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ASSESSMENT OF BACTERIOLOGICAL LOAD OF SELECTED LOCATIONS OF GONDAR TOWN WATER SOURCES

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Abstract

Samples of water from Gondar town water supply (tape water), Shinta and Keha rivers were taken. The corresponding microbial (bacterial) load was studied carefully. To undergoing such analysis, both spread plate count and membrane filtration methods were used. Total bacteria indicator organisms (total coli form, E.coli and fecal streptococci),bathing contaminant (staphylococcus aures) for the river water samples and pathogen analysis (salmonella species) were observed after incubating them on their appropriate media and incubation period for the specified incubating temperature. To this end, our result indicated that most of the bacteria common to the contaminated water sample were indeed found in two our samples (i.e., river water samples) and slightly less number in the tape water sample. The average colony forming unit per milliliter (cfu/ml) was calculated for each of identified bacteria of the three samples and based on this, in both of the river samples, the colony forming unit per milliliter was found above 107 and less than 102 for the tape water sample .For the case of membrane filtration method, the average results showed that total counts ranged from 102 to 104 colony forming unit per 100 milliliter for the samples taken from both rivers , where as for the tape water sample was less than 102 colony forming unit per 100 milliliter for each microorganism.

Keywords: Total bacteria, Indicator organisms, Bathing contaminant, pathogen.

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INTRODUCTION

Water is a chemical substance with the chemical formula H2O.It is a liquid at ambient condition, but it often co-exists on earth with solid state, ice and gaseous state water vapor or steam [1]. Clean drinking water is essential to humans and other life forms. Water for human consumption is called drinking or potable water. Accessibility and Availability of fresh clean water is key to sustainable development and essential element in health [2].Unfortunately, almost half of the world population is without access to improved sanitation facilities and almost one billion people still lack accesses to improved drinking water and supplies [3]. Around 2.2 million deaths per year which can either be prevented or minimized by improving access to drinking water and sanitation are traceable to diarrhea disease. The bulk of the burden is felt in developing nations such as ours where provision of public supply of drinking water is deficient owing partly to the lack of sufficient financial commitment towards existing infrastructure. People in rural settlement often result to other alternatives as reliable source of water for drinking and meet other domestic standards.

The environmental risk assessment studies reveal that the exposure to biological contaminates especially water borne microbial pathogen needs to be given as a higher priority in treatment and regulatory programs for domestic water supplies .Generally, the presence of fecal pathogens in water is demonstrated by recoveries using assays or conventional laboratory media of indicator organisms [4].

The most commonly tested fecal bacteria indicators are total coli forms, fecal coli forms, Escherichia coli, fecal streptococci and enterococci. All but E.coli are composed of a number of species of bacteria that share common characteristics such as shape, habitat or behavior; E.coli is single species in the fecal coli form group [5].

Total coli form groups of bacteria that are wide spread in nature. For recreational waters, total coli forms are no longer recommended. For drinking water, total coli forms are the standard test because their presence indicates contamination of water supply by outside source. E.coli is a species of fecal coli forms bacteria that is specific to fecal material from humans or other warm-blooded animals .Fecal streptococci generally occur in the digestive systems of human and /or other warm–blooded animals. A ratio of fecal coli forms to fecal streptococci is used to determine whether the contamination was human or non human origin. Enterococci are recommended as the best indicator of health risk in salt water [6].

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Currently there are about five techniques for the routine detection and enumeration of indicator and opportunistic microorganisms. They are the most probable number (MPN), membrane filtration, ATP testing, spread plate count and pour plate count methods. The membrane filtration method takes several different sized samples and filters them with the same filter. The filter is then placed in a Petri dish and allowed to sit at a particular temperature for a set period of time .After the time is up, lab technicians observe and count the number of colonies on the dish. The multiple tube fermentation method involves adding specified quantities of the sample to tubes containing a nutrient broth incubating the tube at a specified temperature for a specified period of times then looking for the development of gas and/or turbidity that the bacteria produce. The presence or absence of gas in each tube is used to calculate an index known as the most probable number. The plate count method relies on bacteria growing a colony on a nutrient medium so that the colony becomes visible to the naked eye and the number of colonies on the plate can be counted. Each of the method is described in detail on the literature review part.

