Bacteriological Characteristics of Spring Water in Ambo Town, West Shoa Zone, Oromia Region, Ethiopia

P. Sekhar

Environmental science division, Biology department, Bule Hora University, Bule Hora, Ethiopia

Abstract— *Present study was carried out to assess the quality of spring waters in terms of microbiological and chemical characters from Ambo. The results of the study revealed that chemical parameters such as pH (6.36-7.94),TDS(407-1041mg/L), DO(1.5-5.85mg/L),TS (1170-495mg/L), total alkalinity (313-1277mg/L), total hardness (38-1274 mg/L),COD (70.5-9mg/L)in the "Hora" water were higher than the maximum permissible levels of WHO standards for drinking waters. Total aerobic mesophilic bacterial counts tested were found to be as0.977×10^4 cfu/ml for SFWS, 2.35 $\times10^4$cuf/ml for CDSTRM,1.14×10^4cfu/ml for HB,0.553×10^4 cfu/ml for HD and$2.72\times10^4$for Huluka streams samples. The "Hora" water contained different coli forms when tested by most probable number (MPN) method found to be in the order of 0.66 × 10^2 cfu/ml for SFWS, 39 x 10^2 cfu/ml for CDSTRM, 0×10^2 for HB, 0×10^2forHDand28×10^2cfu/ml for Huluka stream. The water samples from the different "Hora" water sources showed significant variations with respect to bacteriological and chemical characteristics during study period. Statistical analysis showed significant difference ($p < 0.05$) in the distribution of total coli form, and aerobic mesophilic heterotrophic bacteria at various sampling locations. The study concluded poor water quality in terms of bacteriological and chemical characteristics of "Hora"water sources as all the parameters were well above WHO prescribed standards.*

Keywords— Ambo town, bacteriological & chemical characteristics, Hora spring water, Huluka river, water quality, WHO standards.

I. INTRODUCTION

Water is very essential to life and it is undoubtedly the most precious natural resource that exists on our planet. Several forces have been continued to seriously affect the quality of water resources. Many of these are result of human activities and natural processes and include ecosystem and landscape changes, sedimentation, pollution, over- abstraction and climate change. Besides the following natural and human induced factors also affects the quality of water including geology, hydrology, natural hazards, sedimentation/ erosion, agricultural activities, industrial, mining, fishing, sewage discharging/ disposal, deforestation, and other commercial activities. These activities aggravate the pollution of water body and greatly influence the quality of water [1]. Physicochemical and biological water quality indicators will be affected by various ways [2]. The health of aquatic ecosystem is depended on the physico- chemical and biological characteristics [3].The presence of certain microorganisms in water is used as an indicator of possible contamination and an index of water quality [4]. Natural hot and mineral springs water can be defined as water that, while circulating underground, undergoes changes in its composition through heat, pressure and time caused by interaction with the surrounding rock [5].Natural mineral water is characterized by its chemical and microbiological compositions which distinguishes it from drinking water and may not be treated in any way that alters these properties [6]. Besides, the temperature and other essential characteristics of natural mineral water must remain stable over time. Constituents may be present in the natural state in certain natural mineral waters because of their hydro geological origin may present risk to public health above a certain concentration. It is therefore deemed necessary to establish concentration limits for these constituents in natural mineral water [6].

Horas are used as a source of mineral supplement for livestock (cattle, sheep and goats). Water from the "Hora" is perceived to enhance fat, fertility and resistance to diseases [7].In every country that has been investigated natural hot springs mineral water have historically been attributed with therapeutically benefits due to their individual mineral compositions. Ambo "Hora" water (mineral water) is one of water sources which have been used by the city of Ambo, Ambo district and others for drinking purpose (both for humans and animals). It has been considered as having medicinal, bathing recreational, cleaning, cultural values, economic importance (income generation), and other related purpose. People believe that the Ambo "Hora" water has the medicinal value to protect the animals from different diseases, very important for their health and growth. The Ambo "Hora" water has been used throughout the history of human population, but (still no one knows the

composition of the water) there was no study carried to determine the bacteriological and physico-chemical characteristics of this "Hora" water. So, study was aimed at assessing the bacteriological and chemical characteristics of Ambo Hora water. This study will also help concerned authorities to understand the status of these spring waters and take appropriate measures to protect these spring waters from being polluted from anthropogenic activities as these are important in terms of its tourism value.

