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Microbiological Safety Assessment of Groundwater Wells in Bugesera and Muhanga Districts of Rwanda

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Abstract This study was conducted with the aim to monitor the suitability of the groundwater wells in Bugesera and Muhanga districts of Rwanda. As the quantity of water supplied by Rwandan Water and Sanitation Corporation ltd (WASAC) is not sufficient for the whole population in these districts, the people depend on groundwater as the main source of water. The microbial quality assessment made by this research on Bugesera district's groundwater, obtained a number of total coliforms equaling to 132 MPN/100ml during dry season and 399 MPN/100ml during the rainy season with the fecal coliforms estimated to be 125 CFU/ml. In Muhanga district, total coliforms were estimated to be 125 MPN/100ml with the fecal coliforms estimated to be 115 CFU/ml. The microbial analysis results revealed the presence of *Klebsiella*, *Escherichia coli*, *Shigella*, *Salmonella*, *M. morganii* and *Serratia spp* in the groundwater wells of both districts confirming that groundwater wells are contaminated. Therefore, in order for this water to meet the standards of drinking water, it should be boiled or treated with chlorine before consumption and local authorities should be informed about the threats. Community participation and sensitization programs on how to obtain clean and safe water supply from groundwater need to be implemented by responsible stakeholders.

Keywords: *Groundwater wells, microbial safety, Most Probable Number (MPN), Colony Forming Unit (CFU), Muhanga district, Bugesera district*

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1. Introduction

Groundwater is the water located below the earth's surface held in between soil and rocks. It is the *world's* most extracted raw material with *withdrawal rates* currently estimated to be 982km3/year [1]. Groundwater is an important source of drinking water worldwide; it is also the only source of water supply in some countries in the world. In most European countries, ground water use exceeds 70% of the total water consumption [2]. In countries with arid and semiarid climate, groundwater is widely used for irrigation as about one-third of their landmass is irrigated by groundwater [1,2]. Ground water is also a reliable resource for industries [3].

Under most conditions, groundwater is safer than surface water because surface water is more exposed to pollutants like those from industries. However, groundwater can be contaminated by various pathogens like bacteria, viruses, parasites, and chemicals which could lead to sickness and disease. Septic tanks, sewage and municipal wastewater treatment, wildlife, grazing animals, and other agricultural activities can also be sources of groundwater contamination. The most common pathogens identified in groundwater outbreaks included Shigella spp., Hepatitis A virus, norovirus, Giardia intestinalis, Campylobacter spp, and Salmonella spp. [4].

Fecal coliforms and fecal streptococci are the most commonly used bacterial indicators of fecal pollution [5]. They are found in water that is contaminated with fecal wastes that are of human and animal origin. The ratio between fecal coliforms (FC) and fecal streptococci gives a fecal index, which indicates the origin of pollution. High FC and total coliform counts in water are also indicative of the presence of enteropathogens in the water [5].

In Rwanda, groundwater is used mostly in rural areas for various purposes including drinking, preparing food, and bathing, washing clothes and dishes and irrigations. Many people living across the rural areas rely on the use of untreated water so there is an urgent need to identify the type of contaminants and the source of contamination. The purpose of this study was to assess the water quality of groundwater wells accessible in Muhanga and Bugesera districts.

2. Materials and Methods

2.1. Study Area

Two provinces, South and East, were selected randomly from four provinces (East, South, Kigali and North provinces), and then from those two therefore, two

districts (one district from each province) which are Bugesera and Muhanga were randomly selected. From these two districts, 12 sites of groundwater wells, 6 sites from each district, were considered to conduct the study.

Muhanga district population estimate, by national census done in 2012, was about 319,141 people on a 648km^2 of total surface area [6]. Whereas, Bugesera district population was estimated to be 361,914 people with 1288km^2 of total surface area.

Table 1. Show the study design implemented for the collection of water samples

Province	District	Sector	Groundwater well	N° of samples collected
South	Muhanga	1. Shyogwe	Safari, Kabeza, Ruhina1, Ruhina2, Byerwa and Murambi.	3
		2. Nyamabuye	Merani, Nyarucyamu1, Nyarucyamu2, Rwansamira, Nyabisindu and Rutenga.	3
East	Bugesera	1. Nyarugenge	Nyakabingo,Kadogori,Rwakiromba, Cyahafi, Tubumba and Kanogo	8
		2.Ruhuha	Gatare1, Gatare 2, Cyizanye, Nyaburiba, Nyabunogo and Ntagugura.	4

2.2. Study Design and Sample Size

From the selected districts, two sectors were randomly selected. In each sector, six different groundwater wells were selected as illustrated in Table 1. From both districts a total of 18 water samples were collected and analyzed for this study.

