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ASSESSMENTS OF THE TOTAL HETEROTROPHIC BACTERIAL POPULATION DENSITY FROM WABE RIVER, SOUTH-CENTRAL ETHIOPIA

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Abstract

Water pollution is a major global problem which requires ongoing evaluation and revision of water resource policy at all levels (international down to individual aquifers). It has been suggested that water pollution is the leading worldwide cause of deaths and diseases, and that it accounts for the deaths of more than 14,000 people daily. Health education to disseminate the knowledge and practice of hygiene is equally important to prevent the water borne disease. In this study, total heterotrophic bacteriological analysis are carried out using standard plate count technique on Wabe river at various stations viz., Anige, Kokir, Chancho and Wabe bridge of Guraghe zone, Ethiopia. Wabe river is selected because this runs about nearly 150 km and also is utilized by many villages for drinking, house hold purpose, irrigation and bathing purposes. The results obtained are compared with reports of standards for river water. Total heterotrophic bacterial counts varied from too low to count to 79±2 x 10^2 all the sampled sites. Among the total heterotrophic bacterial isolates, Bacillus sp., Staphylococcus sp., Shigella sp., and Escherichia coli are reported. The bacterial counts from all the stations not exceeding acceptable limits are indicative of pollution free from domestic wastes. The results are according to the standards of the World Health Organization (WHO) for River water Quality.

Keywords: Wabe River, Acceptable limit, Heterotrophic bacterial count, Hygiene, Pollution.

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INTRODUCTION

Water is very precious value to sustain humanity and life. Good quality of water is one of the most human necessities (Howard and Bartram, 2003). Rivers carry water and nutrients to areas all around the earth. They play a very important part in the water cycle, acting as drainage channels for surface water. It provides excellent habitat and food for many of the earth's organisms. It is unfortunate in many developing countries water-related diseases are a problem's result from contaminated river water, hence pose a high risk to human health, if it contains pathogens (WHO, 2006).

Water pollution is the contamination of water bodies (e.g. lakes, rivers,oceans, aquifers and groundwater). Water pollution occurs when pollutants like animal waste, open field defecations, waste from factories and residential areas are discharged directly or indirectly into water bodies without adequate treatment to remove harmful compounds. Water pollution affects plants and organisms living in these bodies of water. Water pollution occurs when unwanted materials enter in to water, changes the quality of water (Alrumman SA et al 2016) and harmful to environment and human health (Briggs D 2003).

In developing countries 70% of the water is seriously polluted and 75% of illness and 80% of the child mortality is attributed to water pollution (Raja et al., 2007). It is a well established fact that domestic-sewage and industrial effluent discharged into natural water result in deterioration of water quality and cultural eutrophication (Prabhu et al., 2012). The other important sources of water pollution include mass bathing, rural and domestic sewage, agricultural run-off and solid waste disposal (Prabhu et al., 2012).

Hence, this study would be helpful to understand the status of river Wabe, Ethiopia through the heterotrophic bacterial analysis and their relationship with pH and temperature. And also there is a need of awareness about the water quality. It will reduce the number of deaths in the selected area by avoiding water borne diseases at a right time (Rheinheimer, G., 1980.)

Heterotrophic bacteria are uniquely involved in the mineralization and metabolization of the organic matter, nutrients, trace metals and even the anthropogenic substances. The role of microbial communities in the process of decomposition, nutrient regeneration, cycling and production of particulate matter and relationship with other organisms in marine environment is well known (Rheinheimer, G., 1980.)

MATERIALS & METHODS

2.1 Study Area

The present study was conducted in the four selected stations of Wabe River that are located in Gurage Zone. The four stations are well known by the local community and the distance

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between the stations are determined. The River travel and joined with Omo River. The four stations are shown in thetable1andFig1withtheirgeographicallocations.

