RESEARCH ARTICLE

ASSESSMENT OF MICROBIOLOGICAL AND CHEMICAL QUALITY OF SPRINGWATER IN RIVERSTON OF KNUCKLES MOUNTAIN RANGE IN SRI LANKA

*A. T. Herath**

Department of Botany, Faculty of Natural Sciences, Open University of Sri Lanka

ABSTRACT

Safe and readily available water is important for public health, whether it is used for drinking, domestic use, food production or recreational purposes. The main objectives of this study were to assess the microbiological and chemical quality of spring water in Riverston, situated in the Knuckles Mountain range, Sri Lanka and to identify bacteria isolated from spring water. Water samples were collected from ten springs from different locations. Microbiological and chemical analysis were carried out according to standard protocols. Isolated bacteria were identified using biochemical tests and API identification system. According to the results, Total coliforms (TC) (ranged from 0-27 per 100 ml) and Fecal coliforms (FC) (ranged from 38-326 per 100 ml) bacteria were detected in all water samples tested, and the detected numbers exceeded permitted levels for drinking water. There are four TC species, *viz; Escherichia vulneris, Serratia marcescens*, *Serratia liquefaciens* and *Proteus mirabilis* and one FC species, *viz; Escherichia coli* (dominant species), were identified during the study. All chemical parameters tested were within the permitted levels. This study reveals that spring water in Riverston, Knuckles Mountain Range is not a safe drinking water source. Hence, it is important to take necessary precautions, especially as spring waters from these areas are consumed by many and is the main source water for the bottling industry in Sri Lanka.

Keywords: *Drinking water quality, fecal coliforms, Riverston, spring water, total coliforms*

DOI. *http://doi.org/10.4038/jsc.v12i2.38*

1. INTRODUCTION

Natural freshwater is the most valuable resource which is distributed unevenly around the globe. A major portion of the available water is trapped in areas where humans cannot utilize it for their daily needs. Globally, around 785 million people do not have access to

*Corresponding author: whher@ou.ac.lk

adequate water supply sources [1]. Drinking water is defined as one which does not contain disease producing organisms and chemical substances deleterious to health [2]. Drinking water comes from two major sources; surface water and groundwater. The natural surface water sources include rivers, streams and lakes and groundwater sources are wells and springs.

In Sri Lanka, it is challenging to realize the trend of water quality in public water bodies due to a shortage of monitoring data [3]. As the natural water bodies are polluted with toxic substances and microorganisms, this unsafe water can cause health issues in humans and numerous adverse impacts on aquatic organisms. The Sri Lanka National Water Development Report stated a variety of quality concerns in Sri Lanka, including contamination by nitrate and bacteria in underground and surface waters mainly due to poor sanitation and untreated wastewater or inadequate wastewater treatment, toxic substances from industrial and agricultural activities, and eutrophication in lakes and reservoirs [3, 4].

Groundwater is a major vital natural resource because of its purity and availability. They provide most of the water for individual homes in small towns and rural areas in Sri Lanka. Further, natural springs, shallow and deep wells are commonly used as sources for bottling, natural springs from the mountain regions being the most popular source in Sri Lanka. Most bottled water and mineral water brands in Sri Lanka indicate natural spring water as their water sources. The public believes that these waters originate from protected underground water sources and must be safe to drink at source, in their natural state, without disinfection or chemical treatment. Natural mineral water can only come from specific designated groundwater sources, such as natural exiting or boreholes.

Knuckles mountain range in Sri Lanka is a popular area for spring water. It lies in central Sri Lanka, in the district of Matale and Kandy (33.6819° S, 150.8594° E). Moreover, some bottled water companies are situated at the Knuckles Mountain range. With this background, the main objectives of this study were to assess microbiological and chemical quality of springwater in Riverston, situated in the Knuckles Mountain range, Sri Lanka and to identify bacteria isolated from springwater.

2. MATERIALS AND METHODS

2.1 *Sample collection*

Water samples were collected from ten springs from different locations in Riverston, in the Knuckles Mountain range, Sri Lanka to determine the microbiological and chemical parameters. Water samples were collected in sterilized bottles, brought to the laboratory, and stored at refrigerated temperature (4^{o} C) until the time of analysis. Analysis was carried out within 24 hours after collection.

