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Assessment of Bacteriological Quality of Drinking Water from Various Sources in Tukarah Town, NE Libya.

Evaluación de la calidad bacteriológica del agua potable de varias fuentes en la ciudad de Tukarah, noreste de Libia.

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ABSTRACT

The aim of this study was to evaluate drinking water quality in 21 water sources categorized in three levels. Samples of water were collected from each source for bacteriological examination. The results show there was a significant difference between the three levels 1, 2, and 3 for total coliform and fecal coliform bacteria with *p*-values (0.026) and (0.003) respectively. Presence of total coliform and fecal coliform bacteria were not reported from level 3 and was zero MPN per 100 ml. However, the high contamination by total coliform and fecal coliform bacteria were observed in samples collected from levels 1 and 2, these were in the range of 2 to 350 MPN/100 ml, 2 to 26 MPN/100 ml respectively. On the other hand, the biochemical identification process using Phoenix identified technique for the six isolated strains as *Cedecea lapagel* (DW4), *Citrobacter freundii* (DW9), *Ochrobacterum anthroi* (DW10) *Pseudomonas aeruginosa* (S10), *Stenotrophomonas maltophilia* (DW4) and *Streptococcus anginosus* (DW2), with confidence value identities of 90%, 99%, 90%, 95%, 99% and 91% respectively. The findings showed that water from levels 1 and 2 did not conform to the world health organization (WHO) standard in terms of suitability for drinking purpose.

Keywords: drinking water quality, coliform and fecal coliform bacteria, MPN/100ml.

RESUMEN

El objetivo de este estudio fue evaluar la calidad del agua potable en 21 fuentes de agua categorizadas en tres niveles. Se recogieron muestras de agua de cada fuente para

su examen bacteriológico. Los resultados muestran que hubo una diferencia significativa entre los tres niveles 1, 2 y 3 para las bacterias coliformes totales y coliformes fecales con valores de p (0,026) y (0,003) respectivamente. La presencia de bacterias coliformes totales y coliformes fecales no se informó desde el nivel 3 y fue cero MPN por 100 ml. Sin embargo, la alta contaminación por bacterias coliformes totales y coliformes fecales se observó en las muestras recolectadas de los niveles 1 y 2, estas estuvieron en el rango de 2 a 350 NMP/100 ml, 2 a 26 NMP/100 ml respectivamente. Por otro lado, el proceso de identificación bioquímica mediante la técnica Phoenix identificó para las seis cepas aisladas como *Cedecea lapagel* (DW4), *Citrobacter freundii* (DW9), *Ochrobacterum anthroi* (DW10), *Pseudomonas aeruginosa* (S10), *Stenotrophomonas maltophilia* (DW4) y *Streptococcus anginosus*. (DW2), con identidades de valores de confianza del 90%, 99%, 90%, 95%, 99% y 91% respectivamente. Los hallazgos mostraron que el agua de los niveles 1 y 2 no se ajustaba al estándar de la Organización Mundial de la Salud (OMS) en términos de idoneidad para beber.

Palabras clave: calidad del agua potable, bacterias coliformes y coliformes fecales, MPN/100ml.

INTRODUCTION

Safe water is essential for health and development, and is a basic human right. Water-related diseases caused by insufficient safe water supplies, combined with poor sanitation and hygiene, cause deaths mostly in children (Malhotra Sita *et al*., 2015). Water is one of the most important and abundant compound in 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 fecal pollution (Gopinathl *et al.,* 2012). Nevertheless, due to increased human population, industrialization, use of fertilizers in the agriculture and man-made activity it is highly polluted with different harmful contaminants. Sewage is one of the most dangerous sources of pollutants that contaminate groundwater. Change the physiochemical properties of drinking water spread disease-causing microorganisms and different types of pollutant emitted from wastewater discharge which includes household chemicals such as insect repellents, surfactants and pharmaceuticals (Roohul-Amin *et al.,* 2012). Indicator organisms are commonly used to assess the microbiological quality of surface waters were fecal coliforms (FC) are the most commonly used bacterial indicator of fecal pollution they are found in water that is contaminated with fecal 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* survive for about 4-12 weeks in water containing a moderate microflora at a temperature of 15-18°C (Bumadian M. *et al.,* 2013). Fecal coliform bacteria indicate the presence of sewage contamination of the waterway and the possible presence of other pathogenic organisms. Presence of fecal coliform shows that the source of water may be contaminated by pathogens or disease producing bacteria or viruses (Mashiatullah et *al.,* 2010). It has been estimated that the rate of mortality of water associated diseases exceeds 5 million people per year around the world, there are reports indicate that more than 50% of these deaths are associated with microbial intestinal infections, particularly with cholera and typhoid especially in developing countries (Pesewu *et al.,* 2015), Therefore it is necessary that the quality of drinking water should be checked at regular time intervals, because due to the use of contaminated drinking water, human population suffers from varied of water-borne diseases (P.N *et al*., 2012).