2.2 Sterilization

All the glass wares were soaked in the cleaning solution (100g of potassium dichromate was added to 1L of distilled water followed by 500 ml of conc. H_2SO_4) for about 12 hours and washed in tap water. Finally, they were washed in distilled water, dried and used. All the chemicals and medium used in this work were of Uni-chem limited and glass distilled water was used throughout the study. The glass wares were sterilized at 180 °C for half an hour in hot air oven and media were autoclaved at 15 pounds per square inch pressure for 15 min.

Table 1.List of sample collection Stations from the river Wabe

S.No.	Station	Name of the Station	Latitude	Longitude
1	I	Anige	8° 19' 22.8" N	38° 21' 16.56 E
2	II	Kokir	8° 7' 17.4" N	38° 4' 34.32 E
3	III	Chancho	9° 18' 28.44" N	38° 45'18.36" E
4	IV	Wabe Bridge	8° 14' 58.3512" N	37° 45' 40.2408" E

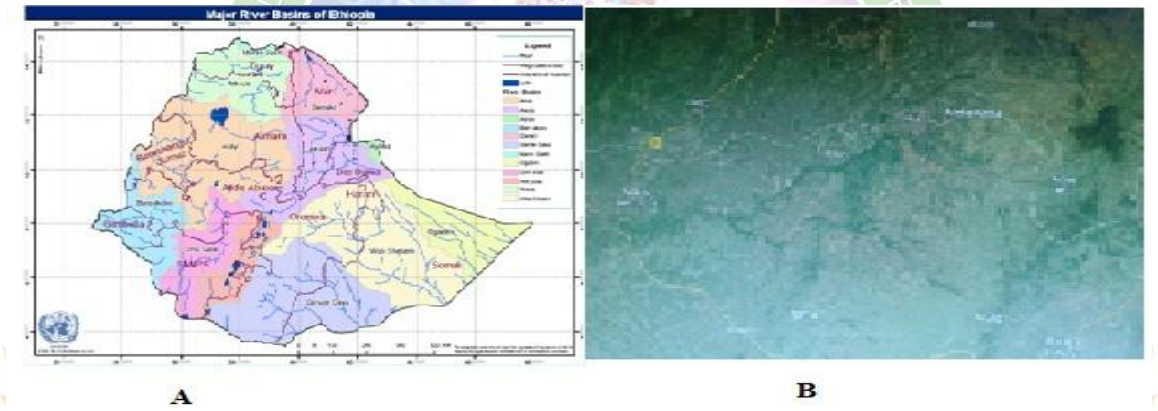

Fig.1: shows major river basins of Ethiopia (A) and map of sample collection stations (B)

2.3 Sample collection

Water samples for microbiological examination, were collected in non reactive borosilicate glass bottles of 500ml capacity each that had been cleansed and rinsed, given a final rinse with distilled water and sterilized. Grab sampling procedure was adopted a recommended by Standard Method for microbiological analysis (Antony and Renuga, 2014). Samples were taken from the river by holding the bottle near its base in the hand and plunging it, neck downward, below the surface (Fig 3). Then turning the bottle until neck points slightly upward and mouth is directed toward the current. The sampling bottle was not filled up to the brim and 20 mm to 30 mm space was left for effective shaking of the bottle (APHA, 1998). Microbiological analysis of water samples was started immediately after collection to avoid unpredictable changes in the microbial population (Gaudy, 1998).

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Fig. 1: Three of the four sample collection areas, Kokir (a), Chancho (b) and Wabe bridge (c)

2.4 Total Heterotrophic Bacterial Population

To determine the total heterotrophic bacterial population in the river water, the samples were collected in sterile plastic bags and immediately transported to the laboratory. Bacteria are to be enumerated as colony forming units (CFU) employing the standard spread plate technique following methods described in APHA (2005). Plate count agar medium are to be used for enumeration purposes. The agar medium is to be autoclaved prior to use. The collected water samples are to be serially diluted upto 10^{-6} using sterile distilled water before inoculation. Plating has to be done employing spread plate technique with the help of an L-rod, and the inoculated plates are to be incubated at 37^0 C in an incubator. After 24-48 hours of incubation, colony counts are to be made using a colony counter. total heterotrophic bacterial plate count are to be expressed as number of bacteria per 100 ml. Based on its colony characterization, the bacterial isolates are separated and inoculated in sterile peptone water and nutrient agar slants and incubated at 37ºC for 24 – 48 hours.