II. METHODOLOGY

2.1 Description of the Study Area

This study was carried out in Ambo town; West Showa located 115km west of Addis Ababa, the capital city of Ethiopia. The town has a latitude and longitude of 8°59′N 37°51′E and an elevation of 2101 meters above sea level. Ambo town is known for its mineral water from natural springs, which is bottled and the most popular brand in Ethiopia.

2.2 Sampling Methods

Samples were collected from five different locations of natural springs which include sites for human bathing (HB), cattle drinking stream (CDSTRM), human drinking (HD), spring filewuha (SFW) and Huluka stream (HUSTR).

2.3 Sampling Size

Total 30 samples were collected from all the five different "Hora"water sources taking two representative samples from each site.

2.4 Sampling Collection procedure

The water samples were collected by using sterilized bottles in order to avoid contamination of the bottles following standard protocols of sample collection. All samples were collected from different locations of upstream and downstream of confluences or point sources with at least 1m intervals. After collection of samples they were brought to microbiology and chemistry laboratories of biology department, Ambo University.

2.5 Chemical Analysis of the Ambo "Hora" Water

Measurement of selected chemical parameters were carried out according standard procedures [8].

2.6 Bacteriological Analysis of the Ambo "Hora" Water

Bacteriological analysis of the water samples was carried out using multiple tube fermentation method for enumeration of total coliform count. Decimal dilution series up to $10^{-7}$from the original sample were prepared and inoculated into five lactose broth tubes. Durham's tubes were introduced in all tubes and sterilized them at a time. 1ml, 0.1mland 0.01ml water samples were added to each of 5 lactose broth tubes and labeled correspondingly. Lactose broth tubes were incubated at37^{o}C and examined the gas formation in Durham tubes at 24hour and 48-hour intervals. Numbers of positive tubes in each dilution were counted and a loop full of cultured from the lactose broth tube from the highest dilution that still showed positive test was taken and streaked it on EMB and ENDO agar plates. The Petri dishes were incubated upside down at the appropriate temperatures 37^{o}C for total coli forms for 24 hours. After incubation, typical colonies were identified and observed for the typical coli form colonies showed a greenish metallic sheen [9].

2.6.1 Plate Count Method

Surface plate count method was used to quantify the bacteria in the samples. Serial dilutions were prepared with sterilized water. Seven tenfold serial dilutions of water samples were prepared and 1ml of each of the dilution was evenly spread on nutrient agar (PCA) by using a sterilized bacterial spreader under laminar flow hood. This was repeated for each sample. The inoculated plates were then incubated at 28^{o}C for three days. Colonies were counted visually after three days of incubation and then stored in the refrigerator for further analysis.

Plates with less than 300 colonies and more than 30 colonies were utilized for data analysis. Results were recorded in units of CFU/1 ml of initial sample water. The concentration of bacteria in the original sample was determined as follows:

CFU = total colonies counted x dilution factor / volume plated

2.6.2 Motility Test [10].

A straight needle was slightly touched to a young colony of 24 hrs and stabbed to a depth of only 1/3 to 1/2 inch slant culture growing on agar medium. The slants were incubated at 37°C and examined daily for up to 7 days. A positive result indicates diffuse, hazy growths that spread throughout the medium rendering it slightly opaque whereas negative result indicates growth that is confined to the stab-line.

2.6.3 Biochemical characterization

2.6.3.1 Oxidase test [9].

Oxidase test was conducted according to the following procedure. A fresh culture (18 to 24 hours) of bacteria was grown in 4.5mL of nutrient broth and kept for overnight. A 0.2mL of 1% α-naphthol, and 0.3mL of 1% p-amino dimethylaniline oxalate (Gaby and Hadley reagents) to the overnight broth culture and then mixed vigorously. The culture was observed for color changes within 10s to 30s. It was interpreted an oxidase positive when the color changed to dark purple within 5 to 10 seconds and oxidase negative if the color did not change or it was taken longer than 2 minutes.

