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Evaluation of Physico-chemical and Antimicrobial Susceptibility Patterns of Microorganisms Isolated from Awetu River, Jimma Town

Shiferaw Demissie and Anbessa Dabassa
College of Natural Sciences, Jimma University, P.O. Box 378, Jimma, Ethiopia

Corresponding Author: Anbessa Dabassa, College of Natural Sciences, Jimma University, P.O. Box 378, Jimma, Ethiopia Tel: 251 911070477

ABSTRACT

Water quality is the great public health concern in developing countries and for the study area at large. This study is aimed to evaluate the Physico-chemical and antibiotic resistance activities of some bacterial isolates obtained from Awetu River. In this study, 284 bacterial strains were isolated and evaluated for their antimicrobial resistance patterns. All of the isolates tested were not sensitive to Nor, Chl, Kan, Gen and Tet. Among the bacterial isolates, the most frequent resistance was noted for methicillin (96%), followed by resistance to penicillin (85%). The most frequent resistance among (Stapylonocci and Micrococci isolates was observed for Methicillin and Penicillin and streptomycin (96% each), followed by resistance to kanamycin (84%), gentamicin (55%) and methicillin (30%). A total of 9 Multiple Drug Resistance (MDR) patterns were detected. About 42% of the isolates showed MDR to four drugs, 29% to five drugs and 2% to six drugs. The most frequent MDR pattern was Van/Str/Kan/Gen/Met and was seen in 42% of the isolates. This pattern was seen in *Lactobacillus*, *Pediococcus* and *Weissella* isolates, but was the most frequent pattern in *Pediococcus* isolates (41%). The results obtained from physico-chemical and antimicrobial susceptibility patterns of the isolate were the health risk for the study area community. The study also found the coliform contamination to be the key problem with drinking water.

Key words: Antimicrobial susceptibility, biochemical activities, physico-chemical parameter, multiple drug resistance

INTRODUCTION

Water is the most important natural resources in the world. Accelerated pollution and eutrophication of rivers and streams is because of human activity throughout the world. The situation is more severe in African countries (Armstrong *et al*., 1981; Beyene *et al*., 2009). According to Bekele and Butako (2010) reports, the coverage and sanitation of the available water supply unswervingly affecting the health and the wellbeing of a population. Microbial and chemical contaminants of river are common in developing countries. Furthermore, the reports of Ramirez *et al*. (2010) indicates from the community problem that, the land disposal of sewage effluents, sludge and solid waste, septic tank effluent, urban runoff and agricultural, mining and industrial practices were some of the most common sources of water contamination in different areas. Awetu River is highly contaminated by microorganisms like; Enterobacteriaceae and coliform bacteria. The river was serving as sidewise for Jimma town residents. So, it was health risks on

humans due to consummation through irrigational product, taking shower and others service. Jimma town is situated near a surface water that is, Awetu River. The river starts from source found near the villages of Limugenet (Faris and Kaba, 1999). It crosses many agricultural fields and forests till it reaches Jimma town. The river crosses at the center of the town from north to south and joins kito stream at "Dedober" bridge out of the town. Both are drain into Boye pond 7 km away from the town. The total length of Awetu River in the town is 4.5 km. Although, nature has conveniently put Awetu River for recreational activities, unfortunately, it has been an eye sore for Jimma residents due to contamination by solid and liquid wastes. Household and commercial wastes also significantly contribute to health hazards in the urban centers. Problems are especially severe in traditional residential areas with high densities of population and housing (Kifle and Gadisa, 2006). Water sources like River and groundwater have often looked and managed as separate resources; although, they are used for different purposes. The establishment and use of different varieties of waste disposal units should be established in the town. Such "waste disposal units" should occur at the local Council, as well as the State and the Federal Government levels (Florez *et al.*, 2005).

MATERIALS AND METHODS

Study area and period: The study was conducted in Jimma town from September 2008 to July 2009. Jimma is found 346 km south-western Ethiopia in Oromia regional state. The tow has population of 200,000 and it covers a total area of 18,412.54 km^2. The mean annual rainfall of the area is between 1800-2300 mm with maximum rainfall between months of June and September. The altitude of the area ranges from 1300-2100 m. The town has bordered by Southern Nations Nationalities and Peoples Region (SNNPR), Illubabor Zone, Welega and west Shewa; in south, northwest, north and northeast, respectively. The town lies within 7°15'N-8°45'N Latitude and 35°3°'E-37°3°'E Longitude. The annual mean temperature of the area is between 15 and 22°C (CSA, 2005).