2.5 Isolation and identification bacteria

The predominant bacterial colonies were isolated from plate count agar; these isolates were purified by streaking twice on nutrient agar and stored in a refrigerator, then further the identification done through biochemical tests (Barrow and Gelthan, 1993) and cultural characterization was done according to Cappuccino and Sherman, (1999)

2.6 Simple staining

Bacterial smear was prepared and heat fixed prior to staining. Then the smear was flooded with crystal violet staining solution. The smear was washed with distilled water to remove excess stain. Then the slide was air dried and examined under microscope.

2.7 Gram staining

Bacterial smear was prepared and heat fixed. Then the slide was flooded with crystal violet staining reagent for 1 min. Smear was washed in a gentle and direct stream of water for 2

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seconds. The slide was flooded with Gram's iodine mordant for 1 min. Smear was washed in a gentle and direct stream of water for 2 seconds. Then the smear was blotted and dried with absorbent paper. The smear was immersed in 95% ethanol for 30 seconds with gentle agitation. Then the smear was blotted and dried with absorbent paper. The smear was immersed for 2 min with counter stain. The smear was washed in a gentle and in-direct stream of water until no color appears in the wash water. The smear was blotted and dried with the absorbent paper and finally the smear was examined with high power oil immersion objective.

2.8 Motility determination-Hanging drop experiment

A cover glass and a cavity slide were cleaned with ethanol alcohol. A thin film of vaseline was placed around the rim of the cover glass. A loopful of overnight incubated culture was placed in the center of cover glass. The cavity slide was held inverted, with cavity slide facing down. The cover glass was then pressed over the cavity slide so that the vaseline adhered to the slide. The slide was turned carefully down to make the drop hanging in the cavity. Then the edge of the drop was observed under microscope.

2.9 Biochemical Characterization (Cappuccino and Sherman, 1999)

Urease test

The bacterial cultures were inoculated into the Christensen's urea agar slants and uninoculated slants may serve as color control. It was then incubated at 30^{o}C and 37^{o}C for 48hours. Formation of pink color is indicative of positive result whereas no change in color of the medium indicates negative result.

Test for Citrate Utilization

The cultures were inoculated in a tube containing Simmon's Citrate agar and incubated at 30^{o}C for 48 hours. Formation of blue colour is indicative of positive result whereas no change in colour of medium indicates negative result.

TSI agar test

The cultures have been inoculated in a tube of triple sugar Iron agar and incubated for 48hrs at 37^{o}C. Formation of black precipitate is indicative of H2S positive result, yellow color is indicative of acid production and pink colour is indicative of alkaline.

Statistical Analysis

The data obtained was subjected to descriptive statistics using mean and standard error of mean. The result output was known by an overview through diagrams with significance. The diagrams are constructed using Microsoft office excels.

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RESULTS

Enumeration of Aerobic Heterotrophic Bacteria in the Wabe River

The total heterotrophic bacterial count from stations I, II and III of Wabe river during the month of May 2015, the number of colonies were in the order of 450 ±10, 3900 ±20 and 7900 ± 20 CFU per mL respectively. station I yielded less number of colonies (Table 2). The pH of the water samples ranged from 7.2 to 7.3 while the temperature of water samples ranged from 20^{o}C – 22^{o}C for all the water samples (Table 2).

Isolation of Bacteria from Wabe River

The aerobic heterotrophic bacterial isolates from the samples, when subjected to the purification, yielded bacterial colonies with different size and colour such as greyish white, white, bluish green, creamy. Based on the colony size and colour, four different bacterial isolates could be recognized and were given the code name based on the name Wabe River (Table 3). The percentage of bacterial isolates in the station 4 is summarized in (Table 5).