2.6.3.2 KliglirsIron Agar Test

A 57.52g of Kliglirs iron agar medium was take in one liter of purified water and heated with frequent agitation and boiled for one minute to completely dissolve the medium. And then distributed into test tubes and autoclaved for 15 minutes at 121^{o}C. After autoclaving, medium was allowed to solidify in a slanted position. Culture response in Kligler Iron Agar media incubated aerobically at 35°C was examined for 24 hours. Results were interpreted as: an alkaline slant-acid butt (red/yellow) indicates fermentation of dextrose only. An acid slant-acid butt (yellow/yellow) indicates fermentation of dextrose and lactose. An alkaline slant-alkaline butt (red/red) indicates dextrose and lactose did not ferment (non-fermented). Cracks, splits, or bubbles in the medium indicates gas production [10].

2.6.3.3 Catalase test

A 24-hour old culture was used to make a homogenous suspension on the slide. A one drop of hydrogen peroxide (3%) was added to the suspension of pure culture isolates and the observed gas formation (effervescence) [11].

2.6.3.4 Simmon citrate agar Test

Simmon citrate agar was used to test citrate utilization. Using a sterile straight wire loop, a saline suspension of the test organism was first streaked on the slant and then stabbed (to create anaerobic condition). All the slants were incubated for 4 days at 37^{o}C in an aerobic atmosphere to detect the production of a positive reaction as indicated by growth with an intense blue cooler in the slant [9].

2.6.3.5 Urea agar test

Urea agar test was used for detection of urease production by proteus species and other members of Enterobacteriaceae. A 24 gm of urea powder was added in distilled water and brought the volume to 950 ml and mixed thoroughly. Slants were prepared with urea broth and slants were inoculated with isolates and incubated at 37^{0}C for 24 hours. The development of pink color colonies indicates a positive result [12].

2.7 Data Analysis

Data analysis was carried out using SPSS 21 version. Parameters like descriptive statistics and 2-tailed correlation analysis was done to know any significant relation exists among various biological and chemical parameters.

III. RESULT & DISCUSSION

Results of Physico-chemical parameters of water samples from “Hora” waters and Huluka stream were analyzed and results were represented in the following table.

TABLE 1
PHYSICO-CHEMICAL PARAMETERS OF HORA WATER SOURCES

S.No.	Parameter	Sample sites				
		SFWS*	CDSTRM*	HB*	HD*	HULSTRM*
1	Temp.	40^0C±0.50	35^0±0.50	40.83^0C±O.28.	39.76^oC±1.07	27.33^0C±1.00
2	P^H	6.37±0.00	6.68±0.01	6.34±0.01	6.38±0.00	7.93±0.01
3	Electrical conductivity (µS/cm)	1791 ±3.0	1933.33±2.08	1913±5.13	1910±8.0	403.66±2.08
4	TDS (ppm)	972.5±2.50	1039±6.55	812.5±2.51	986.53±2.62	402±2.64
5	DO (ppm)	1.6±0.10	1.2±0.04	1.6±0.95	1.8± 0.8	5.8±0.09
6	TS (ppm)	977.66±2.51	1167.00±2.00	926.83±2.36	1045.00±2.00	495.33±3.01
7	TA (ppm)	1260.66±3.05	1253.33±7.63	1256.60±7.63	1261±11.53	312±10.58
8	TH (ppm)	1269.50±10.75	1255±5.00	1277±2.00	1270±17.32	35.33±2.30
9	COD (ppm)	69.5±0.76	80.33±1.04	84.33±0.76	72.93±0.51	92±0.70

SFWS - Spring file wuha sources, HB-human drinking, HULSTRM - Huluka stream, CDSTMR - cattle drinking stream, HD–human drinking