The Fig. 1 shows that Ethiopia and study area, Jimma town where microbiological study took place for this study (Dabassa and Demissie, 2013).

Physico-chemical parameter: Awetu river has a vital role for people around Jimma town for drinking, irrigation, cooling, cleaning, preparation of food, swimming and recreation. So, the physicochemical determination of the study river were as follow (Table 1).

Table 1: Susceptibility of bacterial genera from Awetu river isolates to broad spectrum antimicrobial agent October 2008-January 2009, Jimma town

Bacterial spp.	Pen	Nor	Meth	Tetra	Chlo	Kana	Gent
Antimicrobial susceptibility tested							
Aeromonas (80)	4 (5.0%)	71 (88.8)	2 (2.5)	64 (80.0)	38 (47.5)	69 (86.3)	80 (100)
Micrococci (55)	2 (3.6)	53 (96.4)	18 (32.7)	48 (47.3)	15 (27.3)	53 (96.4)	55 (100)
Staphylococci (42)	2 (4.8)	40 (95.2)	2 (4.8)	18 (42.9)	16 (38.1)	33 (78.6)	42 (100)
Bacillus (38)	7 (18.4)	36 (94.7)	7 (18.4)	36 (94.7)	9 (23.7)	29 (76.3)	38 (100)
Pseudomonas (20)	2 (10.0)	20 (100.0)	4 (20.0)	13 (65.0)	9 (45.0)	16 (80.0)	20 (100)
Moraxella (20)	0 (0.0)	20 (100.0)	2 (10.0)	18 (90.0)	2 (10.0)	20 (100.0)	20 (100)
Enterobacteriaceae (13)	0 (0.0)	13 (100.0)	0 (0.0)	9 (69.2)	4 (30.8)	13 (100.0)	13 (100)
Alcaligenes (9)	0 (0.0)	9 (100.0)	5 (55.6)	9 (100.0)	2 (22.2)	9 (100.0)	9 (100)
Chromobacterium (7)	5 (71.4)	7 (100.0)	0 (0.0)	7 (100.0)	2 (28.6)	7 (100.0)	7 (100)

Pen, penciling; Nor, Norfloxaxin; Met, Methicillin; Tet;/tetracycline; Chlo, chloramphenicol; kan, kanamycin; Gen: Gentamycin. Number in parentheses represents percentage of susceptibility of isolates