2.3. Sample Collection

The collection of water samples was done carefully and with proper care. During sample collection, the following were taken into consideration: - Taking a short note survey of the area around the groundwater wells, with the help of a questionnaire, in order to spot possible ways of contamination of the water and collection of water sample by using a clean and sterile bottle.

2.4. Laboratory Methods

2.4.1. Multiple Tube Fermentation Method

This method gives a statistical estimate of the most probable number (MPN) of total coliforms or fecal coliform population. It involves 3 steps: - Presumptive test, Confirmed test and Completed test. It is a standard technique approved by the Standard Methods Committee, 1994 [7].

2.4.2. Gram Stain Procedure

The Gram stain was used to permit the separation of all bacteria into two large groups, those which retain the primary dye (gram-positive) and those that take the color of the counter-stain (gram-negative). The protocol followed was the test originally developed by Christian Gram in 1884, and was later modified by Hucker in 1921 [8].

2.4.3. Serial Dilution

This procedure was employed to identify the number of viable micro-organisms in a fixed amount of liquid. From each sample, serial dilution was made to reduce the microorganism's concentration in the sample. It was carried out using sterile saline water as diluent, 10 ml was aseptically measured and added in the cotton swab applicator and shaken thoroughly. A one in ten dilution of that solution was prepared by adding 1 ml of that solution to 9 ml of sterile saline water with a sterile pipette and mixing them thoroughly.

2.4.4. Biochemical Tests

Biochemical tests are tests that investigate the enzymatic activities of microorganisms and application of different carbon sources to obtain their energy. They are highly effective tests in the identification of bacteria [9,10].

Aerobic bacterial colonies from nutrient McConkey agar, suspected to be either Gram positive or Gram negative, were picked separately and inoculated on the following biochemical reagents: Simmons citrate agar, Difco^{TM} malonate, urease broth, and Triple sugar iron agar, incubated at 37°C for 24 hours. Catalase test and coagulase test were also done.

2.5. Questionnaire

The people who were using the ground water and those living around each well under our study were asked a number of questions. Volunteers, who responded to the questions asked, were of assistance to us in presenting handy recommendations to various stakeholders.

2.6. Data Analysis

The data was analyzed after the different microscopic examinations and biochemical tests were completed. The Most Probable Number (MPN) was estimated by the number of positive tubes for different microorganisms observed using graphics and tables.

3. Results

The results were acquired according to the different experiments performed.

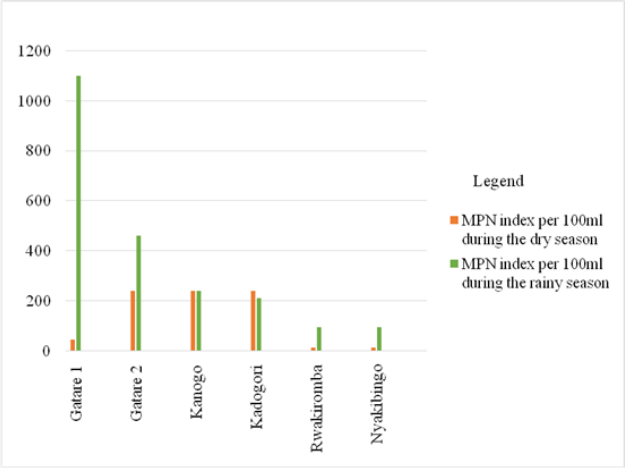

Figure 1. Results of total coliforms in MPN from the presumptive test that show a comparison between dry and rainy seasons in Bugesera district groundwater wells

3.1. Presumptive Test Results for Bugesera District

Figure 1 shows that the total coliforms in dry season were 132 MPN/ml and then there was a great increase during the rainy season up to 366 MPN/ml.

3.2. Confirmed Test Results for Bugesera District

Fecal coliforms in Bugesera district were analyzed using the confirmed test. Only 6 plates from this test during the dry season and 9 from the rainy season showed growth with colonies that were nucleated with dark centers while the rest were green metallic sheen as shown in Table 2 for the dry season and Table 3 for rainy season. The results showed an overall average of 106 CFU/ml numbers of fecal coliforms for dry season and 133 CFU/ml numbers of fecal coliforms for the wet season.