The table 1 shows mean values of the temperature between 27.33^{o}C to 40.83^{o}C. The lowest value (27.33^{o}C) was found in sample site Huluka stream and the highest temperature value (40 .83^{O}C) at sample site of human bathing. During the present investigation, there was no great difference among the temperatures of Hora waters and all of them were above WHO standards of 15^{o}C. The temperature range during the present study was 27.33 to 40.83^{o}C. This range was also considered as above the maximum limit of temperature for drinking water specified by WHO which is 15^{o}C. During the present investigation, there was no great difference among the temperatures of Hora waters and all of them were above WHO standards of 15^{o}C. but temperatures are higher than the study conducted in Bahir Dar town (15–20 °C) spring waters [13]. Higher water temperatures promote the growth of microorganisms in the water, which may increase the taste, odor, turbidity and cause corrosion problems and also it decreases the solubility of gas in the water [14]. The temperature range during the present study was 27.33 to 40.83^{o}C. This range was also considered as above the maximum limit of temperature for drinking water specified by WHO which is 15^{o}C.

Mean pH values of water samples obtained from five sources were shown in table 1 and they are in the range of 7.93 to 6.34. Human bathing recorded the lowest pH value of 6.34 followed by spring File wuha 6.37. Other sources also shown more or less same range of pH as evident from the table no 1. But Huluka stream has registered highest pH value of 7.93. The Huluka stream, CDSTRM and other samples recorded pH within the permissible value of 6.5 to 8.5 for drinking water [15]. The high value of pH may be the result of waste discharge, microbial decomposition of organic matter in the water body [16]. The lower pH values obtained in the study from the SFWS, HB and HD samples could be attributed to mineral salts dissolved in the water. The low pH values observed in most wells and springs could be associated with carbon dioxide saturation in the ground water [17]. The relatively higher pH of the streams could be attributable to the large surface area of the stream which exposes it to sunlight thus increasing the temperature and photosynthetic activities which in turn increases alkalinity of the water [18].

Data in Table 1 shows that the mean of the conductivity of water samples from the five water sources ranged from 403.66 to1933.33µs/cm. The least conductivity was observed in Huluka stream (403.66). Conductivity is a measure of the ability of aqueous solution to carry an electric current that depends on the presence and total concentration of ions their mobility and valance and on the temperature [19]. There is a very good positive correlation exists among conductivity, total solids and total dissolved solids which indicates higher chemical pollutant character of the waters in the study sites.

The mean TDS content values ranged from 402 to 1039 mg/l as depicted in table 1. Huluka stream had the lowest TDS value of 402 mg/L followed by HD (986.53 mg/l), SFW(972.5mg/l), HB (812.5 mg/l) and the CDSTRM (1039 mg/l). The highest level was observed in CDSTRM (1039 mg/l). High values of TDS indicate that the "Hora "water is unfit for human consumption. In the present study, it is found that almost all samples have TDS values more than the prescribed standards

and is unfit for drinking. The Total Dissolved Solids (TDS) represents the amount of inorganic substances present in water. It shows the general nature of water quality or salinity [19]. High values of TDS indicate that the "Hora "water is unfit for human consumption. In the present study, it is found that almost all samples have TDS values more than the prescribed standards and is unfit for drinking.

The data in table 1 indicated that the minimum DO values of 1.2 mg/L, 1.6mg/L,5.8mg/L, 1.6mg/L. 1.8mg/L were observed at sites CDSTRM, SFWS, Huluka STRM, HB and HD in respectively. The lowest values observed may be as a result of the increased mineral solution that place high demand on the DO. The maximum DO values 5.8mg/L and 1.8mg/L were seen at sites Huluka STRM and HD. The overall result shows that the DO of all "Hora" water samples and Huluka water stream were below the water quality standards. The low values of dissolve oxygen observed in the study sites may be as a result of the increased mineral solution that place high demand on the DO. The maximum DO values (5.8mg/L and 1.8mg/L) were seen at sites Huluka STRM and HD. With the progress in summer, dissolved oxygen decreases due to increase in temperature and also due to increased microbial activity [20]. The overall result shows that the DO of all "Hora" water samples and Huluka water stream were below the water quality standard so this water was not suitable for drinking. The low values observed in the present study may be as a result of the increased run off agricultural wastes and industrial effluents discharged into the drains that place high demand on the DO.