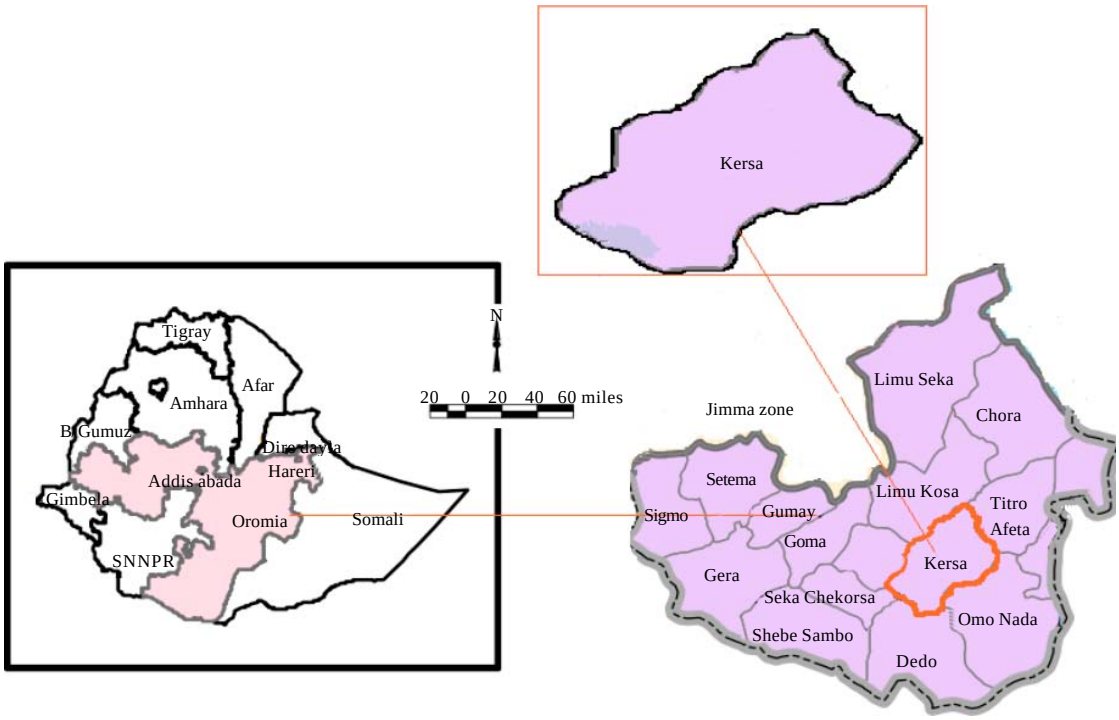

Fig. 1: Study area map

The suitable microbial environment was cheeked by using physico-chemical test. The media used for this purpose is Glucose agar with 25% of glucose. Ingredients (g L^{-1}): Glucose: 250 g, Agar: 15 g, Peptone: 5 g, Malt extranet: 3.0 g, Yeast extract: 3.0 g in 1000 mL of distilled water.

Effects of osmotic pressure: After dividing the bottom of the plate by marking the pure culture broth was streaked on presolidified surface of glucose agar quadrant plate which each contain 0.5, 5, 12 and 17% NaCl, and incubated at 30°C for 48 h and presence or absence of growth and amount of growth of each organisms at different salt concentration was observed.

pH effect: After dividing the bottom of the plate the pure culture broth was streaked on pre solidified surface of Glucose Agar quadrant plate, each having pH 3, 5, 7, 10 and incubated at 30°C for 48 h to observation of presence/absence of growth and amount of growth of organisms at different pH.

Temperature effect: The pure culture broth was streaked on presolidified surface of Glucose Agar quadrant plate and incubated at six different temperature (5,15,25, 33,45,55 and above 58°C) and amount and growth of organism was observed.

Microbiological analysis: A one mililitter of sampled water mixed with 9 mL of saline solution by vortex mixer and then appropriate dilutions were spread plated on various types of solid media for microbial count. The cultures were purified by repeated plating in order to characterize for colony morphology and differiated into various bacterial group such as genus level by using standard microbiological techniques (cell morphology and biochemical test).

Antimicrobial susceptibility test: Antimicrobial susceptibility tests of pure culture were done by inoculating in to nutrient broth and incubated at 32°C for 18-24 h. After incubation the turbidity of the culture was adjusted to 0.5 McFarland standards to bring the cell density to approximately 10^8 CFU mL^{-1}. The McFarland turbidity standard was prepared by mixing 0.1 mL $BaCl_2$ {1%} (Kifle and Gadisa, 2006), neatly, from standardize cultured spreaded by ampplicater swamp on pre solidified surface of Mueller Hinton agar plate.

The isolates were tested for their susceptibility to different antibiotics on Mueller Hinton agar plate for 7 Oxoid drug discs: Gentamycin (Gen-10 ug); Tetra-cycline (Tet-30 ug); Methicillin (Meth-5 ug); Norfloxaxin (Nx-30 ug); Chloramphenicol (Chlo-30 ug); kanamycin (kan-3o ug); pencillinG G,(pen-10 ug).

RESULTS AND DISCUSSION

Fresh water has a vital resource for human being. Currently, new strategic plan have been arranged for the growing water scarcity and contamination to alleviate and meet the water requirements of rapidly growing populations (Cheesbrough, 2001). Awetu River serves the community for irrigation and also causes different problems on the people around Jimma town. It affects the health of plant and humans being.

Antimicrobial susceptibility tests: All isolates of nine general sensitive to Gentamycin (100%) while three) genera such as *Moraxella*, Enterobacteriaceae and *Alcaligenes* were 100% sensitive to Norfloxacin and kanamycin. In addition *pseudomonas* 100% sensitive to Norfloxaxin) while, most isolates resistant to pencillin G and Methicillin. The result was shown (Table 1 and Fig. 2).