Identification of bacterial isolates

The colony, cultural and biochemical characteristics of bacterial isolates are analyzed for all the four different isolates. Microscopic characters like cell shape, cell size, motility, gram reaction and capsular staining were observed in all the isolates. Three of the bacterial isolates were rod shaped and other one was cocci, two of the bacterial isolates were motile and rest was nonmotile. With regard to Gram staining, two isolates stained positive to Gram's reaction and the rest stained negative (Table 5).

Table 2: Enumeration of total heterotrophic bacterial population at four different stations

S. No	Station	pH	Temperature	No. of Bacterial population*
1	I	7.2	20	TLTC**
2	II	7.2	21	45 ±1 x 10^1
3	III	7.3	20	39±2 x 10^2
4	IV	7.3	22	79±2 x 10^2

*- Mean of duplicates
** - too low to count

Table 3.Bacterial isolates from four different stations of Wabe River

S. No	Station	Bacterial isolates
1	I	WB1
2	II	WB1, WB2
3	III	WB1, WB3, WB4
4	IV	WB1, WB2, WB3, WB4

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Table 4: Percentage of bacterial isolates of Wabe River at station IV

S. No	Bacterial isolates	CFU/ml	% Bacterial isolates
1	WB1	12	15
2	WB2	5	6
3	WB3	14	17
4	WB4	50	62*

*** - Significant value found**

Table 5: Colony, cultural and biochemical characteristics of bacterial isolates

Bacterial isolates	Colony Characteristics	Motility	Simple Staining	Gram Staining	C	U	TSI	Suspected bacterial name
WB1	Spreading Colony, White colour	NM	R	G+	-	±	A/K	*Bacillus sp.*
WB2	Round, Convex, Yellow colour	NM	C	G+	-	-	A/K	*Staphylococcus sp.*
WB3	Circular, Convex, smooth and	NM	R	G-	-	-	A/K	*Shigella sp.*
WB4	Opaque, Sticky and grayish white	M	R	G-	-	-	A/A G+	*Escherichia coli*

DISCUSSION

The present study indicates the condition of the Wabe river water which will have serious effects in station III and IV. PH was found to be more or less similar for each sample, where values were ranging from 7.2- 7.3(Fig.3). The admissible pH should be in the range of 6.5 to 7.5 for drinking and domestic purposes (Ring, 2003). The station III and station IV river water was found to have the highest pH value (7.3) and the lowest (7.2) was for station I and station II as observed in table II. The fluctuations in optimum pH ranges may lead to an increase or decrease in the toxicity of poisons in water bodies (Ali, 1991).

The pH obtained in the river waters was within the ranges suitable for aquatic life (Ell-Amin etal.,2012). Based on these guidelines, the pH of the river water would not adversely affect its use for domestic and recreational purposes. The well buffered nature of the station I and II can be attributed to the fact that, normally, running waters are influenced by the nature of deposits over which they flow. In general high levels of free CO2 might be the reason for low pH values obtained in the river water samples, which may consequently affect the bacterial counts (Hammad and Dirar, 1982).

The result of the total heterotrophic bacterial viable count was shown in Table 2. Total heterotrophic viable bacterial count was ranged from very few to 79±2 ×102 bacterial cells per

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ml. There are no such big differences were noticed between the different samples of river Wabe. However, the highest count of 79±2 ×102 was recorded in Wabe bridge samples while the lowest count was recorded in Kokir figure 2. One of the reasons may be due to less human intervention (Binnie et al., 2002; Griffith et al., 2003).

Data presented in this study, Table 2 showed that the total heterotrophic count of downstream water sample was increased when compared with upstream water. This is reflecting that the amount of contamination increased while passing the towns. Because of the most frequent of usage for cleaning the clothes, vehicles and also taking bath, which facilitates microorganisms to introduce into water from the surrounding.