Table 2. Confirmed test results from EMB plates' colony count with colonies of green metallic sheen and with nucleated centers (characteristics of *E coli*) during dry season, Bugesera district

Sampling area	Amount in ml	Media used	N^0 of colonies	Size and color	Fecal coliforms (CFU/ml)*
Gatare 1	10	EMB	100	Small and green metallic sheen	100
	10	EMB	93	Small and green metallic sheen	93
	10	EMB	97	Small and green metallic sheen	97
	Average				**97**
Gatare 2	10	EMB	110	Small and green metallic sheen	110
	10	EMB	119	Small and green metallic sheen	119
	10	EMB	120	Small and green metallic sheen	120
	Average				**116**

Table 3. Confirmed test results from EMB plates' colony count with colonies of green metallic sheen and with nucleated centers (characteristics of *E coli*) during rainy season, Bugesera district

Sampling area	Amount in ml	Media used	N^0 of colonies	Size and color	Fecal coliforms (CFU/ml)*
Gatare 1	10	EMB	120	Small and green metallic sheen	120
	10	EMB	117	Small and green metallic sheen	117
	10	EMB	121	Small and green metallic sheen	121
	Average				**142**
Gatare 2	10	EMB	140	Small and green metallic sheen	140
	10	EMB	130	Small and green metallic sheen	130
	10	EMB	145	Small and green metallic sheen	145
	Average				**138**
Kanogo	10	EMB	115	Small and green metallic sheen	115
	10	EMB	120	Small and green metallic sheen	120
	10	EMB	125	Small and green metallic sheen	125
	Average				**120**

CFU/ml*: colony forming units per milliliter.

3.3. Completed Test Results for Bugesera District

The completed test was done as a confirmation of the results obtained in previous tests (presumptive and confirmed tests). All the 6 EMB plates during dry season and 9 plates during rainy season showed growth on nutrient slants and gas production on lactose broth. Results obtained from the staining procedure showed that the bacteria were gram negative, rod shaped (bacilli), small and in clusters, and that there was no formation of spores.

Table 4. Results from EMB plates' colony count with colonies of green metallic sheen and with nucleated centers (properties of *E coli*), Muhanga district

Sampling area	Amount in ml	Media used	N^0 of colonies	Size and color	Fecal coliforms (CFU/ml)*
Merani	10	EMB	119	Mixed and metallic sheen	119
	10	EMB	115	Mixed and metallic sheen	115
	10	EMB	110	Mixed and metallic sheen	110
	Average				**115**

CFU/ml*: colony forming unit per milliliter.

3.4. Confirmed Test Results for Muhanga District

Fecal coliforms in Muhanga district were analyzed using the confirmed test. Only 3 plates from this test showed growth with colonies that were nucleated with dark centers while others were green metallic sheen as shown in Table 4. The results showed an average of 115 CFU/ml numbers of fecal coliforms in the groundwater sample.

3.5. Completed Test Results for Muhanga District

Completed test was done as a confirmation of the results using the organisms which grew on the confirmed test media. All the three plates from the confirmed test showed growth on all nutrient slants and gas production on lactose broth. Results from staining showed that the bacteria were gram negative, rod shaped (bacilli), were small and in clusters, and that there was no formation of spores.

3.6. Biochemical Tests Results from Bugesera District

These tests were performed by using samples Gatare 1 and Gatare 2 that were positive during the confirmed tests. The results are shown in Table 5. *E coli* bacteria were identified through performing triple sugar iron test and malonate test which were positive. *Klebsiella, Salmonella,* and *Serratia* were identified through triple sugar iron tests and citrate tests being positive. *Proteus* was identified through triple sugar iron test and urease test being positive. *M. morganii*was was identified through triple sugar iron, indole and urease test being positive. *Shigella* was identified by positive triple sugar iron test.

Table 5. Biochemical test results of Gatare 1 and Gatare 2 in Bugesera district

Identified bacteria	TRIPLE SUGAR IRON AGAR				CITRATE	INDOLE	CATALASE	MALONATE	UREASE
	GLU	LAC	H_2S	GAS					
E.coli	+	+	-	+	-	-	-	+	-
Klebsiella	+	+	-	+	+	-	-	-	-
Proteus	+	-	+	+	-	-	-	-	+
M. morganii	+	-	-	+	-	-	-	-	+
Serratia	+	-	-	+	+	-	-	-	-
Salmonella	+	-	+	+	+	-	-	-	-
Shigella	+	-	-	-	-	-	-	-	-

Legend: + = Positive, - = Negative.