MATERIAL AND METHODS

Collection of samples: Water samples were collected from different sources (ground water, treated and untreated water) in the Tukarah town (It was combined from June to October) and placed in autoclaved sterile bottles for microbial examination. Collected water samples were stored and transported in a sterile plastic boxes with ice packs to keep them cool (but not frozen).

The heterotrophic plate count (HPC): The standard plate count technique for the enumeration of microorganisms is one of the most widely used technique in microbiology (Ptak *et al*., 1977 and Lechevallier *et al*., 1980). The HPC test is another method for monitoring the overall bacteriological quality of drinking water. Collected water samples 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 nutrient agar medium (three replicates of petri dish for each test tube) then incubated at 37℃ for 24-48 h. The average number of colonies calculated as CFU/100µl.

Enumeration of bacteria

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), was used to an enumeration of coliform and fecal coliform bacteria as follows in three steps:

1-Presumptive test: water sample bottles were thoroughly shaken. 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 contained strength lactose broth) and then incubated at 37 C° for 24-48 hours. After incubated for 24 h, each test tube was examined for gas production (coliform bacteria produce gas from the lactose in medium, and some of them were trapped in the inverted Durham tube). A number of positive tubes (with gas production) were counted and MPN determined from the standard table.

2-Confirmed test: 100µl were transferred from the positive presumptive test and speared on EMB plate and incubated at 37 C° for 24-48 hours. 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 was examined for gas production. A number of positive tubes (with gas production) were counted and MPN determined from the standard table. 10µl were transferred from the positive completed test and speared on EMB plate and incubated at 37 C° for 24-48 hour. Isolated bacterial that grow at 37°C and 45°C were identified using Phoenix identified technique.

Identification of Microorganisms

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 were 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 the indicator dye, 25µl of the ID suspension was transferred to the AST broth to achieve a final inoculum density of 1.5×10^8 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).

Preparation of a 0.5 McFarland Standards: 85 ml of 1% sulfuric acid (H_2SO_4) and 0.5ml of 1.175% anhydrous barium chloride ($BaCl_2$) will add to a 100ml volumetric flask. Mix for 5 minutes by using a magnetic stirrer until the solution appears homogeneous and free of clumps. And Check the optical density (OD) of the McFarland standard at a wavelength of 625nm and record results. The acceptable range for a McFarland 0.5 standard is 0.08 to 0.10.OD (Zamora and Gracia, 2012).

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 around sample cell with 10 ml of a sample. (this is the blank). And then wiped the blank and place it into the cell holder. Then touched zero the display was showing: 0.00 mg/l CL2. Then filled a second-round cell with 10ml 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.