2.2 *Sample analysis*

Microbiological analysis

Total coliform (TC) and fecal coliform (FC) were enumerated by membrane filtration method [5] passing 100 ml volumes of each sample through the membrane filtration apparatus (Pyrex, Germany) using sterilized membrane filters (Sartorius, Germany) with a pore size of 0.45 µm. Membrane filters were aseptically placed on pre sterilized absorbent pads (Sartorius, Germany), saturated with 3 ml of M-Endo broth (HI-media, India) and 3 ml of M-FC broth base (HI-media, India) and were incubated for 24-48 hours at 36 ± 1 °C and at 44.5 °C for the detection of total coliforms and fecal coliforms respectively. Sterilized distilled water and typical coliforms (*Serratia marcescens* -NCTC 11935, *Escherichia coli* -ATCC 25922) were used as a negative control and positive controls respectively in the detection of coliform bacteria.

Isolation of bacteria for identification

Selected presumptive coliforms colonies were sub cultured by streaking on Tryptone Soy Agar (TSA, Oxoid, UK). Subsequently, well isolated colonies from TSA plates were subjected to identification. The three basic standard preliminary tests viz; Gram's test, oxidase test and catalase test were conducted for each isolate. Stock cultures of all isolates were made for further identification as follows. Pure colonies of isolates were inoculated into microcentrifuge tubes, containing Brain Heart Infusion Broth (BHIB, Oxoid, UK) and the tubes were incubated for 24 hours at 37^{o} C. Three replicate stock cultures were prepared from each isolate and stored at -20^{o} C, after overlaying with 60 % glycerol.

Identification of bacteria

One or two microcentrifuge tubes from each stock culture were thawed, and the tubes were centrifuged for a few seconds to obtain a concentrated cell mass. Subsequently, the TSA (Oxoid, UK) plates were streaked from the concentrated cell mass and the plates were incubated at 37^{o} C for 24 h to obtain pure colonies required for identification tests. The three basic preliminary tests, the Gram's test, Oxidase test and the Catalase test were repeated. Subsequently, other standard biochemical tests used for identification of Gram's negative bacteria were performed. These tests included Triple sugar iron (TSI), Urease, Citrate utilization, Methyl Red and Voges-proskauer tests (MR-VP), Indole production and Motility tests. Further, identification of bacteria was performed by using commercially available Analytical Profile Index (API) 20E (bioMeriex) rapid identification strips as follows.

Identification using API identification strips

Pure colonies (well isolated) on Tryptone Soy Agar (TSA) plates were selected and emulsified in 5 ml sterilized distilled water, in a sterilized tube and mixed well using a vortex machine (VELP Scientifica, Europe) to obtain a homogenous suspension. Using a

micropipette, 2 ml of this bacterial suspension was inoculated into twenty mini test tubes of the API 20E strips.

API 20E strips

Both the tube and the cupule were filled for the tests marked as CIT, VP and GEL. Only the tube was filled in the remaining tubes; anaerobiosis was created for the tests ADH, LDC, ODC, H_2S and URE by overlaying with sterilized mineral oil. To create a humid atmosphere, 5 ml of sterilized distilled water was distributed in the honey-combed wells on the tray. The incubation box was closed with the lid and incubated at 37^o C for 18-24 hours. After incubation, the strip was referred to the 'reading table' provided by the Biomerieux, Inc, USA and spontaneous reactions were recorded. The TDA, VP and IND tests were performed by addition of TDA, VP 1 + VP 2 and James reagents respectively. On the result sheet, the tests were separated into groups of 3 sets and values were summarized as instructed. By adding together, the values corresponding to positive reactions within each group, a 7-digit profile number was obtained for the 20 tests of the API 20 E strips. The numerical profile was entered into the identification software and submitted, and identification performed.

Chemical Analysis

Physiochemical parameters were analyzed following the standard guidelines and procedures [6]. The alkalinity, hardness and the chloride (Cl^-) contents were determined by titration methods using Hach digital titrator and Hach standard reagent cartridges. Calcium (Ca), iron (Fe), manganese (Mn) and zinc (Zn) were measured by the atomic absorption spectrometer (Varian 240FS Inc., Australia) and spectrophotometer (Hach DR-2400 with standard reagents) was used to determine nitrate (NO^{3-}), nitrite (NO^{2-}), phosphate (PO_4^{3-}), fluoride (F^-), ammonium (NH_4^+), sulphate (SO_4^{2-}) and sulfide (S^{2-}). All instruments were calibrated using commercially available standard solutions (BDH, Fulka) before performing the measurements.