Table 1 indicated that high TS of 1167.00ppm was observed in CDSTRM water samples while relatively lowest level (495.33ppm) was registered in Huluka stream water. Significant variations were noted among the five different water sample sources ($P < 0.05$) and variation within sample was significant. The concentration of TS was well above the WHO standards of 500ppm.

All the samples from Hora water have shown high levels of total hardness exceeding WHO standard of 500mg/L but Huluka stream hardness was found to be well below the standard limit as shown in Table 1. The least level was found in Huluka stream (35.33 mg/l), followed by cattle drinking "Hora" water (1255mg/l), spring file wuha (1269.5mg/l), human bathing "Hora" water (1277 mg/l) and human drinking "Hora" water (1770 mg/l) in their increasing order. Hard water is the water that contains high levels of dissolved calcium, magnesium, and other minerals salts such as iron and the greater the amount of dissolved minerals in the water, the harder it is [21]. All the samples from Hora water have shown high levels of total hardness exceeding WHO standard of 500mg/L but Huluka stream hardness was found to be well below the standard limit as shown in table 2. The variations in total hardness may be due to decomposition and mineralization of organic materials [21].

COD is the amount of oxygen that is required to oxidize organic compounds in the water and indicated in table 1. The COD value of the Ambo "Hora" water sources as shown in table 1 were in between 92-69.33mg/L which are above the standard limit of 10mg/L. COD is the amount of oxygen that is required to oxidize organic compounds in the water. The COD value of the Ambo "Hora" water sources as shown in table 2 were in between 92-69.33mg/L which are above the standard limit of 10mg/L and these high values can be attributed to anthropogenic pollution related activities in the study sites. Based on the high values of COD well above the standards, this Ambo "Hora" water is not recommendable for drinking purpose [22].

TABLE 2
THE MEAN COUNTS OF TOTAL HETEROTROPHIC BACTERIA, AND COLI FORM BACTERIA FROMDIFFERENT HORA WATER SOURCES

Sample location /site code	Total heterotrophic bacteria count (CFU/ml)	TC (MPN/100ml)
SFWS	0.977×10^4	0.66×10^2
CDSTRM	2.35×10^4	$39x10^2$
HB	1.14×10^4	0×10^2
HD	0.553×10^4	0×10^2
HULUSTRM	2.72×10^4	$28x10^2$

SFWS - Spring file wuha sources HB - human drinking HULSTRM - Huluka stream CDSTMR - cattle drinking stream HD – human drinking

The total heterotrophic counts as showed in table 2 ranged from 0.977×10^4 to 2.72×10^4 cfu/ml. The Huluka stream samples recorded the highest count of 2.72×10^4 cfu while HD had the least bacterial count of $0.553\times10^{4,}$ The CDSTRM, HB and SFW samples had bacterial count of $2.35\times10^{4,}$ 1.14×10^4 and 0.977×10^4 cfu/ml respectively. Out of all samples of "Hora" water, CDSTRM water and Huluka stream water samples contained higher total heterotrophic bacterial counts than the others. Heterotrophic bacterial count of some samples in the present study was high exceeding standard permissible limit for drinking water. The most probable number (MPN/100ml) values were recorded (Table 2) as 0.66×10^2 MPN/100ml in SFWS, 39 x 10^2 MPN/100ml in CDSTRM and 28 x 10^2 MPN/100ml in Huluka stream water samples. Except HD and HB "Hora" water all samples such as CDSTRM, Huluka stream, and SFW water samples were not within the standards of WHO for coli forms. From all samples of "Hora" water, CDSTRM water and Huluka stream water samples contained higher total heterotrophic bacterial counts than the others. Heterotrophic bacterial count of some samples in the present study was high exceeding standard permissible limit for drinking water. This finding was in good agreement with similar studies by other workers who reported that the sources of heterotrophic bacteria in water are human and animal wastes, run off pasture, natural soil or plant bacteria, sewage, and other unsanitary practices [23]. Runoffs, sewage, agricultural waste are usually high inorganic matter and nutrients and could cause increase in the bacteriological load of the water bodies thereby resulting in high heterotrophic bacteria counts [24]. The higher number of bacterial count recorded in Huluka stream water samples could probably be as a result of the increased surface area of the stream which exposes the water to contaminants as well as human activities like swimming, washing, dipping of dirty legs or hands and inside the stream while fetching water [25].