RESULTS AND DISCUSSION

The HPC value showed a regular trend figure (1). The values increased in level-1 Which has taken water samples from wells area Tukarah, The highest HPC was noted in DW8 (well in Yarmouk mosque it's depth about 30 meters) and DW9 (well about 25 meters in the depth) were as high as 275× 10^3 and 224 ×10^3, respectively. The lowest value 0.66 × 10^3 were recorded in S2, S4, S6 and 1 × 10^3 in S5, respectively in Level-3 samples it's considered to be of good quality and is used for drinking purposes, but in Level-1 and Level-2 the result showed that the different drinking water sources are highly contaminated because the heterotrophic plate count which is far more than the recommended value of 1.2×10^2 of WHO (1995) Ibiene *et al.* (2012). These results are consistent with the result of Ibiene *et al.,* (2012), where their results of the heterotrophic plate count HPC ranged from 1.6×10^3 to 1.5×10^6 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 were showing significant differences (p = 0.000) between the three levels of drinking water samples, where full swing be in level-1 and in level-2 of drinking water samples, The very high contamination may be due to the non-hygienic disposal of fecal waste in the pit.

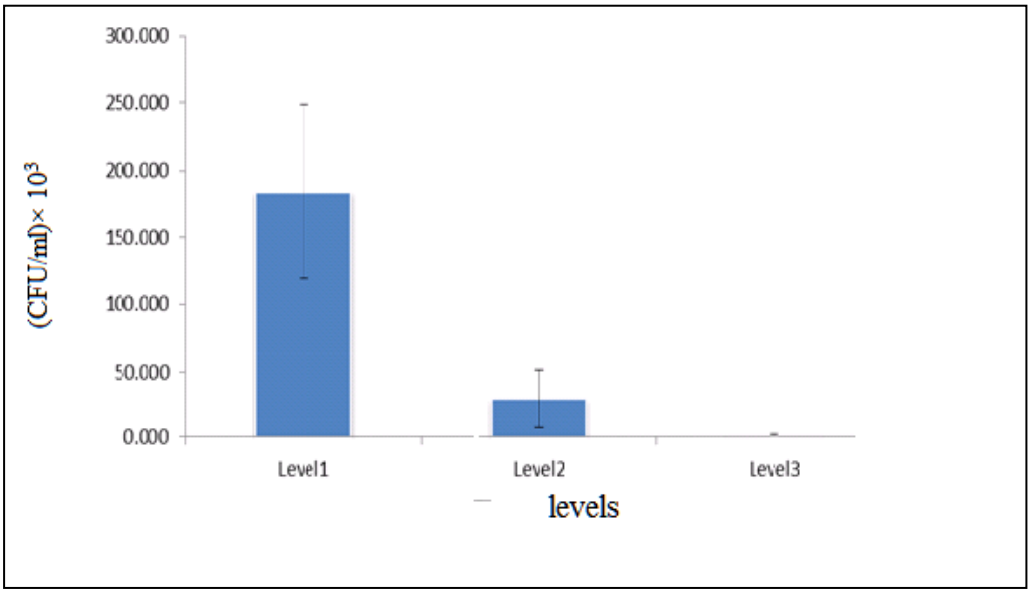

Figure 1. The heterotrophic plate count (HPC) in the three levels of drinking water samples

Total coliform and fecal coliform counts: The most probable number (MPN) for a presumptive total coliform count of the water samples for level-1 ranged from 14 to 350 MPN/100 ml, the maximum total coliform colonies (350 MPN/100ml) was recorded for

DW5 and DW7 and the minimum (14 MPN/100ml) for the DW11 sample, table (2). Most probable number (MPN) for a completed fecal coliform count of the water samples for level- 1 ranged from >2 to 21 MPN/100ml, And the maximum fecal coliform colonies (21 MPN/100ml) was recorded for DW7 and the minimum (>2 MPN/100ml) for DW11 sample, table (2). This is an indication that the sources of drinking water may be prone to a pathogenic organism including *Vibrio* and *Salmonella etc*. These values deviated from the standard recommended by WHO which is zero total coliform count per 100ml for WHO (Isa *et al.,* 2013). These results indicated that level one all the samples had the total coliform counts and fecal coliform counts above the WHO guideline for drinking water. This may be due to the location of the wells beside or around the wells sewage, or it can be due to the lack in depth of the wells. These results confirmed with Haruna *et al* (2005). And also in the table (2) indicate the coliform bacterial population for water samples for level-2 shows that total coliform levels are in the range from >2 to 220 MPN/100ml, The higher values (220 MPN/100ml) of total coliform are observed for DW3 and the minimum (>2 MPN/100ml) for S7 sample. Most probable number (MPN) for completed fecal coliform count of the water samples for level-2 ranged from >2 to 26 MPN/100ml, and the maximum fecal coliform colonies (26 MPN/100ml) was recorded for DW3 and the minimum (>2 MPN/100ml) for S7, S8, S9, S10, DW1, DW2 samples. These values exclusive S7 sample deviated from the standard recommended by WHO which is zero total coliform count per 100 ml for WHO (Isa *et al.,* 2013). This is 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 are an indication that the source of the various water samples had contaminated with the substance of fecal origin.