3. RESULTS AND DISCUSSION

Microbiological analysis

Pollution of water is a major national and global issue, and billions of people do not have access to water that is safe to drink [1]. There is no pure water in nature as water is naturally polluted by-products of rock, deposition of leaf and animal wastes, and solution of minerals [7]. Coliforms have been identified as reliable indicator organisms in water quality testing. They are present in and throughout the environment. Coliforms are found in soil, water, and human or animal waste. Therefore, the numbers of TC should be higher than FC, which are generally present as a contaminant from fecal matter [8]. When animal and human fecal matter enter the water bodies, water can get contaminated with harmful pathogens such as bacteria, viruses and parasites. *Escherichia coli*, a member of the coliform group, had been found as an ideal indicator organism to detect faecal contamination of any water source. The presence of *E. coli* indicates that the water

is fecally contaminated and thus has the possibility of the presence of other pathogenic microorganisms such as, *Salmonella* spp., *Shigella* spp. and *Vibrio* spp., which may pose an immediate health risk to anyone consuming the water.

In the current study, total coliforms and fecal coliforms bacteria were detected in all water samples tested. M-Endo and M-FC media were used to detect total and fecal coliforms respectively. Both red colonies with a green metallic sheen, and red colour colonies with a sheen were enumerated separately for TC. Red colonies with a sheen were too numerous to count (TNTC) in all samples analysed, while the red colonies with a green metallic sheen ranged from 0-27. Typical blue clones indicating fecal coliforms ranged from 38-326 per 100 ml of water (Table 1). According to Sri Lankan Standards [9] and the Health Ministry regulation in Sri Lanka [10], the TC and FC counts should be zero per 100 ml for drinking water which indicated that the water samples tested are not suitable for drinking purposes in its present state.

Bacterial Identification

Results obtained for bacteriological identification, with conventional biochemical test and the API 20E rapid identification systems are shown in Table 2. As depicted in the Table 2 by using the two identification systems five bacterial species were identified including four total coliforms species and one fecal coliform species.

Table 1 Summary of total and fecal coliform counts in Riverston, the Knuckles Mountain range water samples

Sample no	Location (Mile posts/ culvert no; along the road)	Average counts of presumptive TC / 100 ml		Average counts of presumptive FC / 100 ml
		Red colonies with a green metallic sheen	Red colonies with sheen	Blue colonies
1	23/2	0	TNTC	38
2	23/5	1	TNTC	66
3	24/6	23	TNTC	229
4	26/2	14	TNTC	238
5	28	9	TNTC	128
6	28/2	5	TNTC	140
7	29/3	8	TNTC	247
8	29/4	9	TNTC	326
9	29/5	27	TNTC	127
10	29/6	0	TNTC	47

Escherichia vulneris, *Serratia marcescens*, *Serratia liquefaciens* and *Proteus mirabilis* were identified as total coliform species (Figure 1 – a, b, c and d), while *Escherichia coli* which was the dominant species, was identified as the fecal coliform species (Figure 1 – e). The presence of these pathogenic species in drinking water may cause diseases such

as, diarrhoea, bacterial infection and urinary infections. Similarly, research done in Sri Lanka by [11] indicated that ground water samples of Jaffna peninsula were contaminated with total coliform and *E. coli.* Further it revealed that 38% sampling locations were positive for *Salmonella* spp. and out of them six sampling sites were used for drinking purposes. Another study done in Jaffna peninsula reported

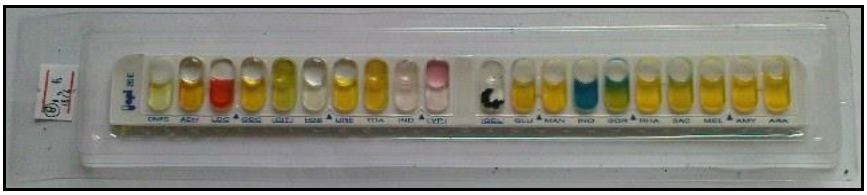
Table 2 Bacterial identification: Biochemical tests and API 20 identification system

