنيسيوم والحديد والفلور والبوتاسيوم و الامونيا , النترات و النيترات.

اظهرت النتائج ارتفاع معدلات قيم وتركيز الخصائص الفيزيائية والكيميائية للنماذج التي جمعت من الابار الخاصة وآبار التجميع مقارنة مع النماذج التي جمعت من محطة التحلية بوترابة , كما اشتركت نماذج الابار الخاصة والعامة بتسجيلها اعلى قيم من الناحية الميكروبية وسجلت نماذج في حين اشتركت النماذج ( p = 0.026)(الابار الخاصة اعلى المعدلات وبفروقات معنوية عالية التالية: البكتيرية الأنواع عزل تم التابعة لمحطة التحلية بتسجيلها ادنى المعدلات, وقد *Escherichia coli, Staphylococcus aureus, Staphylococcus albus, Pseudomonas aeruginosa, Cedecea lapagei, Stenophomonas maltophilia, Citrobacter freundii, Streptococcus anginosus, Ochrobacterum anthropi* .

كما اظهرت نتائج الدراسة ان هناك ارتباط موجب عالي المعنوية بين العكارة و الموصلية الكهربائية(r = 0.993), العكارة و TDS (r = 0.992) ,ا العكارة وا لكالسيوم (r = 0.907) , العكارة و العسر الكلي (r = 0.982), و بين الموصلية الكهربائية مع الكالسيوم (r = 0.950)و العسر الكلي (r = 0.997) و TDS(r= 1.00), وارتباط موجب عالي المعنوية بين TDS و العسر الكلي(r = 0.997) وبين TDS وا لكالسيوم (r = 0.952) .

جامعة بنغازي قسم النبات

# التقييم البكتريولوجي والفيزيائي-الكيميائي لجودة مياه الشرب لمنطقة توكرة, ليبيا


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## Dedication

To big heart at support my dear father , to each the following in of god and messenger my mother, and a lot a lot of thanks to my husband **Abdel Rahim** who help me too much and give me his time and support, to those who have demonstrated me whets beautiful and nice my sisters and brother, too my friend **Mariam** who encourage me and help me in a lot of things.

## Summary

The aim of present study was to evaluate the drinking water quality in 21 drinking water sources category in three levels ( Patrick *et. al.*,2011) in Tukrah town at Libya. Samples of water were collected from each source for both bacteriological and physical-chemical examination.

The results show there were significant difference between the three levels 1, 2 and 3 for total coliform and faecal coliform bacteria with p-vales (0.026) and (0.003) respectively. Presence of total coliform and faecal coliform bacteria was not reported from level 3 and was zero MPN per 100 ml, and total heterotrophic bacteria counts was found between $0.7\times10^3$ and $2.3\times10^3$ CFU per ml. However, the presence of coliform bacteria was reported from levels 1 and 2. The high contamination by total coliform and faecal coliform bacteria were found in level 1 in the range from 14 to 350 MPN/100ml, <2 - 21 MPN/100ml respectively, and from $71\times10^3$ to $275\times10^3$ CFU/ml for total heterotrophic bacteria counts. In contrast, the low contamination by both total coliform and faecal coliform bacteria were found within the level 2 and the range was from <2 to 220 MPN/100 ml, <2 to 26 MPN/100 ml respectively, and from $9\times10^3$ to $68\times10^3$ CFU/ml for total heterotrophic bacteria counts.

On the other hand, the biochemical identification process using Phoenix™ identified technique the six isolated strains as *Psudomonas aeruginosa* (S10), *Streptococcus anginosus* (DW2), *Stenotrophomonas maltophilia* and *Cedecea lapagel* (DW4), *Ochrobacterum anthroi* (DW10) and *Citrobacter freundii* (DW9). with confidence

value identities of 95%, 91% and 99%, 90%, 90% and 99%, respectively. The findings showed that water from these Levels 1 and 2 did not conform to the world health organization (WHO) standard in terms of suitability of drinking purpose.

Chemical analysis illustrated that there were no significant deferent between the three water levels 1, 2 and 3 for all of C, pH, Turbi, $NH_4$ and Fe and (p-vales > 0.05). While, the slightly significant deferent was found only for Rcl (p-vale 0.08). In addition, the significant deferent between water levels were found for EC, TDS , TH, $Mg^{2+}$, NaCl, $Na^{+}$, $Cl^{-}$, $SO_4$, $NO_3$-N (p-vales 0.014 - 0.05). However, the highest significant deferent between water levels were also found for alkali- (p-vale 0.008), $Ca^{2+}$ (p vale 0.002), F (p-vale 0.002), and K (p-vale 0.000). Also, the correlation coefficients were found significantly high between all of EC and Turbidity, EC and TH, TDS and Turbidity, TDS and TH, NaCl and $Cl^{-}$( r- 0.99). And between TH and Turbidity, EC and $Ca^{2+}$, TDS and $Ca^{2+}$, TH and $Ca^{2}$ , $Na^{+}$ and $Cl^{-}$, NaCl and $Na^{+}$ (r- 0.95-0.98). Also between $Ca^{2+}$ and Turbidity, $SO_4$ and $Mg^{2}$, $SO_4$ and $Ca^{2+}$, $NO_3$ and $Mg^{2+}$ (r- 0.85-0.91).

Despite, there was no significant correlation coefficient between Rcl and all elements between water levels, however, there was clearly relationship between Rcl and total coliform bacteria. Coliform bacteria was absent when Rcl present for the first time in level 3 compared with other two levels 2 and 1, Rcl did not present and coliform bacteria was high. On the other hand, there was no clearly significant relationship between total coliform bacteria and slight increase of turbidity in all water levels.

All chemical parameter was high in water Level 1 compared with other two levels 2 and 3 and then decreased with increase the levels. Chemical parameter in Level 3 was less than guideline values that recommended by WHO and EPA for drinking water. However, levels 1 and 2 were found high than recommended for all of EC (5.23 and 1.63 times), TDS (5.39 and 1.69 times), TH (3.27 and 1.14 times), $Ca^{2+}$ (1.43 and 1.24 times), $Cl^{-}$ (36.8 and 4.30 times), $Na^{+}$ (8.69 and 5.11 times) respectively. $Mg^{2+}$, K and $SO_4$ were also high only in level 1 (2.46, 1.45 and 2.03 times) respectively.

In all three water levels, ammonium and nitrate were less than guideline values (1.5 mg/l and 50 mg/l respectively) that recommended for drinking water. But, ammonium was high in two samples in level 1, DW6 (2.7 time) and DW12 (3.5 time), and nitrate was found high in samples, DW7 (1.02 time) and DW11 (1.77 time). However, nitrate ($NO^{-}_3$ -N) were also higher than Baseline concentrations of nitrate in groundwater non polluted and are typically below 2 mg/l.

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# Abbreviations

| | |
|---|---|
| Abtc | Aerobic bacterial total count |
| ANOVA | Analysis Of Variance |
| API | Analytical profile index |
| $C^o$ | Degrees Celsius |
| Ca | Calcium |
| cfu | Coli form unit |
| Cond | Conductivity |
| DW | Deep well |
| *E.* coli | *Escherichia coli* |
| EC | Electrical Conductivity |
| EDTA | Ethylene Diamin Tetra Acetic acid |
| K | Potassium |
| L.S.D | Least Significant Difference |
| FC | Fecal Coliform |
| HPC | Heterotrophic plate count |
| Mg | Magnesium |
| MPN | Most probable number |
| Na | Sodium |
| NH3 | Ammonia |
| NO3 | Nitrate |
| NTU | Nephelometric Turbidity Unit |
| pH | Power of hydrogen |
| PHO | Provincial Health Office |
| Rcl | Residual Chlorine |
| S | Station |
| Sal | Salinity |
| SO4 | Sulphate |
| Std. Deviation | Standard Deviation |
| SPSS | Statistical package for social sciences |
| TC | Total Coliform |
| TDS | Total Dissolved Solids |
| TH | Total Hardness |
| Turb | Turbidity |
| UN | United Nations |
| Vol/vol | Volume/volume |
| WHO | World Health Organization |

# CHAPTER ONE

# 1. Introduction

Water is one of the most important and abundant compounds of the ecosystem. All living organisms on the earth need water for their survival and growth (Kakaraddi *et al.*,2014). As per World Health Organization standards, drinking water should not contain any microorganisms known to be pathogenic or any bacteria indicative of faecal pollution (Gopinathl *et al.,* 2012). But due to increased human population, industrialization, use of fertilizers in the agriculture and man-made activity it is highly polluted with different harmful contaminants, Therefore it is necessary that the quality of drinking water should be checked at regular time interval, because due to use of contaminated drinking water, human population suffers from varied of water borne diseases (P.N *et al*., 2012). Sewage is one of the most general sources of drinking water pollution increase entry the distribution system of drinking water through leakage, change the physiochemical properties of drinking water spread disease causing microorganism and Different types of pollutant emit from waste water discharge which include household chemicals such as insect repellents, surfactants and pharmaceuticals (Roohul-Amin *et al.,* 2012).

Good quality of water resources depends on a great number of physical chemical parameters and biological characteristics, To asses that monitoring of these parameters is essential to identify magnitude and source of any pollution load ( Thirupathaiah *et al.*,2012). Thus many infectious diseases are transmitted by water through faecal oral contamination (Isa *et al.,* 2013). Diseases contacted through

drinking water kill about 5 million children annually and make Sixth of the world population sick (Shittu *et al.*, 2008 ). Water pollution results in transmission of infectious diseases such as dysentery, cholera, diarrhea, typhoid, shigellosis, salmonellosis, and varieties of other bacteria as well as fungi, viral, and parasitic infection (Okereke *et al.*, 2014).

Although poor sanitation and food are the main sources for contamination with pathogen of gastrointestinal tract, drinking water is the major source of microbial pathogens in developing regions (Yasin *et al.*, 2015), People affected by diarrheal diseases are those with the lowest financial resources and poorest hygienic facilities. Children under five, primarily in Asian and African countries, are the most affected by microbial diseases transmitted through water (Seas *et al.*, 2000). Acute microbial diarrheal diseases are a major public health problem in developing countries. People affected by diarrheal diseases are those with the lowest financial resources and poorest hygienic facilities. Children under five, primarily in Asian and African countries, are the most affected by microbial diseases transmitted through water ( Dimri, *et al* ., 2014).

Indicator organisms are commonly used to assess the microbiological quality of surface waters and faecal coliforms (FC) are the most commonly used bacterial indicator of faecal pollution they are found in water that is contaminated with faecal wastes of human and animal origin.(Antony *et al.*, 2012). *Escherichia.coli* is the most common coliform among the intestinal flora of warm-blooded animals and its presence might be principally associated with fecal contamination.( Rompre *et al.*, 2002). In

general terms, *E. coli* survives for about 4-12 weeks in water containing a moderate microflora at a temperature of 15-18°C ( Bumadian *et al.,* 2013).

Fecal coliform bacteria indicate the presence of sewage contamination of a waterway and the possible presence of other pathogenic organisms, Presence of fecal colifom shows that the source water may be contaminated by pathogens or disease producing bacteria or viruses ( Mashiatullah *et al.,* 2010). It has been estimated that the mortality of water associated diseases exceeds 5 million people per year around the world, of these, there are reports that more that 50% of these deaths are associated with microbial intestinal infections, particularly with cholera and typhoid more especially in developing countries (Pesewu *et al.,* 2015).

Physical chemical parameter study is very essential and important to test the water before it is used for drinking, domestic, agricultural or industrial purpose. Water must be tested with different physic-chemical parameters, Selection of parameters for testing of water is solely depends upon for what purpose his going to use that water and what extent we need its quality and purity ( P.N *et al.,* 2012), The Total Dissolved Solids is the term used to describe the inorganic salt and small amount of organic matter present in solution or water. The principal constituents are usually calcium, magnesium, sodium and potassium cation, carbonate, hydrogen carbonate, chloride, sulphate and nitrate anion, the presence of TDS in water may affect its taste.

In soil, fertilizers containing inorganic nitrogen and wastes containing organic nitrogen are first decomposed to give ammonia, which is then oxidized to nitrite and nitrate. The nitrate is taken up by plants during their growth and used in the synthesis of organic nitrogenous compounds. Surplus nitrate readily moves with the groundwater (WHO., 2003). For the nitrate concentration in groundwater and surface water is normally low but can reach high levels as a result of agricultural runoff, refuse dump runoff, or contamination with human or animal wastes (Nas *et al.*,2006).

A wide variety of materials have been identified as contaminants found in ground water. These include synthetic organic chemicals, hydrocarbons, inorganiccations, inorganic anions, pathogens, and radionuclides (Nas *et al.*,2006).

**Statement of the Problem:**

Tukrah Libyan town is under Albakur slope, away from the city of Benghazi, about 70 km to the east, and on the prairie 20 km to the west with a population of 15,000 thousand people, its like most developing countries struggles to improve access to potable water and sanitation by its urban population. The major sources of drinking water in the Tukrah town are water desalination station Putrabh, The main tank Tokra and deep well with pump. In this research has been the quality of water sources that feed the Tukrah area and its suburbs analysis of a desalination plant and tank main Tukrah and own wells in government institutions The houses.

**1.1 Aims of study:**

1- The general purposes of this work were to carry out a set of chemical and microbiological analyses for drinking water in Tukrah town in Libya.

2- Detection and enumeration of coliform and fecal coliform bacteria in chlorinated and de-chlorinated water of Tukrah town in Libya by using MPN test.

3- Identification of waterborne bacteria will grown at 37 C and 44.5 C by using biochemical tests and phoenix device.

4- Detection some of physical and chemical elements : pH, Rcl, EC, TDS, total alkalinity, turbidity, temperature, $Cl^-$ , F, $NO_3$, $NO_2$, $NH_3$, $S_{O4}$, Fe, K, Na, salinity, total hardness, Ca, Mg.

5- Finding correlation coefficients between the physic-chemical parameters for three levels of water samples.

# CHAPTER TWO

## 2. Reviw of Literature

Safe drinking water is very importance for human life because of this conducted a lot of studies to determine the extent of water available for drinking in this study, the validity of the focus was on water analysis of the microbial, physical and chemical in Tukrah area after it has been the division of water resources to levels based on several studies, including Coupling microbiological testing and sanitary surveys in drinking water quality programs: results from Capiz Province, Philippines. Studied by Patrick ***et al.,*** (2011) , This paper provides the results of an initiative by the Provincial Health Office in Capiz, Philippines and the United Nations (UN) see table 2.1, to conduct a first ever, provincial, microbiological water quality test program. Which aimed to identify sources most at risk, to test field-based analytical methods against standard methods. The results showed that there was an increasing trend in water quality from 'unimproved' to treated and/or piped supplies, but that many 'improved' point sources were contaminated. Less than 20% of the samples tested for chlorine residual were above the World Health Organization guideline. Sanitary surveys identified potential sources of contamination and were used to recommend priorities for remedial action.

Haruna ***et al.,*** (2005), studied the quality of water from protected springs in Katwe and Kisenyi parishes, Kampala city, Uganda. This study were to examine the bacteriological quality of water from ten springs in Katwe and Kisenyi parishes of Kampala, and to identify and quantify risks for spring water contamination with faecal

**Table (2.1). Capiz PHO water source designation and corresponding United Nations (UN) designation category**

| UN designation Category | Capiz PHO designation Category | Source type |
|---|---|---|
| Improved | Level 3 (L3) (piped connection on premises) | • Water district<br>• Local water utilities administration<br>• Barangay (village) waterworks system |
| | Level 2 (L2) | • Gravity protected spring with pipe distribution to communal tap stands<br>• Deep well with pump, with pipe distribution to communal tap stands |
| | Level 1 (L1) | • Shallow well pump<br>• Jetmatic pump with or without motor<br>• Deep well pump<br>• Protected dug well<br>• Protected spring without distribution<br>• Rainwater catchment (ferro-cement tank) |
| AUnimproved | Doubtful (D) | • Open dug well<br>• Unprotected spring<br>• Surface water (rivers, streams, creeks |

bacteria. A cross sectional sanitary risk assessment using a standardised format was carried out in ten randomly selected springs in the parishes of Katwe and Kisenyi parishes in Kampala. A total of 80 samples of water from these springs were collected from December 2001 to March 2002. The samples were analysed for indicators of faecal contamination: total coliforms, faecal coliforms. Physical chemical parameters were measured. It was results Aggregate qualitative sanitary risk scores ranged from medium to high. The total coliform counts in 90% of the samples exceeded the WHO guideline for drinking water. All the samples had faecal coliform counts above the WHO guideline. A strong correlation was observed between the median faecal coliform counts and the sanitary risk score. Sixty percent of the samples had nitrate levels above the WHO recommended limit. There was no correlation between the levels of chlorides and nitrates and levels of indicators of faecal bacterial contamination.

Sulieman, *et al.,* (2009) studied chemical and microbiological assessment of drinking water quality in central Sudan. the present study were to carry out a set of chemical and microbiological analyses for the drinking water samples to match the results with the Sudanese and international standards for drinking water quality, as well as the identification of the dominating microflora in these samples. The water samples (groundwater, treated and untreated surface water) were collected monthly from different places in Wadmedani town. The microbiological analyses revealed that , and the Biological oxygen demand levels were highly detected in the water samples, however; these levels were extremely high in the groundwater samples.

Mashiatullah *et al.,*(2010) studied coliform bacterial pollution in Rawal lake, Islamabad and its river, Coliform bacteria in Rawal lake and feeding streams water were determined by membrane filtration technique. The results indicated that *E. Coli* population in four streams (input waters) feeding the Rawal Lake ranged from 25 - 57 (mean 36) fecal coliform per 100 mL. The Kurang River, one of the feeding streams, hosted the largest population of fecal coliform (57 fecal coliform per 100 mL). The highest population of fecal coliform (105 fecal coliform per 100 mL) in Rawal Lake surface water was observed at the confluence of Kurang River and the Lake in the vicinity of village. While in the Rawal Lake water columns, it ranged from 12 - 65 (mean 25) fecal coliform/ 100mL. The measured levels of fecal coliform bacteria are much higher than the maximum permissible levels for drinking water as recommended by WHO and The United States Environmental Protection (No fecal coliform in drinking water). It is concluded that the indiscriminate amount of pollution from domestic sewage and poultry industry has seriously affected the biological quality of stream waters and the Rawal Lake waters.

Physical chemical and bacteriological investigation on the river Cauvery of Kollegal Stret Karnataka, as reported by Venkatesharaju *et al.*, (2010), six sampling stations over a distance of 2.5Km were selected for the study. Totally 144 surface water samples were collected from six different locations. Two liter capacity of plastic cans for physical chemical samples, 100ml autoclavable plastic bottles for bacteriological samples were used to collect surface water samples. 21 various physicochemical and bacteriological parameters including pH, Temperature, Turbidity, Electrical Conductivity, Total

Dissolved Solids, Total Hardness, Calcium Hardness, Magnesium Hardness, Total alkalinity, Sodium, Potassium, Sulphates, Phosphates, Nitrates, total coliforms and faecal coliforms selected for study were analyzed using standard methods (APHA, 2005). Reported that river water of the study area was not polluted in respect to physical chemical assessment. But bacteriological studies attributed river water was both total and faecal coliforms yearly averages showed increasing trend at S1, S2, S3 and S4. while mixing zones S5and S6 showed slight decrease in their counts.

Tabor *et al.,* (2011) studied bacteriological and physicochemical quality of drinking water and hygiene-sanitation practices of the consumers in Bahir Dar city, Ethopia. A cross sectional prospective study was conducted in Bahir Dar City from October-December, 2009. Water samples were collected from 35 private taps and 35 household water containers for bacteriological analysis. The turbidity, pH, temperature and turbidity were measured immediately after collection. Finally, the hygiene sanitation practices of the consumers were surveyed using interview. The results was twenty seven (77.1%) of the household water samples had high total coliforms counts. Twenty (57.1%) household water samples and 9 (25.7%) of the tap water samples had no residual free chlorine. Sixteen (45.7%) household water samples had very high risk score to thermotolerant coliforms. Eight (22.9%) tap water samples had low risk score for total coliforms whereas 21(60%) tap water had very low risk score for thermotolerant coliforms. Twelve (34.3%) of the consumers collect water without contact with their hand and 9(25.7%) wash their hands with soap after visiting toilet.

Sati *et al.,* (2011) reported bacterial indicators of faecal pollution and physiochemical assessment of tributaries of Ganges River in Garhwal Himalayas, India, Water samples were analyzed for various bacteriological parameters including total viable count (TVC), total coliform (TC), faecal coliform (FC) and faecal streptococci (FS). Also, physicochemical attributes viz. Total viable count exceeded the maximum permissible limits in all the samples irrespective to different seasons. The high most probable number (MPN) values and presence of faecal coliforms and streptococci in the water samples suggests the potential presence of pathogenic microorganisms which might cause water borne diseases. A direct effect of season and human activities on the pollution status was observed at all the water sampling sites. The overall objective of this work was to investigate the incidence of these indicator organisms, coliform, faecal coliform, faecal streptococci and physiochemical parameters during different seasons in two main tributaries of Ganges river.

Temgoua (2011) studied chemical and bacteriological Analysis of drinking water from alternative sources in the Dschang Municipality, Cameroon. In the poor zones of sub-saharan Africa. There are many pollutants in groundwater due to seepage of organic and inorganic pollutants, heavy metals, etc. Seventeen alternative water points created in 2008, for drinking water in Dschang municipality were examined for their physicochemical and bacteriological characteristics. The results revealed that water from managed points in Dschang is of poor quality. Most of the water samples were below or out of safety limits (standards) provided by WHO. The water is characterized by high turbidity and presence of feacal coliforms. It can be used for drinking and

cooking only after prior treatment. This situation shows that water point management was limited only to the drawing up comfort. These water points require installation of suitable surfaces of filtration and the development of a chlorination follow-up plan. Specific concerns of well water were raised and the management options to be taken proposed.

Ibiene *et al.,* (2012) studied bacteriological assessment of drinking water sources in Opuraja community of delta State, Nigeria. The total heterotrophic count ranges from $1.45\times10^3$ to $1.5\times10^6$ for all sources of water. The MPN values of the water samples ranged from 2 to 17 MPN/100ml. The total coliform count of water samples ranged from 14 to 198 MPN/100ml and the faecal coliform count ranged from 5 to 56 MPN/100ml. The temperature ranges from 22 to 28oC. The pH varies from 5.0 to 7.6 which are quite acidic. The bacteria isolated were *Escherichia coli, Salmonella* sp., *Shigella* sp., *Citrobacter* sp., *Proteus* sp., *Klebsiella* sp., *Vibrio sp., Bacillus* sp. *and Enterobacter* sp. All the water sources fell far below the standards approved by WHO and NAFDAC.

Oku *et al.,* (2012) studied evaluation of fecal coliforms and other heterotrophic bacteria in the Great Kwa River, Calabar, Cross River state, Nigeria. Eight water samples were collected (one sample per week) from the Great Kwa river and analyzed for total heterotrophic bacteria and fecal coliform. The water samples showed a heavy presence of bacterial contamination. The fecal coliform count on the samples ranged from 18 to 34 colonies/100 mL and the total coliform ranged from 42 to 76 colonies per

100ml. The results obtained showed the presence of *Escherichia coli* (33.3%), *Staphylococcus aureus* (12.5%), Bacillus spp (4.17%), *Clostridium* spp (8.33%), *Enterobacter* spp (12.5%), *Corynebacterium* spp (4.17%), *Pseudomonas* spp (8.33%), *Serratia marcesan* (8.33%), *and Streptococcus* spp (8.33%).

Thirupathaiah *et al.,* (2012) reported analysis of water quality using physico-chemical parameters in lower manair reservoir of Karimnagar district, Andhra Pradesh, Monthly changes in physico- chemical parameters such as water temperature, pH, turbidity, transparency, total dissolved solids, total hardness, chlorides, phosphate, nitrates, dissolved oxygen and biological oxygen demand were analyzed for a period of one year from September 2009 to August 2010, The results indicated that physico-chemical parameters of the water were within the permissible limits and can be used for domestic, irrigation and pisciculture.

Uwah *et al.,* (2014) studied physicochemical and bacteriological analyses of sachets water samples in Kano metropolis, Nigeria. were carried out using standard procedures to assess the quality of such water consumed in the area. Samples were collected from four different water depots in different parts of Kano metropolis. The results showed variations in the concentrations of the analyzed parameters in the water samples. The pH values ranged from 6.97±0.20 to 7.25±0.33; Electrical Conductivity ranged from 176 ±0.02 to 282±0.25µS/cm; Alkalinity ranged from 0.17±0.02 to 0.69±0.28 mg/l; Total solids were in the range of 100.30±0.25 to 157.34±0.30mg/l. Total Dissolved Solids ranged from 67.80±0.30 to 84.70±0.23mg/l; Total Suspended Solids ranged from

15.60±0.36 to75.84±0.02mg/; Total Hardness ranged from 85.00±0.03 to 103.00±0.20 mg/ and turbidity ranged from 0.60±0.21 to 2.23±0.32 NTU. *Escherichia coli* were not detected in all the samples. The levels of some of the anions analyzed ranged from 0.03±0.00 mg/l $NO_2$- to 7.06 ±0.02 mg/l $SO_4$2-. Similarly, the levels of some of the heavy metals analyzed ranged from 0.12±0.02mg/l Cu to 0.71±0.01mg/l Fe. Accordingly, the water samples were colourless and odourless. In general, the concentrations of all the parameters analyzed in the samples were below or within the World Health Organization (WHO) permissible limits.

Bumadian *et al.,* (2013) reported detection and enumeration of coliform bacteria in drinking water at hospital of Benghazi/Libya, at three different seasons. Samples were collected every month from two points viz surgery department (tapwater) and kidneys department (dialysis water) and examined by MPN and plate count methods. Presence of faecal coliform bacteria was not reported from both sources. However, the presence of coliform bacteria was reported from both source and it was slightly higher than the recommended one from both sources. Chemical analysis of water indicates the presence of organic matter like $NO_3$ but the level was lower than the recommended by both world health organization (WHO) and environmental protection agency (EPA).

Homaida *et al*., (2013) studied microbiological quality assessment of drinking water at Ed-Dueim Town, Sudan. The bacterial load was determined according to the pour plate standard methods and most probable number techniq ues for coliform, fecal coliform and fecal streptococci. Asbestos pipes were ranged from $0.3 \times 10^4$ to $9.3 \times 10^7$

cfu/ml. Total coliform MPN values were ranged from 0.0 to 11MPN/100 ml. Faecal coliform 0.0 to 7MPN/100 ml, while for faecal streptococci MPN were ranged from 0.0 to 3/100ml. The most predominant bacterial genera found in drinking water were *Bacillus sp.* (44%), *Corynebacterium sp.* (31%), *Micrococcus sp.* (13%), *Staphylococcus sp.* (6%) and *Streptococcus sp.* (6%). In addition, in this study, the physicochemical parameters such Turbidity, electrical conductivity, pH, temperature, total dissolved solid, chloride, fluoride, calcium, iodine, magnesium and sulfate were investigated, and the results show all the values except turbidity falls below the maximum limit of Sudanese Standard Metrology Organization and WHO guideline standard. From the results, it may be concluded that the drinking water in Ed-Dueim town has adequate physical and chemical quality and suitable for drinking.

Gopinath *et al.*,(2012) studied physical and bacteriological quality of well water samples from Kanakkary panchayath, Kottayam district, Kerala state, India. In the present study, a comparative analysis were carried out on the physical and bacteriological quality of well water samples collected from ten different locations of Kanakkary Panchayath, Kottayam district, Kerala state. The pH of water samples collected ranged from 5.24 to 7.13. The results showed that the MPN values of samples collected from five areas exceeds the World Health Organization (WHO) standards and these when subjected to confirmatory and biochemical tests showed that *Escherichia coli* was present only in one sample. Out of ten well water samples, three samples showed the presence of *Salmonella typhi* and six showed the presence of *Vibrio cholerae.* The Biological Oxygen Demand (BOD) values of all samples except 2 exceed

the WHO standards whereas the Total Dissolved Solids (TDS) values were all as per the standards.

Sorlini *et al.,* (2013) studied assessment of physical-chemical drinking water quality in the Logone Valley (Chad-Cameroon). Water supplies were sampled throughout the villages of this area mostly from boreholes, open wells, rivers and lakes as well as some piped waters. The samples were analysed for their physical chemical and microbiological quality in order to identify the contamination problems and suggest appropriate solutions. Results of the assessment confirmed that in the studied area there are several parameters of health and aesthetic concern. Elevated lead levels were detected both in aquifers and in surface waters, confirming that further investigations of the occurrence of lead contamination in the Logone valley are warranted. In addition, many groundwater sources are negatively impacted by parameters of aesthetic concern, such as turbidity, iron and manganese. Even though they do not affect human health, elevated levels of these parameters cause consumers to abandon improved water supplies, often in favour of surface water sources that are microbiologically contaminated. The use of alternative sources, improvement of water supply structures and water treatment are possible solutions to improve the quality of drinking water in the Logone valley.

Mishra *et al.,*(2013) studied occurrence and distribution of microbiological and physical chemical indicators in ground water contaminated by Drainages, north India. Ground water samples collected from different locations in winter and summer at

increasing distances (5 to 70 m) from the drainages were assessed for their suitability for human consumption. The samples were analyzed for various bacteriological parameters including total viable count, total coliforms, faecal coliforms and faecal streptococci. Additionally, physic chemical [pH, dissolved oxygen (DO), biological oxygen demand (BOD) and chemical oxygen demand (COD)] were assessed. Heavy metals like $Al^{3+}$ was detected in 83% and Cd, Cu, Zn in 75% and Pb in 41% water samples in winter while during summer season the percentage was slightly higher. Total viable as well as coliforms count exceeded the maximum permissible limits in most water samples irrespective of distance from drainages. The higher most probable number (MPN) values and presence of antibiotic resistant faecal coliforms and streptococci in the water samples suggest the presence of pathogenic microorganisms, heavy metals as well as organic load decreased with increase in distance.

# CHAPTER THREE

# 3. Material and Methods

## 3.1. Area of Study :-

This study is built about the polluted drinking water Tukrah town, its Libyan town is under Albakur slope, away from the city of Benghazi, about 70 km to the east, and on the prairie 20 km to the west with a population of 15,000 thousand people, one from villages in struggles to improve access to potable water and sanitation by its urban population.

## 3.2. Collection of samples :-

Water samples were collected from different areas in the Tukrah town (table 3.1) and placed in one bottle for microbial analysis and one bottle for chemicals analysis. Collected water stored for transport in plastic boxes with icepacks to keep them cool (but not frozen). Water samples were collected in autoclaved sterile bottles for both chemical and microbial examination.