In level-3 tables shows negative results for the total and fecal coliform counts, all water samples in level3 was >2 MPN/100ml, 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 physical 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 data were further analyzed using Kruskal-Wallis Test for total coliform count and for fecal coliform count levels between the three levels of drinking water samples and the results showed significant differences ($p = 0.026$, $p = 0.003$ respectively) between the three levels of drinking water samples were full swing be in level and in level-2 of drinking water samples, The very high contamination may be due to the non-hygienic disposal of fecal waste in pit figure (2 and 3). These results 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 fecally contaminated (Shittu *et al.,* 2008).

Table 2: Total coliform and fecal coliform counts in the water samples of three Levels

Water levels	Water samples	Total coliform MPN/100 ml	95% Confidence Limits		Fecal coliform MPN/100 ml	95% Confidence Limits	
			Lower	Higher		Lower	Higher
Level-3	S1	< 2	0	6	< 2	0	6
	S2	< 2	0	6	< 2	0	6
	S3	< 2	0	6	< 2	0	6
	S4	< 2	0	6	< 2	0	6
	S5	< 2	0	6	< 2	0	6
	S6	< 2	0	6	< 2	0	6
Level-2	S7	<2	0	6	<2	0	6
	S8	20	7	40	<2	0	6
	S9	17	7	40	<2	0	6
	S10	64	11	93	<2	0	6
	DW1	6.8	1	17	<2	0	6
	DW2	11	5	35	<2	0	6
	DW3	220	70	440	36	7	40
	DW4	140	52	400	14	3	28
Level-1	DW5	350	100	710	9.2	2	21
	DW6	17	6	36	10	1.8	15
	DW7	350	100	1100	21	7	40
	DW8	33	9	78	9	2	21
	DW9	39	9	78	4	0.7	10
	DW10	32	7	40	6.8	1	17
	DW11	14	6	36	<2	0	6