3.7. Biochemical Test Results from Muhanga District

These tests were performed by using samples from Merani that was positive during the confirmed tests. The results are shown in Table 6. *E coli* bacteria was identified through performing triple sugar iron test, indole and malonate tests which were positive. *Klebsiella, Salmonella,* and *Serratia* were identified through triple sugar iron tests and citrate tests being positive. *Proteus* was identified through triple sugar iron test being positive. *M. morganii* was identified through triple sugar iron test and urease test being positive. *Shigella* was identified by positive triple sugar iron test.

Table 6. Biochemical test results of Merani in Muhanga district

Identified bacteria	TRIPLE SUGAR IRON AGAR				CITRATE	INDOLE	CATALASE	MALONATE	UREASE
	GLU	LAC	H_2S	GAS					
E.coli	+	+	-	+	-	-	-	+	-
Klebsiella spp.	+	+	-	+	+	-	-	-	-
Proteus spp.	+	-	+	+	-	-	-	-	+
M. morganii	+	-	-	+	-	-	-	-	+
Serratia spp.	+	+	-	+	+	-	-	-	-
Salmonella spp.	+	-	+	+	+	-	-	-	-
Shigella spp.	+	-	-	-	-	-	-	-	-

Legend: + = Positive, - = Negative.

4. Discussion

This study showed total coliforms from Bugesera district equaling to 132 MPN/ml during the dry season and 366 MPN/ml during the rainy season. The increase in coliforms in the rainy season could be due to poor construction or cracks in the wells and since during this season the sub-standard pit latrines and open sewers have their contents overflow, microorganisms may enter the water supply around defective wells. Soil wetness also facilitates contaminants' travel through seepage hence more microbes in wastes from poorly constructed pit-latrines find their way into wells [11]. Furthermore, the groundwater sources are constructed downhill and close to sanitation facilities as well as surface water. Consequently, runoff of human and domestic wastes and seepage of contaminants from the streams may pollute the water. A research performed in Kenya [11], stated that seasonal variation in bacterial contamination is attributable to wet

weather (rainfall). Water and soil characteristics facilitate bacterial movement, for this reason more contamination is evident in wet weather compared to dry conditions.

All the wells sampled yielded coliform colonies. The results obtained exceeded the limits stipulated by WHO maximum limits for drinking water –0 colonies/100ml water sample [12]. If only total coliform bacteria are detected in drinking water, the source is probably environmental [13]. If fecal coliform counts are high (over 200 colonies per 100 ml of water sample), there is a greater chance that pathogenic organisms are also present. Diseases and illnesses such as typhoid fever, hepatitis, gastroenteritis, dysentery, and ear infections can be contracted from consuming water with high fecal coliform counts [14]. According to WHO drinking water must have zero total coliforms, fecal coliforms and *E. coli* [15].

E. coli was also identified in water samples from Bugesera and Muhanga districts where its presence emphasizes that there has been fecal contamination of the drinking water sources. *E. coli* is abundant in human and animal feces, and in fresh feces it may attain concentrations of 109 per gram [16]. It has been suggested that *E. coli* may be present or even multiply in tropical water and is not restricted to human fecal pollution. However, even in the most remote regions, fecal contamination by wild animals including birds can never be excluded [17]. *E.coli*'s presence in water indicates not only recent fecal contamination of the water, but also the possible presence of intestinal-disease causing bacteria, viruses, and protozoa [18]. Fecal coliform bacteria can enter rivers through direct discharge of waste from mammals and birds, from agricultural runoff practices such as allowing animal wastes to wash into nearby streams during the rainy season, spreading manure and fertilizer on fields during rainy periods, allowing livestock watering in streams, and from untreated human sewage [19]. Individual home septic tanks can become overloaded during the rainy season allowing untreated human wastes to flow into drainage ditches and nearby waters [19].

The detection of bacteria of fecal origin in groundwater could be attributed to the fact that the groundwater (wells) have similar features: they lack proper physical barriers like concrete sanitary seals, concrete plinths, concrete aprons, well linings, sanitary covers, lockable sanitary lids, et cetera which could prevent overland runoff containing human, animals and domestic wastes from contaminating the water sources [20]. The WHO (2006) reported that groundwater is less vulnerable to contamination due to the barrier effect, and that once the protective barrier is breached direct contamination may occur [15]. Chapman (1996) noted that due to the relatively slow movement of water through the ground, once polluted, a groundwater body could remain so for decades, or even centuries [21].