The high total heterotrophic count is indicative of the presence of high organic and dissolved salts in the water. The primary sources of these bacteria in water are animal and human wastes as majority of the community are using open field defecation and keep their animal in field (personal communication) these sources of bacterial contamination include surface runoff, pasture, and other land areas where animal wastes are deposited. Additional sources include seepage or discharge from septic tanks, sewage treatment facilities and natural soil /plant bacteria (EPA, 2002).

Environmental Protection Agency (EPA, 2003) establishes heterotrophic plate count as a primary standard, which are based on health considerations. The British Standard Institute (BSI, 1993) specified that counts greater than 104are considered unsatisfactory for Enterobacteriacea. The reason for high number of bacterial colonies might be due to inadequate maintenance of water reservoirs and the mixing of sewage into the reservoirs or directly into the rivers. In the Station IV the high total bacterial count may be indicative of the presence of high organic compounds in the water. Because this place is very close to the place of Wolkite town. In station II and III the primary sources of these bacteria in water are animal and human wastes. These sources of bacterial contamination include surface runoff, pasture, and other land areas where animal wastes are deposited. Additional sources include seepage or discharge from septic tanks, sewage treatment facilities and natural soil /plant bacteria (EPA, 2003).

From table 3, it was noted that the water samples had four different types of bacteria. Distinct colonies could be identified using biochemical analysis given in table 5. The results of the bacteriological analysis of water of Wabe River showed that the two areas namely Chancho and Wabe bridge water are contaminated with human waste.

The bacterial species identified were members of the Enterobacteriaceae family (Table 4). The most predominant bacterial genera found in Wabe river water were Escherichia coli (62%),

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Shigella sp. (17%), Bacillus sp. (15%) and Staphylococcus sp. (6%).Enteric pathogens cannot normally multiply in water hence water is not its mode of transmission to humans (WHO, 1996). However, the presence of enterobacteriae would be enough infective doses in people whose local or general natural defense mechanisms are impaired to significantly low. The people likely to be at risk would be the very old or the very young as well as patients undergoing immunosuppressive therapy. Other immuno-compromised individuals suffering from AIDS would also be at risk. Also, water polluted by bacteria when permitted to contaminate food would lead to the multiplication of the pathogens to very large doses.

According to a study by Baxter-Potter and Gilliland (1988) on straight river water shed when precipitation and stream flows are high, the influence of continuous sources for pollution. It is a common practice for people living along the river catchment to discharge their domestic and agricultural wastes as well as human excreta/wastes into rivers. In addition to using the river as a source of drinking water people use the source for bathing, washing of clothes and for recreational purposes such as swimming. Wild and domestic animals seeking drinking water can also contaminate the water through direct defecation and urination (Karikari and Ansa-Asare, 2006).

Several developing and developed countries have embarked on programmes to endeavour to reduce contamination of rural water sources by waterborne diseases (Ganozaet al., 2005; Roe and Cardinale, 2005; Hörman, 2005; Fenwick, 2006). This work showed the clear status of river Wabe and helpful for the prevention of water borne diseases around Wabe river zone.

CONCLUSIONS AND RECOMMENDATIONS

The present study indicates that the heterotrophic bacterial populations of the river Wabe which will have some amount of microbial load. However, the presence of enterobacteriae would be enough infective doses in people whose defense mechanisms are impaired to significantly low. The people likely to be at risk would be the very old or the very young as well as patients undergoing immunosuppressive therapy. Other immuno-compromised individuals suffering from AIDS would also be at risk. It is evident that water borne diseases are due to improper disposal of refuse, contamination of water by sewage, surface runoff, therefore programmes must be organized to educate the general populace on the proper disposal of refuse, treatment of sewage and the need to purify our water to make it fit for drinking because the associable organisms are of public health significance being implicated in one form of infection or the other. In areas lacking in tap water as in rural dwelling, educative programmes must be organized by researchers and government agencies to enlighten the villagers on the proper use of surface water.

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Typical methods normally advised for inactivating microbes for rural water treatment at such levels are disinfection treatment (use of hypochlorite) and boiling (use of heat) that have some efficiency on microbial content reduction.

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