## Table (3.1). Type and code of sample water points for quality analysis

| U.N. Designation Category | Capiz PHO Designation | | Code sample |
|---|---|---|---|
| | Category | Source Type | |
| Improved | Level 3 (L3) | Water desalination station | |
| | | 1- A sample of Aozou School. | S1 |
| | | 2- Sample directly from the station. | S2 |
| | | 3-One of the houses Tukrah. | S3 |
| | | 4-Mosque within the region. | S4 |
| | | 5- One of the houses Tukrah. | S5 |
| | | 6- Al-Zahrawi Specialized Clinic. | 56 |
| | Level 2 (L2) | The main tank Tukrah | |
| | | 7- Main tank | S7 |
| | | 8- Haita Park | S8 |
| | | 9- Tukrah Hospita | S9 |
| | | 10- Tukrah Security Directorate. | S10 |
| | | Deep well with pump, with pipe distribution to communal tap stands | |
| | | 11- One of the wells that feed the reservoir. | DW1 |
| | | 12- Tukrah University. | DW2 |
| | | 13- Romanian company | DW3 |
| | Level 1 (L1) | Deep well pump | |
| | | 14- Hospital Tukrah village well inside the hospital | DW4 |
| | | 15- Well above a depth of 200 meters | DW5 |
| | | 16-Well depth of nearly 30 meters. | DW6 |
| | | 17-Well depth of 23 meters nearly. | DW7 |
| | | 18- Battle of Yarmouk mosque well depth of 30 meters | DW8 |
| | | 19- Well depth of 25 meters nearly. | DW9 |
| | | 20- Well depth of 40 meters nearly. | DW10 |
| | | 21- Well depth of between 55-70 meters. | DW11 |

## 3.3. Bacteriological Experiments :

### 3.3.1. The heterotrophic plate count (HPC):

The standard plate a count agar technique for the enumeration of microorganisms is one of the oldest and most widely used techniques in microbiology. The HPC test is another method for monitoring the overall bacteriological quality of drinking water. described by (Bumadian *et al.,* 2013) Therefore Collected water sample made diluted up to $10^{-9}$ by serial dilution method in normal saline (8.5 g/l NaCl solution) and 0.1 ml solution from each test tube was spread on top of the plate a count medium (three replicates of petri dish for each test tube) then incubated at 37C° for 24-48 h. The average number of colonies calculated as CFU/100µl (figure 3.1).

### 3.3.2. Enumeration of bacteria :

**3.3.2.1. Most probable number (MPN)** :

MPN counts are statistical best estimates ( hence the name, most probable number) obtained by culturing a number (usually five) of sample volumes and/or dilutions of such sample. MPN method which described in standard method (Andrew *et. al.,* 1995), were used to enumeration of coliform and faecal coliform bacteria as follows in three steps.

**1-Presumptive test:** water sample bottles were thoroughly shaked. 10 ml, 1 ml and 0.1 ml (1ml of the 1:10 dilution) of water samples were inoculated into three sets of sterile test-tube. Each set containing on five test tube containing an inverted Durham tube and

9 ml of Lactose broth (the first five set were contain strength lactose broth) and then incubated at 37 C° for 24-48 hours.

After incubated for 24 h, each test tube were examined for gas production (coliform bacteria produce gas from the lactose in medium, and some of the was trapped in the inverted Durham tube). Number of positive tubes (with gas production) were counted and MPN determined from standard table (Andrew ***et. al.,*** 1995).

**2-Confirmed test:** 100µl were transferred from positive Presumptive test and speared on EMB plate and incubated at 37 C° for 24-48 hours (Andrew ***et. al.,*** 1995).

**3-completed test**: Lactose broth was inoculated by positive confirmed test and incubated at 44.5 C° for 24-48 hours. After incubated for 24 h, each test tube were examined for gas production (faceal coliform bacteria produce gas from the lactose in medium, and some of the was trapped in the inverted Durham tube). Number of positive tubes (with gas production) were counted and MPN determined from standard table. 10 µl were transferred from positive completed test and speared on EMB plate and incubated at 37 C° for 24-48 hour (Andrew ***et. al.,*** 1995).

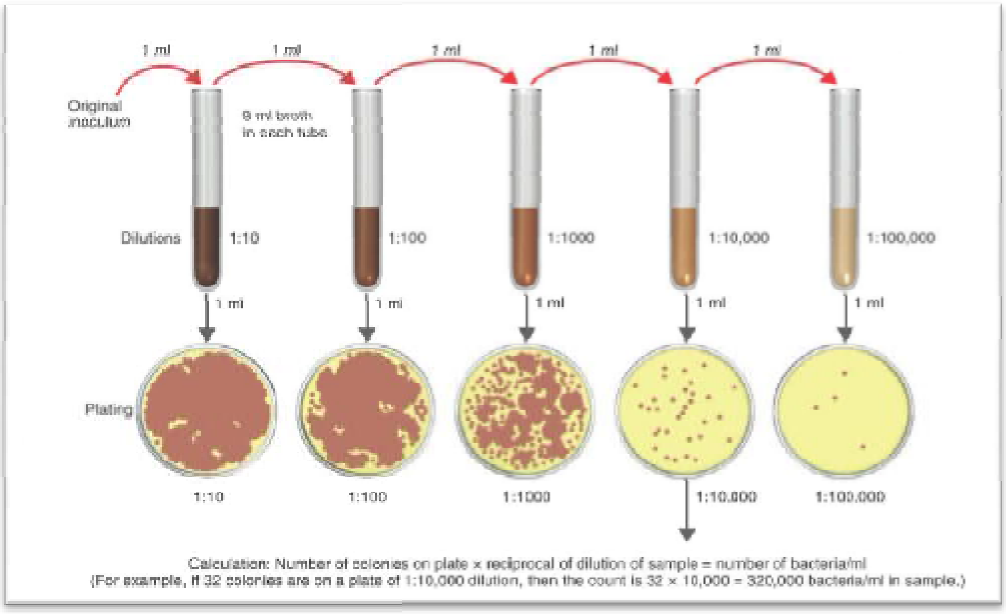


Figure (3.1). Description of the Heterotrophic Plate Count (HPC)

Tortora J.G; Funke R. B.; Case L. C. (2010). An introduction microbiology. Tenth Edition. San Francisco, Boston, Newyork.

### 3.3.3. Isolation and identification of isolates bacteria :

### 3.3.3.1: Isolation of bacteria:

Isolated bacterial was growth in 37 C° and 45 C° in the MPN test by use biochemical tests:

It was cultured the specimen at Blood agar medium , Maconky agar medium and Chocolate agar medium, then incubated both plates at 35 to 37 C° over night. Then examined the specimens by microscopic after gram stain prepared: The most common and useful staining procedure is the gram stain which separates bacteria into 2groups. It has to take the light tinge of bacteria that its character and to be placed in a clean slide and then added one drop of water using the drops. By Loop sample mixed well with water and then distributed until almost half the slide area. I left to dry in the air and then were installed by flame benzene, then it became a swab ready to dye. Then immered dye crystal violet swab with a solution of one minute for then washed with water. And then flooded with a solution of iodine swab one minute period, then wash by water. After that alcohol was added until the color disappeared for a period of 15-30 seconds, depending on the intensity of the swab, then washed with water. After that immersion dye safranin swab for 30 seconds, then washed with water. Then dried slide by filter paper, then reading the result by microscopic bacteria, Gram Positive appear light colored purple and gram Negative bacteria, show red color.

### 3.3.3.2: Identification of isolates bacteria:

**1-API test:** After Gram stain for choosing the right partner, detected by API for watch results It took a similar colony or colonies of bacterial growth to be examined, and added to sodium chloride solution 0.85% and mix until the degree of homogeneity. Then measured the density of the solution using a bacterial density measuring device Densimat, and in this case must be equal to 0.5 MacFarland (McF). And then it was taked the tape strip API 20A It contains a 20 room and each room is a vital chemical examination, was added to each compartment 55 microliter using pipette, some cabins have been added two drops of mineral oil in order to provide an atmosphere not antenna. Then put the lid on the tape (strip). And put tape (strip) in the incubator at 37° C for 24 hours, After that, read the results of the samples, by read the result based on a special table called a table reading as it shows the color of each chamber if the result is negative or positive, and the results were recorded on a private papers (Biomerieux, 2002).

**2-DNase test:** To distinguish between *Staphylococcus aureus* and *Staphylococcus albus* bacteria We used DNase test by cultured the specimen in DNase media way line for each sample of the samples he wanted to differentiate them, and put it on the dish (Hcl 15%) and then placed in the incubator at 37° C for 24 hours, after that if formed zone this means unknown sample is *Staphylococcus aureus* but if it's not formed zone this mean unknown sample is *Staphylococcus albus.*

**3-Device BD Phoenix™:** For the Phoenix system, the combined ID and AST NMIC/ID 14 panel for Gram-negative bacilli and the PMIC/ID 13 panel for Gram-positive cocci were used. The setup of the panels was performed according to the manufacturer's instructions. The Phoenix ID broth was inoculated with bacterial colonies from blood agar and adjusted to a 0.5 to 0.6 McFarland standard using the Crystal Spec Nephelometer (BD Diagnostic Systems). After supplementing the AST broth with one drop of indicator dye, 25µl of the ID suspension was transferred to the AST broth to achieve a final inoculum density of $1.5 \times 10^8$ CFU/ml. The ID and the AST broths were poured into the respective side of the panel placed on the Phoenix inoculation station. The inoculated panels were closed and placed into the transport caddy, and, after entering the accession number, the panels were placed into the Phoenix instrument (Salomon *et al.*,1999).

## 3.4. Physical and Chemical Experiments :by Instrument and Procedure Manual:

### 3.4.1. Determination of pH and Temperature:

In the laboratory, pH meter (inoOLab pH 720) for measuring acidity and alkalinity of water by measuring the degree of proportion ph we can recognize the water (acidic - alkaline – neutral) and Buffer solutions of pH 4.0, 7.0 and 9.0 were used to calibrate the pH meter. About 50ml of water sample was poured into a clean glass beaker and the electrode inserted into it. The button selector of the pH meter was turned and the pH and temperature were read and recorded.

### 3.4.2. Determination of Conductivity:

Conductivity meter ( inolab cond 720) was used to determine the conductivity of water samples in the laboratory. It was calibrated by using standard solution of 1413 μS/cm at a temperature of 25C°. About 50ml of water sample was poured into a clean glass beaker and the conductivity meter electrode was then inserted into the water. The value was read and recorded after five minutes in μS/cm. The same procedure was repeated three times for all other water samples.

### 3.4.3. Determination of Turbidity:

Turbidity of water samples was determined with turbidity meter ( 2100P ISO Turbidity meter ). The turbidity meter was first calibrated with Formazin standard solutions of 0.2 NTU, 10 NTU, 100 NTU and 1000 NTU by filling consecutively a clean dry cuvette with the well mixed standard solutions. It was then returned to the measurement mode and used. A clean dry cuvette was rinsed three times with the water sample to be tested. The cuvette was filled with the water sample to be analysed and then covered with light shield cap. The outer surface of the cuvette was wiped dry with a clean tissue paper. It was then pushed firmly into the optical well and the lid closed. The NTU values were measured by pressing and releasing the arrow and the value was recorded after the display has stopped flashing.

### 3.4.4. Determination of Total Dissolved Solids:

A multifunctional Conductivity meter ( inolab cond 720) was used to determine the total dissolved solids of water samples in the laboratory after calibration. About 50ml of

water sample was poured into a clean glass beaker. The electrode was then immersed into the sample and stirred to ensure uniform mixture. After the reading stabilised the value was read and recorded in mg/L.

### 3.4.5. Determination of Ammonia:

Spectrophotometer (DR2800 ) was used to determine the concentration of ammonia in the water samples after calibration. Filled on the sample cell to the mark with 10 mL sample, then filled a around sample cell to the 10ml mark with deionized water (this is the blank). After that added the contents of one Ammonia Salicylate powder pillow to each cell. Then Stoppered and shaked until it is completely dissolve powder. After three minutes of interaction passage, added the contents of one ammonia Cyanurate Reagent Powder Pillow to each cell. Stopper and shake until completely melted Detector. After 15 minutes of interaction passage. Agreen color will develop if ammonia-nitrogen is present. Then place the blank into cell holder. Touching zero even appeared on the screen: 0.00 mg / L NH3-N. Withdrawn and placed in the sample cell holder. So that the results appeared to mg / L NH3-N. Test result are measured at 655nm.

### 3.4.6. Determination of Nitrite-Nitrogen ($NO_2$):

Spectrophotometer (DR2800 ) was used to determine the concentration of Nitrite By following these steps: I'm working on programming device (373 N, Nitrate HR PP). then presse start, then selected the test. Programs stored press (373 N, nitrate HR PP).It never is then pressure, Then we define the test. after that filled a square sample cell with

10ml of sample. then prepared sample by added the contents of one NitriVer2Nitrite Reagent Pillow. Stopper and shake to dissolve. then press timer>ok a ten minute reaction period will begin. To prevent low results, leave the sample on a flat surface and do not disturb it during the reaction period. blank preparation: filled a second square sample cell with 10ml of sample. Then press zero the display will show: 0 mg/L $N_{O2}$-. after the timer expires, cap and gently invert the prepared sample twice. Avoid excessive mixing, or low results may occur. Then wiped the prepared sample and inserted it into the fill line facing right. Then press Read. Results are in mg/L NO2-. Nitrate is measured at a wavelength of 585 nm in the case of the presence of nitrite in the sample interacts with the guide to be a greenish-brown color, which increases its focus to increase nitrite.

**3.4.7. Determination of Nitrate-Nitrogen ($NO_3$-N):**

Spectrophotometer (DR2800 ) was used to determine the concentration of Nitrate By following these steps: touch stored programs (355 N, Nitrate HR PP). then touched startup then selected the test. Then filled a square sample cell with 10ml of sample, Then prepared sample by added the contents of one Nitrate Reagent powder Pillow Stopper. Then press timer>ok. (one minute reaction period will begin). After that shake the cell vigorously until the timer expires. When the timer expires. Press timer>ok again. A five minute reaction period will begin. (an amber color will develop) if nitrate is present. blank preparation: when the timer expires, filled a second square sample cell with 10ml of sample. Then wiped the blank and insert it into the cell holder with the fill line facing right. Then Click zero. The display showed: 0.0 mg/L NO3-_N. within one

minute after the timer expires, wipe the prepared sample and inserted it into the cell holder with the filled line facing right. Click Read Results are in mg/L NO3-_N.

### 3.4.8. Determination of Sulphat:

By Spectrophotometer (DR2800 ) touched Hach Programs and Selected program 680 sulfate touch start. A clean sample cell of 10ml filled sample, added the contents of one SulfaVer4 Reagent Powder Pillow to the sample cell (the prepared sample). Swirl to mix. then press timer>ok. A five-minute reaction period will begged. then Filled a second sample cell with 10 ml of sample (the plank). When the timer beeps, placed the plank into the cell holder. And touched Zero. The display were show: 0 mg/l $SO4^{2-}$. Within five minutes after the timer beeps, placed the prepared sample into the cell holder. Results was appear in mg/l $SO4^{2-}$, Sulfation in the sample react with barium in the sulfaVer 4 and form a precipitate of barium sulfate. The amount of turbidity formed was proportional to the sulfaVer 4 also contains a stabilizing agent to hold the precipitate in suspension. Test results are measured at 450 nm.

### 3.4.9. Determination of Chlorine residual:

By Spectrophotometer (DR2800 ) By Hach Programs, were touched Hach programs and selected program 80 Clor.F&T. And then touched start. Filled a round sample cell with 10 ml of sample. (this is the blank). And then wiped the blank and place it into the cell holder. Then touched zero the display was show: 0.00 mg/l CL2. Then Filled a second round cell with 10 ml of sample, then added the contents of one DFD Free Chlorine powder Pillow to the sample cell (this is the prepared sample). And swirl the sample

cell for 20 seconds to mix. Within one minute of adding the reagent, place the prepared sample into the cell holder. Results will appear in mg/l CL2. Test results are measured at 530 nm.

**3.4.10. Determination of Chloride:**

(Digital Titrator at Hach Model 16900) (10 to 10000 mg/l as Cl-): Selected the sample volume and Silver Nitrate Titration Cartridge that corresponds to the expected chloride concentration from Table 3.2. Then were inserted a clean delivery tube into the titration cartridge. Attached the cartridge to the titrator body. Then hold the digital titrator with the cartridge tip pointing up. And turn the delivery knob umtil a few drops of titrant are expelled. Reset the counter to zero and wipe the tip. Use a graduated cylinder or pipet to measure the sample volume from Taple1. And transfer the sample into aclean 250 ml Erlenmeyer flask. Dilute to about the 100 ml mark, if necessary. And then add the contents of one Chloride 2 indicator powder pillow and swirl to mix.( result will still be accurate if a small amount of powder does not dissolve. And then place the deliver tube tip into the solution and swirl the flask will titrating with silver nitrate from a yellow to red-brown color. Record the number of digits required. Finally calculate: digits required × digit multiplier = mg/l Chloride. The sample is titared with Silver Nitrate Standard in presence of potassium chromate (from the Chloride 2 Indicator Powder). The silver nitrate reacts with the chloride present to produce insoluble white silver chloride. After all chloride has been precipitated, the selver ions react with the excess chromate present to form a red-brown silver chromate precipitate, marking the end point of the titration.

### 3.4.11. Total Hardness Determination:

By Digital Titrator at Hach Model 16900 Total Hardness, (10 to 4000 mg/l as CaCo3) Using EDTA: used table 3.3 for selected sample size and EDTA Titration Cartridge corresponding to the expected total hardness as calciu carbonate (CaCO3) concentration, insert a clean delivery tube into the titrator body. Then turn delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip. Then use pipet to measure the sample the sample volume from table 3.3. Transfer the sample into a clean 250 ml Erlenmeyer flask. Dilute to about the 100 ml mark with deionized water, if necessary. After that add 2 ml of hardness 1 Buffer Solution and swirl to mix. Then added the content ManVer2 Hardness Indicator Powder Pillow and swirl to mix. Placed the delivery tub tip into the solution and swirl the flask while titrating with EDTA from red to pure blue. Record the numper of digits required.

Calculate the final concentration:

Digits Required × Digit Multiplier (Table 3.3) = mg/l Total hardness as CaCO3.

### 3.4.12. Determination of Calcium hardness and Magnesium:

Digital Titrator at Hach Model 16900 . Measured by adding potassium hydroxide solution and calcium guide to the sample and were calibrated with a solution (EDTA 0.08 M) and end the calibration at a turning pink to blue.

Calcium = Calcium hardness*0.4.

Magnesium harness = Total hardness – Calcium.

### 3.4.13. Determination of Fluoride:

Spectrophotometer DR 2800 By Hach Programs Selected program 190 Fluoride. Touch start. Pipet 10 ml of sample into dry, around sample cell (this was the prepared sample), then pipet 10 ml of deionized water into a second dry, round sample cell (this is the blank). After that carefully pipet 2 ml of SPADNS Reagent into each cell. Swirl to mix. After one minute reaction period will begin, place the blank into the cell holder, touched zero the display were showed: 0.00 mg/l F-. Then placed the prepared sample into the cell holder. The results were appear in mg/l F-.

### 3.4.14. Determination of Iron:

Spectrophotometer DR 2800 By Hach Programs, select program 265 Iron, FerroVer. Then touch start. Filled a clean, round sample cell with 10 ml of sample, then was added the contents of one FerroVer Iron Reagent Powder Pillow to the sample cell (the prepared sample). Swirl to mix. Touch the timer icon. Touch OK. A three minute reaction period will begin. Then filled another sample cell (the blank) with 10 ml of sample, when timer beeps, place the blank into the cell holder, then touch zero the display will show: 0.00 mg/l Fe, finally placed the prepared sample into the cell holder. Results were appear in mg/L Fe.

### 3.4.15: Determination of Sodium:

By Flame photometer BWB technologies. The measurement of sodium in 200 ml of the samples after the device has calibration information at three concentrations of sodium (60ppm, 200ppm, 400).

### 3.4.16: Determination of Potassium:

By Flame photometer BWB technologies. The measurement of Potassium in 200 ml of the samples after the device has calibration information at three concentrations of Potassium (50ppm, 100ppm, 200ppm).

**Table (3.2). The sample volume and Silver Nitrate Titration Cartridge that corresponds to the expected chloride concentration**

| Range (mg/l as Cl-) | Sampe Volume (ml) | Titration Cartridge (N AgNO3) | Catalog Number | Digit Multiplier |
|---|---|---|---|---|
| 10-40 | 100 | 0.2256 | 14396-01 | 0.1 |
| 25-100 | 40 | 0.2256 | 14396-01 | 0.25 |
| 100-400 | 50 | 1.128 | 14397-01 | 1.0 |
| 250-1000 | 20 | 1.128 | 14397-01 | 2.5 |
| 1000-4000 | 5 | 1.128 | 14397-01 | 10.0 |
| 2500-10000 | 2 | 1.128 | 14397-01 | 25.0 |

**Table (3.3). The sample volume and EDTA Titration Cartridge that corresponds to the expected Total hardness concentration**

| Range (mg/l as CaCO3) | Sample Volume (ml) | Titration cartridge (M EDTA) | Catalog Number | Digit Multiplier |
|---|---|---|---|---|
| 10-40 | 100 | 0.0800 | 14364-01 | 0.1 |
| 40-160 | 25 | 0.0800 | 14364-01 | 0.4 |
| 100-400 | 100 | 0.800 | 14399-01 | 1.0 |
| 20-800 | 50 | 0.800 | 14399-01 | 2.0 |
| 500-2000 | 20 | 0.800 | 14399-01 | 5.0 |
| 1000-4000 | 10 | 0.800 | 14399-01 | 10.0 |

### 3.4.17. Determination of Alkalinity:

by Digital Titrator at Hach Model 16900 Phenolphthalien and Total using Sulfuric Method. Analysis has been using the device Digital Titration (10 to 4000 mg/L as CaCO3). Selected the sample volume and Sulfuric Acid (H2SO4) Titration Cartridge that correspond to the expected alkalinity concentration as mg/L calcium carbonate (CaCO3) from table3. Then inserted a clean delivery tube into titration cartridge. Attach the cartridge to the titration body. After that turned the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip. By using a graduated cylinder or pipit to measure the sample volume from Table3.4, then transferred the sample into a clean, 250ml Erlenmeyer flask. Diluted to the 100ml mark with deionized water, if necessary. Then added the contents of one Phenolphthalein Indicator Powder Pillow and swirl to mix. If the solution turns pink, titrate to a colorless end point. Placed the delivery tube tip into the solution and swirl the flask while titrating with sulfuric acid. Record the number of digits required. . (if the solution is colorless before titrating with sulfuric acid, the phnolphathalein (P) alkalinity is zero. Proceed to step 8).

calculate: Digits Required * Digit Multiplier = mg/L as CaCO3P Alkalinity Add the contents of one Bromcresol Green-Methyl Red Indicator Powder pillow to the flask. Swirl to mix. continued the titration with sulfuric acid to a light pink (pH 4.5) color. As required by sample composition. Record the number of digits required.

calculate: Digits Required * Digit Multiplier = mg/L as CaCO3 Total (T or M) Alkalinity.

Table (3.4). the sample volume and sulfuric acid Titration Cartridge that corresponds to the expected alkalinity concentration

| Range (mg/L as CaCO3) | Sample Volume (ml) | Titration Cartridge (N H2SO4) | Catalog Number | Digit Multiplier |
|---|---|---|---|---|
| 10-40 | 100 | 0.1600 | 14389-01 | 0.1 |
| 40-160 | 25 | 0.1600 | 14389-01 | 0.4 |
| 100-400 | 100 | 1.600 | 14389-01 | 1.0 |
| 200-800 | 50 | 1.600 | 14389-01 | 2.0 |
| 500-2000 | 20 | 1.600 | 14389-01 | 5.0 |
| 1000-4000 | 10 | 1.600 | 14389-01 | 10.0 |

## 3.5. Statistical methods used in the study:

After the completion of the data collection process was used by computer based on "statistical Package for sociality science SPSS 21" program, which the Statistical Package for Social Sciences, the study relied on two aspects of the census: descriptive statistics, extract Standard deviation Std. Deviation Mean and inferential statistics, use analysis of variance (ANOVA) follow a normal distribution data to find out significance at 5 % levels., and Kruskal and Las Kruskal-Wallis Test tracking data distribution is not normal. and use the correlation coefficient correlation data follow a normal distribution. The bacteriological counts and chemical parameters and physical recorded were compared with the WHO guidelines for drinking water.

# CHAPTER FOUR

# 4. Resuls and Discution

## 4.1. Results microbial analysis of samples collected:

### 4.1.1. The heterotrophic plate count (HPC) :

The heterotrophic plate count value showed a regular trend (table 4.1, figure 4.1, figure 4.2, figure 4.3 ), indicate the bacterial population for water samples collected from different water sources, Analysis of water samples collected from input water sources in level1(Deep well pump) shows that total bacterial levels are in the range from $67.5\times 10^3$ to $275 \times 10^3$ cfu/ml for water, and in level2 (Gravity protected spring with pipe distribution to communal tap stands ,Deep well with pump, with pipe distribution to communal tap stands) total bacterial levels are in the range from $8.5\times10^3$ to $77\times10^3$ cfu/ml water. And in level3 (Water desalination plant) ranged from $1\times10^3$ to $2.5\times10^3$ cfu/ml water (table 4.1, figure 4.3).

Heterotrophic microorganisms include both members of the natural (typically nonhazardous) microbial flora of water environments and organisms present in a range of pollution sources. They occur in large numbers in raw water sources. The actual organisms detected by HPC tests vary widely between locations and between consecutive samples (Gopinath *et al.*, 2012).

The HPC value showed a regular trend (table 4.1). The values increased in level1 Which has taken water samples from wells area Tukrah, The highest HPC was noted in DW8 (well in Yarmok mosque it's deep about 30 meter nearly) and DW9(well about 25 meter nearly in the deep) were as high as $275\times 10^3$ and $224 \times10^3$, respectively. The

lowest value $0.66 \times 10^3$ were recorded in S2, S4, S6 and $1 \times 10^3$ in S5, respectively in Level3 samples it's considered to be of good quality and is used for drinking purposes, but in Level1 and Level2 the result showed that the different drinking water sources are highly contaminated because the heterotrophic plate count which are far more than the recommended value of $1.2 \times 10^2$ of WHO (1995) (Ibiene *et al.*, 2012) . Theses result are consistent with the result of Ibiene *et al.*, (2012), Where their results of the heterotrophic plate count HPC ranged from $1.6 \times 10^3$ to $1.5 \times 10^6$ for all sources of drinking water in Opuraja community of Okpe Local Government area, Delta State, Nigeria..

The results of the heterotrophic plate count value (table 4.2, figure 4.4) showed significant differences ($p = 0.000$) between the three levels of drinking water samples where full swing be in level1 and in level2 of drinking water sample, The very high contamination may be due to the non-hygienic disposal of fecal waste in pit.