Objectives

General Objective

Evaluation of bacterial load of selected Gondar town water sources

Specific Objectives

- Monitoring the quality of water based on bacteriological load of anaerobic and aerobic mesophilic heterotrophic bacteria
- Isolation of water borne pathogens such as Salmonella, Staphylococcus auerus and E.coli
- Isolation of fecal Streptococci whether origin from human or animal faeces
- Provision of data/information on ways to reduce water safety hazards in selected Gondar town water sources.

MATERIALS & METHODS

Materials and chemicals required

There are different types of media and equipments required for investigation of the bacteria in the laboratory, especially in this study we prepared solid media. For this reason, chemical and equipments needed for the examination of different groups of bacteria in the water described as follows. Generally, media required to undertake our study were SS agar, MacKonkey agar, Mannitol salt agar, Sorbitol MacKonkey agar, Plate count agar and KF agar. The detail of these media is discussed in the media preparation and spread plate technique section. The general

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chemicals and equipments used for these study were distilled water, ethanol, vortex mixer, incubator ,beaker, pipette, rack, test tube, sponge, aluminum foil, colony counter, spatula, Laminar air flow, Autoclave ,flask, membrane filter paper, measuring cylinder ,Petri dish sensitive balance ,pH meter , wire loop, swamp, cotton , funnel, filter paper, Bunsen burner, refrigerator, magnetic stirrer and heater.

Sample collection

Samples of water were collected from Gondar town water supply (Maraki tape water), Shinta River and Keha River aseptically in sterile bottle for studying the bacteriological quality analysis of these water sources.

Media preparation

Before sample processing different biological media were prepared for successful detection and growth of bacteria found in water. About six different media were prepared in which the detail of preparation of the media was described below.

Spread plate technique

- **Serial Dilution**

  After media preparation, 1ml of the sample was transfer to nine ml of sterile distilled water and mixed serial dilutions thoroughly. The dilution was held from 10^{-1} up to 10^{-8} and overtaxing was done at each successive step of the series. The sample was maintained in increasing dilutions.

- **Total bacteria**

  From appropriate dilutions, 0.1 ml aliquots were spread plated in duplicates on pre-dried surfaces of plate Count agar (g/l:yeast extract,5; Glucose, 1;peptone, 5; Agar, 15)plates. Colonies were counted after incubation at 30^0C for 48 hrs.

- **Coli forms**

  The isolation of Coliform according to [7], a volume of 0.1ml appropriate dilutions were spread plated in duplicates on pre-dried surfaces of MacKonkey Agar (g/l:yeast extract ,3;pepton,7;Bilesalt no.3, 1.5;Lactose, 10;Sodium Chloride,5;Agar,15;Neutral red,0.03;Crystal violet,2)plates. The plates were incubated at 35^0C for 24 hours after which purplish red colonies surrounded by reddish zone of precipitated bile were counted as coli forms.

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- **Isolation of E.coli**

  The isolation of E.coli was done according to [8], 100ml samples were collected and E.coli were detected by enriching on pre-warmed (42^0C) modified Tryptone soy broth with Nonbioncin 20mg/l for up to 24 hrs. Then 0.1ml was sub-cultured on Sorbitol MacKonkey Agar (g/l:peptone,20;sodium Chloride ,5;Bilsaltno.0.3,1.5;Sorbitol,10;Crystal violet,0.001;Neutral red,0.03;Agar,15) plates after 24 hrs. The plates were incubated at 37^0C for 24 hrs.