Referring to the Guidelines for Canadian Drinking Water Quality if any Coliform bacteria are detected in drinking water, the source should be immediately investigated. If known or suspected to be fecal Coliform or *E. coli*, the water should not be consumed without treatment such as boiling for one minute [22].

Expectedly, diarrheal-related diseases are among the most reported cases from the survey done with the help of a questionnaire. Studies show that high incidence of diarrhoea is associated with the drinking of contaminated water [23,24]. Additionally, visitors who drink from contaminated water sources are also vulnerable to diarrhea-related diseases. Diarrhea is a major killer among the poor, especially in developing countries, and each year an estimated number of 2.2 million people, most of whom are under 5 years of age, die from diarrhea-related diseases [25].

The results obtained during the biochemical tests identified organisms including *Salmonella spp., Klebsiella spp., Serratia spp., Shigella spp, M. morganii,* and *Proteus spp.* These organisms are under the Enterobacteriaceae family where they are referred to as enterobacteria or "enteric bacteria", as several members of the family live in the intestines of animals [26]. Some members of Enterobacteriaceae are parasites of animals causing waterborne diseases.

Finally, in order to reduce the level of bacterial contamination of drinking groundwater sources in these districts, there is the need for stakeholders to educate inhabitants, particularly women and children, on causes, modes of transmission, and prevention of water and sanitation related diseases. In addition modes of storing water in proper storing facilities with narrow necks, proper handling of stored water, the treatment of collected water and hand-washing, etc which help reduce the consumption of contaminated water need to be sensitized to the community. Also, residents of the area must endeavor to cultivate better sanitation habits and ensure that their surroundings and water sources are not indiscriminately polluted, especially by passersby.

5. Conclusion

The presence of *E.coli* in the samples is an indicator that pathogenic microorganisms are present in the ground water wells of Muhanga and Bugesera districts. Microorganisms, such as *Salmonella spp., Klebsiella spp., Serratia spp., Shigella spp., M. morganii,* and *Proteus spp.* that have the potential to cause waterborne diseases, were identified from the different biochemical tests. It was confirmed through laboratory analysis that coliforms are more pronounced during the rainy season than in the dry season. Thus the study reveals that the raw ground water from these districts is not safe for human consumption. Therefore, from this study it can be concluded that the water should be treated before domestic use. Because the utilization of such water may cause diseases like dysentery, diarrhoea, typhoid, cholera, jaundice, gastroenteritis, shigellosis, enteric fevers, and other ailments.

In order to meet the potability of the groundwater, it is recommended that effective and continuous treatment combined with constant monitoring is essential to ensure that it meets the standards of drinking water. Fecal coliform like other bacteria can usually be killed by boiling water or by treating with chlorine. Washing thoroughly with soap after contact with contaminated water can also help prevent infections.

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Conflict of Interests

No conflict of interests between authors.

References

[1] Facts about Global Groundwater Usage. [Online]. Available: http://www.ngwa.org/Fundamentals/use/Documents/global-groundwater-use-fact-sheet.pdf accessed on 18/08/2015. [Accessed 18/08/2015].

[2] Igor, S.Z. and Lorne, G.E. Groundwater resources of the world and their use. 2004. [Online]. Available*: http://unesdoc.unesco.org/images/0013/001344/134433e.pdf*. [Last Accessed 17/12/2014].

[3] aroslav, V. and Annukka, L. Groundwater Resources Sustainability Indicators. 2007. [Online]. Available at: *http://unesdoc.unesco.org/images/0014/001497/149754e.pdf*. [Last Accessed 17/12/2014].

[4] CDC. Ground Water Awareness Week. 2014. [Online]. Available at: *http://www.cdc.gov/features/groundwaterawareness/*. [Last Accessed 31 August 2014].

[5] Fatoki, O.S., Muyima, N.Y. and Lujiza, N. Situation analysis of water quality in the Umtata river catchments. *Water SA*. 27, 467-473, 2001.

[6] Ministry of Finance and Economic Planning, National Institute of Statistics of Rwanda. Fourth Population and Housing Census, Rwanda, 2012: Thematic Report Characteristics of households and housing. January 2014. [Online]. Available: http://www.lmis.gov.rw/scripts/publication/reports/Fourth%20Rwanda%20Population%20and%20Housing%20Census_Housing.pdf [Accessed 15/08/2015].