**Table (4.1). Heterotrophic plate count (HPC) of samples collected**

| Levels | Number and code sample | Results microbial analysis of samples collected | | | The heterotrophic plate count $\times 10^3$ (CFU/ml) |
|---|---|---|---|---|---|
| Level3 | S1 | 2 | 3 | 2 | 2.33 |
| | S2 | 1 | 1 | 0 | 0.66 |
| | S3 | 1 | 1 | 2 | 1.33 |
| | S4 | 1 | 0 | 1 | 0.666 |
| | S5 | 1 | 1 | 1 | 1 |
| | S6 | 1 | 0 | 1 | 0.66 |
| Level2 | S7 | 40 | 43 | 38 | 40.33 |
| | S8 | 9 | 8 | 11 | 9.33 |
| | S9 | 9 | 8 | 9 | 8.66 |
| | S10 | 12 | 11 | 12 | 11.66 |
| | DW1 | 33 | 30 | 32 | 31.66 |
| | DW2 | 51 | 53 | 48 | 50.66 |
| | DW3 | 65 | 69 | 70 | 68 |
| | DW4 | 12 | 15 | 20 | 15.66 |
| Level1 | DW5 | 210 | 214 | 205 | 209.67 |
| | DW6 | 170 | 165 | 172 | 169 |
| | DW7 | 190 | 188 | 195 | 191 |
| | DW8 | 70 | 65 | 77 | 70.66 |
| | DW9 | 275 | 270 | 280 | 275 |
| | DW10 | 223 | 230 | 220 | 224.33 |
| | DW11 | 147 | 145 | 150 | 147.33 |

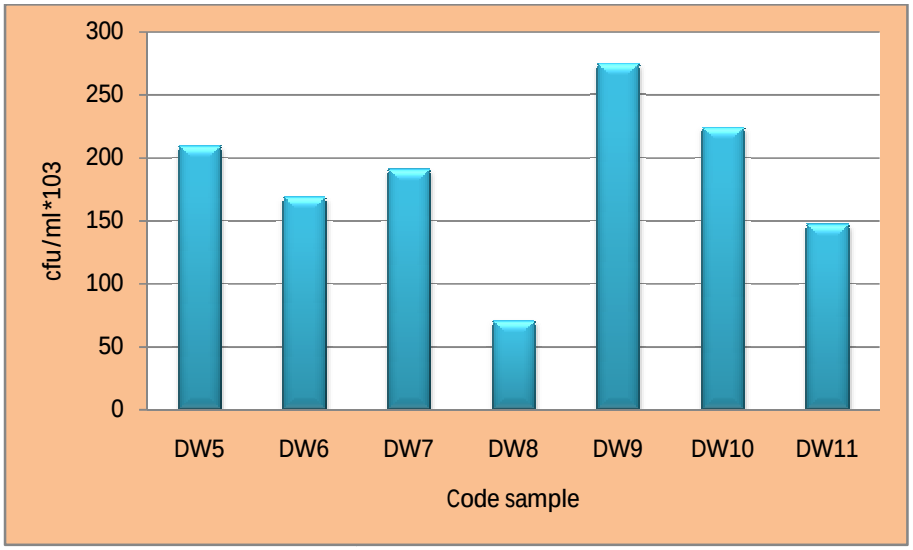


Figure (4.1). Heterotrophic plate count (HPC) for Level 1 samples

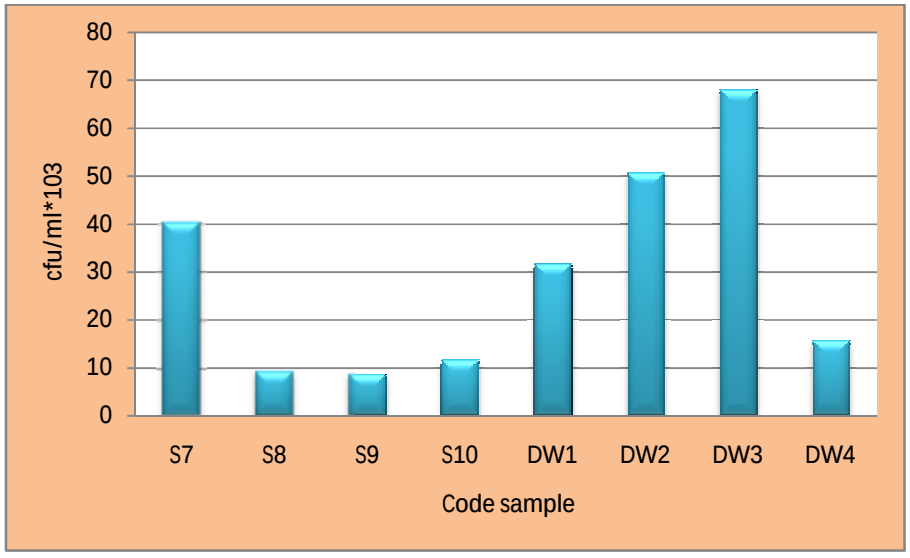


Figure (4.2). Heterotrophic plate count (HPC) for Level2 samples

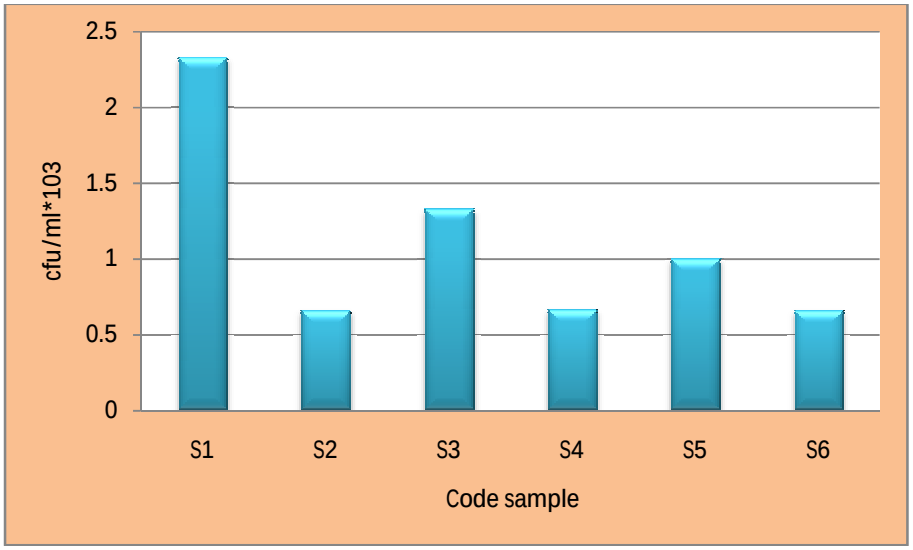


Figure (4.3). heterotrophic plate count (HPC) for Level 3 samples

**Table (4.2). Analysis of variance (ANOVA) showing heterotrophic plate count (HPC) for three levels of drinking water samples**

| Department | N | Mean | Std. Deviation | %95Confidence Interval for Mean | | ANOVA | |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | p-vales | L.S.D |
| Level 1 | 7 | 183.86 | 64.59 | 124.12 | 243.59 | 0.000 | A>B B>C |
| Level 2 | 8 | 29.50 | 22.05 | 11.07 | 47.93 | | |
| Level 3 | 6 | 1.11 | 0.66 | .42 | 1.80 | | |
| Total | 21 | 72.84 | 89.61 | 32.05 | 113.63 | | |

A: Level 1 B: Level 2 C: Level 3

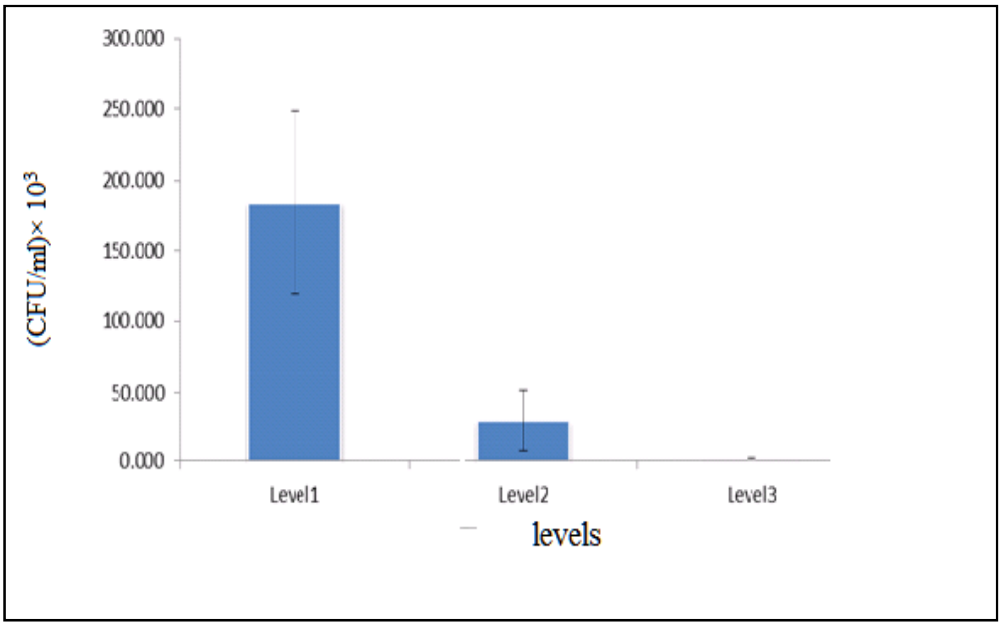


Figure (4.4). The heterotrophic plate count (HPC) in the three levels of drinking water samples

### 4.1.2. Total and faecal coliform count of drinking water:

The most probable number (MPN) for presumptive total coliform count of the water samples for level1 ranged from 14 to 350 MPN/100ml, The maximum total coliform coloneis (350 MPN/100ml) was recorded for DW5 and DW7 and the minimum (14 MPN/100ml) for DW11 sample ( table 4.3, figure 4.5), most probable number (MPN) for completed faecal coliform count of the water samples for level1 ranged from >2 to 21 MPN/100ml, And the maximum faecal coliform coloneis (21 MPN/100ml) was recorded for DW7 and the minimum (>2 MPN/100ml) for DW11 sample (table 4.3, figure 4.5). This is an indication that the sources of drinking water may be prone to pathogenic organism like Vibrio, Salmonella etc. These values deviated from the standard recommended by WHO which are zero total coliform count per 100 ml for WHO (Isa *et al.,* 2013 ).

This results indicated that level one All the samples had The total coliform counts and faecal coliform counts above the WHO guideline for drinking water. This may be due to locale of the wells beside or around the wells sewage, or it can be to see the lack of depth of the wells. This results confermed with Haruna *et al* (2005).

**Table (4.3). The most probable number (MPN) for presumptive total coliform count of the water samples in Level 1**

| No. tested sample | Number of positive tube | | | MPN Per 100 ml | **95% Confidence Limits** | |
|---|---|---|---|---|---|---|
| | 10ml | 1ml | 0.1 ml | | **Lower** | **Higher** |
| DW5 | 5 | 4 | 4 | 350 | 100 | 710 |
| DW6 | 3 | 1 | 2 | 17 | 6 | 36 |
| DW7 | 5 | 5 | 1 | 350 | 100 | 1100 |
| DW8 | 4 | 3 | 1 | 33 | 9 | 78 |
| DW9 | 4 | 3 | 2 | 39 | 9 | 78 |
| DW10 | 3 | 4 | 3 | 32 | 7 | 40 |
| DW11 | 3 | 2 | 0 | 14 | 6 | 36 |

**Table (4.4). The most probable number (MPN) for completed faecal coliform count of the water samples in Level 1**

| No. tested sample | Number of positive tubes | | | MPN Per 100 ml | **95% Confidence Limits** | |
|---|---|---|---|---|---|---|
| | 10ml | 1ml | 0.1ml | | **Lower** | **Higher** |
| DW5 | 2 | 1 | 1 | 9.2 | 2 | 21 |
| DW6 | 1 | 2 | 2 | 10 | 1.8 | 15 |
| DW7 | 3 | 3 | 1 | 21 | 7 | 40 |
| DW8 | 2 | 2 | 0 | 9 | 2 | 21 |
| DW9 | 1 | 0 | 1 | 4 | 0.7 | 10 |
| DW10 | 2 | 0 | 1 | 6.8 | 1 | 17 |
| DW11 | 0 | 0 | 0 | <2 | 0 | 6 |

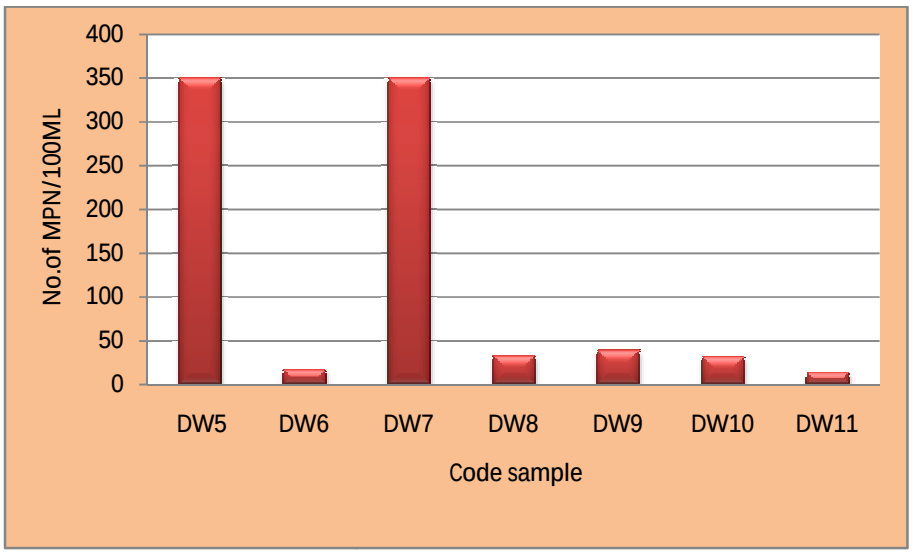


Figure (4.5). The most probable number (MPN) for presumptive total coliform count of the water samples for level1

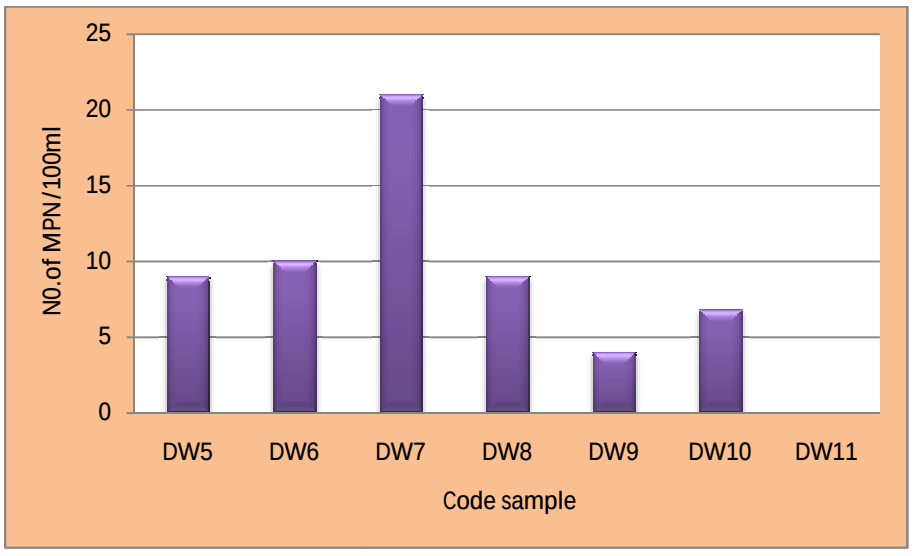


Figure (4.6). The most probable number (MPN) for completed faecal coliform count of the water samples for level1

Table (4.5) and figure (4.7) indicate the Coliform bacterial population for water samples for level2 shows that Total Coliform levels are in the range from >2 to 220 MPN/100ml, The higher values (220 MPN/100L) of Total Coliform are observed for DW3 and the minimum (>2 MPN/100ml) for S7 sample. most probable number (MPN) for completed faecal coliform count of the water samples for level2 ranged from >2 to 26 MPN/100ml, And the maximum faecal coliform colonies (26 MPN/100ml) was recorded for DW3 and the minimum (>2 MPN/100ml) for S7, S8, S9, S10, DW1, DW2 samples (table 4.6, figure 4.8). These values exclusive S7 sample deviated from the standard recommended by WHO which are zero total coliform count per 100 ml for WHO (Isa *et al.,* 2013 ). These in agreement with Oku et al (2012) who found growth enumeration colonies for total coliform count varied from 42 to 76 per 100mls while fecal coliform count varied from 18 to 34 yielded colonies per 100mls in water samples collected from the Great Kwa River in different locations. All the locations had fecal coliforms which is an indication that the source of the various water samples had been contaminated with substance of fecal origin.

**Table (4.5). The most probable number (MPN) for presumptive total coliform count of the water samples in Level 2**

| No. tested sample | Number of positive tubes | | | MPN Per 100 ml | **95% Confidence Limits** | |
|---|---|---|---|---|---|---|
| | 10ml | 1ml | 0.1ml | | **Lower** | **Higher** |
| S7 | 0 | 0 | 0 | <2 | 0 | 6 |
| S8 | 3 | 2 | 2 | 20 | 7 | 40 |
| S9 | 3 | 2 | 1 | 17 | 7 | 40 |
| S10 | 4 | 5 | 3 | 64 | 11 | 93 |
| DW1 | 2 | 0 | 1 | 6.8 | 1 | 17 |
| DW2 | 3 | 1 | 0 | 11 | 5 | 35 |
| DW3 | 5 | 4 | 2 | 220 | 70 | 440 |
| DW4 | 5 | 3 | 2 | 140 | 52 | 400 |

**Table (4.6). The most probable number (MPN) for completed faecal coliform count of the water samples in Level 2**

| No. tested sample | Number of positive tubes | | | MPN Per 100 ml | **95% Confidence Limits** | |
|---|---|---|---|---|---|---|
| | 10ml | 1ml | 0.1ml | | **Lower** | **Higher** |
| S7 | 0 | 0 | 0 | <2 | 0 | 6 |
| S8 | 0 | 0 | 0 | <2 | 0 | 6 |
| S9 | 0 | 0 | 0 | <2 | 0 | 6 |
| S10 | 0 | 0 | 0 | <2 | 0 | 6 |
| DW1 | 0 | 0 | 0 | <2 | 0 | 6 |
| DW2 | 0 | 0 | 0 | <2 | 0 | 6 |
| DW3 | 3 | 4 | 4 | 36 | 7 | 40 |
| DW4 | 2 | 3 | 1 | 14 | 3 | 28 |

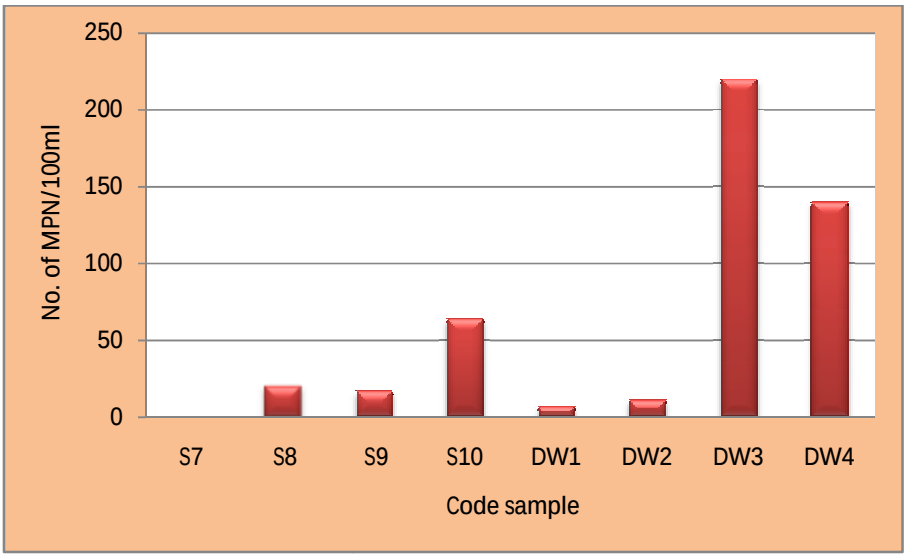


Figure (4.7). The most probable number (MPN) for presumptive total coliform count of the water samples for level 2

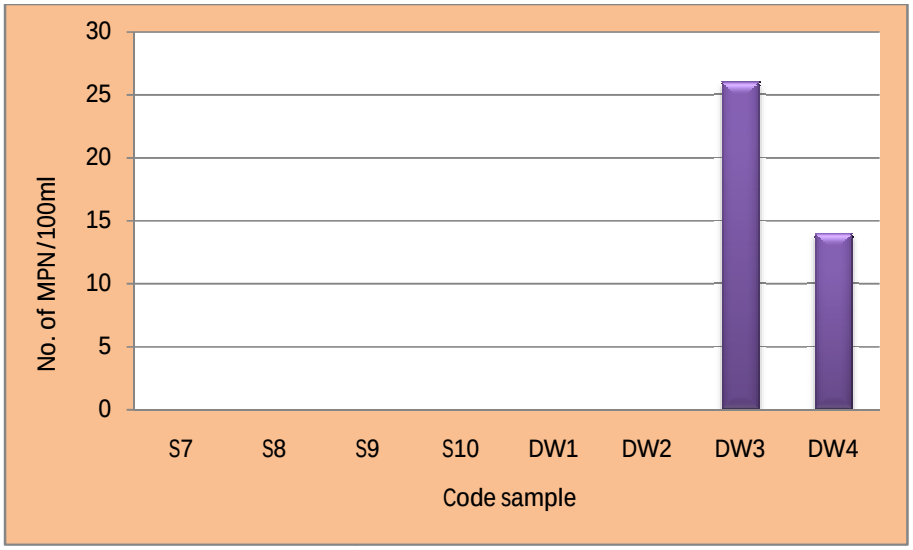


Figure (4.8). The most probable number (MPN) for completed faecal coliform count of the water samples for level 2

In level3 tables shows negative results for the total and faecal coliform counts, all water samples in level3 was >2 MPN/100ml (table 4.7, table 4.8) this water is safe for drinking , This may be due to the efficiency of chlorination. This study agreed with Gopinath *et al.,* (2012) reported that Pysical and bacteriological quality of well water samples from Kanakkary panchayath, Kottayam district, Kerala state, India. The water sample from Cheruvil showed the least MPN value of 7 and this water is safe for drinking.

The results of the total coliform count ( table 4.9, figure 4.9), and faecal coliform account (table 4.10, figure 4.10) showed significant differences ($p = 0.026$, $p = 0.003$ respectively) between the three levels of drinking water samples where full swing be in level1 and in level2 of drinking water sample, The very high contamination may be due to the non-hygienic disposal of fecal waste in pit. Theses result are consistent with the result of Ibiene *et al.,* (2012) bacteriological assessment of drinking water sources in Opuraja community of delta State, Nigeria. where ranged its results of MPN 14 to 192 MPN/100ml . The high coliform count obtained in the samples may be an indication that the water sources are faecally contaminated (Shittu *et al.,* 2008).

**Table (4.7). The most probable number (MPN) for presumptive total coliform count of the water samples in Level 3**

| No. tested sample | Number of positive tubes | | | MPN Per 100 ml | **95% Confidence Limits** | |
|---|---|---|---|---|---|---|
| | 10ml | 1ml | 0.1ml | | **Lower** | **Higher** |
| S1 | 0 | 0 | 0 | < 2 | 0 | 6 |
| S2 | 0 | 0 | 0 | < 2 | 0 | 6 |
| S3 | 0 | 0 | 0 | < 2 | 0 | 6 |
| S4 | 0 | 0 | 0 | < 2 | 0 | 6 |
| S5 | 0 | 0 | 0 | < 2 | 0 | 6 |
| S6 | 0 | 0 | 0 | < 2 | 0 | 6 |

**Table (4.8) The most probable number (MPN) for completed faecal coliform count of the water samples in Level3**

| No. tested sample | Number of positive tubes | | | MPN Per 100 ml | **95% Confidence Limits** | |
|---|---|---|---|---|---|---|
| | 10ml | 1ml | 0.1ml | | **Lower** | **Higher** |
| S1 | 0 | 0 | 0 | < 2 | 0 | 6 |
| S2 | 0 | 0 | 0 | < 2 | 0 | 6 |
| S3 | 0 | 0 | 0 | < 2 | 0 | 6 |
| S4 | 0 | 0 | 0 | < 2 | 0 | 6 |
| S5 | 0 | 0 | 0 | < 2 | 0 | 6 |
| S6 | 0 | 0 | 0 | < 2 | 0 | 6 |

**Table (4.9). Analysis of (Kruskal-Wallis Test ) total coliform count**

| Kruskal-Wallis Test | | | | | |
|---|---|---|---|---|---|
| Levels | N | Mean Rank | df | Chi-square | p- vales |
| Level 1 | 7 | 14.93 | 2 | 7.323 | 0.026* |
| Level 2 | 8 | 10.94 | | | |
| Level 3 | 6 | 6.50 | | | |

**levels between the three levels of drinking water samples**

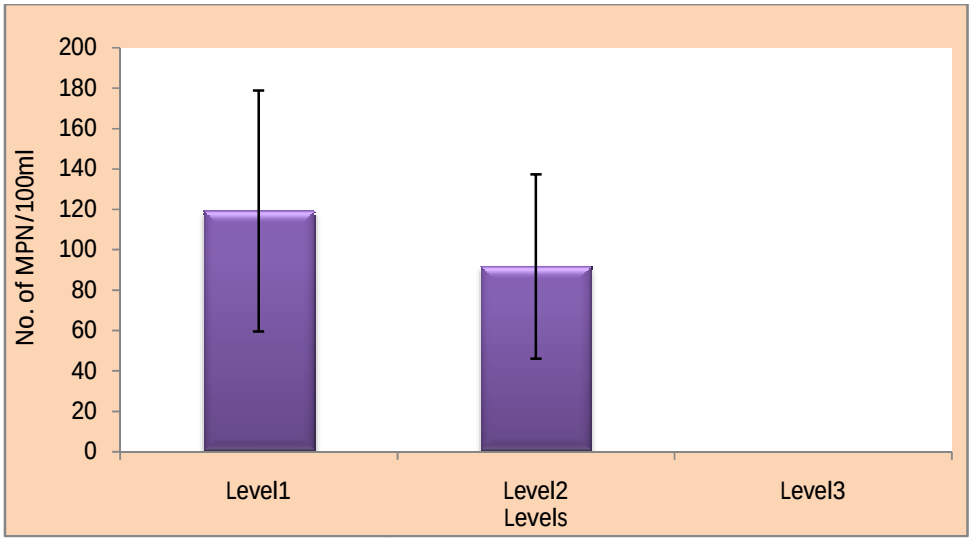


Figure (4.9). Total coliform count levels in the three levels of drinking water samples

**Table (4.10). Analysis of (Kruskal-Wallis Test ) faecal coliform count levels between the three levels of drinking water samples**

| Kruskal-Wallis Test | | | | | |
|---|---|---|---|---|---|
| Levels | N | Mean Rank | df | Chi-square | p- vales |
| Level 1 | 7 | 15.21 | 2 | 11.842 | 0.003** |
| Level 2 | 8 | 12.56 | | | |
| Level 3 | 6 | 4 | | | |

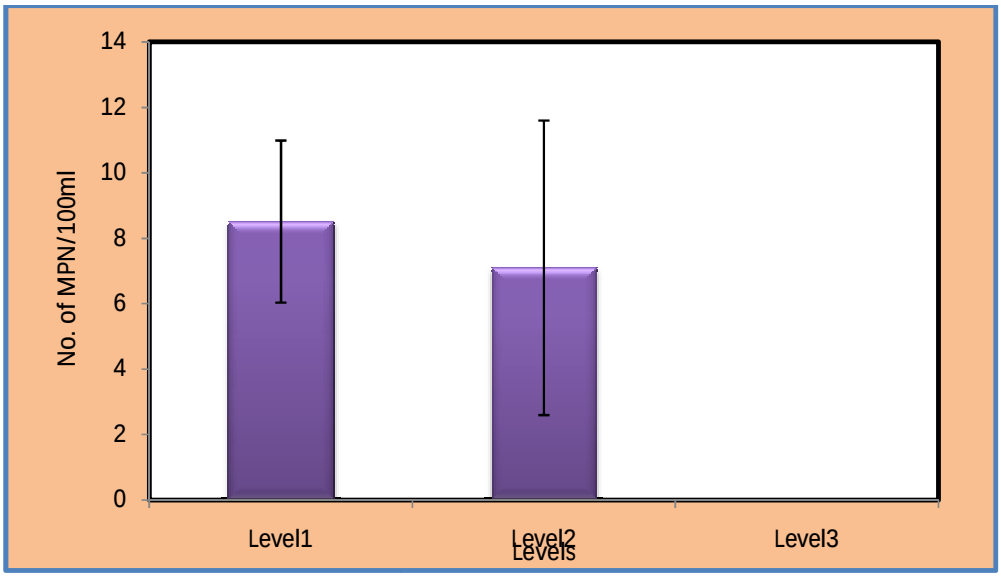


Figure (4.10). Faecal coliform count levels in the three levels of drinking water samples

## 4.1.3. Isolation and identification of isolates:

### 4.1.3.1. Bacterial identified by API tests:

The bacteria isolated from water samples in this work included *Staphylococcus aureus, Staphylococcus albus* and *Escherichia coli ,* in level 1 and level 2 water samples (table 4.11). theses in agreement with Alam and Pandey (2014), They've isolated *Staphylococcus, E.coli* from water samples of river Barak and Its tributaries, Assam, India. And in agreement with Shittu *et al.,* (2008), They also isolatedated *Staphylococcus aureus* from river water used for drinking and swimming purposes in Abeokuta, Nigeria.

*Staphylococcus aureus* is a Gram positive coccal bacterium that is frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies. *Staphylococcus aureus* described in one drinking water sample for level 2 (DW3), and one drinking water sample for level 1 (DW6) (table 4.11).

S*taphylococcus epidermidis* (*Staphylococcus albus*) is a Gram-positive bacterium, and one of over 40 species belonging to the genus Staphylococcus . It is part of the normal human flora, typically the skin flora, and less commonly the mucousal flora. Although *S. epidermidis* is not usually pathogenic, patients with compromised immune

systems are at risk of developing infection. These infections are generally hospital-acquired. *Staphylococcus albus* described in four drinking water samples for level 2 ( S8, S9, DW1), and five drinking water sample for level 1 (DW5, DW7, DW9, DW11) see table (4.11).

*Escherichia coli* also known as *E.coli* is a Gram-negative, facultative anaerobic, rod shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E.coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination. it's a member of the total coliform group of bacteria and is the only member that is found exclusively in the faeces of humans and other animals. Its presence in water indicates not only recent faecal contamination of the water but also the possible presence of intestinal disease causing bacteria, viruses, and protozoa. In this study *E. coli* isolated from only one sample its DW6 (Well depth of 60 meters nearly) see table (4.11). This may be due to Its located near or in the vicinity of well Sanitation. These *E. coli* strains may belong to recently identified pathogenic serotypes such as *E. coli* O157:H7 and *E. coli* O104:H4 that have been reported to cause diseases in humans (Palamuleni and Mercy Akoth., 2015). The isolation of E. *Coli* is a strong indication that the water samples contain pathogenic organisms and are not potable for drinking.

**Table (4.11). Bacteria isolated from drinking water samples in level 1 and level 2 and identification by (API) test**

| Levels | Sample sites | Bacterial isolated |
|---|---|---|
| Level 1 | DW5: Well above a depth of 200 meters | *Staphylococcus albus* (at 45C$^{O}$) |
| | DW6: Well depth of 60 meters nearly (It located near or in the vicinity of well Sanitation) | *Staphylococcus aureus, Escherichia coli* (at 45C$^{O}$) |
| | DW7: Well depth of nearly 23 meters | *Staphylococcus albus* (at 45C$^{O}$) |
| | DW9: Well depth of nearly 25 meters | *Staphylococcus albus* (at 45C$^{O}$) |
| | DW11: Well depth of nearly 70 meters | *Staphylococcus albus* (at 37C$^{O}$) |
| Level 2 | S8: Haita park | *Staphylococcus albus* (at 37C$^{O}$) |
| | S9: Tukrah hospital | *Staphylococcus albus* (at 37C$^{O}$) |
| | DW1: One of the wells that feed the reservoir. | *Staphylococcus albus* (at 37C$^{O}$) |
| | DW3: Romanian company | *Staphylococcus aureus* (at 45C$^{O}$) |

### 4.1.3.2. Bacterial identified by the BD Phoenix system:

the Phoenix systems (BD Diagnostic System) is automated instruments for rapid organism identification and susceptibility testing. Some bacterial isolates device definition Al Phoenix The resulting species are *Pseudomonas aeruginosa, Citrobacter freundii, Ochrobacterum anthropi, Cedecea lapagie, Streptococcus anginosus, Stenophomonas maltophilia.*

*Pseudomonas aeruginosa:* is a genus of Gram-negative, belonging to the family Pseudomonadaceae containing 191 validly described species. The members of the genus demonstrate a great deal of metabolic diversity, and consequently are able to colonize a wide range of niches. *Pseudomonas sp.* are very common in water systems due to their ease of colonization and they form thick biofilms which consequently has an effect on turbidity, taste and odour of drinking water (Kurup *et al.*, 2010). presence of *P. aeruginosa* in drinking water in high volumes may be associated with complaints about taste, odour and turbidity (Okereke1 *et al.*, 2014). In these study its isolated from S10 (Tukrah security directorate  well within the Directorate) and DW2 (Tukrah university: well within the Directorate).

*Streptococcus anginosus:* Streptococci are facultatively anaerobic, Gram positive organisms that often occur as chains or pairs and are catalase negative (in contrast, staphylococci are catalase positive). is part of the human bacteria flora, but can cause diseases including brain and liver abscesses under certain circumstances. The habitat of

*S. anginosus* is a wide variety of sites inside the human body. In these study its isolated from DW2: Tukrah university (well within the Directorate).