Except HD and HB "Hora" water all samples such as CDSTRM, Huluka stream, and SFW water samples were not within the standards of WHO for coli forms. The high coli forms obtained may be an indication that the water samples were contaminated by fecal matter [26]. The presence of bacteria was not only making the water unsuitable for human consumption, but also poses serious health concerns [15]. Similar studies reported the presence of these bacteria in drinking water sources [27] and attributed it to indiscriminate human and animal defecation and general poor sanitation.

TABLE 3
DESCRIPTIVE STATISTICS

	Mean	Std. Deviation	N
TEMP	36.5840	5.65531	5
PH	6.7400	.67937	5
EC	1590.1320	665.61654	5
TDS	842.5060	260.38296	5
TS	922.3640	255.10880	5
TA	1068.5860	422.95521	5
TH	1021.3660	551.26897	5
COD	79.8180	8.98509	5
DO	2.4000	1.91311	5

Table 3 shows descriptive statistics such as mean and standard deviations. From the above table mean DO was found to be 2.4 and COD has a mean value of 79.818. Mean values of total dissolved and suspended solids were found to be 842.50 and 922.36 ppm respectively. Significant correlation among various chemical parameters was evaluated using 2-tailed Pearson correlation matrix at different sites and depicted in table 4.

TABLE 4
CORRELATION MATRIX OF CHEMICAL PARAMETERS

		TEMP	PH	EC	TDS	TS	TA	TH	COD	DO
TEMP	Pearson Correlation	1	-.978**	.897*	.771	.721	.917*	.920*	-.745	-.869
	Sig. (2-tailed)		.004	.039	.127	.169	.028	.027	.148	.056
	N	5	5	5	5	5	5	5	5	5
PH	Pearson Correlation	-.978**	1	-.968**	-.882*	-.850	-.980**	-.982**	.776	.952*
	Sig. (2-tailed)	.004		.007	.048	.068	.003	.003	.123	.012
	N	5	5	5	5	5	5	5	5	5
EC	Pearson Correlation	.897*	-.968**	1	.941*	.946*	.996**	.996**	-.715	-.993**
	Sig. (2-tailed)	.039	.007		.017	.015	.000	.000	.175	.001
	N	5	5	5	5	5	5	5	5	5
TDS	Pearson Correlation	.771	-.882*	.941*	1	.982**	.946*	.942*	-.826	-.954*
	Sig. (2-tailed)	.127	.048	.017		.003	.015	.017	.084	.012
	N	5	5	5	5	5	5	5	5	5
TS	Pearson Correlation	.721	-.850	.946*	.982**	1	.934*	.931*	-.706	-.956*
	Sig. (2-tailed)	.169	.068	.015	.003		.020	.022	.183	.011
	N	5	5	5	5	5	5	5	5	5
TA	Pearson Correlation	.917*	-.980**	.996**	.946*	.934*	1	1.000**	-.761	-.993**
	Sig. (2-tailed)	.028	.003	.000	.015	.020		.000	.135	.001
	N	5	5	5	5	5	5	5	5	5
TH	Pearson Correlation	.920*	-.982**	.996**	.942*	.931*	1.000**	1	-.758	-.992**
	Sig. (2-tailed)	.027	.003	.000	.017	.022	.000		.138	.001
	N	5	5	5	5	5	5	5	5	5
COD	Pearson Correlation	-.745	.776	-.715	-.826	-.706	-.761	-.758	1	.721
	Sig. (2-tailed)	.148	.123	.175	.084	.183	.135	.138		.169
	N	5	5	5	5	5	5	5	5	5
DO	Pearson Correlation	-.869	.952*	-.993**	-.954*	-.956*	-.993**	-.992**	.721	1
	Sig. (2-tailed)	.056	.012	.001	.012	.011	.001	.001	.169	
	N	5	5	5	5	5	5	5	5	5

*****. Correlation is significant at the 0.01 level (2-tailed)***
****. Correlation is significant at the 0.05 level (2-tailed)***

Table 4 shows correlation between different pollutants in the sites. Dissolved oxygen shows a positive correlation with pH (significant at 0.01 level) and COD. A strong correlation exists between total dissolved solids and parameters like electrical conductivity, total solids and total hardness as evident from table 4. Hardness has a very strong positive correlation with parameters like EC, TDS and TS.