[7] Standard Total Coliform Fermentation Technique. [Online]. Available: https://www.norweco.com/html/lab/test_methods/9221bfp.htm [Accessed 15/08/2015].

[8] Gram Stain Protocol. [Online]. Available: http://www.med-chem.com/pages/lab_procedures/pdf/gram_stain.pdf [Accessed 12/08/2015].

[9] MacFaddin, J.F. Biochemical tests for identification of medical bacteria. 3rd ed. Lippincott Williams & Wilkins. Philadelphia, PA, 2000.

[10] Identifying Unknown Bacteria Using Biochemical and Molecular Methods. [Online]. Available: http://www.nslc.wustl.edu/elgin/genomics/Bio3055/IdUnknBacteria06.pdf [Accessed 12/08/2015].

[11] Mutuku, S. C. and Matolo, M. P. Temporal variation in coliform densities in groundwater supplies in a County Residential Estate. *Annals of Biological Research*, 5 (8):30-35, 2014. [Online]. Available at: (http://scholarsresearchlibrary.com/archive.html) [Accessed 12/08/2015].

[12] WHO. Guidelines for drinking-water quality. (2nd ed.). MALTA, 1997. [Online]. Available: http://www.who.int/water_sanitation_health/dwq/gdwqvol32ed.pdf [Accessed 12/08/2015].

[13] DOH. (2011). Dohwagov. [Online]. Available: *http://www.doh.wa.gov/Portals/1/Documents/Pubs/331-181.pdf* [Accessed 7 June, 2015].

[14] Hassan, A. and Nawaz, M. Microbiological and physicochemical assessments of groundwater quality at Punjab, Pakistan. *African Journal of Microbiology Research*, 8(28), 2672-2681, 2014.

[15] WHO. Microbial aspects. Guidelines for drinking water quality, Microbial aspects. 2006. [Online]. Available: http://www.academia.edu/6051932/Guidelines_for_Drinking-water_Quality_THIRD_EDITION_INCORPORATING_THE_FIRST_AND_SECOND_ADDENDA_Volume_1_Recommendations [Accessed 19/08/ 2015].

[16] Micro Biological analysis of drinking water.pdf - auroville.info. [Online]. Available: www.auroville.info/ACUR/.../report_microbiological_tests_of_water.pdf [Accessed 19/08/ 2015].

[17] Rutanga, J.P. Assessment of microbiological and physicochemical parameters of groundwater: A case study of Gikondo Industrial Park, Kigali, Rwanda. *Ethiopian Journal of Environmental Studies & Management*, 7(2), 153-159, 2014.

[18] Health Canada. Guidelines for Canadian Drinking Water Quality: Guideline Technical Document - Total Coliforms; 2013. [Online]. Available: *http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/coliforms-coliformes/index-eng.php* [Accessed May 19, 2015].

[19] Water Research Center. Water-research.net. 2014. [Online]. Available: http://www.water-research.net/index.php/e-coli-in-water. [Accessed 7 June, 2015].

[20] Omari, S. D. and Yeboah-Manu. The Study of Bacterial Contamination of Drinking Water Sources: A Case Study Of Mpraeso, Ghana. *The Internet Journal of Microbiology*. Volume 10, Number 1, 1-6, 2012.

[21] Chapman, D. Water Quality Assessments. A guide to use of biota, sediments and water in environmental monitoring, 2nd (ED). E&FN Spon; 417, 1996.

[22] Guidelines for Canadian Drinking Water Quality. 2010. [Online]. Available: http://www.dieppe.ca/en/hoteldeville/resources/Canadiandrinkingwaterguidelines.pdf [Accessed 18/08/ 2015].

[23] Howard, G., Chave, P., Bakir, P. and Hoque, B. Socioeconomic, institutional and legal aspects in groundwater assessment and protection. In: WHO. Protecting Groundwater for Health: Managing the Quality of Drinking-water Sources, IWA Publishing; 142, 2006.

[24] Mahvi, A.H. and Karyab, H. Risk assessment for microbial pollution in drinking water in small community and relation to diarrhoea disease. *American-Eurasian Journal of Agricultural and Environmental Science*; 2 (4): 404-406, 2007.

[25] WHO/UNICEF. Meeting the MDG drinking water and sanitation target: mid-term assessment of progress; 4, 6, 2004.

[26] From Wikipedia, the free encyclopedia. 2015. Enterobacteriaceae. [Online]. Available: https://en.wikipedia.org/wiki/Enterobacteriaceae [Accessed 18/08/ 2015].