- **Test for Fecal Streptococci**

  A volume of appropriate dilutions were spread plated in duplicates on pre –dried surfaces of KF (kenner fecal) Streptococcus Agar (g/l:protease,10;Yeast extract,10;Sodium Chloride,5;Sodium Glycerol phosphate,10;Maltose,1;Lactose,1;Sodium Azide,0.4;Sodium Carbonate, 0.636;Bromocresol,0.4) plates. The plates were incubated at 35^0C for 48hrs. Red to pink colonies was formed.

- **Test for Staphylococci**

  Staphylococci are generally assumed to serve as indicators of water pollution deriving from bathers (i.e. by shedding from their body surface).To test the presence of Staphylococci in the selected Gondar town water sources, the following experiment was done. A volume of 0.1ml of appropriate dilutions were spread plated in duplicates on Pre-dried surfaces of Mannitol salt Agar (g/l:protease,10;Beef Extract,1;D-Mannitol,10;Sodium Chloride,7.5;Agar15;Phenol red,25) plates. The plates were incubated at 30^0C for 36 hrs. Yellow colonies were counted as Staphylococci.

- **Pathogen Analysis**

  When samples show elevated levels of indicator microorganisms (bacteria) further analysis is often undertaken to look for specific pathogenic bacteria [9]. Species commonly investigated are Salmonella typhi, salmonella typhimurium and Vibrio cholera. For the case of this study,

  SSAgar(g/l:LabLemcopoweder,0.5;peptone,0.5;Lactose,1;Bilesalt,0.85;SodiumCitrate,1;SodiumThiosulphate,0.85;Ferric-Citrate,0.1;Brilliantgreen,0.000033;Neutral red,0.025;Agar,1.8)was used as a growing medium.

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Membrane Filtration Technique

To undergoing membrane filtration technique and to assess the bacterial load of the water samples from three sources of water under investigation, the following procedures were held .filtration equipment was set up [10]. The water sample was shacked well to re suspend all materials and a measured volume was poured in to the funnel (11ml sample was transferred into 89ml of sterile distilled water and 100ml of this dilution was filtered to obtained the 10 ml sample for membrane filtration method. The filter paper with 0.45µm pore size was carefully removed from the filter holder using sterile forceps after filter has stopped (ended).The filter was carefully placed on the culture medium. The culture medium used was prepared in similar fashion to that of spread plate technique for each test microorganism. The filter was placed on the agar media as it was in the filter holder. The plates were inverted and incubated at a temperatures mentioned above the spread plating method.

Gram Staining of Bacteria

Gram stain is very useful stain for identifying and classifying bacteria in to two major groups: the gram-positive and gram-negative. To undergoing these process the fixed bacterial smear was subjected to four different reagents added sequentially these are crystal violate (primary stain). Iodine solution (Mordant). Alcohol (decolorizing agent) and Safranin (counter Stain).Then finally oil was added and observed under oil immersion of objective lens microscope [11].

Calculating colony forming unit

The standard plate count is used to determine the total number of viable bacteria in a water sample .The presence of large number of bacteria is undesirable in most water sources, because it increases the likely hood that pathogens will be present and it enhances the potential for water pollution [12].In a standard plate count, the colony forming unit was calculated .each colony may arise from a group of cells rather than individual cell. The initial sample was diluted through serial dilution in order to obtain visible colonies on each plate. After incubation the number of each colony was counted. Plates between 30-300 colonies are favorable for counting. The microbial population in the original water sample can be then evaluated using the following equation [10].

Cfu/ml = number of colonies / Amount plated X dilution factor used

And for the membrane filtration method,

N= (N colony count/volume of sample used) x100, where, N-is the total number of colonies per 100 milliliter.

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RESULT AND DISCUSSION

In this study, bacteriological analysis results of selected location of Gondar town water, i.e., Maraki tape water, Shinta River and Keha River streams that are used for drinking purpose in Gondar town and Gondar areas were undergoing. The average bacteriological analysis (evaluation) of load of fresh water sites (samples) is present in table 1 below.