*Citrobacter freundii:* is a species of facultative, anaerobic Gram negative bacilli of the Enterobacteriaceae family. The bacteria are long bacterial rods with a typical length of 1–5 μm. Most *C. freundii* cells generally have several flagella used for locomotion, but some do not and are non-motile. *C. freundii* is a soil organism, but can also be found in water, sewage, food and in the intestinal tracts of animals and humans. As an opportunistic pathogen, *C. freundii* is responsible for a number of significant infections. It is known to be the cause of nosocomial infections of the respiratory tract, urinary tract, blood, and many other normally sterile sites in patients. *C. freundii* represents about 29% of all opportunistic infections. *C. freundii* in theses study isolated from DW9 (Well depth of nearly 25 meters) and agreement with Antony and Ferdinand Brisca Renuga (2012) them also isolated *C. freundii* from Ananthanar channel of Kanyakumari district, Tamil Nadu, India.

*Ochrobacterum anthropi: Ochrobactrum anthropi* is a Gram-negative, motile, non-fermentative, oxidase and urease positive, aerobic bacillus, formerly classified as Achromobacter species, that belongs to the new genus Ochrobactrum. The organism is widely distributed in soil, environmental and water sources, including antiseptic solutions and dialysis fluid, and it has been recognized as part of the normal human flora of the large intestine. *O. anthropi* has been rarely described as a human pathogen. in theses study isolated from DW10 (Well depth of nearly 40 meters).

*Cedecea* bacteria are gram negative, oxidase negative bacilli that include 5 species. This genus was designated by the Centers for Disease Control (CDC) in 1981 as a separate genus in the *Enterobacteriaceae* family. In humans, *Cedecea*has been located in the blood and saliva, wounds and abscesses, and in ulcerated tissue the medical literature there are very few reports that describe infections such as pneumonia, soft tissues infections, urinary tract infections and sepsis, which were caused by different species of the *Cedecea* genus such as *C. neteri* and *C. lapagei.* however, This genus resembles no other group of Enterobacteriaceae. in theses study isolated from DW4 (Hospital Tukrah town well inside the hospital).

*Stenophomonas maltophilia* is an aerobic, nonfermentative, Gram egative bacterium. It is an uncommon bacterium and human infection is difficult to treat. grouped in the genus *Xanthomonas* before eventually becoming the type species of the genus *Stenotrophomonas* in 1993. in theses study isolated from DW4 Hospital Tukrah town (well inside the hospital).

**Table (4.12). Bacteria isolated from drinking water samples in level 1 and level 2 and identification by by the BD Phoenix system**

| Levels | Sample sites | Bacterial isolated | Confidence Value |
|---|---|---|---|
| Level 2 | from S10 (Tukrah security directorate well within the Directorate) | *Pseudomonas aeruginosa* | 95% |
| | DW2: Tukrah university (well within the Directorate) | *Streptococcus anginosus* | 91% |
| | DW4: Hospital Tukrah town (well inside the hospital). | *Cedecea lapagei*<br>*Stenophomonas maltophilia* | 90%<br>99% |
| Level 1 | DW9 (Well depth of nearly 25 meters) | *Citrobacter freundii* | 99% |
| | DW10 (Well depth of nearly 40 meters). | *Ochrobacterum anthropi* | 90% |

## 4.2. Physical and chemical Characteristics of Water samples:

### 4.2.1. The temperature :

Water Temperature ranged between 19.3 to 25°C in level3 and in level2 ranged between 19 to 24.5°C, in level1 water temperature ranged between 19 to 24.8°C the maximum temperature (25°C) was recorded for level3 in and the minimum (19°C) for level1 and level2 samples (table 4.13, figure 11). Thirupathaiah *et al* (2012) observed that water temperature fluctuate between 24.75°C to 28.5°C during studies of Monthly changes in physico-chemical parameters of Karimnagar district, Andhra Pradesh .Temperature of water may not be as important in pure water because of the wide range of temperature tolerance in aquatic life, but in polluted water, temperature can have profound effects on dissolved oxygen (DO) and biological oxygen demand (BOD).The fluctuation in river water temperature usually depends on the season, geographic location, sampling time and temperature of effluents entering the stream (Venkatesharaju *et al*., 2010).The results showed not significant differences ($p = 0.847$) ( table 4.14, figure 13).

### 4.2.2. pH:

pH of water samples ranged from 7.1 to 7.4 in level3 and from 6.85 to 7.45 in level2 and in level1 ranged from 6.66 to 7.5 (table 4.13, figure 4.12) . This near neutrality of most of the waters examined in this study poses no health risk to consumers WHO use the water for cooking, washing, drinking, bathing and for other domestic purposes. pH is most important in determining the corrosive nature of water. Lower the pH value

higher is the corrosive nature of water ( Kakaraddi *et al*, 2014). The pH of water is extremely important.

The fluctuations in optimum pH ranges may lead to an increase or decrease in the toxicity of poisons in water bodies (Okonko *et al.,* 2008). The pH values of the samples are given in (table 4.15, Figure 4.14). Table showing there was no significant statistical difference ($p = 0.201$) of average PH in the three levels. The pH of all the water samples were in agreement with pH assigned by Shittu *et al* (2008) as the pH of water samples ranges from 6.5 – 8.5.

**Table (4.13). Temperature setting and the values of pH and the free chlorine and turbidity of the samples that have been collected**

| LEVELS | Number and code sample | $C^o$ | pH | Rcl | turbid | Bicarbonate |
|---|---|---|---|---|---|---|
| | | 25 | 6.5-8.5 | 0.2-0.5 | <5 | <500 |
| Level 3 | S1 | 19.3 | 7.4 | 0.01 | 0.16 | 21 |
| | S2 | 24 | 7.1 | 0.1 | 0.27 | 18 |
| | S3 | 25 | 7.3 | 0.02 | 0.2 | 18 |
| | S4 | 24 | 7.34 | 0.01 | 0.24 | 18 |
| | S5 | 20.3 | 7.4 | 0 | 0.1 | 16 |
| | S6 | 22.8 | 7.2 | 0 | 0.12 | 20 |
| Level 2 | S7 | 20.7 | 7.25 | 0 | 0.2 | 150 |
| | S8 | 19.5 | 7.35 | 0 | 0.51 | 120 |
| | S9 | 24.4 | 7.4 | 0 | 0.45 | 165 |
| | S10 | 19.5 | 7.45 | 0 | 0.74 | 135 |
| | DW1 | 19 | 7 | 0 | 0.19 | 68 |
| | DW2 | 23.5 | 7.15 | 0 | 0.29 | 210 |
| | DW3 | 23.6 | 7.1 | 0 | 0.51 | 180 |
| | DW4 | 24.5 | 6.85 | 0 | 3 | 97 |
| Level 1 | DW5 | 22 | 7.38 | 0 | 0.55 | 142 |
| | DW6 | 24.5 | 7.55 | 0 | 0.18 | 208 |
| | DW7 | 19.3 | 6.96 | 0 | 0.62 | 210 |
| | DW8 | 20.5 | 6.8 | 0 | 0.2 | 205 |
| | DW9 | 24.8 | 7.2 | 0 | 0.32 | 125 |
| | DW10 | 24 | 6.66 | 0 | 0.58 | 240 |
| | DW11 | 19 | 6.72 | 0 | 31.6 | 200 |

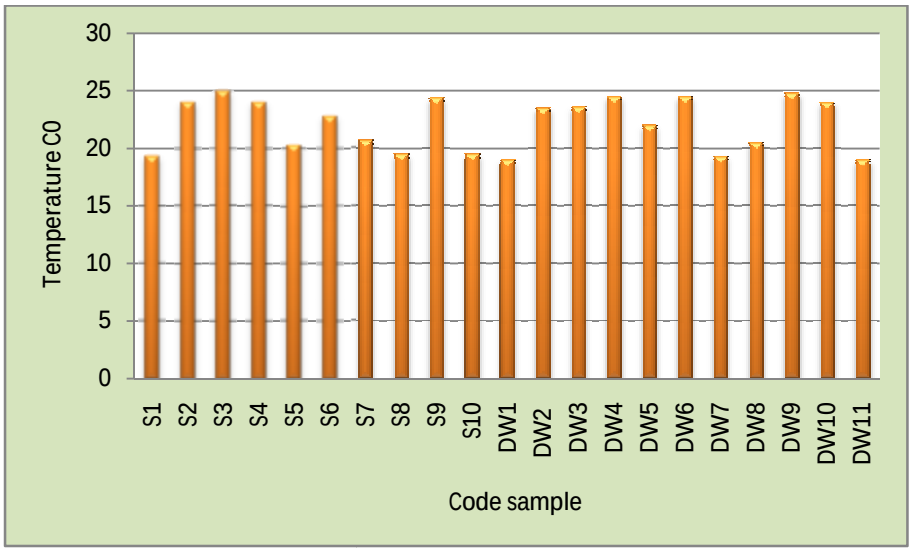


Figure (4.11). Temperature of three levels of drinking water samples

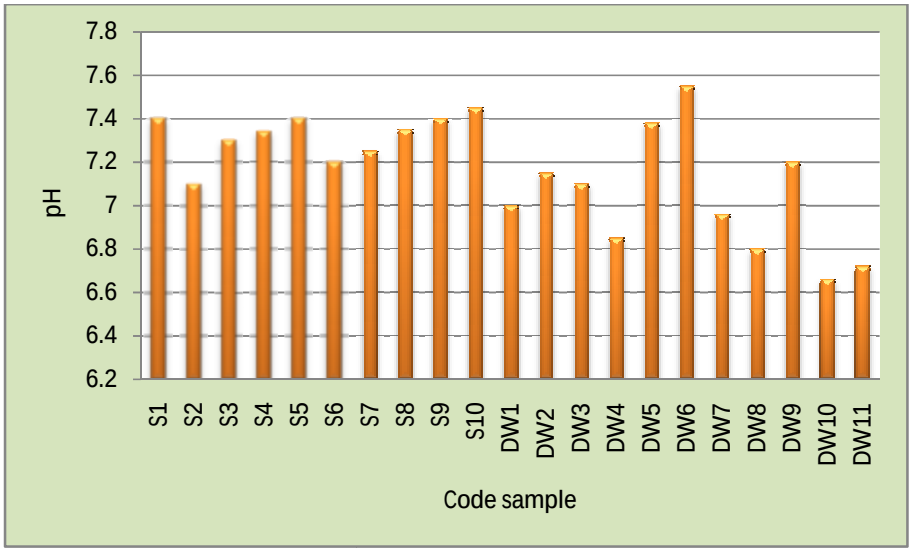


Figure (4.12). pH of three levels of drinking water samples

**Table (4.14). Analysis of variance (ANOVA) showing temperature of three levels of drinking water samples**

| Department | N | Mean | Std. Deviation | %95Confidence Interval for Mean | | ANOVA | |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | p-vales | L.S.D |
| Level 3 | 6 | 22.566 | 2.27 | 20.178 | 24.954 | 0.847 | - |
| Level 2 | 8 | 21.837 | 2.38 | 19.844 | 23.830 | | |
| Level 1 | 7 | 22.014 | 2.47 | 19.729 | 24.299 | | |
| Total | 21 | 22.104 | 2.28 | 21.065 | 23.143 | | |

N: Number of drinking water samples for each level.

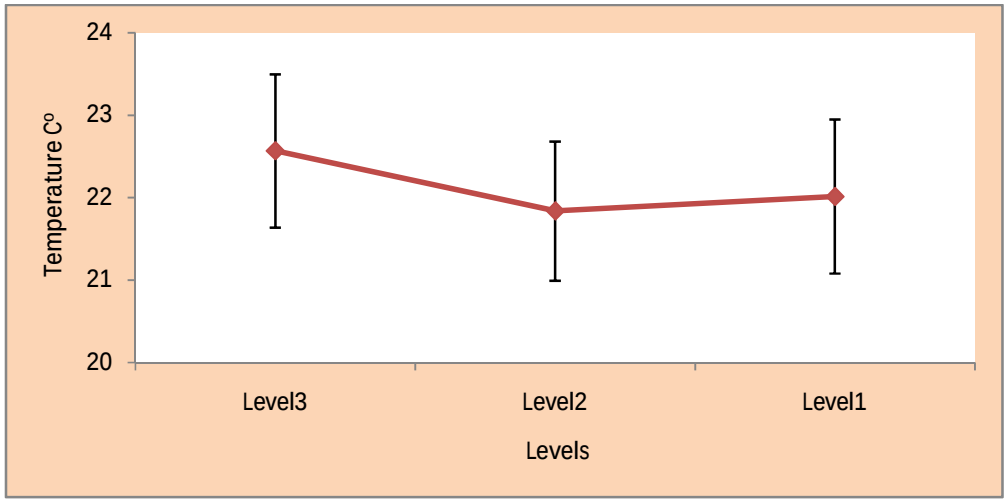


Figure (4.13). Variance of temperature levels of the three levels of drinking water samples

**Table (4.15). Analysis of variance (ANOVA) showing difference of average pH in the three levels**

| Department | N | Mean | Std. Deviation | %95Confidence Interval for Mean | | ANOVA | |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | p-vales | L.S.D |
| Level 3 | 6 | 2.27 | 0.11 | 7.164 | 7.415 | 0.201 | - |
| Level 2 | 8 | 2.38 | 0.20 | 7.02 | 7.367 | | |
| Level 1 | 7 | 2.47 | 0.34 | 6.72 | 7.357 | | |
| Total | 21 | 2.28 | 0.25 | 7.053 | 7.285 | | |

N: number of drinking water samples for each level.

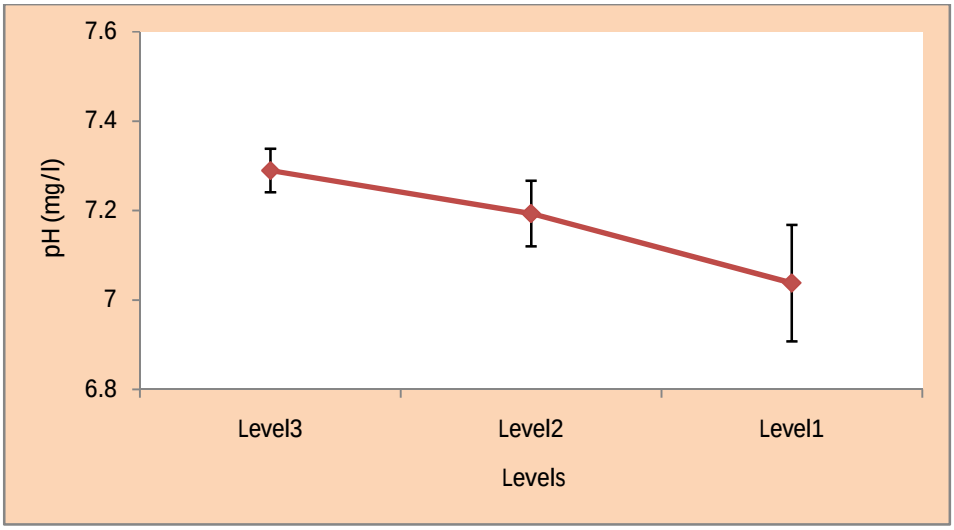


Figure (4.14). Difference of average pH in the three levels

### 4.2.3. Chlorine residual (Rcl):

According to the WHO, after at least 30 min of contact time the minimum residual concentration of free chlorine at the point of use should be 0.2 mg/L (Patrick *et al.*, 2011) In this study, the concentration of residual free chlorine in most water samples were below the recommended limit of WHO (0.2-0.5 mg/l), which indicates the inefficiency of disinfection in the distribution system. Where the residual concentration of free chlorine find only in three samples for level 3 where S1,S4 were 0.01 mg/L and in S2 was 0.1 mg/L, S3 was 0.02 mg/L. but in level 1 and level 2 drinking water sample is measured not in them (table 4.13, figure 4.15). It is either not duplicate or ercentage of chlorination very low. The results showed not significant differences ($p > 0.01$) between the three levels it's described in (table 4.16, figure 4.16).

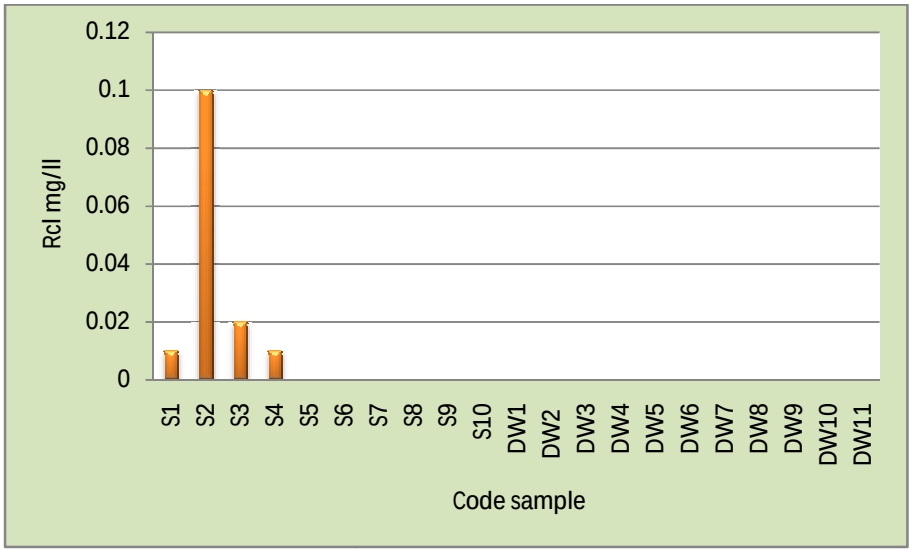


Figure (4.15). Chlorine residual of three levels of drinking water samples

**Table (4.16). Analysis of variance (ANOVA) showing difference of Chlorine residual average in the three levels**

| Department | N | Mean | Std. Deviation | %95Confidence Interval for Mean | | ANOVA | |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | p-vales | L.S.D |
| Level 3 | 6 | 0.023 | 0.038 | -0.016 | 0.063 | 0.083 | - |
| Level 2 | 8 | 0 | 0 | 0 | 0 | | |
| Level 1 | 7 | 0 | 0 | 0 | 0 | | |
| Total | 21 | 0.006 | 0.0219 | -0.003 | 0.016 | | |

N: Number of drinking water samples for each level.

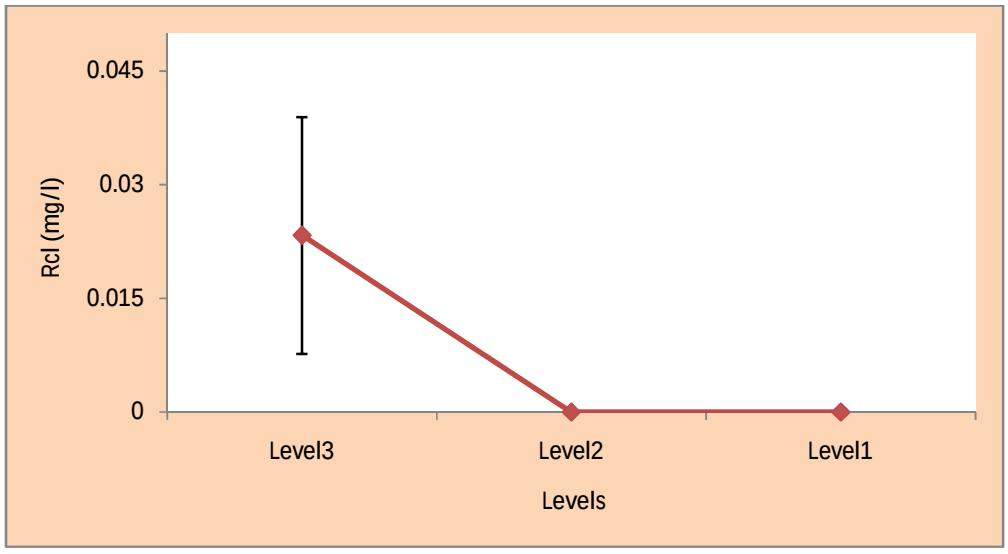


Figure (4.16). Difference of average chlorine residual in the three levels

### 4.2.4. Turbidity:

Turbidity measurements ranged from 0.1 NTU to 0.2 neophelometric turbidity units (NTU), for samples water in level 3, and from 0.2 to 3 NTU for sample water in level 2, 0.18 to 31.6 NTU for samples inn level1 (table 4.13, figure 4.17), Generally the high turbidity observed in some of the water sources did not agree with WHO standards (5 NTU). But for level 3 drinking water samples the turbidity is in agreement with WHO standard. Water turbidity is very important because high turbidity is often associated with higher level of disease causing microorganism, such as bacteria and other parasites ( Isa *et al.,* 2013). Variations were statistically no significant of different water samples (P = 0.4) (table 4.17, figure 4.18). this is not agreement with turbidity assigned by Yasin *et al* in (2015) The mean turbidity value of water samples was the highest (24.22 NTU) for unprotected wells and the least (1.87 NTU) for tap water.

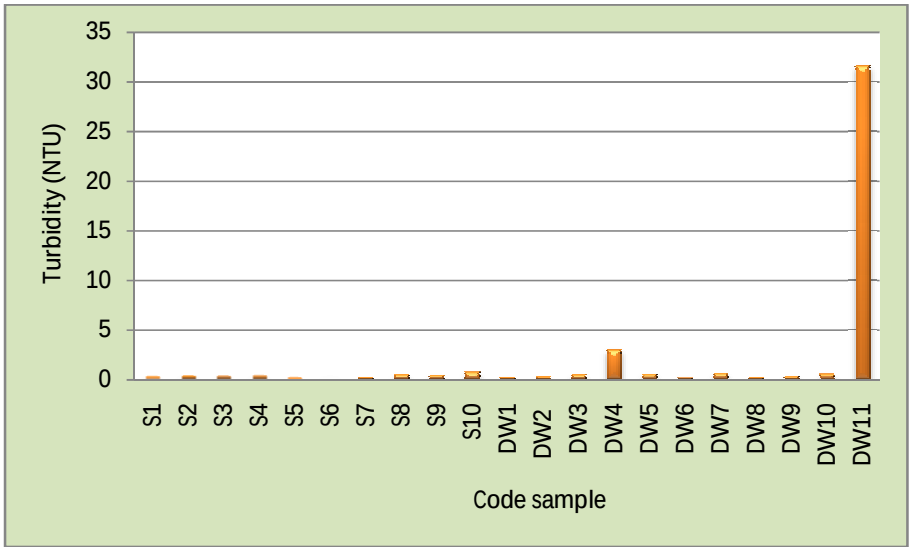


Figure 4.17: Turbidity of three levels of drinking water samples.

**Table (4.17). Analysis of variance (ANOVA) showing difference of turbidity average in the three levels**

| Department | N | Mean | Std. Deviation | %95Confidence Interval for Mean | | ANOVA | |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | p-vales | L.S.D |
| Level 3 | 6 | 0.181 | 0.067 | 0.111 | 0.252 | 0.400 | - |
| Level 2 | 8 | 0.736 | 0.933 | -0.043 | 1.516 | | |
| Level 1 | 7 | 4.864 | 11.790 | -6.040 | 15.768 | | |
| Total | 21 | 1.953 | 6.819 | -1.150 | 5.058 | | |

N: Number of drinking water samples for each level.

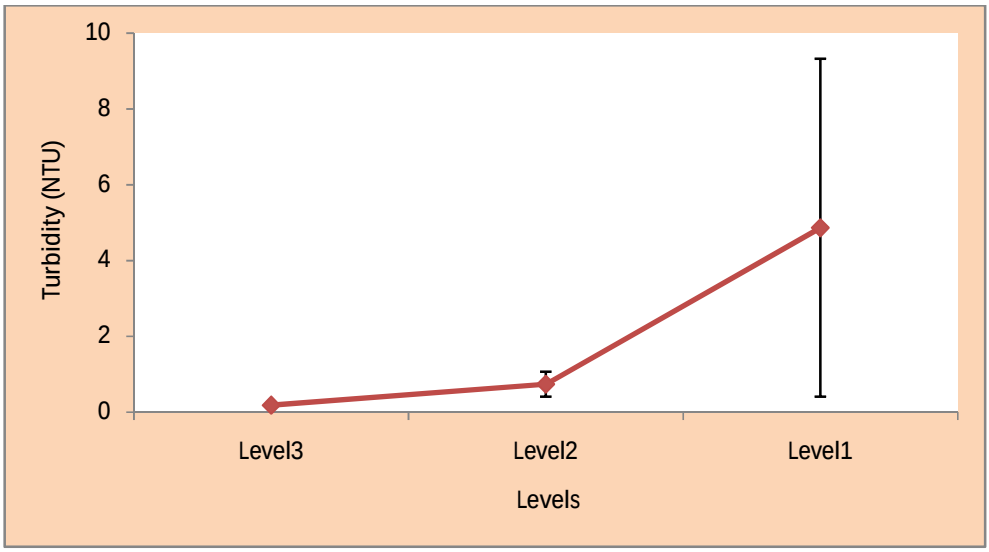


Figure (4.18). Difference of average turbidity in the three levels

**4.2.5. Alkalinity:**

Total alkalinity is the sum of carbonates and bicarbonates. The values of bicarbonates are also used to express alkalinity, in the absence of carbonates (Temgoua., 2011). of water samples ranged from 16 to 21 mg/l in level3 and from 68 to 210 ml/l in level2 and in level1 ranged from 125 to 240 (table 4.13, figure19). the concentration of bicarbonate in all water samples were below the recommended limit of WHO (500 mg/l). The results showed significant differences ($p \leq 0.01$) between the three levels it's described in (table 4.18, figure 4.20).

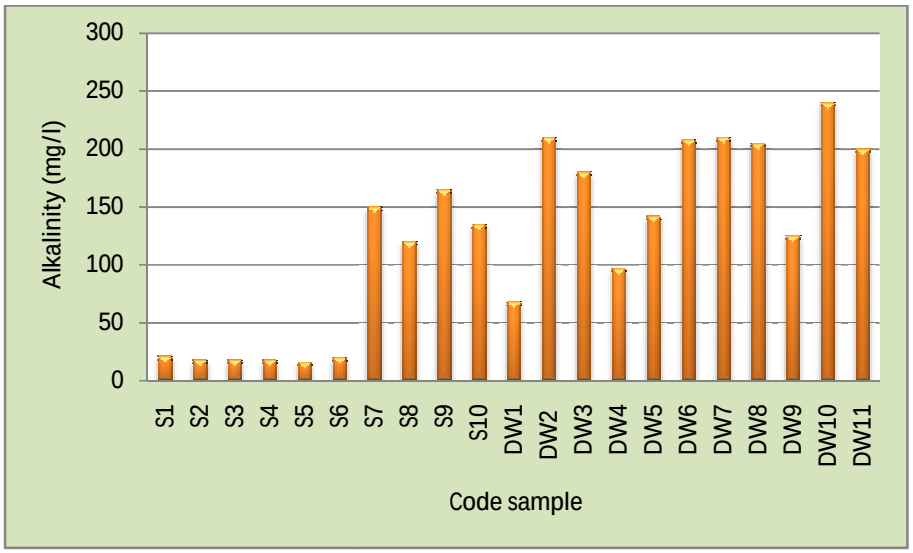


Figure (4.19). Alkalinity of three levels of drinking water samples

**Table (4.18). Analysis of variance (ANOVA) showing difference of average alkalinity in the three levels**

| Department | N | Mean | Std. Deviation | %95Confidence Interval for Mean | | ANOVA | |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | p-vales | L.S.D |
| Level 3 | 6 | 18.5 | 1.76 | 16.65 | 20.34 | 0.008* | C>B B>A |
| Level 2 | 8 | 140.62 | 45.79 | 102.33 | 178.91 | | |
| Level 1 | 7 | 190 | 40.98 | 152.09 | 227.90 | | |
| Total | 21 | 122.195 | 78.80 | 86.32 | 158.05 | | |

A: Level 3 B: Level 2 C: Level 1

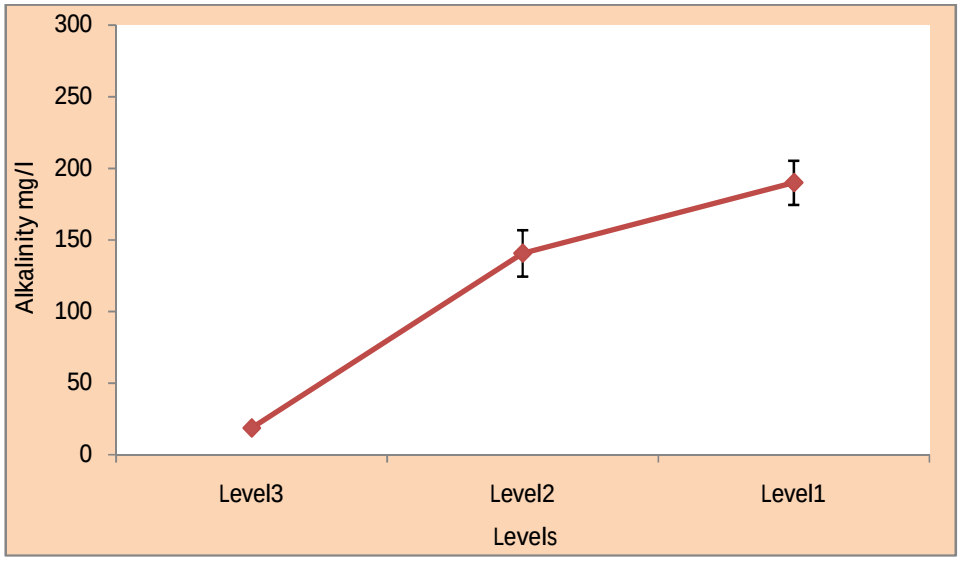


Figure (4.20). Difference of average alkalinity in the three levels

### 4.2.6. Electical Conductivity:

The electrical conductivity in the water samples ranged from 131μs/cm (microSiemens per centimetre) to 187 μs/cm in level3 , its agreement with WHO standers (<2500 μs/cm) and arise in level2 to range from 1825 μs/cm to 8760 μs/cm a. It in level1 ranged from 1660 μs/cm to 41000 μs/cm did not agree with WHO standards (table 4.19, figure 4.21). Higher conductivity of 41000 μs/cm was observed in the water sample (DW11: Well depth of nearly 70 meters) for level 1 sample, although there is no disease or disorder associated with conductivity of drinking water (Isa ***et al.,*** 2013). There was a statistically significant difference (P = 0.021) among mean electric conductivities of different between levels of drinking water samples(table 4.20, figure 4.23).

### 4.2.7. Total dissolved solid:

Total dissolved solids for drinking water samples ranged from 66 mg/L to 95 mg/L in level3 and 912 mg/L to 4380 mg/L for samples of water in level2, in level1 total dissolved solids for drinking water samples ranged from 829 mg/L to 20000 mg/L (table 4.19, figure 4.22). the high TDS observed in some of the water sources in level 1and level 2 did not agree with WHO standards (<1200 mg/L). But for level 3 drinking water samples the TDS is in agreement with WHO standard. Dissolved Solids in natural water are generally consistence from bicarbonate, chloride, calcium, magnesium, sodium and sulphate where the primary sources for TDS in receiving waters are; leaching of soil contamination, agricultural and residential runoff and point source water pollution discharge from industrial or sewage treatment plants ( Al-Obaidy ***et al.***, 2015).