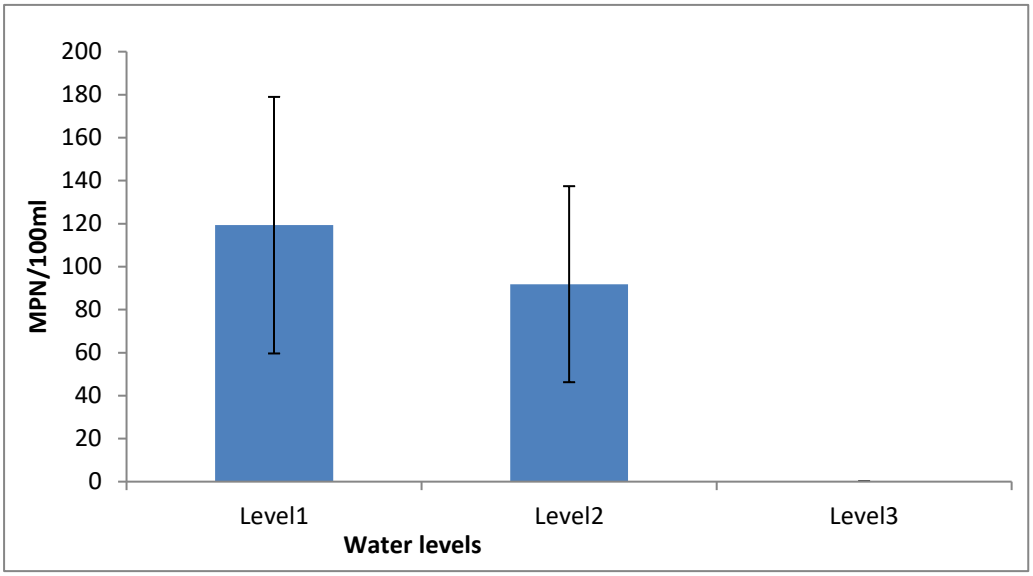

Figure 2. Total coliform count in the three levels of drinking water samples

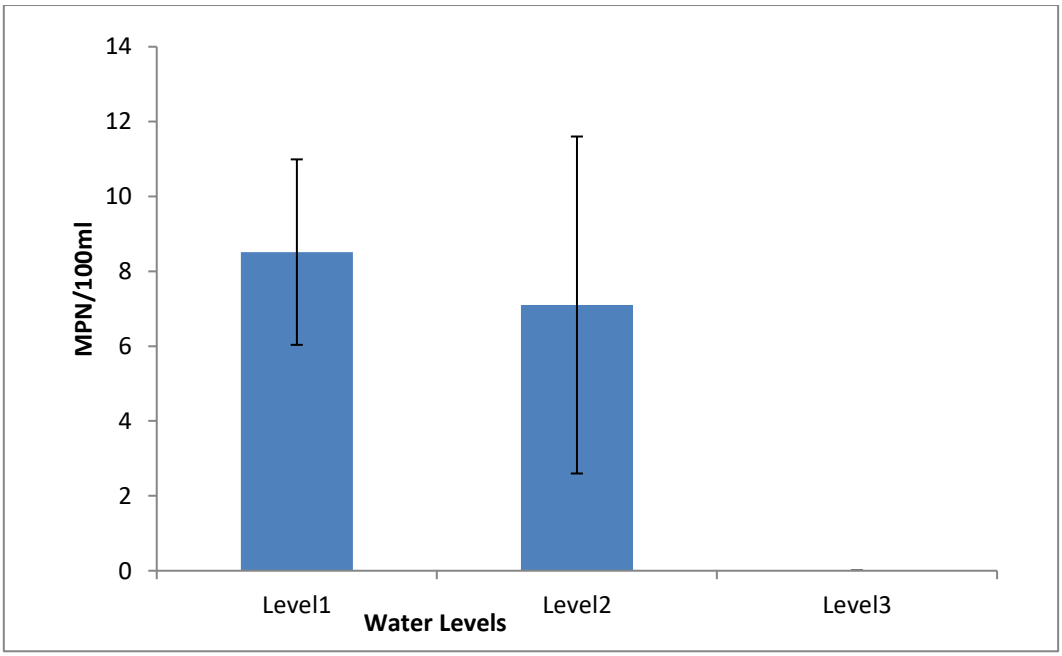

Figure 3. Fecal coliform count in the three levels of drinking water samples

The Phoenix systems (BD Diagnostic System) is automated instruments for rapid organism identification and susceptibility testing. Table (3) present the bacterial isolates device definition Al Phoenix, the resulting species are *Pseudomonas aeruginosa, Citrobacter freundii, Ochrobacterum anthropi, Cedecea lapagie, Streptococcus anginosus, Stenophomonas maltophilia*.

Table 3. Bacteria isolated from drinking water samples in level 1 and level 2 and identification by the BD Phoenix system

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

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. It is either not duplicate or percentage of chlorination very low. The results showed no significant differences ($p > 0.01$) between the three levels it's described in figure (4).

It was to clarify the relationship between total coliform bacterial and chlorine residual in all drinking water samples represented graphically found that figure (5), 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 .