Table 1: Average bacteriological load of samples of selected water sources of Gondar town (cfu/ml).

Sample site	Water sample	Total count in colony forming unit per milliliter					
		Total bacteria	Coli form	E.coli	Fecal Streptococci	Staphylococci	Salmonella
Shinta river	1	$7.23x10^7$	$6.31x10^8$	$5.03x10^7$	$1.52x10^7$	$3.83x10^7$	-
	2	$7.25x10^7$	$6.32x10^8$	$5.07x10^7$	$1.59x10^7$	$3.86x10^7$	-
	3	$7.26x10^7$	$6.37x10^8$	$5.09x10^7$	$1.56x10^7$	$3.87x10^7$	-
Maraki tap water	1	$5.92x10^2$	$1.68x10^1$	$1.02x10^1$	$1.12x10^1$	-	-
	2	$5.90x10^2$	$1.61x10^1$	$1.03x10^1$	$1.14x10^1$	-	-
	3	$5.95x10^2$	$1.65x10^1$	$1.07x10^1$	$1.09x10^1$	-	
Keha river	1	$7.32x10^9$	$6.05x10^9$	$2.63x10^8$	$1.01x10^7$	$2.04x10^8$	-
	2	$7.30x10^9$	$6.01x10^9$	$2.60x10^8$	$1.02x10^7$	$2.01x10^8$	-
	3	$7.33x10^9$	$6.03x10^9$	$2.65x10^8$	$1.05x10^7$	$2.07x10^8$	-

Table 2: Membrane filtration method of total bacteria, total coli forms, E.coli, fecal Streptococci, and Salmonella typhi present in the water collected from selected locations of Gondar town water sources.

Sample site	Water sample	Total count in colony forming unit per milliliter					
		Total bacteria	Coli form	E.coli	Fecal Streptococci	Staphylococci	Salmonella
Shinta river	1	2464	427	200	149	117	-
	2	2461	422	193	143	115	-
	3	2467	429	201	152	121	-
Maraki tap water	1	27	18	9	27	-	-
	2	21	13	7	22	-	-
	3	25	15	13	23	-	
Keha river	1	25814	973	1736	1954	722	-
	2	25813	971	1732	1951	770	-
	3	25817	975	1733	1953	773	-

Cultural characteristics and Staining result of bacteria

The bacteria which retain the primary stain (appear dark blue or violate) are gram positive bacteria ,where as those that lose crystal violet and counter stained by safranin are gram negative bacteria. It was observed that gram staining results of six different biological media. The colony that was grown on MacKonkey agar retains safranin red color, so that coli forms are gram negative ,rod shaped, on-spore forming bacteria and purplish red colonies surrounded by reddish zone of precipitated bile were counted as coli forms; colonies that was grown on plate count agar

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retained dark blue or violet and safranin red color were observed and different shapes and colors were appeared ;bacteria plated on sorbittol MacKonkey and KF Agar retained safranin red and crystal violet for E.coli and streptococci respectively ,so E.coli is gram-negative, road shape, non spore forming and purplish colony was observed .streptococci are gram positive ,spherical shaped and red to pink colonies was formed. Bacteria that was grown on mannitol salt agar was retained dark blue or violet color observed .Staphylococci are gram positive, spiral shaped and yellow colonies were counted as Staphylococci.

To this end, our result indicated that most of the bacteria common to the contaminated water sample were indeed found in two of our samples (i.e., river water samples) and slightly in the tape water. The average colony forming unit per milliliter (cfu/ml) was also done for each of the identified bacteria of the three samples and based on this, in both of the river samples, the Cfu/ml was found above 10^7 and less than 10^2 cfu/ml for the tape water sample. For the case of membrane filtration method, the average results showed that total count ranged from $10^2$to 10^4cfu/100ml for the samples taken from both of rivers where as for the tape water sample was less 10^2 cfu/100ml for each microorganism.