Table 4.21, figure 4.24 showed strong significant deferential (p = 0.018) between levels of drinking water samples.

**Table (4.19). Measurement of electrical conductivity and the proportion of dissolved solids in the samples collected**

| Levels | Number and code sample | Electical Conductivity | Total dissolved solid |
|---|---|---|---|
| | | Limit <2500 µs/cm | Limit <1200mg/l |
| Level 3 | S1 | 131.5 | 66 |
| | S2 | 134 | 67 |
| | S3 | 145 | 72 |
| | S4 | 168.4 | 84 |
| | S5 | 186 | 93 |
| | S6 | 187 | 95 |
| Level 2 | S7 | 2830 | 1414 |
| | S8 | 2840 | 1418 |
| | S9 | 4320 | 2160 |
| | S10 | 2860 | 1428 |
| | DW1 | 5690 | 2845 |
| | DW2 | 3500 | 1749 |
| | DW3 | 1825 | 912 |
| | DW4 | 8760 | 4380 |
| Level 1 | DW5 | 1660 | 829 |
| | DW6 | 6560 | 3280 |
| | DW7 | 6600 | 3300 |
| | DW8 | 7360 | 3680 |
| | DW9 | 12740 | 6370 |
| | DW10 | 15630 | 7815 |
| | DW11 | 41000 | 20000 |

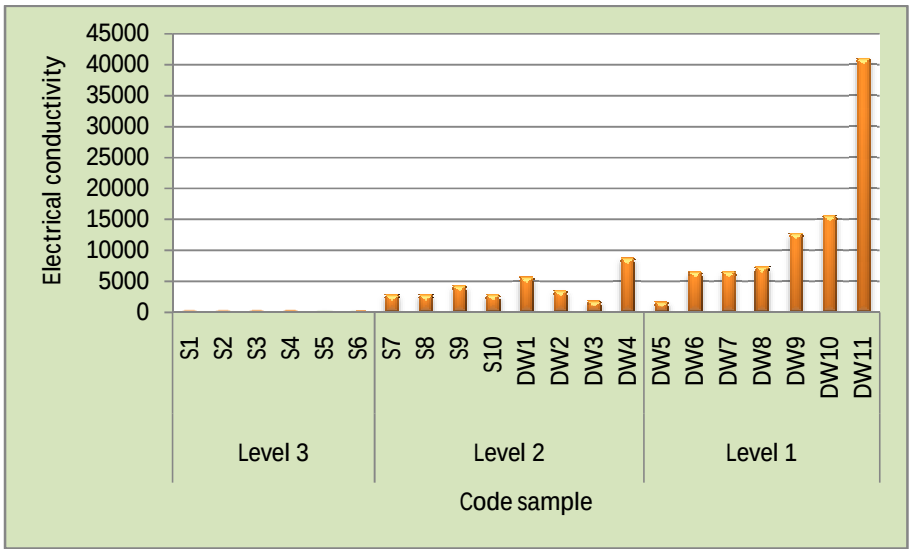


Figure (4.21). Electrical conductivity of three levels of drinking water samples

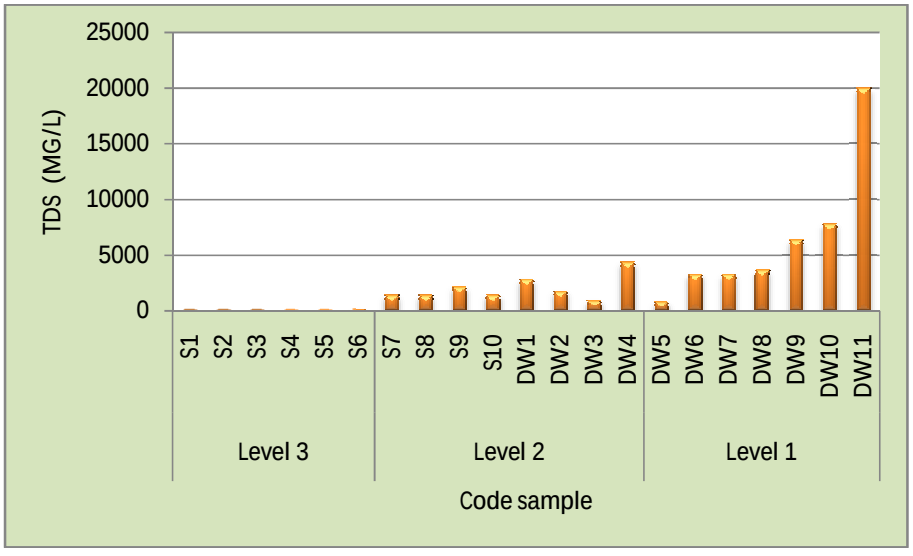


Figure (4.22). Total dissolved solids of three levels of drinking water samples

**Table (4.20). Analysis of variance (ANOVA) showing difference of electrical conductivity average in the three levels**

| Department | N | Mean | Std. Deviation | %95Confidence Interval for Mean | | ANOVA | |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | p-vales | L.S.D |
| Level 3 | 6 | 158.65 | 25.210 | 132.193 | 185.107 | 0.021 * | C>A C>B |
| Level 2 | 8 | 4078.1 | 2222.03 | 2220.45 | 5935.79 | | |
| Level 1 | 7 | 13078 | 13126.18 | 938.877 | 25218.2 | | |
| Total | 21 | 5958.4 | 9092.11 | 1819.74 | 10097.1 | | |

A: Level 3 B: Level 2 C: Level1

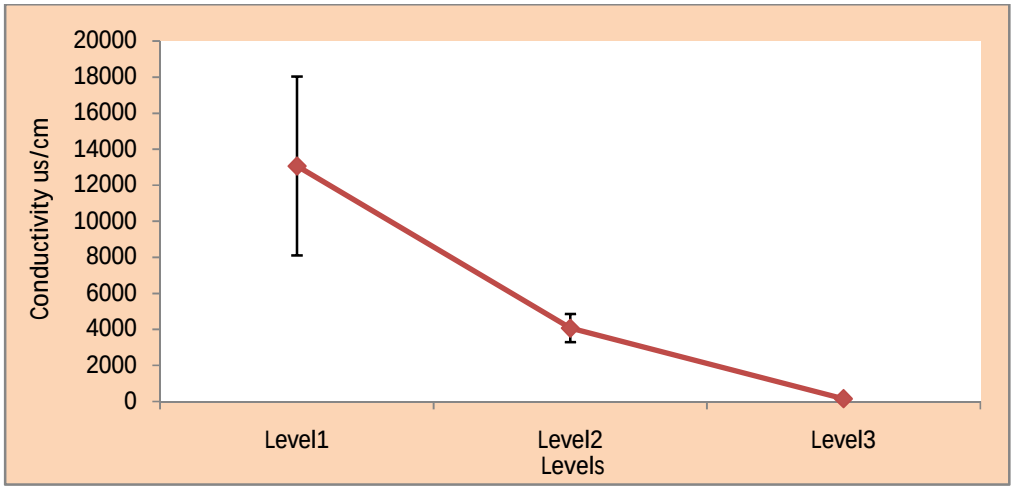


Figure (4.23). Difference of average electrical conductivity in the three levels

**Table (4.21). Analysis of variance (ANOVA) showing difference of Total dissolved solids average in the three levels**

| Department | N | Mean | Std. Deviation | %95Confidence Interval for Mean | | ANOVA | |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | p-vales | L.S.D |
| Level 3 | 6 | 79.5 | 12.942 | 65.92 | 93.08 | 0.018* | C>A C>B |
| Level 2 | 8 | 2038.2 | 1111.522 | 1108.99 | 2967.51 | | |
| Level 1 | 7 | 6467.7 | 6386.31 | 561.36 | 12374.07 | | |
| Total | 21 | 2955.1 | 4450.09 | 929.43 | 4980.76 | | |

A: Level 1 B: Level 2 C: Level 1

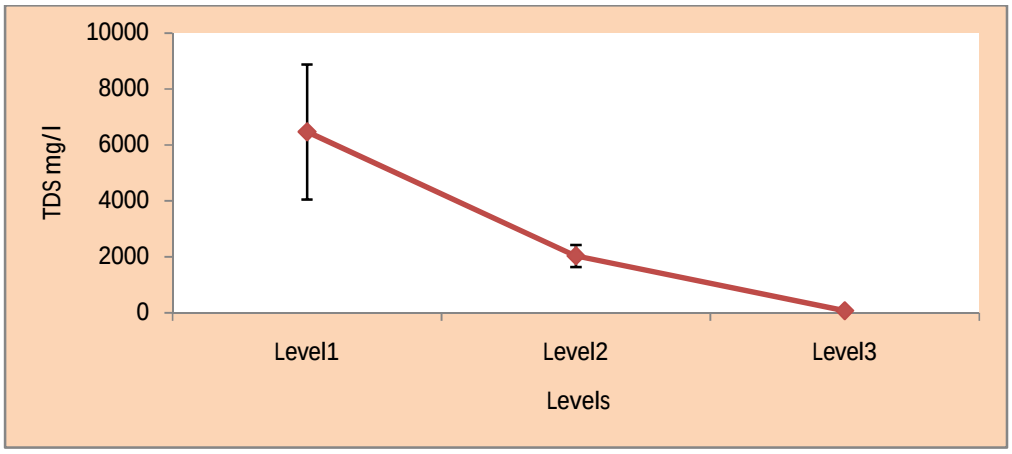


Figure (4.24). Difference of average Total dissolved solids in the three levels

### 4.2.8. Nitrites and Nitrates:

The nitrate-nitrogen in the water samples for all levels were zero mg/l (table 4.20), and the nitrates in the drinking water samples were zero mg/l for level3 samples, and in level2 ranged from 1.77 mg/l to 64.19 mg/l, and the nitrates in the drinking water samples ranged from 0 mg/l to 88.54mg/l for level1 (table 4.22, figure 4.25). The **nitrites**-nitrogen in all drinking water sample in this study is in agreement with WHO standard (<3 mg/L), but high nitrates observed in two of the water sources in level 1 (DW10: well depth of nearly 40 meters ) and level 2(DW4: hospital Taucheira village well inside the hospital). did not agree with WHO standards (<50 mg/L). But for level 3 drinking water samples the nitrates is in agreement with WHO standard. These results was agreement with results Temgoua (2011) where In Dschang, values of nitrates were between 0.9 and 3.5 mg/l for the majority of management points. The Fongo Ndeng spring water, and Fiankop wells have raised rates (17 and 12 mg/l respectively). Significant variations were observed for nitrates in the three levels of drinking water samples ($P < 0.05$) (table 4.23, figure 4.27).

### 4.2.9. Ammonia:

Ammonia concentration in water samples was zero mg/l in level3, but in level2 ranged from 0 mg/l to 0.29 mg/l, in level1 ammonia concentration ranged from 0 mg/l to 5.25 (table 4.22, figure 4.26). Ammonia concentration in level 3 and level2 of drinking water samples were agreement with WHO standard(>1.5 mg/L), but in level 1 drinking water samples high ammonia concentration observed in two samples (DW5: well above a depth of 200 meters, DW11: well depth of nearly 70 meters) were 4mg/l, 5.25mg/l

respectively. Table 4.24and figure 4.28 showed variations were not statistically significant among means of different water samples (P = 0.121). $NH_3$, $NO_2$ and $NO_3$ are naturally occurring ions in water system that are part of the nitrogen cycle. The sources of nitrogen Compounds in aquatic environments include the decomposition or breakdown of organic waste matter, gas exchange with the atmosphere, animal waste, nitrogen fixation processes, domestic wastewater, Fertilizer and sewage, Nitrate is the stable form of combined nitrogen for oxygenated systems, and can be reduced by microbial action. Nitrite ion contains nitrogen in a relatively unstable oxidation state; many chemical and biological processes can further reduce nitrite to various compounds or oxidize it to nitrate under oxygenation or reduce it to ammonia under Deoxygenation (Al-Obaidy *et al.,* 2015**).**

**Table (4.22). Analysis of each of nitrites and nitrates and ammonia in the samples collected**

| Levels | Number and code sample | $NO_2$ <3 mg/l | $NO_3$ <50 mg/l | $NH_3$ <1.5 mg/l |
|---|---|---|---|---|
| Level 3 | S1 | 0 | 0 | 0 |
| | S2 | 0 | 0 | 0 |
| | S3 | 0 | 0 | 0 |
| | S4 | 0 | 0 | 0 |
| | S5 | 0 | 0 | 0 |
| | S6 | 0 | 0 | 0 |
| Level 2 | S7 | 0 | 1.77 | 0 |
| | S8 | 0 | 3.1 | 0 |
| | S9 | 0 | 11.51 | 0 |
| | S10 | 0 | 1.77 | 0 |
| | DW1 | 0 | 3.98 | 0 |
| | DW2 | 0 | 13.28 | 0.29 |
| | DW3 | 0 | 6.19 | 0.01 |
| | DW4 | 0 | 64.19 | 0 |
| Level 1 | DW5 | 0 | 0 | 4 |
| | DW6 | 0 | 32.75 | 0 |
| | DW7 | 0 | 9.73 | 0.06 |
| | DW8 | 0 | 50.91 | 0.08 |
| | DW9 | 0 | 42.94 | 0 |
| | DW10 | 0 | 88.54 | 0.024 |
| | DW11 | 0 | 0 | 5.25 |

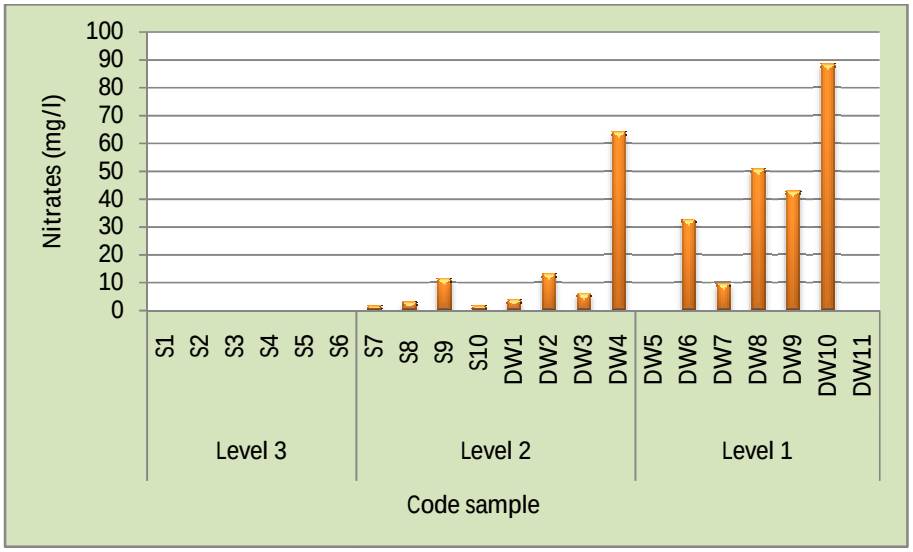


Figure (4.25). Nitrates of three levels of drinking water samples

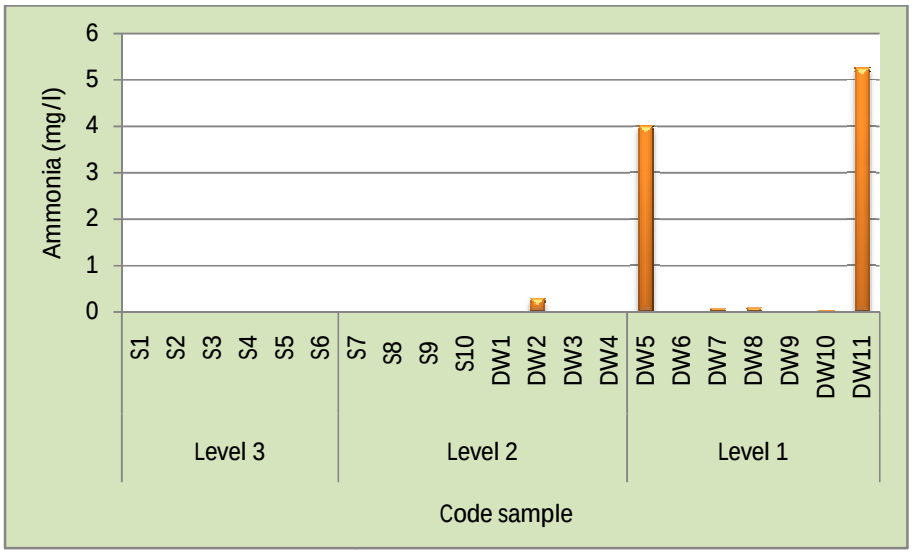


Figure (4.26). Ammonia of three levels of drinking water samples

**Table (4.23). Analysis of variance (ANOVA) showing difference of nitrates average in the three levels**

| Department | N | Mean | Std. Deviation | %95Confidence Interval for Mean | | ANOVA | |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | p-vales | L.S.D |
| Level 3 | 6 | | | | | 0.049* | C>B |
| Level 2 | 8 | 13.223 | 21.045 | -4.370 | 30.81 | | |
| Level 1 | 7 | 32.124 | 32.193 | 2.350 | 61.89 | | |
| Total | 21 | 15.745 | 25.2338 | 4.259 | 27.23 | | |

A: Level 3 B: Level 2 C: Level 1

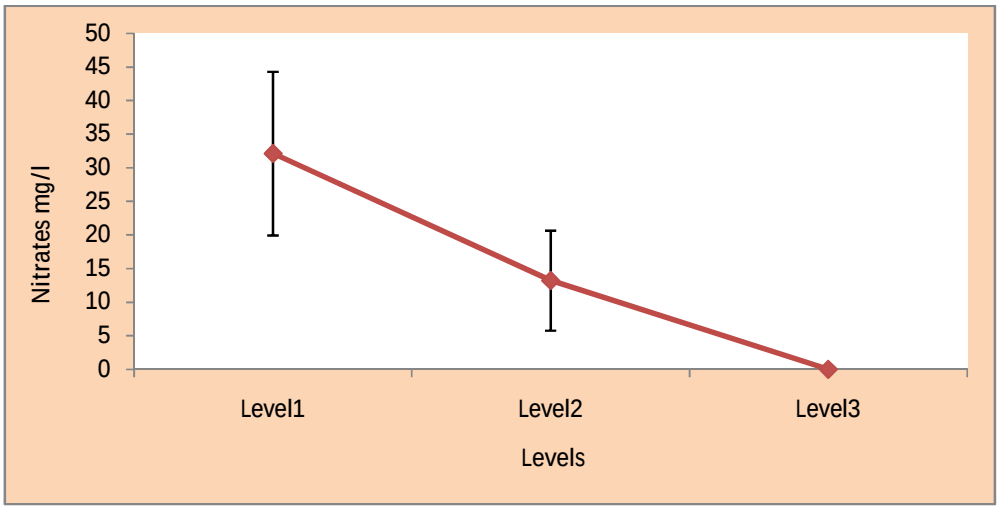


Figure (4.27). Difference of average nitrates in the three levels

**Table (4.24). Analysis of variance (ANOVA) showing difference of ammonia average in the three levels**

| Departme nt | N | Mean | Std. Deviation | %95Confidence Interval for Mean | | ANOVA | |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | p-vales | L.S.D |
| Level 3 | 6 | 0 | 0 | 0 | 0 | 0.121 | - |
| Level 2 | 8 | 0.037 | 0.1020 | -0.0478 | 0.1228 | | |
| Level 1 | 7 | 1.3448 | 2.2698 | - 0.7543 | 3.4440 | | |
| Total | 21 | 0.4625 | 1.3993 | -0.1744 | 1.0995 | | |

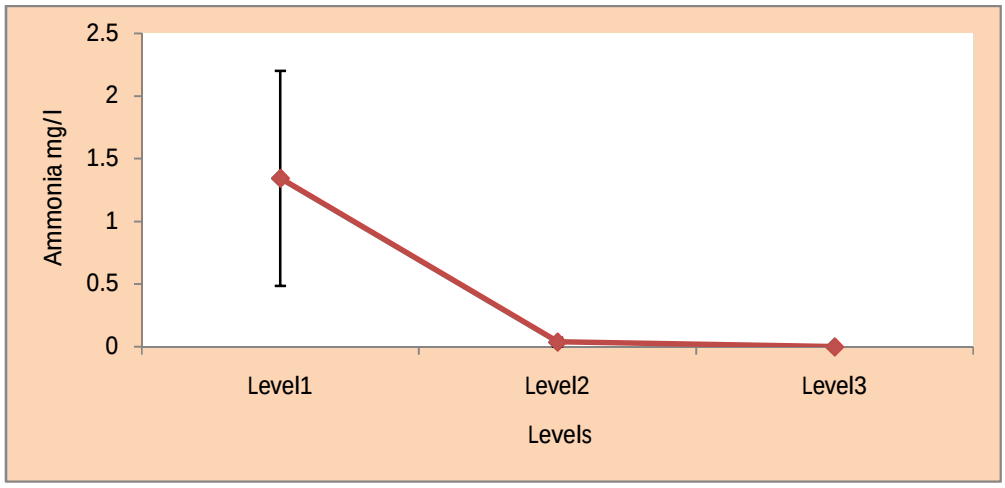


Figure (4.28). Difference of average ammonia in the three levels

**4.2.10. Chloride:**

Chloride concentration of the samples ranged from 28.36 to 35.45 mg/l for level 3 drinking water sample and for level 2 drinking water samples ranged from 411 to2300 mg/l, in level 1 drinking water samples ranged from 355 to 37000 mg/l(table 4.25, figure 29). high chloride observed in all of the water sources in level 1 and level 2 did not agree with WHO standards (<250 mg/L). But for level 3 drinking water samples the chloride is in agreement with WHO standard and thus of good quality with respect to chlorides. These is result concerted with results Temgoua (2011). Where Its results In water of Dschang, the values obtained were between 0 and 3 mg/l. ,Significant variations were observed for chloride in the three levels of drinking water samples (P = 0.05) (table 4.26, figure 4.31). occur naturally in all types of waters. High concentration of chlorides is considered to be the indicators of pollution due to organic wastes of animal or industrial origin. Chlorides are troublesome in irrigation water and also harmful to aquatic life (K *et al*., 2010).

**4.2.11: Sodium:**

sodium concentration of the samples ranged from 7.7 to 11.3 mg/l for level 3 drinking water sample and for level 2 drinking water samples ranged from 125 to 1182 mg/l, in level 1 drinking water samples ranged from 125 to 6710 mg/l (table 4.25, figure 30). high sodium observed in most of the water sources in level 1 and level 2 did not agree with WHO standards (<200 mg/L), These is result concerted with results Venkatesharaju *et al.,* (2010). But for level 3 drinking water samples the sodium is in

agreement with WHO standard. Significant variations were observed for sodium in the three levels of drinking water samples (P = 0.048) (table 4.27, figure 4.32).

**Table (4.25). Determine the proportion of chloride, sodium, sulfates and salinity in the samples collected**

| Levels | Number and code sample | Cl | Na | So4 | Salinity |
|---|---|---|---|---|---|
| | | <250 mg/l | <200 mg/l | <250mg/l | Mg/l |
| Level 3 | S1 | 30 | 11.3 | 2 | 0 |
| | S2 | 28.36 | 10.1 | 2 | 0 |
| | S3 | 35.45 | 10.2 | 2 | 0 |
| | S4 | 28.36 | 9.7 | 1 | 0 |
| | S5 | 30 | 7.7 | 2 | 0 |
| | S6 | 29 | 9 | 2 | 0 |
| Level 2 | S7 | 740 | 261 | 160 | 1300 |
| | S8 | 1099 | 497 | 140 | 2200 |
| | S9 | 744 | 250 | 72 | 1300 |
| | S10 | 1631 | 540 | 169 | 3000 |
| | DW1 | 780 | 238 | 91 | 1400 |
| | DW2 | 904 | 308 | 35 | 1700 |
| | DW3 | 411 | 108 | 81 | 800 |
| | DW4 | 2300 | 1182 | 302 | 4900 |
| Level 1 | DW5 | 2092 | 806 | 54 | 4000 |
| | DW6 | 355 | 125 | 397 | 700 |
| | DW7 | 4609 | 2006 | 508 | 9200 |
| | DW8 | 16396 | 6710 | 1594 | 26000 |
| | DW9 | 2003 | 716 | 157 | 3600 |
| | DW10 | 37000 | 1140 | 568 | 7400 |
| | DW11 | 1900 | 675 | 275 | 3600 |

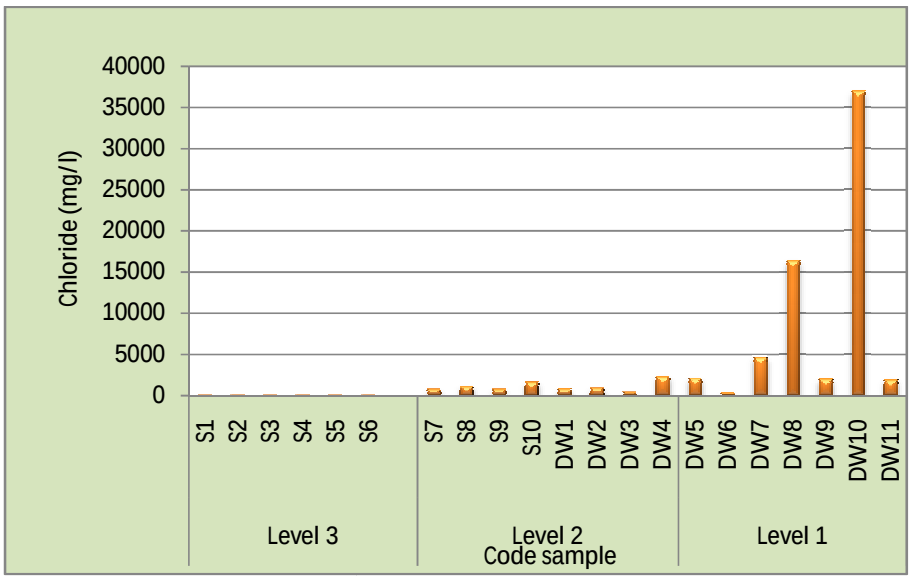


Figure (4.29). Chloride of three levels of drinking water samples

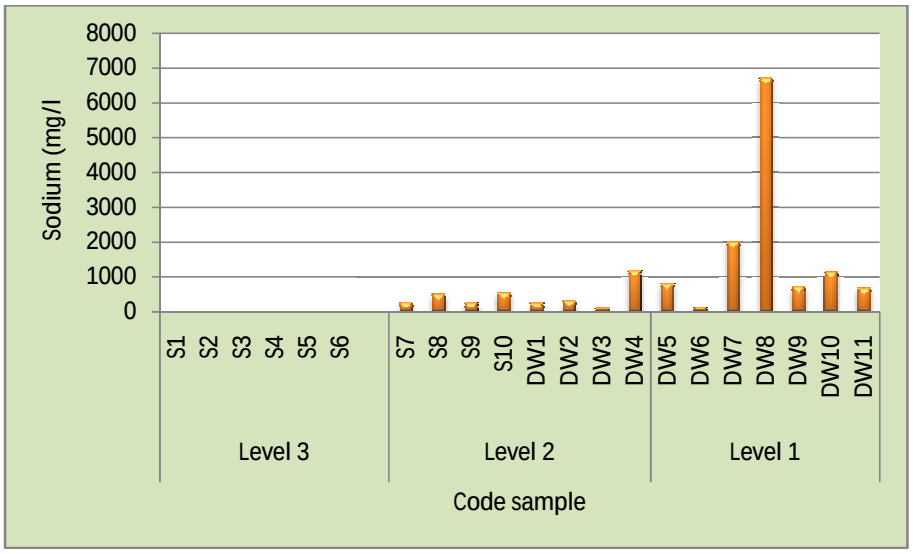


Figure (4.30). Sodium of three levels of drinking water samples

**Table (4.26). Analysis of variance (ANOVA) showing difference of chloride average in the three levels**

| Department | N | Mean | Std. Deviation | %95Confidence Interval for Mean | | ANOVA | |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | p-vales | L.S.D |
| Level 3 | 6 | 30.195 | 2.67782 | 27.3848 | 33.0052 | * 0.05 | C>A |
| Level 2 | 8 | 1076.1 | 608.191 | 567.664 | 1584.58 | | |
| Level 1 | 7 | 9193.5 | 13413.57 | 3211.92 | 21599.06 | | |
| Total | 21 | 3483.1 | 8450.689 | -363.605 | 7329.81 | | |

A: Level 3 B: Level 2 C: Level 1

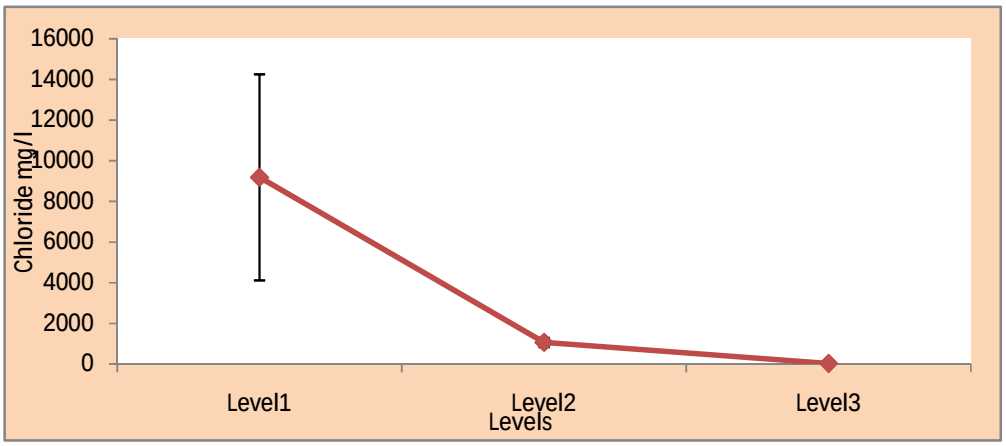


Figure (4.31). Difference of average chloride the three levels

**Table (4.27). Analysis of variance (ANOVA) showing difference of sodium average in the three levels**

| Department | N | Mean | Std. Deviation | %95Confidence Interval for Mean | | ANOVA | |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | p-vales | L.S.D |
| Level 3 | 6 | 9.667 | 1.2209 | 8.385 | 10.948 | *0.048 | C>A |
| Level 2 | 8 | 423 | 337.658 | 140.71 | 705.29 | | |
| Level 1 | 7 | 1739.7 | 2265.503 | 355.52 | 3834.95 | | |
| Total | 21 | 743.81 | 1459.33 | 79.529 | 1408.09 | | |

A: Level 3 B: Level 2 C: Level 1

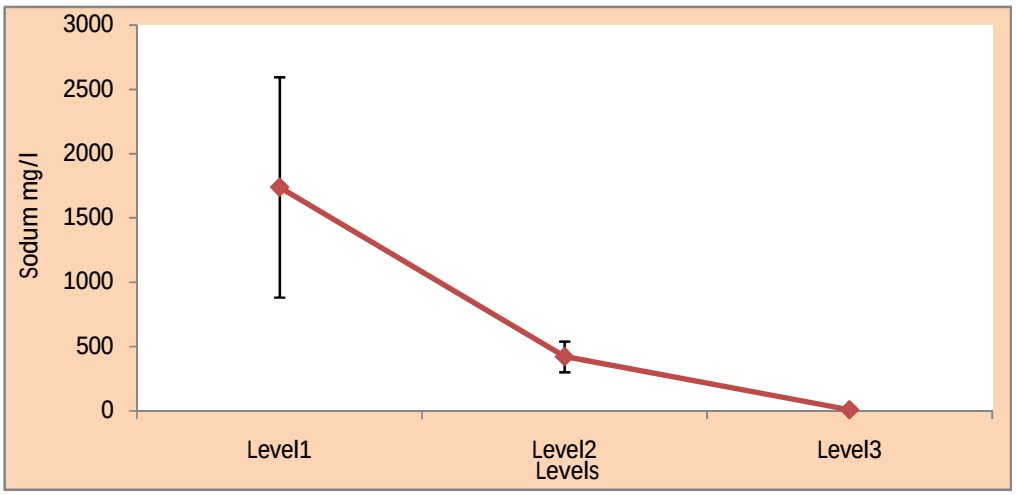


Figure (4.32). Difference of average sodium in the three levels

**4.2.12. Sulphates:**

the sulphates in water represent agricultural pollution (Temgoua ., 2011). the sulphates concentration of the samples ranged from 1 to 2 mg/l for level 3 drinking water sample and for level 2 drinking water samples ranged from 35 to 302 mg/l, in level 1 drinking water samples ranged from 54 to 1594 mg/l(table 4.25, figure 33). high sulphate observed in all of the water sources in level 1 did not agree with WHO standards (<250 mg/L) except DW5 sample in agreement with WHO standard. and for level 2 (except DW4) and level 3 drinking water samples the sulphate is in agreement with WHO standard. these results similar result's reporter by Kakaraddi *et al* (2014), they find Sulphate concentration ranged from 7 to 238ppm, which is within the permissible limit(400mg/l). High concentration of sulphate has laxative effect. Significant variations were observed for sulphate in the three levels of drinking water samples (P = 0.017) (table 4.28, figure 4.35).