All isolated nine genera were sensitive (100%, 88.8-100%, 76.3-100%) to Gentamycin, Norfloxalin, Kanamycin, respectively, Similar to the recent report of Pato-Mesola and Donaldo (1997). However, 93.3% (22) and 85.9% (40) isolate were resistant to antimicrobial agent of pencillin G. and Methicillin. Moreover, *Moraxella*, Enterobacteriaceae and *Alcaligenes* were 100% resistant to pencillin G. and chromobacterium and Enterobacteriaceae were 100% resistant to Methicillin, respectively (Table 2). In generally antimicrobial agent such as, gentamycin, Norfloxain

Fig. 2: Antibiotic susceptibility test for the microbial isolate

Table 2: Multiple drug resistance patterns of bacterial isolates from Awetu River Jimma town south western Ethiopia

Genus	No. of tested isolate	MDR pattern	No. of the pattern
Chromobacterium	7	pen/Met/Chl	3
Alcaligenes	9	pen/Met/Chl	3
Enterobacteriaceae	13	pen/Met/Tet/Chl	4
Morixalla	20	pen/Met/Tet/Chl	4
Pseudomonas	20	pen/Met/Tet/Chl/Kan	5
Bacillus	38	pen/Met/Tet/Chlo/Kan/Nor	6
Staphylococci	42	pen/Met/Tet/Chlo/Kan/Nor	6
Micrococci	55	pen/Met/Tet/Chlo/Kan/Nor	6
Aeromonas	80	pen/Met/Tet/Chlo/Kan/Nor	6

Gen: Gentamycin, Kan: Kanamycin, Met: Methicillin, Pen: Penicillin, Tet: Tetracycline, Chl: Chloramphenicol and Nor: Norphloxacin

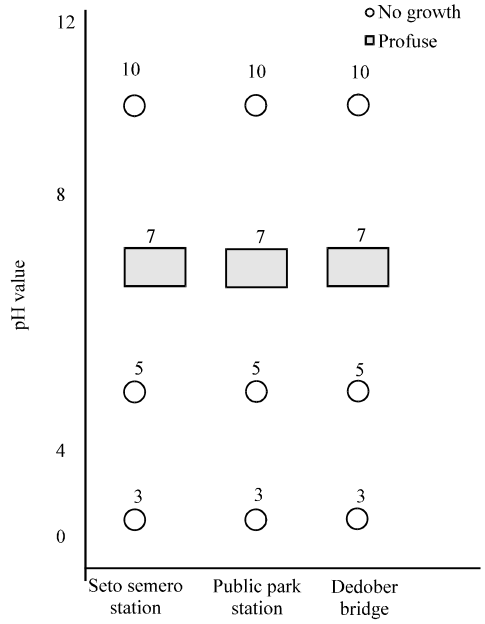

Fig. 3: Microbial growth vs. pH difference on isolates from Awetu river

and kanamycin were effective antibiotic against for both gram positive and negative bacterial isolate from Awetu stream (Fig. 2). The emergency of antimicrobial resistance bacteria has compromised control of many bacteria pathogen (Armstrong *et al*., 1981).

Although, increment of multidrug resistant bacteria in the river used by local people for drinking, irrigation and other activities can create a big challenge in the community. The reports from Al-Bahry *et al*. (2007), support that the antibiotic resistance of bacterial strains appears to be increasing which is of the world health concern.

pH and temperature of fresh sample: Physico-chemical parameters have great impacts on the distribution of microorganisms in the actual habitat. It also has an indicator value in determining microbial safety of the sampled water. The pH and temperature ranges of Awetu River range 6.76-7.85 and 21.1-20°C (Table 3).

The results of the pH indicate that all the isolates were highly grown at the neutral pH (Fig. 3). While, none of the isolates were grown at the pH, 12, 5 and 3. This indicates the isolated microorganisms were neutrophils.