TABLE 5
CORRELATION MATRIX BETWEEN BIOLOGICAL AND CHEMICAL PARAMETERS

		THB	TC	TEMP	DO	PH
THB	Pearson Correlation	1	.914*	-.896*	.615	.818
	Sig. (2-tailed)		.030	.040	.269	.091
	N	5	5	5	5	5
TC	Pearson Correlation	.914*	1	-.756	.336	.607
	Sig. (2-tailed)	.030		.140	.581	.278
	N	5	5	5	5	5
TEMP	Pearson Correlation	-.896*	-.756	1	-.869	-.978**
	Sig. (2-tailed)	.040	.140		.056	.004
	N	5	5	5	5	5
DO	Pearson Correlation	.615	.336	-.869	1	.952*
	Sig. (2-tailed)	.269	.581	.056		.012
	N	5	5	5	5	5
PH	Pearson Correlation	.818	.607	-.978**	.952*	1
	Sig. (2-tailed)	.091	.278	.004	.012	
	N	5	5	5	5	5

. Correlation is significant at the 0.05 level (2-tailed)
****. Correlation is significant at the 0.01 level (2-tailed)***

Table 5 shows correlation between heterotrophic, total coliform bacteria and some selected chemical parameters. Results revealed that there exists a positive correlation between total heterotrophic bacteria and dissolved oxygen as well as with pH. This is true in case of total coliforms which showed a positive relation with both dissolved oxygen and pH. But both bacterial counts (THB & TC) exhibited a negative trend with temperature as evident from the table 5.

IV. CONCLUSIONS

Based on the foregoing analysis, it was concluded that bacteriological and physico-chemical quality of "Hora" and Huluka water samples in the current study did not meet the standards set for drinking water. Some of the physicochemical parameters such as temperature (26.91- 0.160◦C), pH (6.34-7.94), EC (404.66-1934.89 µS/cm), TDS (402-1067.50 mg/L), DO (1.2-5.8mg/L), TS (1167-1168mg/L) in the "Hora" water was found to be higher than the maximum permissible levels set for drinking water. Bacteriological parameters like total coliform and total heterotrophic bacteria were also much above the recommended standard values of WHO. In the wake of high pollution status of these waters, it was highly recommended that concern authorities who are responsible for maintaining the water quality of Hora waters in Ambo town should implement stringent rules to protect these spring water sources from being polluted further.

REFERENCES

[1] Zinabu G. The Ethiopian Rift Valley Lakes: Human interactions and water quality changes as a possible threat to the biodiversity, Awassa, Ethiopia. 2002

[2] Chatterjee C. and Raziuddin M. Determination of water quality index of a degraded river in Asansol Industrial area, Rangiganj, Burdwan, West Bengal. Environment and pollution Technology. 2002, 1 (20): 181-189.

[3] Venkatesharaju K, Ravi Kumar P, Somashekar R.K, and Prakash. K.L. Physic- chemical and bacteriological Investigation on the river Cauvery of Kollegal stretch in Karnataka, Journal of science Engineering and technology. 2001, 6 (1):123-160.

[4] Hurst C. J, Mclnerney M.J, Stezenbach. L.D, and Walter M.V. Manual of Environmental microbiology. American society for Microbiology. American Society of microbiology press, Washington, DC, 2002, 265-284.

[5] Erfurt Patricia J. An assessment of the role of natural hot and mineral springs in health, wellness and recreational tourism. PhD thesis. James Cook University. 2011

[6] Commission of the European communities (CEC). Establishing the list of concentration limits and labeling requirements for the constituents of natural waters. Journal of the Europe and Union 2003, 6 (2) : 146-150.