No of isolates	Gram test	Oxidase test	Catalase test	TSI test	Citrate test	Urease test	Indole test	Motility test	MR test	VP test	Identification API 20E	Bacterial type Total coliforms (TC) Fecal coliforms (FC)
3	-	-	+	+	-	-	-	-	-	-	*Escherichia vulneris*	TC
6	-	-	+	+	-	-	-	-	-	+	*Serratia liquefaciens*	TC
4	-	-	+	+	+	+	-	-	-	+	*Serratia marcescens*	TC
3	-	-	+	+	-	-	-	-	+	+	*Proteus mirabilis*	TC
9	-	-	+	+	-	-	+	-	+	-	*Escherichia coli*	FC
Total 25												

that most (90%) public water sources were microbiologically unacceptable [12]. Further, a study revealed that surface water and groundwater of the Kelani River Basin were contaminated with total coliform and *E. coli* bacteria and also it is stated that all the sampling locations exceed the permitted values for drinking water given by the SLS guideline [13]. As most bottled water and mineral water brands in Sri Lanka indicate natural spring water as their water sources, the results of the current study are alarming. Though filtration and UV radiation are employed before bottling, many studies undertaken in Sri Lanka revealed that even bottled water samples exceeded permitted levels for microbiological parameters [14, 15, 16].

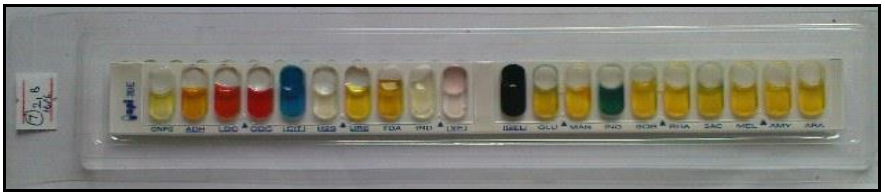
Chemical Analysis

When considering chemical parameters of drinking water, pH is one of the most important water quality parameters. According to WHO guidelines and Sri Lanka



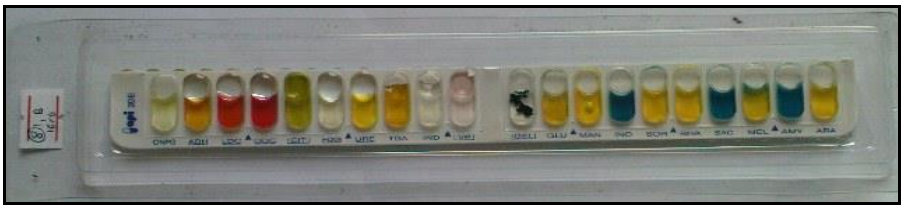
(a)Escherichia vulneris

(b) Serratia macescens



(c) Serratia liquefaciens

(d) Proteus mirabilis



(e) Escherichia coli

Figure 1 API profiles of Total coliforms (TC) and Fecal coliforms (FC) identified.
(a) *Escherichia vulneris* (TC) (b) *Serratia macescens* (TC) (c) *Serratia liquefaciens* (TC)
(d) *Proteus mirabilis* (TC) and (e) *Escherichia coli* (FC)

Standards (SLS), the optimum required pH in drinking water is in the range of 6.5 to 9.5 and 6.5–8.5 respectively [17, 18]. In the present study, the minimum and maximum values of pH in spring water was 6.5 and 7.75, respectively. The values were within the WHO and SLS permitted levels. According to SLS, the maximum permitted level for electric conductivity (EC) for drinking water is 750.0 $\mu S \cdot cm^{-1}$ [18]. All water samples tested were within the permitted level for EC, however the values were very low. Hardness and fluoride are also two important water quality parameters of drinking water, and both parameters were within the permitted levels in all samples tested. Considering the other water quality parameters investigated, alkalinity, anions (Cl^-, SO_4^{2-}, S^{-2}, PO_4^{3-},

NO_3^-, NO_2^-) and cations (Zn^{2+}, Ca^{2+}, Mn^{2+} and Fe^{2+}) were found within the Sri Lanka drinking water standards (Table 3).

Table 3 Summary of chemical parameters of spring water in Riverston, the Knuckles Mountain range water samples. Parameter concentrations are given in mg/l unless otherwise specified.

Parameter	Min.	Max.	Mean	SD	Permitted level
pH	6.5	7.75	6.93	0.37	6.5 to 8.5
EC (µS/cm)	12.3	22.1	16.43	3.62	750
Alkalinity	15.2	53.6	21.6	11.48	200.0
Hardness	1.6	7.2	3.28	1.54	10-20
Chloride	6.25	9.75	7.5	1.26	250.0
Fluoride	0	0.09	0.021	0.02	1.5
Sulfate	0	1	0.6	0.46	250.0
Sulfide (µg/L)	3	17	8.3	5.1	50
Phosphate	0.01	0.07	0.034	0.02	5
Nitrate-N	0.9	2.8	1.64	0.56	50.0
Nitrite-N	0.004	0.008	0.0056	0.001	3.0
Ammonium-N	0.03	0.11	0.078	0.03	0.5
Zn	0	0.01	0.002	0.004	3.0
Ca	0.03	0.35	0.191	0.08	150
Mn	0	0.02	0.004	0.01	0.05
Fe	0	0	0	0	0.2