From the investigated water samples, the two river streams were highly contaminated by indicator organisms such as coli forms, fecal Streptococci (Enterococci), Staphylococci. And E.coli than that of the Maraki tape water. But the tape water was also slightly contaminated which indicates whether the distribution source was contaminated with human, animal faeces or the amount of addition of chemicals (Chlorine) was not effectively treated them.

When the load of total bacteria, total coli forms, E.coli, fecal Streptococci and staphylococci in Keha river stream was counted, it was counted, it was highly loaded. The load of coli forms was higher in Keha River than Shinta and Maraki tape water sources.

E.coli which is the most indicator organism is found in large numbers in the faeces of human and all warm-blooded animals. The load was higher as that of coli forms in Keha River than the two water sources. Fecal Streptococci, which originates only in the fecal contents of human beings and animals, were, found in the water sample examined. Staphylococci mainly caused during bathing from the nasal fluids, skin and other parts of the bodies. Salmonella typhi are in the satisfactory condition. They are used for further analysis of specific pathogenic bacteria present in the sample, but they were not found in the selected water sources. As the result indicated, from the selected water sources, Keha River was highly contaminated by indicator organisms, which

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are health hazards for humans and other users. This raises cause for alarm especially when the water source is available as drinking water choice for residents of community that service from it. Membrane filtration method which is more accurate method than the other methods of water analysis was also used for investigation purpose in this study, i.e., the bacteriological load of the water samples under study was checked using it. Especially, the two river samples were highly contaminated with indicator organisms but that of the tape water was slightly less contaminated. In three of the samples taken, salmonella species were not detected.

Various factors are currently considered as responsible for contamination of drinking water .First and for most; most of the sources of drinking water in two rivers are unprotected from human, animal faeces .Secondly, the socio-economic conditions of the settlers of that area. Thirdly, lack of awareness about the importance of safe drinking water and water born disease and lastly, the lack of knowledge about personal hygiene and general cleanliness among the people.

CONCLUSION

It was confirmed that, using plate count and membrane filtration of bacteriological water analysis methods, the bacteriological load of the three water samples were evaluated. The current study presented an evaluation of bacterial load of the selected water sources in Gondar town were conducted on. In addition to the main purpose of the study was to monitor bacteriological quality of water, to isolate water borne pathogens and to reduce water safety hazards at the selected water sources. We could identify and detect bacterial load in water by using bacteriological water analysis methods such as plate count, membrane filtration, etc. During the study, some indicator organisms were found in higher load .Especially, Keha river was contaminated highly with indicator organisms when it was compared with Shinta River .But the Marki tap water was very slightly contaminated with indicator organisms .In the three selected water sources, Salmonella typhi was not found.Generally, according to [13], there should not be any total coli form ,E.coli and fecal Streptococci in 100ml. According to [14], there should not be any pathogenic Staphylococcus species in 100ml of drinking water .However; we did not found the standard of our country with regard to microbial load of water with relative to these standards. These selected location of Gondar town water sources are poorly qualified .This shows that potential health hazards diseases can be outbreak in the society .Furthermore, both the pollution indicators (coli forms) and pathogens are in the unfavorable condition that indicates more samples shall be undergoing to further analyze the samples in order to confirm the results.

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RECOMMENDATION

This indicates that the above selected locations of Gondar town water sources, i.e., Keha River and Shinta River streams are especially highly contaminated than that of the Marki tape water, So that communities /small holder people that settle around those sources can be prone to various water borne diseases. Meaning that we should advice for those communities to use Chlorine and municipal wastes should be disposed properly. They have to use tape water other than those river streams. It may also necessary further sampling and that environmental health officers may wish to undergo a further detection of selected sources of water to determine whether faces, nasal fluids during bathing or others are the origin ones.

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