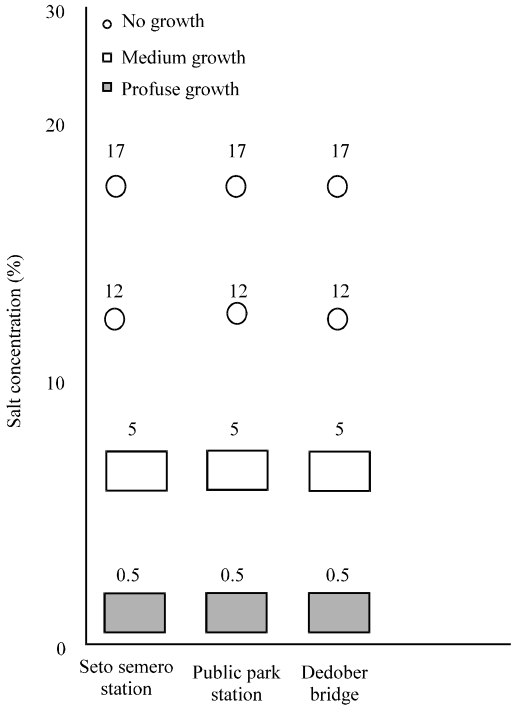

Fig. 4: Microbial growth vs. salt concentration isolates from Awetu river

Table 3: Physico-chemical parameter of fresh water sample

	pH		Temperature (°C)	
Sample code	X	R	X	R
ASFA	7.20	6.76-7.85	25.98	22.8-27.6
APP	7.40	6.76-7.80	25.83	21.1-27.8
ADB	7.23	6.97-7.55	26.17	23.0-28.0

x̄: Mean value, R: Range

The chemical influents discharged by communities and industry to the river affect the pH of the water and the organism in it (Fig. 3) (Aryal *et al*., 2012). pH is also the most important in determining the corrosive nature of water (Patil *et al*., 2012).

The tested isolates were grown profusely grown on 0.5% NaCl followed by medium growth on 5% NaCl concentration (Fig. 4). This could be due to the isolates were less tolerant to salt concentration.

The results of the salt concentration effect on the growth of microorganism were presented in Fig. 4. These values obtained are similar to those reported by Agbalagba *et al*. (2011) and Reimann *et al*. (2003). The idea reported by Rao *et al*. (2012) also support of the result presented.

Relative to the other two physic-chemical parameters, the isolates were grown in wide ranges of temperature. Furthermore, most of the isolates were densely grown at the ambient temperature. This indicates the mesophilic microorganisms were the dominant and were connected with sources of human related pathogen (Fig. 5).

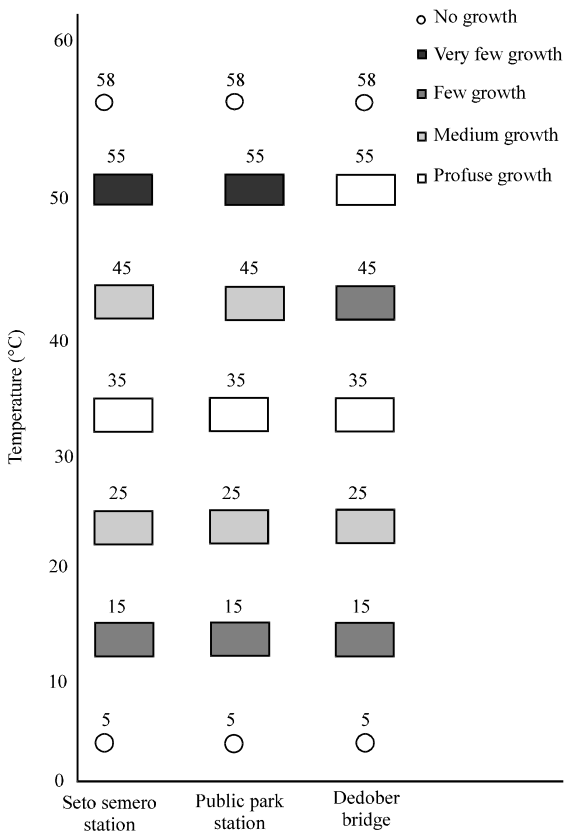

Fig. 5: Microbial growth vs. temperature range of the isolates from Awetu river

According to Patil *et al.* (2012), the rate of life process of the water organism like growth, reproduction and the immunity were affected by the water temperature. Drastic temperature changes can be fatal to fish and other living organisms.