[7] Kabaija E. Non- conventional indigenous mineral supplements used for cattle feeding in the pastoral rangelands of Ethiopia. Trop, Anita. Hlth Prod. 2008, 21: 256- 262.

[8] USEPA. Methods for Chemical Analysis of Water and Wastes, USEPA Publication No. EPA-600/4-79- 020, United States Environmental Protection Agency. 2009

[9] American Public Health Association (APHA). Standard methods for examination of water and wastewater 20th Ed. American Public Health. New York. 1998, 81-85.

[10] Murray, Baron, Jorgensen, Landry and P faller.ed. Manual of clinical microbiology, 9th ed. American Society for Microbiology. Washington D.C., 2007

[11] Fawole M.O. and Oso B.A. *Laboratory Manual of Microbiology* Spectrum Books Limited. Ibadan, Nigeria. 2001, 71-80.

[12] Brock T.D, Madigan M.T, Martinko J.M. and Parker. *Biology of Microorganisms*. 7th edition. Prentice-Hall. New Jersey. 1994, 192-194.

[13] Milkiyas T, Mulugeta K, Bayeh A. Bacteriological and physico-chemical quality of drinking water and hygiene-sanitation practices of the consumers in Bahir Dar city. Ethiopian Health Sci. 2001, 22:19–32.

[14] Gupta B.K. and Gupta R.R. Physicochemical and bacteriological study of drinking water in Satna Maheya prade. 1999, 523–552.

[15] World health organization (WHO) Guidelines for Drinking Water. Quality. 4thEdition. WHO press. 2011; 564.

[16] Patil S.G, Chonde S.G, Jadhav A.S, and Raut P.D. Impact of Physico-Chemical Characteristics of Shivaji University lakes on Phytoplankton Communities, Kolhapur, India, *Research Journal of Recent Sciences.* 2009, 1 (2): 56- 60

[17] Amukama D, Kansime F, Mach R, Farnleitner A. Determination of *Escherichia coli* contamination with Chromo cult coliform agar showed a high level of discrimination efficiency for differing pollution levels in tropical waters of Kampala, Uganda. Appl Environ Microbiol. 1999, 66: 864-846.

[18] John KN, Orish EO, and Linus OE. Some physicochemical parameters of potable water supply in Warri, Niger Delta area of Nigeria. Sci. Res. And Essay. 2008, 3(11): 547-551.

[19] Orama.B. Sources of total Dissolved Solids.Minerals.in Drinking Water, htt://WWW.water-research.net/total dissolved solids. htm, accessed on Oct 18, 2010.

[20] Kataria H. C, Quershi H. A, Iqbal S. A, and Shandilya A. K. Assessment of water quality of Kolar reservoir in Bhopal. M.P. Pollution Research. 1996, 15 (2): 191-193.

[21] Hart. A. Total hardness and water quality, 2007 htt://ezinearticles.com/Total hardness and water quality and & id=516135, accessed on oct 18, 2010.

[22] Pakistan council of Research in water Resources (PCRWR). National Water Quality Monitoring Program. 2001, 1-20

[23] Edema M.O, Omemu A. M, and Fapet O. M. Microbiology and Physicochemical Analysis of different sources of drinking water in Abeokuta. Nigeria. Niger. J. Microbial. 2001, 57

[24] Obire O, Aguda M. Bacterial community of Leachate from a waste dump and an adjacent stream. Appl. Sci. Environ Mgt. 2004, 6 (2): 71-76.

[25] Welch P, David J, Clarke TAPD, Berston S, McDougall L, Adesiyin AA. Microbial sanitation of water in rural communities of Trinidad. Pan Ameri. J. Public Hlth. 2000, 8 (3): 172-180.

[26] Ajayi AO, Akonai KA. Distribution pattern of enteric organisms in the Lagos Lagoon. Afr. J. Biomed.Res. 2005, 8 (3): 163-168.

[27] Okonko I. O, Adejoye O. D, Ogunnusi T.A, Fajobi E. A, and Shittu O. B. Micobiological and Physicochemical Analysis of Different Water Samples Used for Domestic Purposes in Abeokuta and jota, Lagos State, Nigeria. African journal of Biotechnology. 2008, 75: 617-621.