**4.2.13: Salinity:**

Salinity concentration of the samples ranged from 700 to 26000 mg/l for level 1drinking water sample and for level 2 drinking water samples ranged from 800 to 4900 mg/l, in level 3 drinking water samples Salinity concentration was zero for all samples (table 4.25, figure 34). Significant variations were observed for salinity in the three levels of drinking water samples (P = 0.028) (table 4.29, figure 4.36).

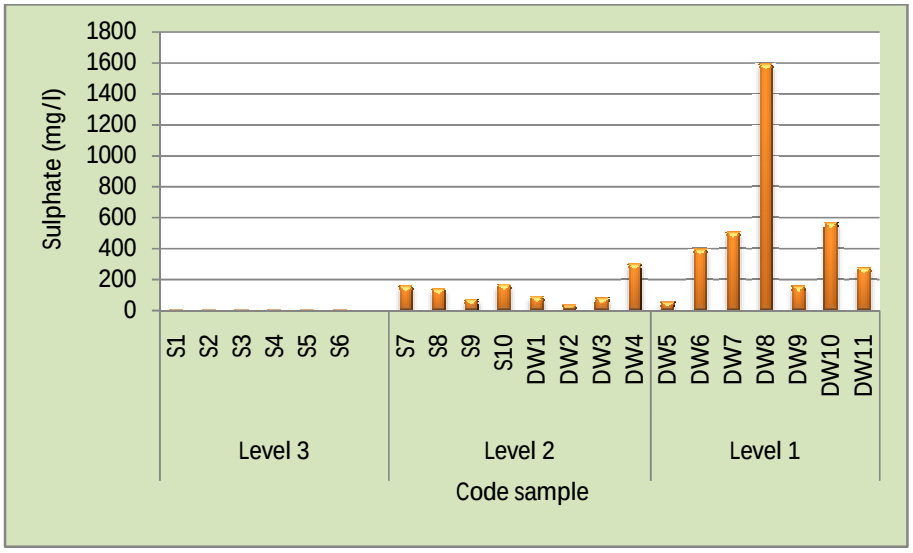


Figure (4.33). Sulphate of three levels of drinking water samples

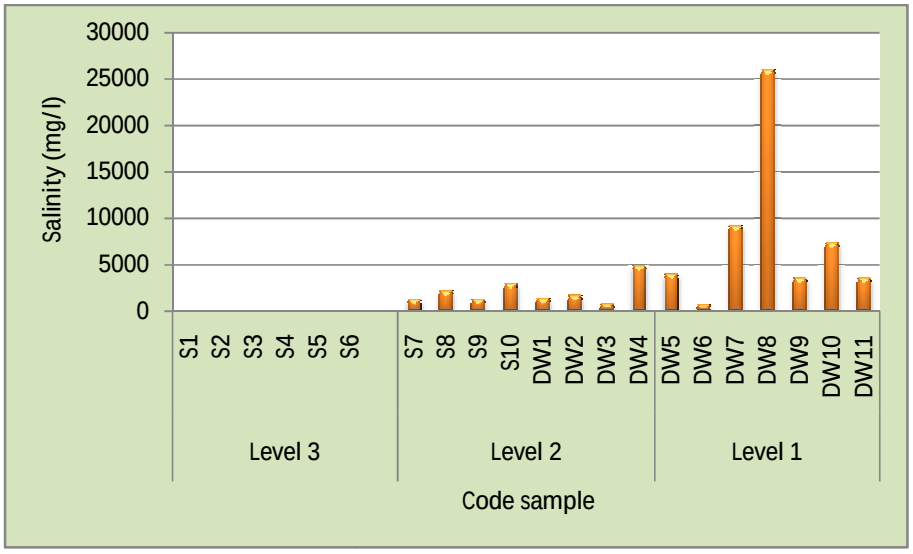


Figure (4.34). Salinity of three levels of drinking water samples

**Table (4.28). Analysis of variance (ANOVA) showing difference of sulphate average in the three levels**

| Department | N | Mean | Std. Deviation | %95Confidence Interval for Mean | | ANOVA | |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | p-vales | L.S.D |
| Level 3 | 6 | 1.83 | 0.408 | 1.4 | 2.26 | * 0.017 | C>A C>B |
| Level 2 | 8 | 131.25 | 83.155 | 61.73 | 200.77 | | |
| Level 1 | 7 | 507.57 | 512.985 | 33.14 | 982 | | |
| Total | 21 | 219.71 | 357.407 | 57.02 | 382.4 | | |

A: Level 3 B: Level 2 C: Level 1

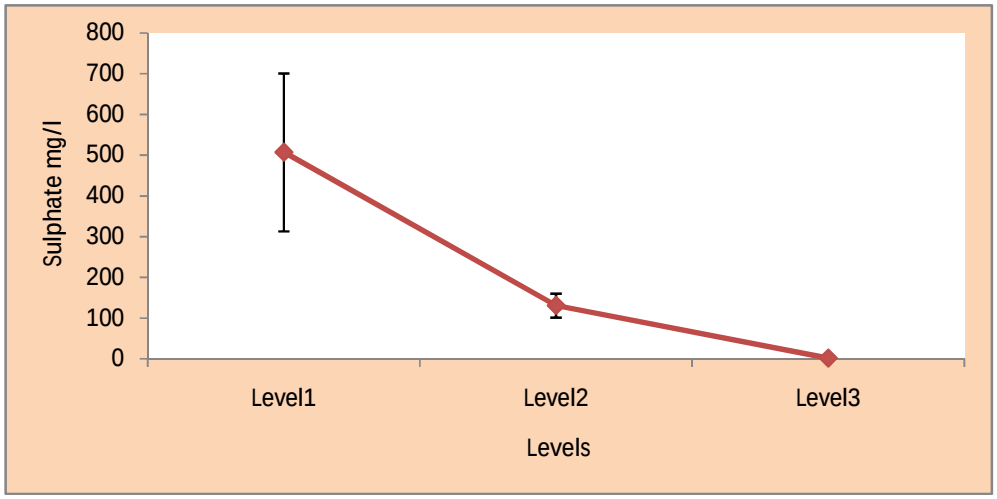


Figure (4.35). Difference of average sulphate in the three levels

**Table (4.29). Analysis of variance (ANOVA) showing difference of salinity average in the three levels**

| Department | N | Mean | Std. Deviation | %95Confidence Interval for Mean | | ANOVA | |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | p-vales | L.S.D |
| Level 3 | 6 | 0 | 0 | 0 | 0 | * 0.028 | C>B |
| Level 2 | 8 | 2075 | 1324.225 | 967.92 | 3182.0 | | |
| Level 1 | 7 | 7785.71 | 8499.88 | -75.37 | 15646.8 | | |
| Total | 21 | 3385.7 | 5761.10 | 763.29 | 6008.14 | | |

A: Level 3 B: Level 2 C: Level 1

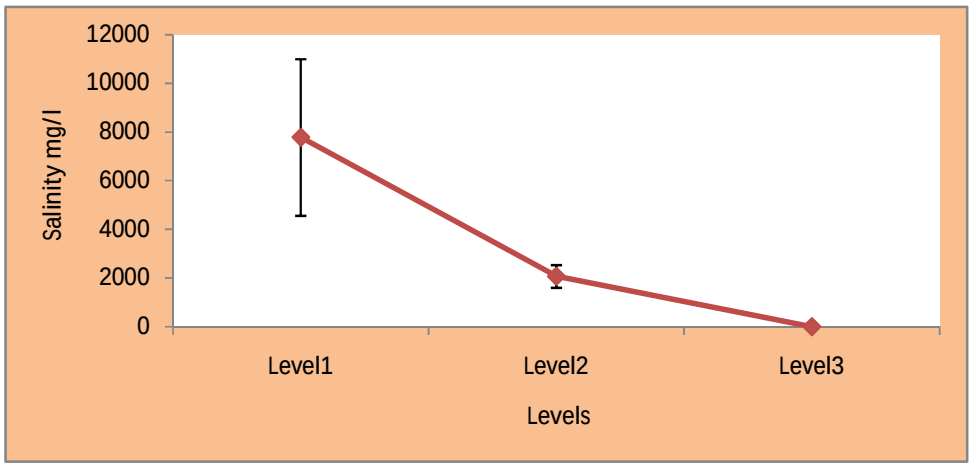


Figure 4.36: Difference of average salinity in the three levels

### 4.2.14. Total Hardness as $CaCO_3$:

The principal natural sources of hardness in water are dissolved polyvalent metallic ions from sedimentary rocks, seepage and runoff from soils. Calcium and magnesium, the two principal ions, are present in many sedimentary rocks, the most common being limestone and chalk (WHO., 2011)**.** total hardness concentration of the samples ranged from 25 to 27 mg/l for level 3 drinking water sample and for level 2 drinking water samples ranged from 350 to 1336 mg/l, in level 1 drinking water samples ranged from 235 to 4600 mg/l(table 4.30, figure 37). high total hardness observed in the water sources in level 1 did not agree with WHO standards (<500 mg/L) except DW5 sample (well above a depth of 200 meters) in agreement with WHO standard. and for level 2 is in agreement with WHO standard except DW1 (One of the wells that feed the reservoir) and DW5 (hospital Tukrah village well inside the hospital) and level 3 drinking water samples the total hardness is in agreement with WHO standard. Significant variations were observed for total hardness in the three levels of drinking water samples (P = 0.014) (table 4.31, figure 4.40).

### 4.2.15. Calcium:

calcium concentration of the samples ranged from 6.8 to 10 mg/l for level 3 drinking water sample and for level 2 drinking water samples ranged from 8 to 228 mg/l, in level 1 drinking water samples ranged from 60 to 640 mg/l(table 4.30, figure 38). All driking water sample for level 3 and level 2 except DW4 are in agreement with WHO standard(<200 mg/l).but for level 1 drinking water samples DW5, DW6, DW7,DW8 are in agreement with WHO standard and for DW9, DW10, DW11 are above of WHO

standard. Significant variations were observed for calcium in the three levels of drinking water samples ($P = 0.002^{**}$ ) (table 4.32, figure 4.41).

**4.2.16. Magnesium:**

magnesium concentration of the samples ranged from 0.8 to 3.2 mg/l for level 3 drinking water sample and for level 2 drinking water samples ranged from 20 to 286.4 mg/l, in level 1 drinking water samples ranged from 34 to 1200 mg/l(table 4.30, figure 39). All drinking water sample for level 3 and level 2 except DW4 are in agreement with WHO standard(<150 mg/l).but for level 1 drinking water samples DW5, DW6, DW7 are in agreement with WHO standard and for DW8, DW9, DW10, DW11 are above of WHO standard. Significant variations were observed for magnesium in the three levels of drinking water samples ($P = 0.039^{*}$) (table 4.33, figure 4.42).

**Table (4.30). Analysis of the total calcium carbonate hardness and measurement of calcium in the samples collected**

| levels | Number and code sample | Total Hardness as $CaCO_3$ | Ca | Mg |
|---|---|---|---|---|
| | | < 500mg/l | <200mg/l | <150mg/l |
| Level 3 | S1 | 25 | 6.8 | 3.2 |
| | S2 | 26.5 | 8.4 | 2.2 |
| | S3 | 27 | 9.4 | 1.4 |
| | S4 | 26 | 8.4 | 2 |
| | S5 | 27 | 10 | 0.8 |
| | S6 | 27 | 9.5 | 1 |
| Level 2 | S7 | 350 | 80 | 60 |
| | S8 | 450 | 110 | 70 |
| | S9 | 500 | 90 | 110 |
| | S10 | 450 | 104 | 76 |
| | DW1 | 600 | 150 | 90 |
| | DW2 | 525 | 140 | 70 |
| | DW3 | 350 | 120 | 20 |
| | DW4 | 1336 | 248 | 286.4 |
| Level 1 | DW5 | 235 | 60 | 34 |
| | DW6 | 800 | 240 | 80 |
| | DW7 | 850 | 192 | 148 |
| | DW8 | 860 | 160 | 184 |
| | DW9 | 1350 | 284 | 256 |
| | DW10 | 2760 | 420 | 684 |
| | DW11 | 4600 | 640 | 1200 |

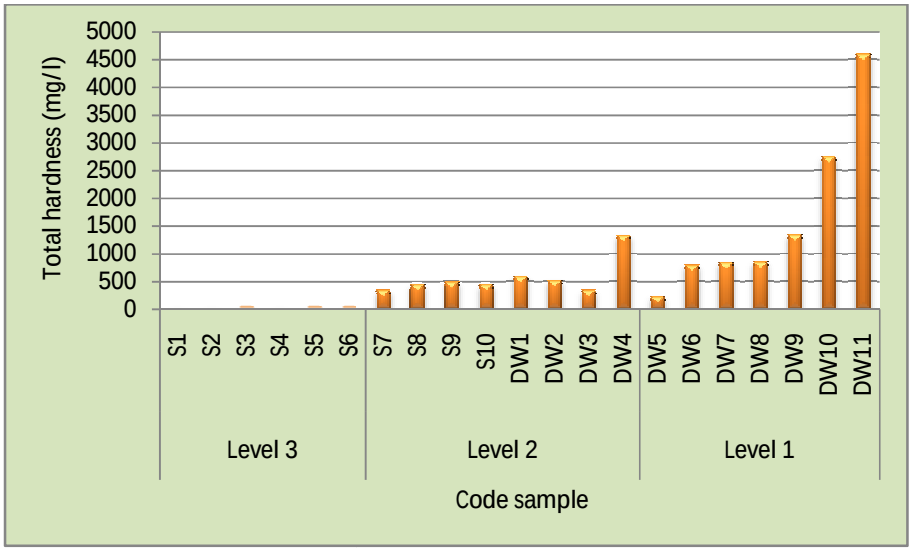


Figure (4.37). Total hardness of three levels of drinking water samples

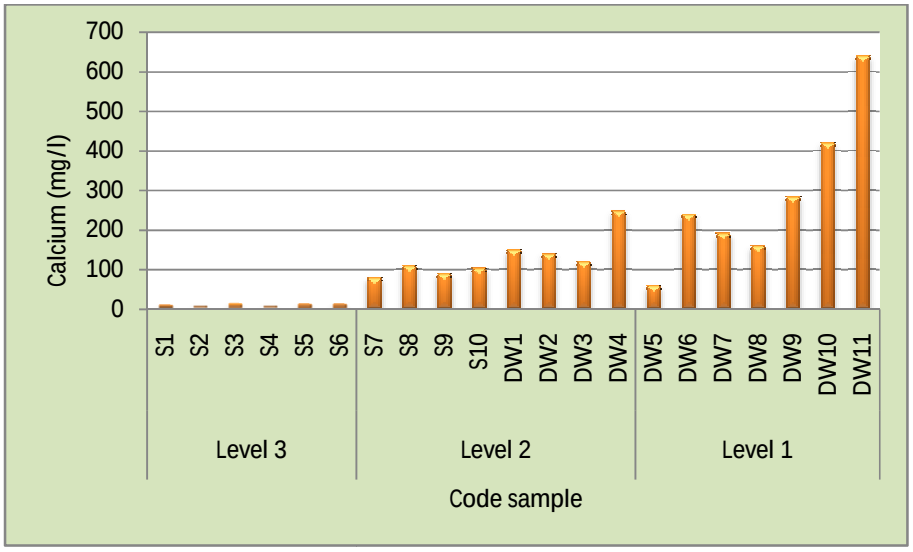


Figure (4.38). Calcium of three levels of drinking water samples

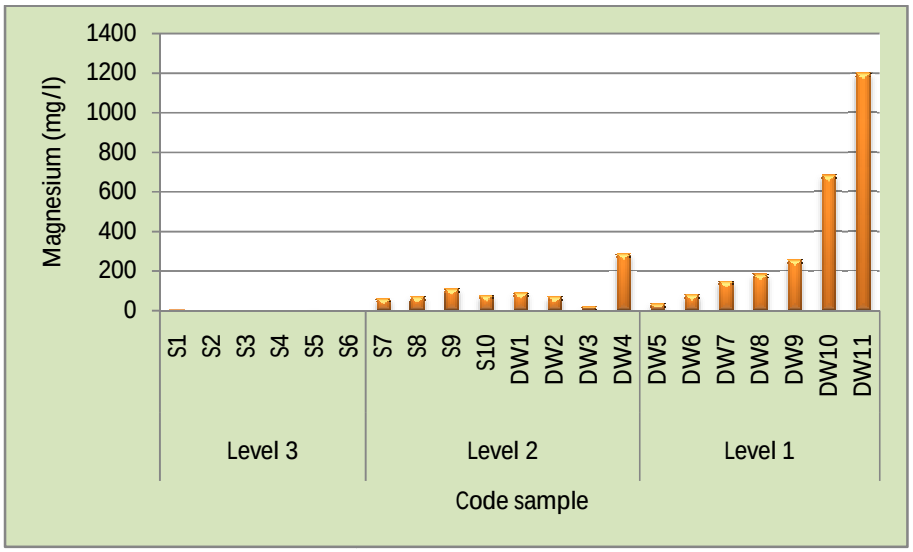


Figure (4.39). Magnesium of three levels of drinking water samples

**Table (4.31). Analysis of variance (ANOVA) showing difference of total hardness average in the three levels**

| Department | N | Mean | Std. Deviation | %95Confidence Interval for Mean | | ANOVA | |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | p-values | L.S.D |
| Level 3 | 6 | 26.417 | 0.801 | 25.57 | 27.25 | 0.014 * | C>A C>B |
| Level 2 | 8 | 570.12 | 320.7 | 301.96 | 838.28 | | |
| Level 1 | 7 | 1636.4 | 1528.1 | 223.09 | 3049.76 | | |
| Total | 21 | 770.2 | 1086.8 | 275.49 | 1264.9 | | |

A: Level 3 B: Level 2 C: Level 1

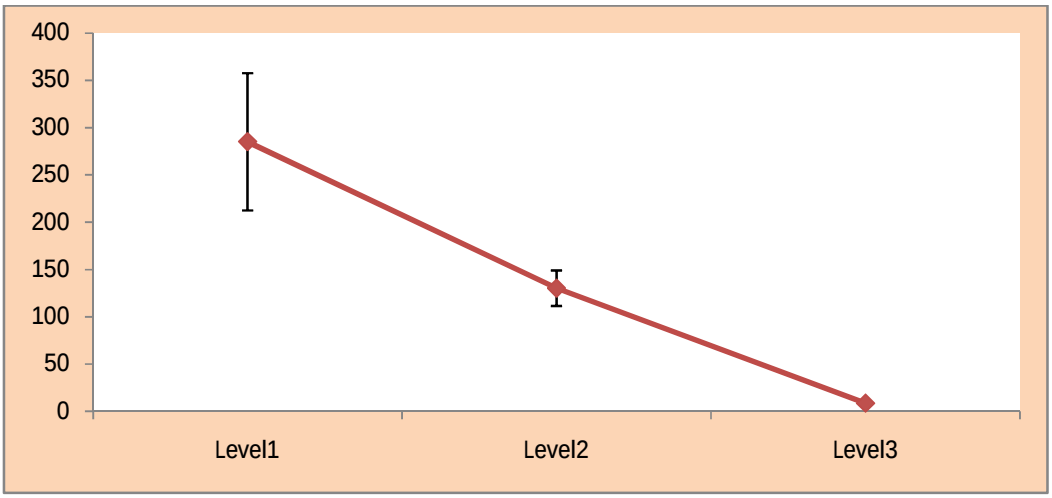


Figure (4.40). Difference of average total hardness in the three levels

**Table (4.32). Analysis of variance (ANOVA) showing difference of calcium average in the three levels**

| Department | N | Mean | Std. Deviation | %95Confidence Interval for Mean | | ANOVA | |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | p-vales | L.S.D |
| Level 3 | 6 | 8.75 | 1.1485 | 7.545 | 9.955 | 0.002** | C>A C>B |
| Level 2 | 8 | 130.25 | 53.049 | 85.9 | 174.6 | | |
| Level 1 | 7 | 285.14 | 191.996 | 107.57 | 462.71 | | |
| Total | 21 | 147.167 | 156.74 | 75.81 | 218.51 | | |

A: Level 3 B: Level 2 C: Level 1

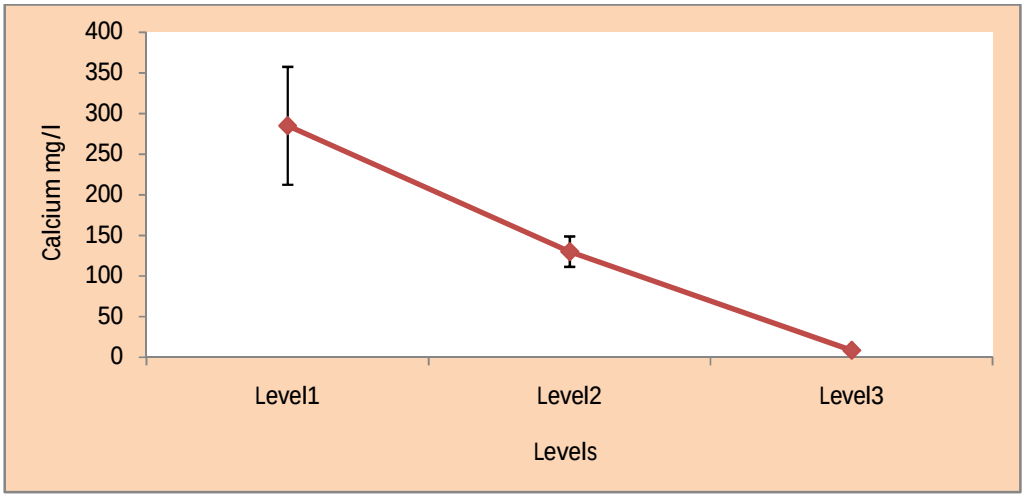


Figure (4.41). Difference of average calcium in the three levels

**Table (4.33). Analysis of variance (ANOVA) showing difference of magnesium average in the three levels**

| Department | N | Mean | Std. Deviation | %95Confidence Interval for Mean | | ANOVA | |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | p-vales | L.S.D |
| Level 3 | 6 | 1.767 | 0.8892 | 0.834 | 2.7 | 0.039* | C>A |
| Level 2 | 8 | 97.8 | 80.42 | 30.55 | 165.04 | | |
| Level 1 | 7 | 369.42 | 424.53 | 23.19 | 762.05 | | |
| Total | 21 | 160.905 | 284.15 | 31.561 | 290.24 | | |

A: Level 3 B: Level 2 C: Level 1

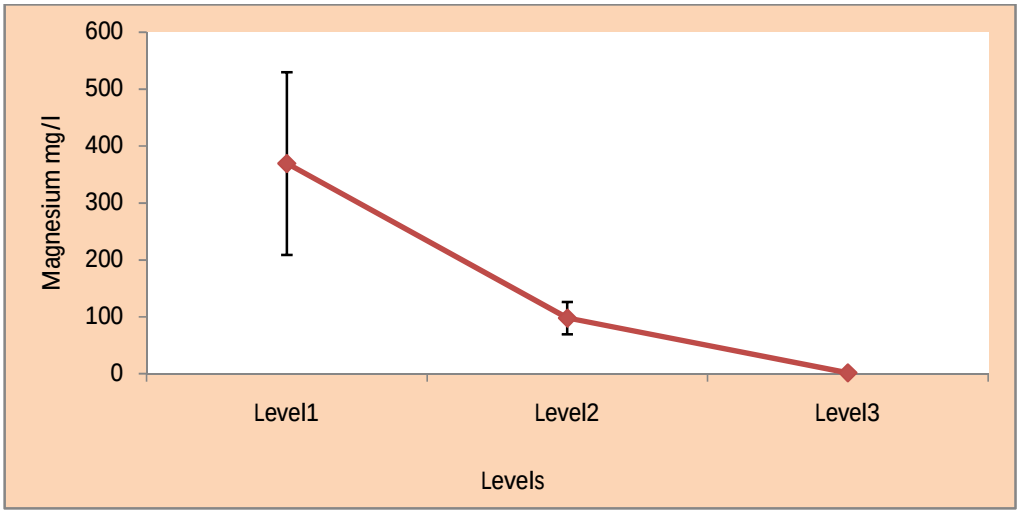


Figure (4.42). Difference of average magnesium in the three levels

**4.2.17. Iron:**

iron content also ranged from 0.02 to 0.05 mg/l for drinking water samples in level 3 and in level 2 ranged from 0.01 to 0.1 mg/l, for drinking water samples in level 1 iron content ranged from 0.06 mg/l (table 4.34, figure 4.423), this mean all of water samples agreement with WHO standards (<0.3). No significant variations were observed for sodium in the three levels of drinking water samples (P = 0.715) (table 4.35, figure 4.46).

**4.2.18. Fluoride:**

fluoride content was zero of all drinking water samples of level 3, this mean water samples in this level agreement with Libyan standard specifications and WHO standards (<1.5 mg/l) but in level 2 water sample the fluoride content ranged from 0.57 to 1.83 mg/l, for level 1 water samples fluoride content ranged from 0.92 to 1.8 mg/l (table 4.34, figure 4.44), this mean most water samples for level 1 and level 2 agreement with WHO standards except S8 (Haita park) for level 2 and DW11(Well depth of nearly 70 meters) for level 1 did not agreement with WHO standards and Libyan standard specifications. Significant variations were observed for fluoride in the three levels of drinking water samples (P = 0.002) (table 4.36, figure 4.47).

**4.2.19. Potassium:**

table 4.34, figure 4.45 show potassium content ranged from 0.2 to 0.4 mg/l for level 3 drinking water samples and for level 2 drinking water samples ranged from 3.1 to 17.7 mg/l . its agreement with WHO standards and Libyan standard specifications, for level 1

drinking water samples fluoride content ranged from 9.1 to 150 mg/l in this level DW7 (Well depth of nearly 23 meters) ,DW11 (Well depth of nearly 70 meters) were high potassium content. High significant variations were observed for potassium between the three levels of drinking water samples (P = 0.000) (table 4.37, figure 4.48).