CONCLUSION

Changes in water quality are reflected in its physical, biological and chemical conditions and these in turn are influenced by physical and anthropogenic activities. The Jimma town residents have no concept about health problem in connection with contaminated water other than drinking. But, this river was highly contaminated by faecal materials, Enterobacteriaceae, coliform bacteria and other different chemicals. Many areas of river bodies near Jimma town are highly contaminated/polluted. This is the result of both garbage dumped by individuals and dangerous chemicals illegally dumped by the town people, health centers, schools and market places. So, this contaminated water is very harmful to human, animal and water life. The effects can be catastrophic to the utilizing people/organisms depending on the kind of organism/chemicals, concentrations of the pollutants and where there are polluted. Eventually, humans are also the one that can be affected by this process as well. People can get diseases by eating food that has been poisoned by contaminated water. There is always outbreak and diseases as a result utility of poor safety water treatment from contaminated waters. An irrigated plant can be severely changed or destroyed by the polluted water. Many areas the water body are now being affected by careless human pollution and microbial contamination and this pollution and contamination is coming back to hurt humans in many ways.

REFERENCES

Agbalagba, O.E., O.H. Agbalagba, C.P. Ononugbo and A.A. Alao, 2011. Investigation into the physico-chemical properties and hydrochemical processes of groundwater from commercial boreholes In Yenagoa, Bayelsa State, Nigeria. Afr. J. Environ. Sci. Technol., 5: 473-481.

Al-Bahry, S.N., A.E. ElShafie, S. Al-Busaidy, J. Al-Hinai and I. Al-Shidi, 2007. Antibiotic-resistant *Salmonella* sp. from human and non-human sources in Oman. Eastern Mediterr. Health J., 13: 49-55.

Armstrong, J.L., D.S. Shigeno, J.J. Calomiris and R.J. Seidler, 1981. Antibiotic-resistant bacteria in drinking water. Applied Environ. Microbiol., 42: 277-283.

Aryal, J., B. Gautam and N. Sapkota, 2012. Drinking water quality assessment. J. Nepal Health Res. Counc., 10: 192-196.

Bekele, J. and B. Butako, 2010. Occurrence and financial loss assessment of cystic echinococcosis (hydatidosis) in cattle slaughtered at Wolayita Sodo municipal abattoir, Southern Ethiopia. Trop. Anim. Health Prod., 43: 221-228.

Beyene, A., W. Legesse, L. Triest and H. Kloos, 2009. Urban impact on ecological integrity of nearby rivers in developing countries: The Borkena River in highland Ethiopia. Environ. Monitor. Assess., 153: 461-476.

CSA, 2005. The federal democratic republic of Ethiopia: National labour force survey. Central Statistical Agency, Addis Ababa, Ethiopia, pp: 56-67.

Cheesbrough, M., 2001. District Laboratory Practice: Tropical Countries Part 2. Cambridge University Press, UK., pp: 4-12.

Dabassa, A. and S. Demissie, 2013. Correlation of traditional knowledge with laboratory based *Salmonella* detection in egg shell and contents of market sold egg. Asian J. Biol. Sci., 6: 168-174.

Faris, K. and M. Kaba, 1999. Hygienic behaviour and environmental conditions in Jimma town, South-Western Ethiopia. Ethiop. J. Health Dev., 13: 77-86.

Florez, A.B., S. Delgado and B. Mayo, 2005. Antimicrobial susceptibility of lactic acid bacteria isolated from a cheese environment. Can. J. Microbiol., 51: 51-58.

Kifle, S. and T. Gadisa, 2006. Microbial quality of Jimma water supply. Ethiop. J. Educ. Sci., 2: 21-36.

Patil, P.N., D.V. Sawant and R.N. Deshmukh, 2012. Physico-chemical parameters for testing of water: A review. Int. J. Environ. Sci., 3: 1194-1207.

Pato-Mesola, V.V. and M.E.S. Donaldo, 1997. Antimicrobial susceptibility of *Salmonella typhi* isolates from government and private hospitals in Cebu city. Phil. J. Microbiol. Infect. Dis., 26: 5-8.

Ramirez, E., E. Robles, M.E. Gonzalez and M. Martinez, 2010. Microbiological and physicochemical quality of well water used as a source of public supply. Air Soil Water Res., 3: 105-112.

Rao, V.S., S. Prasanthi, V. Jagarlapudi, K. Shanmukha and K.R.S. Prasad, 2012. Physicochemical analysis of water samples of Nujendla area in Guntur District, Andhra Pradesh, India. Int. J. ChemTech Res. 4: 691-699.

Reimann, C., K. Bjorvatn, B. Frengstad, Z. Melaku, R. Tekle-Haimanot and U. Siewers, 2003. Drinking water quality in the Ethiopian section of the East African Rift Valley I-data and health aspects. Sci. Total Environ., 311: 65-80.