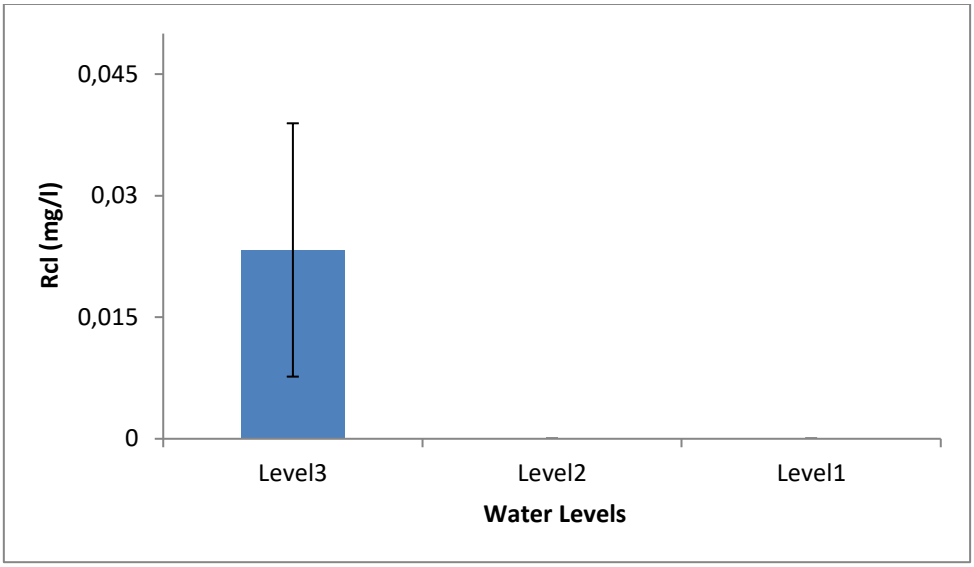

Figure 4. A difference of average chlorine residual in the three levels

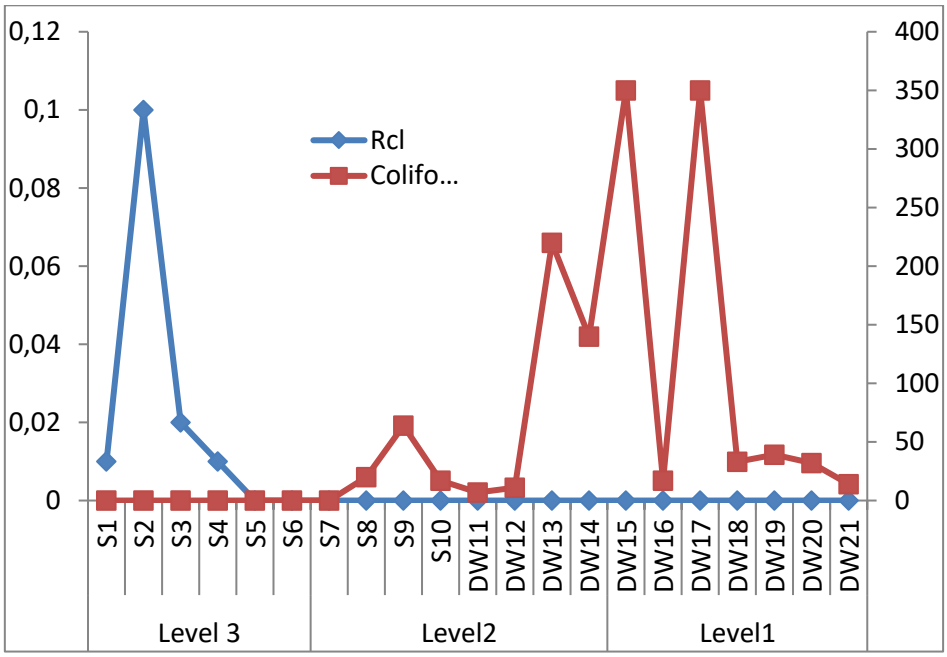

Figure 5. Correlation of total coliform bacterial counts and the levels of chlorine residual

CONCLUSION AND RECOMMENDATION

In this study, 21 drinking water samples taken for the analysis of chemical and bacteriological quality from Taucheira village from random selected wells and from public water distributing points of present study areas were not fit for drinking. 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 disinfection, maintenances and supervisions of water sources; and regular bacteriological assessment of all water sources for drinking should be planned and conducted. Maintain water chlorine levels within the limits recommended by the WHO and EPA.

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