**Table (4.34). Determine the proportion of chemical elements (iron, potassium ,fluoride) in the water samples collected**

| Levels | Number and code sample | Fe <0.3 mg/l | F 1.5 mg/l | K <40 mg/l |
|---|---|---|---|---|
| Level 3 | S1 | 0.02 | 0 | 0.3 |
| | S2 | 0.02 | 0 | 0.3 |
| | S3 | 0.05 | 0 | 0.4 |
| | S4 | 0.05 | 0 | 0.4 |
| | S5 | 0.02 | 0 | 0.2 |
| | S6 | 0.02 | 0 | 0.2 |
| Level 2 | S7 | 0.02 | 1.24 | 8.3 |
| | S8 | 0.02 | 1.83 | 8.5 |
| | S9 | 0.02 | 1.47 | 10 |
| | S10 | 0.03 | 1.51 | 7.2 |
| | DW1 | 0.03 | 1.16 | 17.7 |
| | DW2 | 0.01 | 0.99 | 7.6 |
| | DW3 | 0.02 | 0.57 | 3.1 |
| | DW4 | 0.1 | 1.54 | 15 |
| Level 1 | DW5 | 0.06 | 1 | 9.1 |
| | DW6 | 0.03 | 1.46 | 15 |
| | DW7 | 0 | 1.06 | 20 |
| | DW8 | 0.01 | 1.28 | 150 |
| | DW9 | 0.03 | 0.92 | 30 |
| | DW10 | 0.02 | 1.2 | 39 |
| | DW11 | 0.01 | 1.8 | 136 |

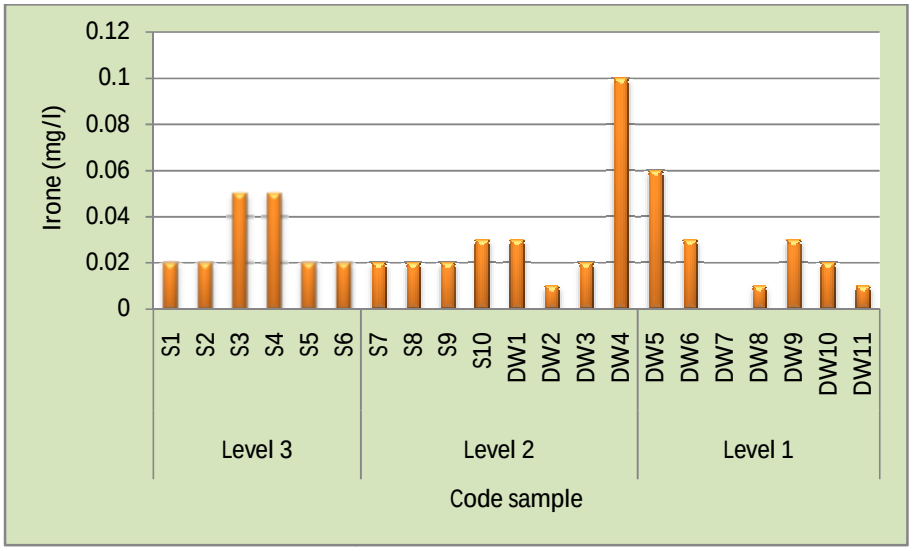


Figure (4.43). Iron of three levels of drinking water samples

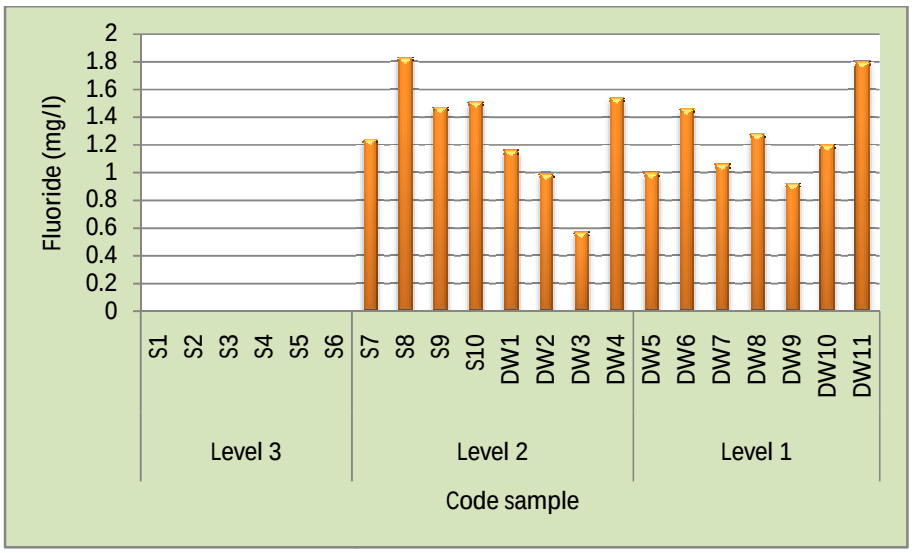


Figure (4.44). Fluoride of three levels of drinking water samples

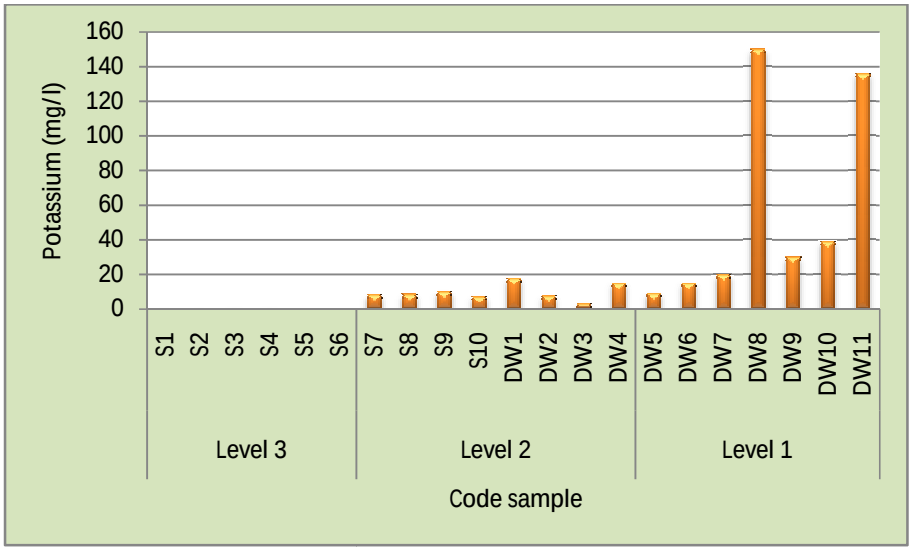


Figure (4.45). Potassium of three levels of drinking water samples

**Table (4.35). Analysis of variance (Kruskal-Wallis Test ) showing difference of iron average in the three levels**

| Kruskal-Wallis Test | | | | | |
|---|---|---|---|---|---|
| Levels | N | Mean Rank | df | Chi-square | p- vales |
| Level 1 | 7 | 9.57 | 2 | 0.671 | 0.715 |
| Level 2 | 8 | 11.38 | | | |
| Level 3 | 6 | 12.17 | | | |

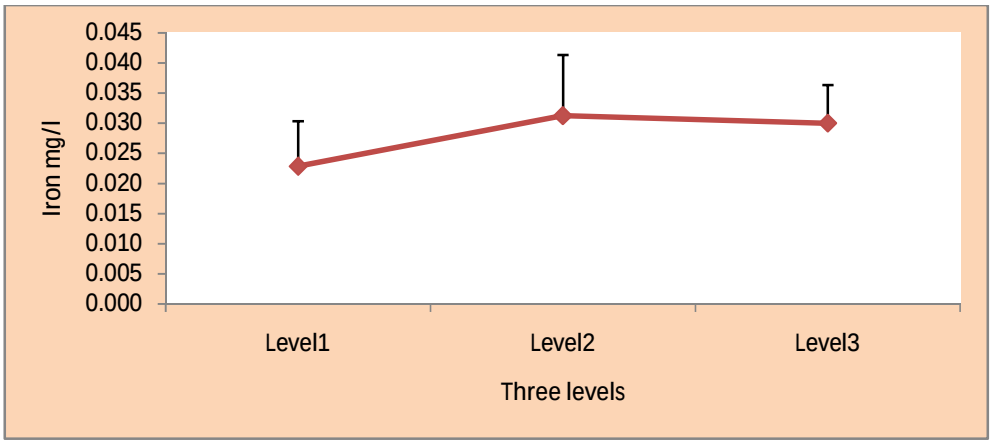


Figure (4.46). Difference of average iron in the three levels

**Table (4.36). Analysis of variance (Kruskal-Wallis Test ) showing difference of florid average in the three levels**

| Kruskal-Wallis Test | | | | | |
|---|---|---|---|---|---|
| Levels | N | Mean Rank | df | Chi-square | p- vales |
| Level 1 | 7 | 13.29 | 2 | 12.736 | 0.002* |
| Level 2 | 8 | 14.63 | | | |
| Level 3 | 6 | 3.50 | | | |

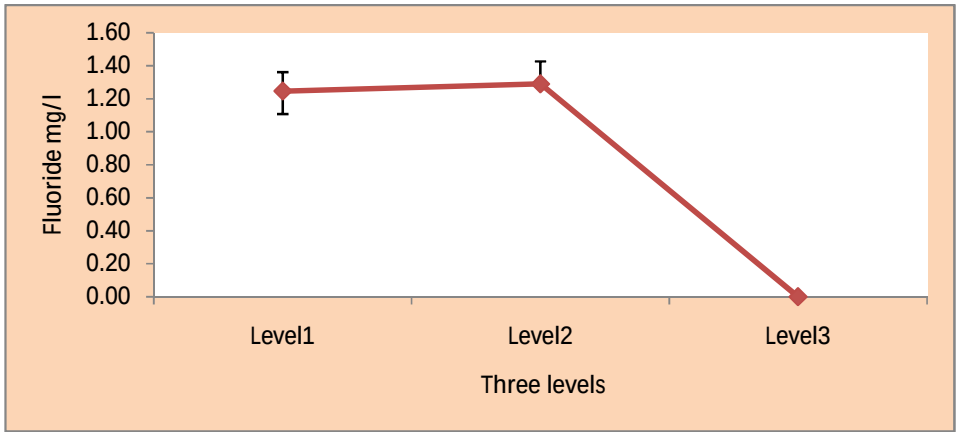


Figure (4.47). Difference of average fluoride in the three levels

**Table (4.37). Analysis of variance (**Kruskal-Wallis Test **) showing difference of potassium average in the three levels**

| Kruskal-Wallis Test | | | | | |
|---|---|---|---|---|---|
| Levels | N | Mean Rank | df | Chi-square | p- vales |
| Level 1 | 7 | 17.36 | 2 | 16.157 | 0.000** |
| Level 2 | 8 | 11.06 | | | |
| Level 3 | 6 | 3.50 | | | |

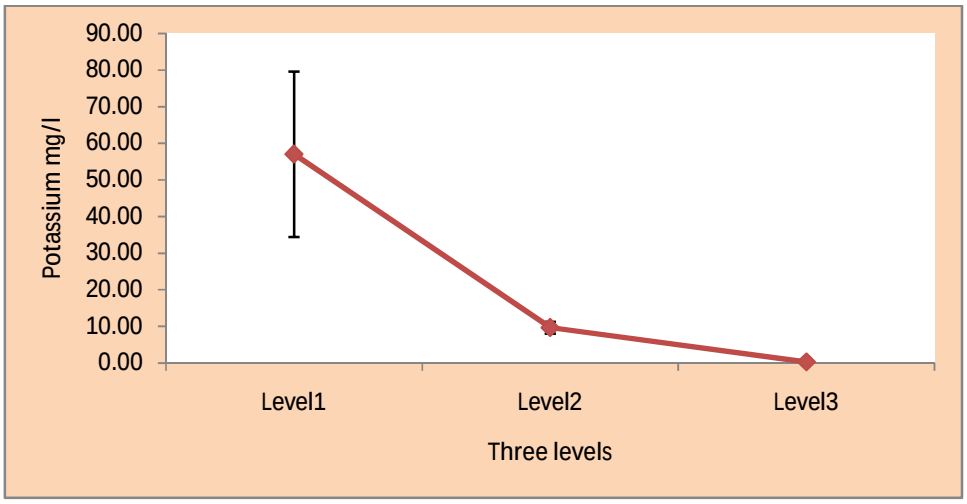


Figure (4.48). Difference of average potassium in the three levels

## 4.3. Correlation coefficients between the physic-chemical parameters for Each level separately from water source:

In the present study the correlation coefficient (r) between seven parameter pairs in computed by taking the average values as shown in table-2. Correlation coefficient (r) between any two parameters, x & y is calculated for parameter such as water temperature, pH. The degree of line association between any two of the water quality parameters as measured by the simple correlation coefficient (r) is presented in table as 7×7 correlation matrix.

For level 1 water samples chloride CL has been found to show positive correlations with nitrate $NO_3$ (r = 0.844), and There is a strong positive correlation (r = 0.960) between calcium and magnesium(table 4.38). And in level two water samples (table 4.39) pH and calcium showed a highly significant negative correlation(r = -0.840). and There is a strong positive correlation (r = 0.968) between chloride and sodium. Strong positive correlation (r = 0.755) between chloride and nitrates and between chloride and magnesium (r = 0.834). and sodium has been found to show positive correlations with nitrate $NO_3$ (r = 0.858), and shows a strong positive correlation (r = 0.903) between him and magnesium also. and there is a strong positive correlation (r = 0.901) between nitrate and calcium. And strong positive correlation (r = 0.841) between calcium and magnesium. Correlation coefficients between the physico-chemical parameters in level3 water samples shows a strong negative correlation (r = -0.850) between sodium and calcium, and strong positive correlation (r = -0.876)

between sodium and magnesium. Calcium and magnesium showed a highly significant negative correlation(r= -0.989)(table 4.40).

**Table (4.38). Correlation coefficients between the physico-chemical parameters in Level1 water samples**

| | | ph | Cl | Na | NO3 | NH | Ca | Mg |
|---|---|---|---|---|---|---|---|---|
| **ph** | Pearson Correlation | 1 | -.637 | -.416 | -.342 | -.072 | -.572 | -.687 |
| | N | | 7 | 7 | 7 | 7 | 7 | 7 |
| **Cl** | Pearson Correlation | | 1 | .296 | .844* | -.360 | .174 | .238 |
| | N | | | 7 | 7 | 7 | 7 | 7 |
| **Na** | Pearson Correlation | | | 1 | .242 | -.289 | -.315 | -.200 |
| | N | | | | 7 | 7 | 7 | 7 |
| **NO3** | Pearson Correlation | | | | 1 | -.674 | .085 | .023 |
| | N | | | | | 7 | 7 | 7 |
| **NH** | Pearson Correlation | | | | | 1 | .363 | .519 |
| | N | | | | | | 7 | 7 |
| **Ca** | Pearson Correlation | | | | | | 1 | .960** |
| | N | | | | | | | 7 |
| **Mg** | Pearson Correlation | | | | | | | 1 |
| | N | | | | | | | |

**Table (4.39). Correlation coefficients between the physico-chemical parameters in Level2 water samples**

| | | ph | Cl | Na | NO3 | NH | Ca | Mg |
|---|---|---|---|---|---|---|---|---|
| **ph** | Pearson Correlation | 1 | -.266 | -.395 | -.673 | -.092 | -.840** | -.550 |
| | N | | 8 | 8 | 8 | 8 | 8 | 8 |
| **Cl** | Pearson Correlation | | 1 | .968** | .755* | -.130 | .702 | .834* |
| | N | | | 8 | 8 | 8 | 8 | 8 |
| **Na** | Pearson Correlation | | | 1 | .858** | -.151 | .784* | .903** |
| | N | | | | 8 | 8 | 8 | 8 |
| **NO3** | Pearson Correlation | | | | 1 | -.004 | .901** | .943** |
| | N | | | | | 8 | 8 | 8 |
| **NH** | Pearson Correlation | | | | | 1 | .072 | -.154 |
| | N | | | | | | 8 | 8 |
| **Ca** | Pearson Correlation | | | | | | 1 | .841** |
| | N | | | | | | | 8 |
| **Mg** | Pearson Correlation | | | | | | | |
| | N | | | | | | | |

**Table (4.40). Correlation coefficients between the physico-chemical parameters in Level3 water samples**

| | | ph | Cl | Na | NO3 | NH | Ca | Mg |
|---|---|---|---|---|---|---|---|---|
| **ph** | Pearson Correlation | 1 | .234 | -.071 | - | - | -.130 | .087 |
| | N | | 6 | 6 | 6 | 6 | 6 | 6 |
| **Cl** | Pearson Correlation | | 1 | .172 | - | - | .256 | -.195 |
| | N | | | 6 | 6 | 6 | 6 | 6 |
| **Na** | Pearson Correlation | | | 1 | - | - | -.850* | -.876* |
| | N | | | | 6 | 6 | 6 | 6 |
| **NO3** | Pearson Correlation | | | | 1 | - | - | - |
| | N | | | | | 6 | 6 | 6 |
| **NH** | Pearson Correlation | | | | | 1 | - | - |
| | N | | | | | | 6 | 6 |
| **Ca** | Pearson Correlation | | | | | | 1 | -.989** |
| | N | | | | | | | 6 |
| **Mg** | Pearson Correlation | | | | | | | |
| | N | | | | | | | |

## 4.4. Correlation coefficients between the physic-chemical parameters for three levels of water samples:

In the present study the correlation coefficient (r) between all parameter pairs in computed by taking two sample from every level to formed sex samples assimilate of all 21 sample by taking the average values as shown in table 4.41. From table 4.41 showed a highly significant negative correlation between pH with calcium(r = -0.838), and with magnesium (r = -0.92) ,highly significant negative correlation between pH with sulphate. There is a strong positive correlation between turbidity and conductivity (r = 0.993), and a strong positive correlation between turbidity and total dissolved solid (r = 0.992), a strong positive correlation between turbidity and calcium (r = 0.907), turbidity and total hardness as CaCO3 (r = 0.982). electrical conductivity total dissolved solid (r = 1.00) this result concurred with Temgoua (2011), and with total hardness (r = 0.997), electrical conductivity has been found to show positive correlations with calcium ( r = 0.950). total dissolved solid had positive correlation with total hardness as CaCO3 (r = 0.997), and with calcium (r = 0.952),There is a strong positive correlation (r = 0.971) between total hardness as CaCO3 and calcium. Also show from table found a strong positive correlations (r = 0.912) between magnesium and nitrate, and chloride has been found to show a strong positive correlations with sodium (r = 0.970). There is a strong positive correlation between sulphate with magnesium and calcium (r = 0.861)(r = 0.840) respectively. Salinity has been found to show positive correlations with chloride and sodium (r = 0.996)(r = 0.987) respectively (table 4.41), Thirupathaiah *et al.,* 2012, found that a strong positive

correlation (r=0.82794) between pH and chloride, pH and turbidity showed a highly significant negative correlation(r=−0.8725).

**Table (4.41). Correlation coefficients between the physico-chemical parameters for three levels of water samples**

| | C | Ph | Rcl | Turb | TA | Cond | TDS | Hd | Ca | Mg | NO3 | NH3 | CL | Na | SO4 | Sal |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | 1 | | | | | | | | | | | | | | | |
| Ph | -.303 | 1 | | | | | | | | | | | | | | |
| Rcl | -.480 | .435 | 1 | | | | | | | | | | | | | |
| Turbid | -.492 | -.583 | -.224 | 1 | | | | | | | | | | | | |
| TA | -.263 | -.378 | -.562 | .646 | 1 | | | | | | | | | | | |
| Cond | -.421 | -.663 | -.275 | .993** | .683 | 1 | | | | | | | | | | |
| TDS | -.417 | -.667 | -.277 | .992** | .684 | 1.00** | 1 | | | | | | | | | |
| Hd | -.364 | -.715 | -.294 | .982** | .684 | .997** | .997** | 1 | | | | | | | | |
| Ca | -.180 | -.838* | -.377 | .907* | .708 | .950** | .952** | .971** | 1 | | | | | | | |
| Mg | .489 | -.92-** | -.291 | .297 | .223 | .395 | .400 | .464 | .646 | 1 | | | | | | |
| NO3 | .718 | -.708 | -.207 | -.121 | -.045 | -.016 | -.011 | .061 | .282 | .912* | 1 | | | | | |
| NH3 | -.390 | -.181 | -.312 | .741 | .730 | .714 | .712 | .692 | .613 | .001 | -.322 | 1 | | | | |
| CL | .307 | -.616 | -.541 | .397 | .702 | .470 | .473 | .515 | .654 | .670 | .522 | .587 | 1 | | | |
| Na | .473 | -.682 | -.495 | .264 | .545 | .352 | .356 | .411 | .589 | .796 | .711 | .388 | .970** | 1 | | |
| SO4 | .146 | -.903*- | -.476 | .583 | .664 | .676 | .680 | .724 | .861* | .840* | .625 | .267 | .727 | .747 | 1 | |
| Sal | .363 | -.650 | -.526 | .362 | .650 | .441 | .445 | .491 | .643 | .723 | .592 | .526 | .996** | .987** | .742 | 1 |

**4.5. Correlation of total coliform bacterial counts and the levels of Chlorine residual (Rcl):**

It was to clarify the relationship between total coliform bacterial and chlorine residual in all drinking water samples represented graphically found that (figure 4.49), there is a strong inverse relationship between them, where the presence of chlorine residual in level 3 drinking water samples (water chlorinated) comes with a lack of presence of total coliform bacteria, but in the level 1 and level 2 and the presence of total coliform bacteria ratio rises with the lack of chlorine level due to lack of water chlorination or a small percentage of chlorine did not reach the source of the one who took him to the water sample .

**4.6. Correlation of total coliform bacterial counts and the levels of turbidity:**

It was to clarify the relationship between total coliform bacterial and turbidity for all drinking water samples in figure 50, there show found positive correlation between them, in most samples, where the more turbidity increased water contaminated with bacteria ratio.

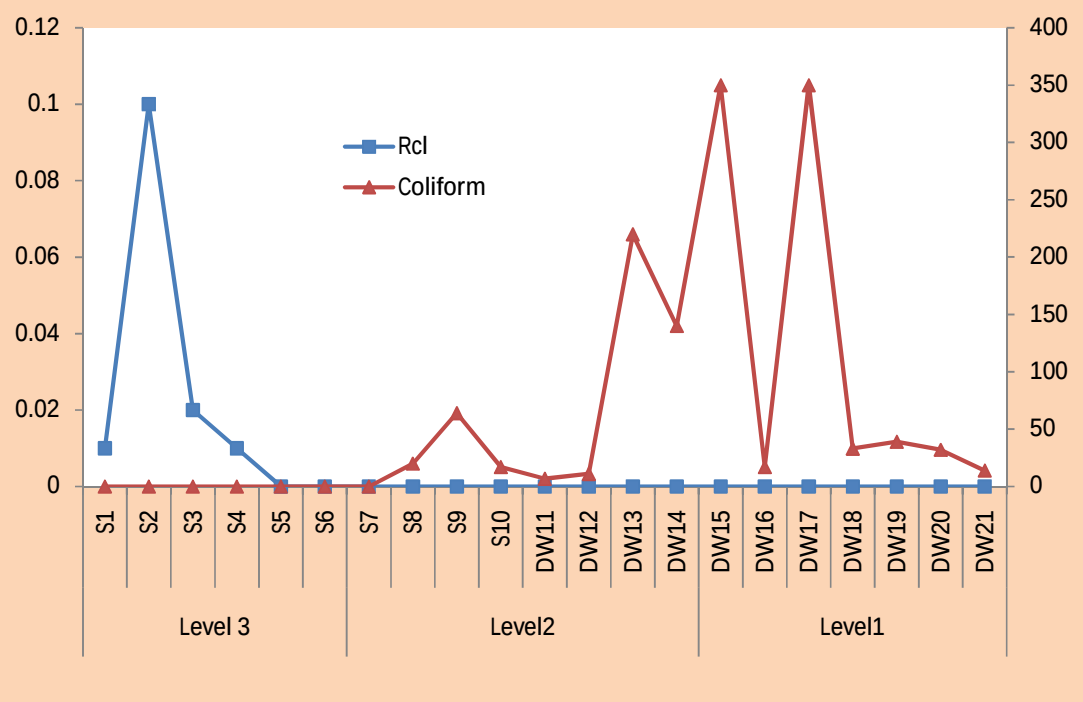


Figure (49). Correlation of total coliform bacterial counts and the levels of Chlorine residual

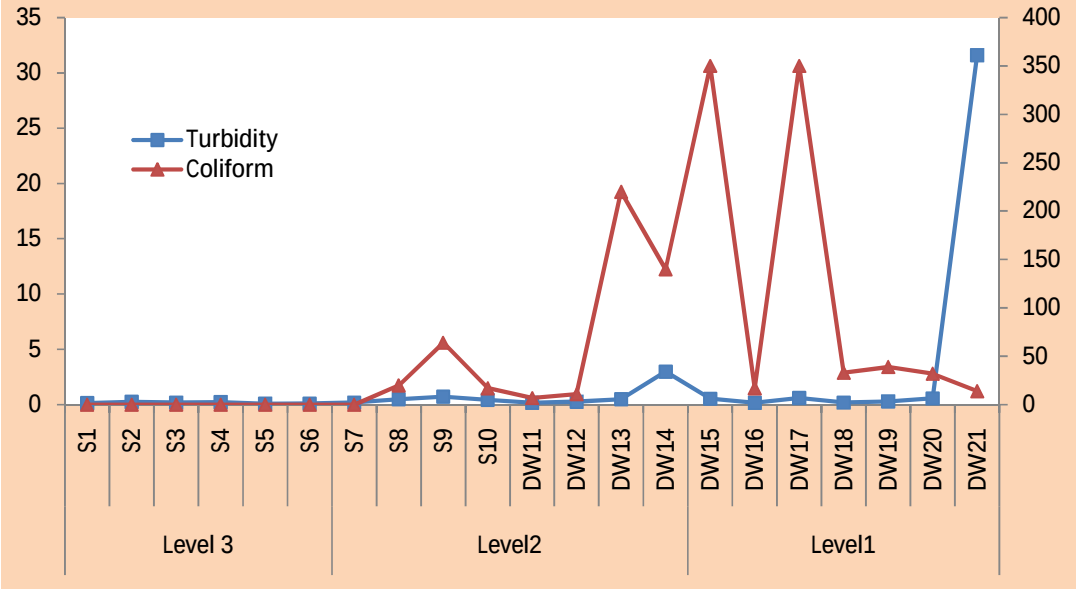


Figure (50). Correlation of total coliform bacterial counts and the levels of turbidity

# CHAPTER FIVE

# 5. CONCLUSION

In this study, 21 drinking water samples taken for the analysis of physical and chemical and bacteriological quality from Tukrah town, Some water of present study areas were not healthy for drinking. Insects or other media may carry bacteria to enter the well, The source of contamination may be septic system, too close to the well or the well casing isn't deep enough to assure that recharge water receives sufficient filtration to remove bacteria. The Newly made wells or tube wells often show contamination because the drill hole was contaminated by dirty tools, pipe or drilling water. The *E.coli* and pseudomonas contaminated water can be treated using chlorine, ultra-violet light, or ozone, all of which act to kill or inactivate *E. coli*. We would like to recommend the following important points: proper sanitary survey, design and implementation of water and/or sanitation projects; regular disinfections, maintenances and supervisions of water sources; and regular bacteriological assessment of all water sources for drinking should be Planned and conducted.

# CHAPTER SIX

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# Appendix

## 1. Bacteriological Experiments

**The ways of preparing media:**

1. Nutrient agar Medium:

Preparing by 28 g of nutrient agar medium were Suspend in 1000 ml distilled water and then heat to boiling to dissolve the medium completely. Sterilized by autoclaving at 15 lbs pressure (121 C°) for 15 min. Used as general culture medium.

2. Lactose Broth Medium:

13 g of lactose broth medium were added to 1000ml distilled water and heat to ensure complete dissolve the medium. Sterilize by autoclaving at 15 lbs pressure (121 C°) for 15 min. used for detection of coliform bacteria in water as described in standard methods.

3. MacConkey Agar Media:

40g of the media powder was dissolved in 1000ml distilled water, boiled for one minute and then autoclaved at 121C° for 15 minutes. The medium was poured into petri dishes.

4. Eosin Methylene Blue Agar Medium (EMB):

37.5 g of EMB agar medium were added to 1000ml distilled water and then heated to boiling to dissolve the medium completely cool to 50C° and shake the medium in order to oxidize the

methylene blue and to suspend the precipitate which is an essential part of the medium. Recommended used for the isolation enumeration and differentiation of enterobacteriaceae .

5. Chocolate agar:

Suspend 45 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C. Aseptically add equal amount of sterile 2% Hemoglobin Solution (FD022). Also add the contents of one vial of Yeast Autolysate Supplement (FD027) or Vitamino Growth Supplement (FD025) reconstituted as directed. Mix well before pouring. When single strength medium is desired, suspend 45 grams in 1000 ml distilled water.

6. DNase Test Agar Base:

Suspend 42gram in 1000ml distilled water . heat with frequent agitation to dissolve the medium completely. Sterilized by autoclaving at 15 lbs pressure (121 C°) for 15 min. cool to 45c$^{o}$ and pour into sterile petri plates.

7. Blood agar:

Suspend 28 g of nutrient agar powder in one liter of distilled water and then heat this mixture while stirring to fully dissolve all components. Autoclave the dissolved mixture at 121 degrees for 15 minutes. When the agar has cooled to 45-50 °C, Add 5% (vol/vol) sterile defibrinated blood that has been warmed to room temperature and mix gently but well and avoid air bubbles. Dispense into sterile plates while liquid.

## Table (1). Most Probable Number of Bacteria Per 100 ml or g of Test Material Using 5 Tubes With 10,1 and 0.1 ml or g of Test Material

| *Pos.* | *MPN* | *Pos.* | *MPN* | *Pos.* | *MPN* |
|---|---|---|---|---|---|
| 10,1,0.1 | | 10,1,0.1 | | 10,1,0.1 | |
| 0.0.0 | ‹1.8 | 1.0.0 | 2 | 2.0.0 | 4.5 |
| 0.0.1 | 1.8 | 1.0.1 | 4 | 2.0.1 | 6.8 |
| 0.0.2 | 3.6 | 1.0.2 | 6 | 2.0.2 | 9.1 |
| 0.0.3 | 5.4 | 1.0.3 | 8 | 2.0.3 | 12 |
| 0.0.4 | 7.2 | 1.0.4 | 10 | 2.0.4 | 14 |
| 0.0.5 | 9 | 1.0.5 | 12 | 2.0.5 | 16 |
| 0.1.0 | 1.8 | 1.1.0 | 4 | 2.1.0 | 6.8 |
| 0.1.1 | 3.6 | 1.1.1 | 6.1 | 2.1.1 | 9.2 |
| 0.1.2 | 5.5 | 1.1.2 | 8.1 | 2.1.2 | 12 |
| 0.1.3 | 7.3 | 1.1.3 | 10 | 2.1.3 | 14 |
| 0.1.4 | 9.1 | 1.1.4 | 12 | 2.1.4 | 17 |
| 0.1.5 | 11 | 1.1.5 | 14 | 2.1.5 | 19 |
| 0.2.0 | 3.7 | 1.2.0 | 6.1 | 2.2.0 | 9.3 |
| 0.2.1 | 5.5 | 1.2.1 | 8.2 | 2.2.1 | 12 |
| 0.2.2 | 7.4 | 1.2.2 | 10 | 2.2.2 | 14 |
| 0.2.3 | 9.2 | 1.2.3 | 12 | 2.2.3 | 17 |
| 0.2.4 | 11 | 1.2.4 | 15 | 2.2.4 | 19 |
| 0.2.5 | 13 | 1.2.5 | 17 | 2.2.5 | 22 |
| 0.3.0 | 5.6 | 1.3.0 | 8.3 | 2.3.0 | 12 |
| 0.3.1 | 7.4 | 1.3.1 | 10 | 2.3.1 | 14 |
| 0.3.2 | 9.3 | 1.3.2 | 13 | 2.3.2 | 17 |
| 0.3.3 | 11 | 1.3.3 | 15 | 2.3.3 | 20 |
| 0.3.4 | 13 | 1.3.4 | 17 | 2.3.4 | 22 |
| 0.3.5 | 15 | 1.3.5 | 19 | 2.3.5 | 25 |
| 0.4.0 | 7.5 | 1.4.0 | 11 | 2.4.0 | 15 |
| 0.4.1 | 9.4 | 1.4.1 | 13 | 2.4.1 | 17 |
| 0.4.2 | 11 | 1.4.2 | 15 | 2.4.2 | 20 |
| 0.4.3 | 13 | 1.4.3 | 17 | 2.4.3 | 23 |
| 0.4.4 | 15 | 1.4.4 | 19 | 2.4.4 | 25 |
| 0.4.5 | 17 | 1.4.5 | 22 | 2.4.5 | 28 |
| 0.5.0 | 9.4 | 1.5.0 | 13 | 2.5.0 | 17 |
| 0.5.1 | 11 | 1.5.1 | 15 | 2.5.1 | 20 |
| 0.5.2 | 13 | 1.5.2 | 17 | 2.5.2 | 17 |
| 0.5.3 | 15 | 1.5.3 | 19 | 2.5.3 | 26 |
| 0.5.4 | 17 | 1.5.4 | 22 | 2.5.4 | 29 |
| 0.5.5 | 19 | 1.5.5 | 24 | 2.5.5 | 32 |

| *Pos.* | *MPN* | *Pos.* | *MPN* | *Pos.* | *MPN* |
|---|---|---|---|---|---|
| 10,1,0.1 | | 10,1,0.1 | | 10,1,0.1 | |
| 3.0.0 | 7.8 | 4.0.0 | 13 | 5.0.0 | 23 |
| 3.0.1 | 11 | 4.0.1 | 17 | 5.0.1 | 31 |
| 3.0.2 | 13 | 4.0.2 | 21 | 5.0.2 | 43 |
| 3.0.3 | 16 | 4.0.3 | 25 | 5.0.3 | 58 |
| 3.0.4 | 20 | 4.0.4 | 30 | 5.0.4 | 76 |
| 3.0.5 | 23 | 4.0.5 | 36 | 5.0.5 | 95 |
| 3.1.0 | 11 | 4.1.0 | 17 | 5.1.0 | 33 |
| 3.1.1 | 14 | 4.1.1 | 21 | 5.1.1 | 46 |
| 3.1.2 | 17 | 4.1.2 | 26 | 5.1.2 | 64 |
| 3.1.3 | 20 | 4.1.3 | 31 | 5.1.3 | 84 |
| 3.1.4 | 23 | 4.1.4 | 36 | 5.1.4 | 110 |
| 3.1.5 | 27 | 4.1.5 | 42 | 5.1.5 | 130 |
| 3.2.0 | 14 | 4.2.0 | 22 | 5.2.0 | 49 |
| 3.2.1 | 17 | 4.2.1 | 26 | 5.2.1 | 70 |
| 3.2.2 | 20 | 4.2.2 | 32 | 5.2.2 | 95 |
| 3.2.3 | 24 | 4.2.3 | 38 | 5.2.3 | 120 |
| 3.2.4 | 27 | 4.2.4 | 44 | 5.2.4 | 150 |
| 3.2.5 | 31 | 4.2.5 | 50 | 5.2.5 | 180 |
| 3.3.0 | 17 | 4.3.0 | 27 | 5.3.0 | 79 |
| 3.3.1 | 21 | 4.3.1 | 33 | 5.3.1 | 110 |
| 3.3.2 | 24 | 4.3.2 | 39 | 5.3.2 | 140 |
| 3.3.3 | 28 | 4.3.3 | 45 | 5.3.3 | 180 |
| 3.3.4 | 31 | 4.3.4 | 52 | 5.3.4 | 210 |
| 3.3.5 | 35 | 4.3.5 | 59 | 5.3.5 | 250 |
| 3.4.0 | 21 | 4.4.0 | 34 | 5.4.0 | 130 |
| 3.4.1 | 24 | 4.4.1 | 40 | 5.4.1 | 170 |
| 3.4.2 | 28 | 4.4.2 | 47 | 5.4.2 | 220 |
| 3.4.3 | 32 | 4.4.3 | 54 | 5.4.3 | 280 |
| 3.4.4 | 36 | 4.4.4 | 62 | 5.4.4 | 350 |
| 3.4.5 | 40 | 4.4.5 | 69 | 5.4.5 | 440 |
| 3.5.0 | 25 | 4.5.0 | 41 | 5.5.0 | 240 |
| 3.5.1 | 29 | 4.5.1 | 48 | 5.5.1 | 350 |
| 3.5.2 | 32 | 4.5.2 | 56 | 5.5.2 | 540 |
| 3.5.3 | 37 | 4.5.3 | 64 | 5.5.3 | 920 |
| 3.5.4 | 41 | 4.5.4 | 72 | 5.5.4 | 1600 |
| 3.5.5 | 45 | 4.5.5 | 81 | 5.5.5 | ›1600 |

## Figures of bacteriological experiments :

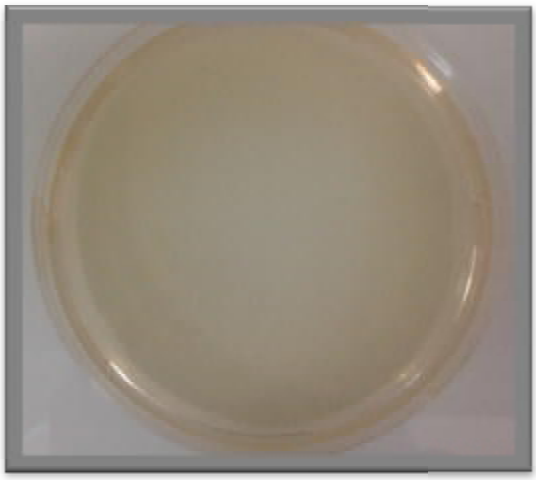

Figure 1: shows HPC × $10^3$ (CFU/ml) for S2 sample

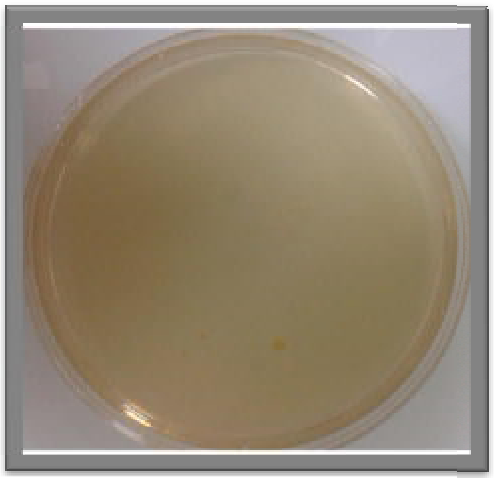

Figure 2: show HPC × $10^3$ (CFU/m for S3 sample

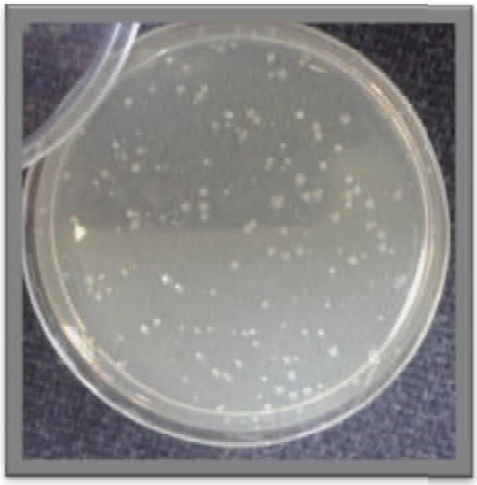

Figure 3: shows HPC × $10^3$ (CFU/ml) for DW11 sample

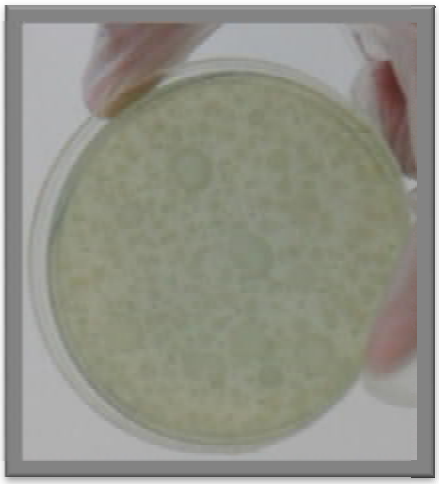

Figure 4: shows HPC × $10^3$ (CFU/ml) for DW9 sample

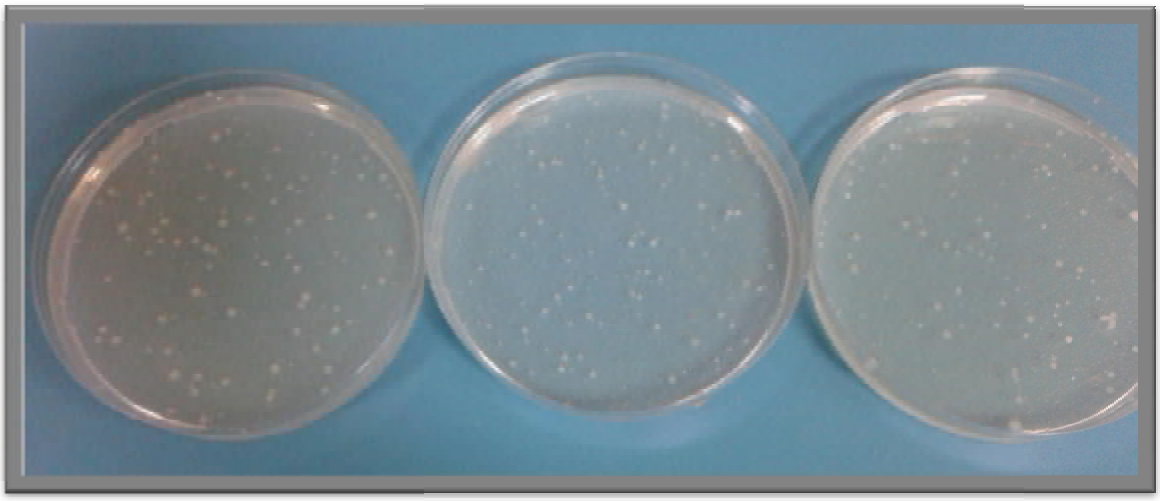

Figure (5). shows HPC $\times 10^1$ and $\times 10^2$ and $\times 10^3$ (CFU/ml) for DW 2 sample

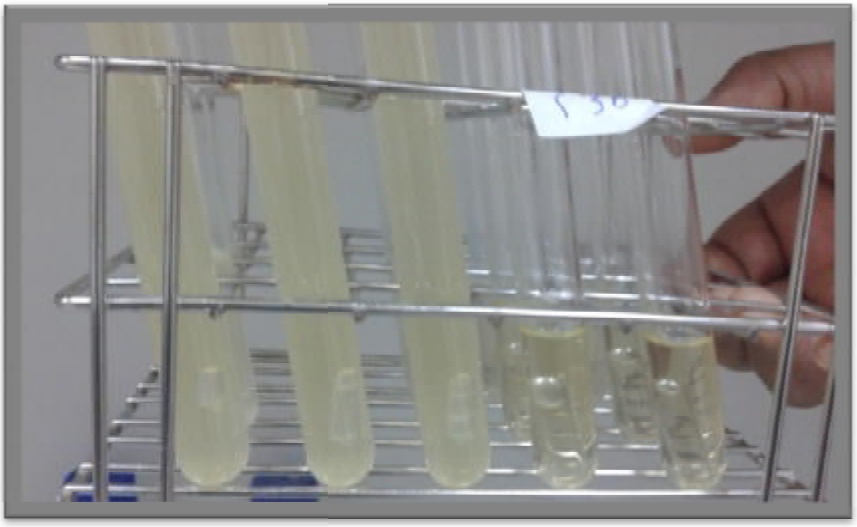

Figure (6). show The most probable number (MPN) for presumptive total coliform count of DW6 the water sample for level1

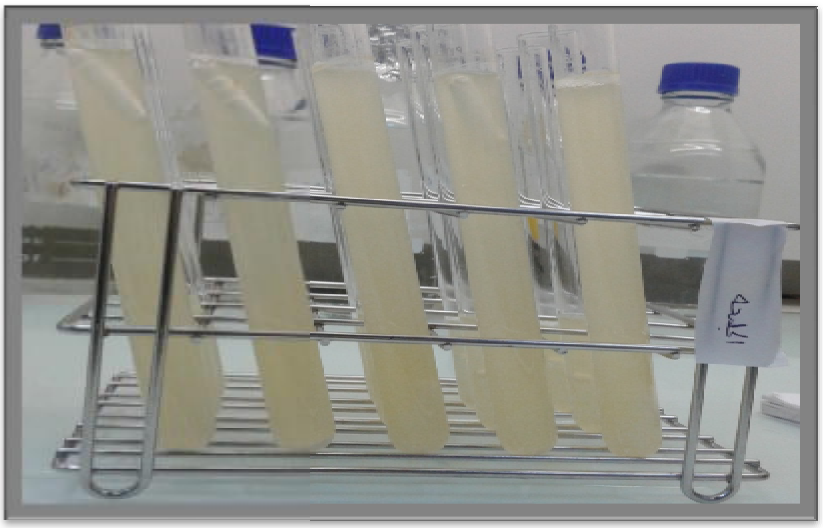

Figure (7). the most probable number (MPN) for presumptive total coliform count of DW2 the water sample for level 2

Figure (8). the most probable number (MPN) for presumptive total coliform count for DW7 and DW11 of the water samples for level1

Figure (9). show faecal coliform bacteria isolated from DW9 sample

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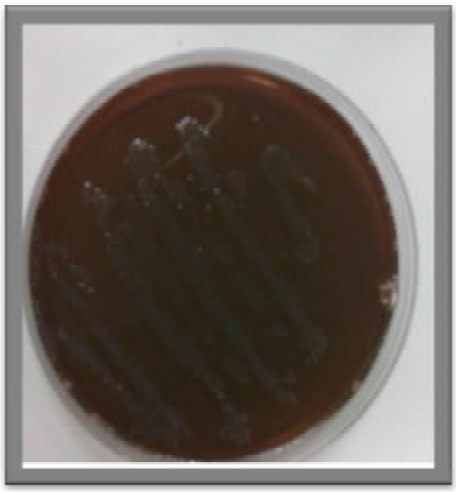

Figure (10). show coliform bacteria isolated from S10 sample

Figure (11). show coliform bacteria isolated from DW4 sample

Figure (12). show faecal coliform bacteria isolated from DW7 sample

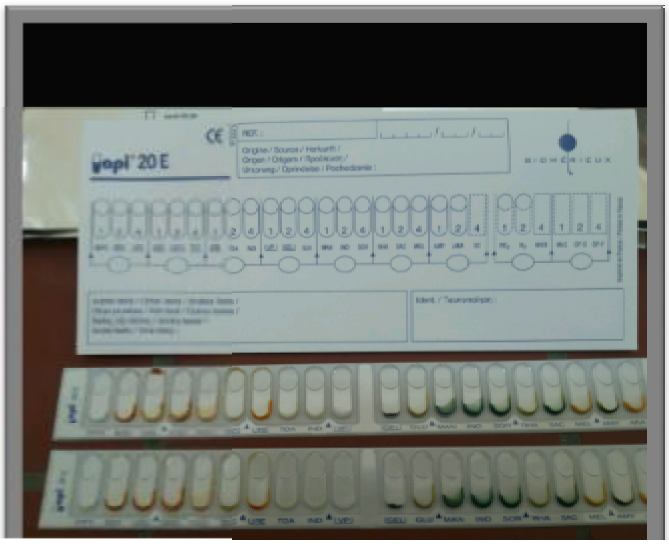


Figure (15). show stripe biochemical API 20E tests used for identification the bacteria

Figure (16). show stripe biochemical API 20E tests used for identification the bacteria

## Table (2). Reading table for stripe biochemical API 20E tests

| TESTS ACTIVE | INGREDIENTS QTY | mg/cup | REACTIONS/ENZYMES | RESULTS | |
|---|---|---|---|---|---|
| | | | | NEGATIVE | POSITIVE |
| ONPG | 2-nitrophenyl-ßDgalactopyranoside | 0.223 | ß-galactosidase (Ortho NitroPhenyl-ßDGalactopyranosidase) | colorless | yellow (1) |
| ADH | L-arginine | 1.9 | Arginine DiHydrolase | yellow | red / orange (2) |
| LDC | L-lysine | 1.9 | Lysine DeCarboxylase | yellow | red / orange (2) |
| ODC | L-ornithine | 1.9 | Ornithine DeCarboxylase | yellow | red / orange (2) |
| CIT | trisodium citrate | 0.756 | CITrate utilization pale | green / yellow | blue-green / blue (3) |
| H2S | sodium thiosulfate | 0.075 | H2S production | colorless / greyish | black deposit / thin line |
| URE | urea | 0.76 | UREase | yellow | red / orange (2) |
| TDA | L-tryptophane | 0.38 | Tryptophane DeAminase | yellow | reddish brown |
| IND | L-tryptophane | 0.19 | INDole production | Colorless pale green / yellow | pink |
| VP | sodium pyruvate | 1.9 | acetoin production (Voges Proskauer) | colorless | pink / red (5) |
| GEL | Gelatin (bovine origin) | 0.6 | GELatinase | no diffusion | diffusion of black pigment |
| GLU | D-glucose (4) | 1.9 | fermentation / oxidation (GLUcose) | blue / blue-green | yellow / greyish yellow |
| MAN | D-mannitol | 1.9 | fermentation / oxidation (MANnitol) (4) | blue / blue-green | yellow |
| INO | inositol | 1.9 | fermentation / oxidation (INOsitol) (4) | blue / blue-green | yellow |
| SOR | D-sorbitol | 1.9 | fermentation / oxidation (SORbitol) (4) | blue / blue-green | yellow |
| RHA | L-rhamnose | 1.9 | fermentation / xidation (RHAmnose) (4) | blue / blue-green | yellow |
| SAC | D-sucrose | 1.9 | fermentation / oxidation (SACcharose) (4) | blue / blue-green | yellow |
| MEL | D-melibiose | 1.9 | fermentation / oxidation (MELibiose) (4) | blue / blue-green | yellow |
| AMY | Amygdalin | 0.57 | fermentation / oxidation (AMYgdalin) (4) | blue / blue-green | yellow |
| ARA | L-arabinose | 1.9 | fermentation / oxidation (ARAbinose) (4) | blue / blue-green | yellow |
| OX | (see oxidase test package insert) | | cytochrome-OXidase | (see oxidase test package insert) | |

(1) A very pale yellow should also be considered positive.

(2) An orange color after 36-48 hours incubation must be considered negative.

(3) Reading made in the cupule (aerobic).

(4) Fermentation begins in the lower portion of the tubes, oxidation begins in the cupule.

(5) A slightly pink color after 10 minutes should be considered negative.

## Table (3). Supplementary rapid biochemical test panel for the API 20E bacterial identification system.

| TESTS ACTIVE | INGREDIENTS QTY | mg/cup | REACTIONS/ENZYMES | RESULTS | |
|---|---|---|---|---|---|
| | | | | NEGATIVE | POSITIVE |
| Nitrate reduction GLU tube | potassium nitrate | 0.076 | NO2 production | NIT 1 + NIT 2 / 2-5 min<br>Yellow | red |
| | | | reduction to N2 gas | Zn / 5 min<br>orange-red | yellow |
| MOB | API M Medium or microscope | / | motility | non-motile | motile |
| McC | MacConkey medium | / | growth | absence | presence |
| OF-F | glucose (API OF Medium) | / | fermentation : under mineral oil | green | yellow |
| OF-O | | | oxidation : exposed to the air | green | yellow |

## 2. Physical and Chemical Experiments

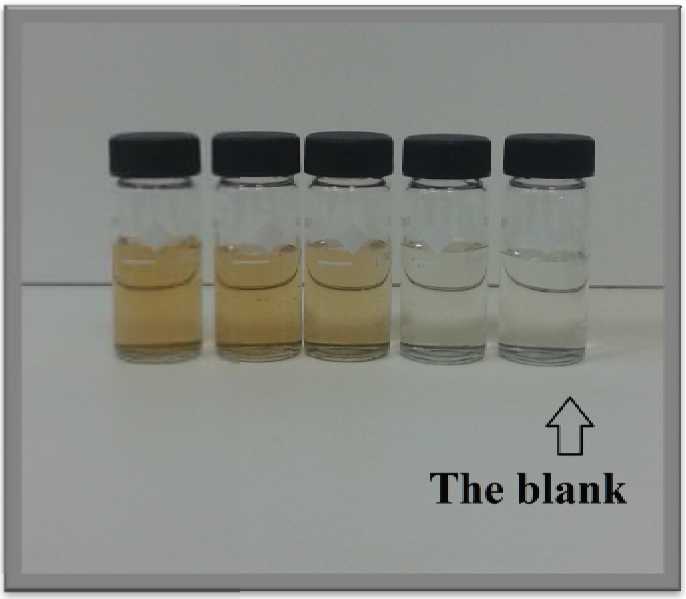


Figure (17). show the action between sample of water and reagent for detect the concentration of nitrate before set in Spectrophotometer

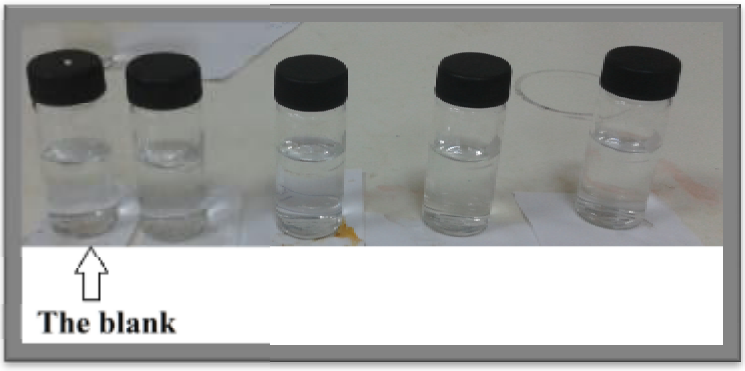


Figure (18). show the action between sample of water and reagent for detect the concentration of nitrite before set in Spectrophotometer

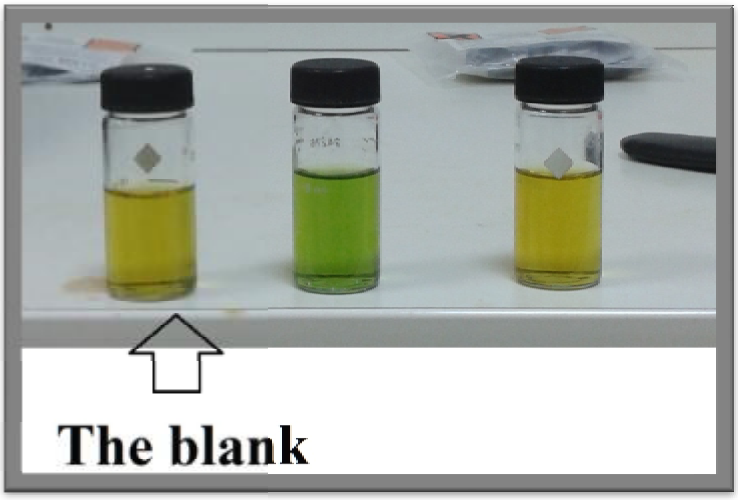


Figure (18). show the action between sample of water and reagent for detect the concentration of ammonia before set in Spectrophotometer

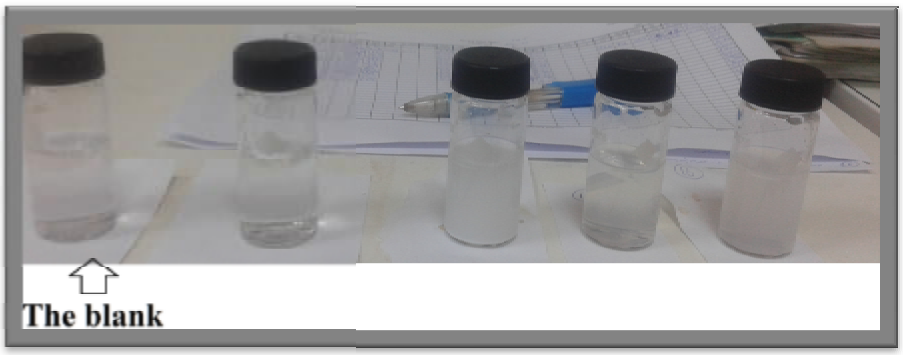


Figure (19). show the action between sample of water and reagent for detect the concentration of sulphate before set in Spectrophotometer

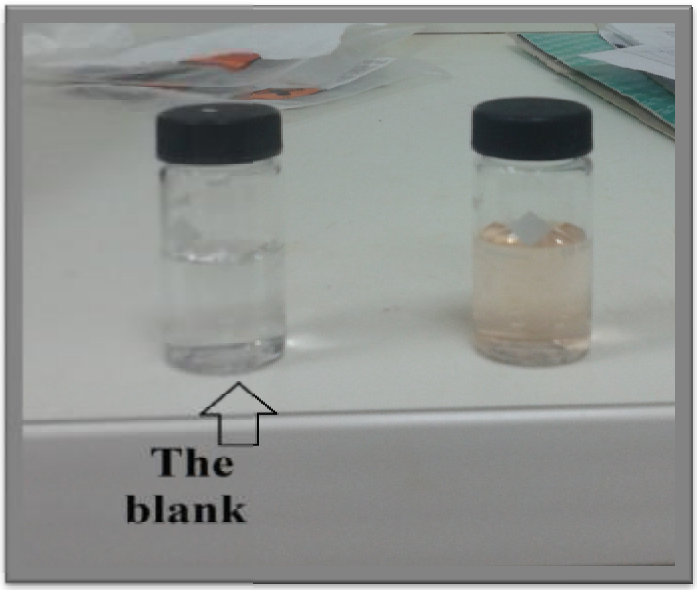


Figure (21). show the action between sample of water and reagent for detect the concentration of iron before set in Spectrophotometer

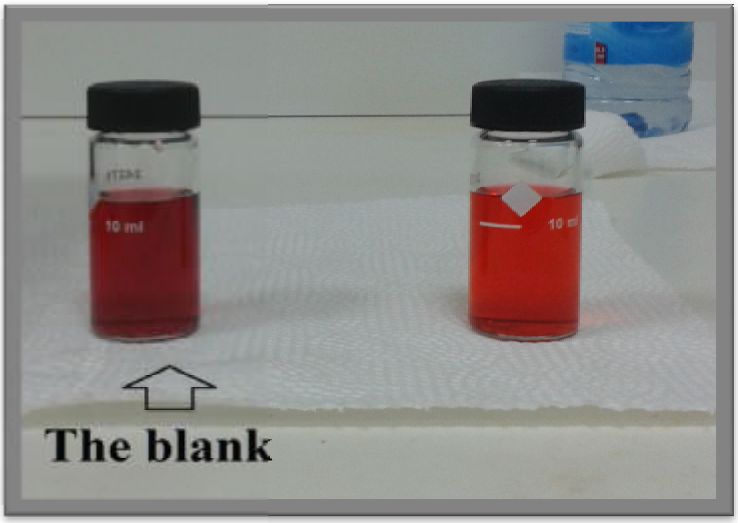


Figure (22). show the action between sample of water and reagent for detect the concentration of fluoride before set in Spectrophotometer

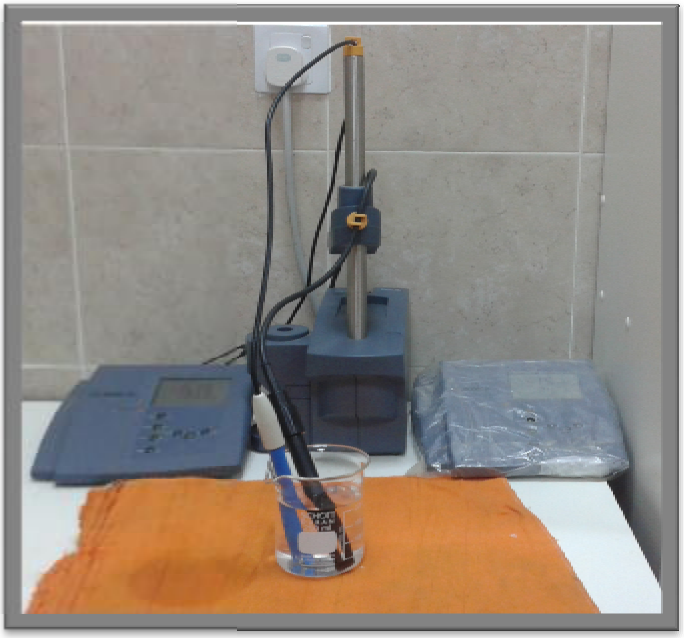

Figure (23). show Conductivity meter (  inolab cond 720) and pH meter (inoLab pH 720)


Figure (24). show Spectrophotometer (DR2800 )

Figure (25). show  flame photometer BWB technologies


Figure (26). show Device BD Phoenix™

## Libyan National Center for Standardization and Metrology:

### LNCSM 82:2008

#### 1- Chemical properties:

It must be chemical components that have an impact on public health in non-bottled drinking water, according to the table:

### Table (4). Inorganic elements measured in mg / l:

| Measurement | The maximum allowable |
|---|---|
| **Ph** | 6.5 |
| **Total dissolved solid** | 1200 |
| **Total hardness** | 500 |
| **Ammonia** | 1.5 |
| **Sodium** | 200 |
| **Iron** | 0.3 |
| **Sulphate** | 250 |
| **Chloride** | 250 |
| **Fluoride** | 1.5 |
| **Nitrite** | 3 |
| **Nitrate** | 50 |

## 2- The vital characteristics:

1- Drinking water must be completely free of algae any stage of the Preliminary animals, including amoeba, as well as insects and their stages or parts.

2- Should drinking water be completely free from micro-algae, fungi and viruses

The bacteria causing the disease, according to the following **table (5).**

| **Standard** | **measuring unit** | **The maximum allowable In treated water Entering the network Distribution** | **The maximum allowable In treated water Within the distribution network** |
|---|---|---|---|
| **Coliform Bacterial Group** | MPN/100ml | Zero | zero |
| **Faecal coliform bacteria** | MPN/100ml | Zero | zero |
| **Escherichia . coli** | MPN/100ml | Zero | zero |
| **The total number aerobic microbes** | CFU/ml | 500 | 500 |

## 3- Free residual chlorine

- Must be free residual chlorine concentration in drinking water is not bottled enough to kill all Microbes in them, not to increase the concentration of free residual chlorine in the water 0.5 mg/l in a million after period touches 30 minutes at a minimum when the pH less than 8.
- Increasing concentration of chlorine in epidemics and in special cases, as determined by a dish The competent authorities to do so.
- In the case of water treatment with chlorine or ozone or UV or by any means other treatments, should this treatment be enough to kill microbes, and water is treatment matching characteristics microbiological water treatment.