CONCLUSION

While the chemical parameters of spring water were within permitted levels, the results of the current study indicate concerns over the microbiological quality of the water of the spring water in Riverston, in the Knuckles Mountain range, as they exceeded the permitted levels of coliform bacteria for drinking water according to the Health Ministry regulation in Sri Lanka, Sri Lanka standards and WHO guidelines. Hence, it is important to take necessary precautions, especially as spring waters from these areas are consumed by many and in addition is the main source water for the bottling industry in Sri Lanka.

REFERENCES

[1] WHO (2019). Drinking water – key facts, Report 16th June 2019, World Health Organization, Geneva.

[2] WHO (2016). Drinking water fact sheets, reviewed November 2016. https://reliefweb.int/report/world/drinking-water-fact-sheet-reviewed-november-2016.

[3] UNESCO (2006). Sri Lanka National Water Development Report. Case Study: Sri Lanka. Report. Imbulana, K. A. U. S., Sri Lanka, National Water Development Report (Ed. by Wijesekara, N. T. S. and Neupane, B. R.), MAI&MD, UN-WWAP, UNESCO and University of Moratuwa, Sri Lanka, Paris and New Delhi.

[4] Athukorala, S. W., Weerasinghe, L. S., Jayasooria, M., Rajapakshe, D., Fernando, L., Raffeeze, M. and Miguntanna, N.P. (2013). Ananlysis of Water Quality Variation in Kelani River, Sri Lanka Using Principal Componet Analysis. *In Proceedings of the SAITM Research Symposium on Engineering Advancements*, Malabe, Sri Lanka **27**, 129–135.

[5] Obiri-Danso, K. and Jones, K. (1999). The effect of a new sewage treatment plant on faecal indicator numbers, campylobacters and bathing water compliance in Moreca- me Bay. *Journal of Applied Microbiology* **86**, 603-14.

[6] Hach (2002). Water analysis handbook. 4th Ed. Hach Company, Colorado, USA.

[7] Fawell, J. and Nieuwenhuiisen, M. J. (2003). Contaminants in drinking water: Environmental pollution and health. *British Medical Bulletin* **68**, 199-208.

[8] Downes, F. P. and Ito, K. (2001). Compendium of methods for the microbiological examination of foods. Washington, DC: APHA.

[9] Sri Lanka Standards 894: Part 2: (2020). Specification for Bottled (Packaged) drinking water (3rd Revision), SLSI.

[10] The Gazette of the Democratic Socialist Republic of Sri Lanka, Extraordinary, Part 1: Section (1) –General. Government Notifications, Food Act, No.26 of 1980. No. 1420/4- Monday November 21, 2005, 1A-9A.

[11] Mahagamage, M.G.Y.L., Manage, P.S. and Manage, P.M. (2019). Water quality and microbial contamination status of groundwater in Jaffna peninsula, Sri Lanka. *Journal of Water and Land Development* **40**, 3–12.

[12] Arulnesan, C.A., Kamsan, S., Rajeshkannam, N., Sivapalan, K. and Murugananthan, K. (2015). Contaminated public well water as a source of sporadic outbreak of enteric infection in Northern Sri Lanka. *Sri Lankan Journal of Infectious Diseases* **5**(2), 64–72.

[13] Mahagamage, M.G.Y.L., Pathirage, M.V.S.C. and Manage, P.L. (2020). Contamination status of *Salmonella* spp., *Shigella* spp. And *Campylobacter* spp. In surface and ground water of the Kelani river basin, Sri Lanka. *Water* **12**(8), 2187.

[14] Herath, A.T., Abayasekara, C.L., Chandrajith, R. and Adikaram, N.K.B. (2012). Temporal variation of microbiological and chemical quality of noncarbonated bottled drinking water sold in Sri Lanka. *Journal of food Science* **77**, 160-164.

[15] Sasikaran, S., Sritharan, K., Balakumar, S. and Arasaratnam, V. (2012). Physical, chemical and microbial analysis of bottled drinking water. *Ceylon Medical Journal* **57**, 111-116.

[16] Herath, A.T., Abayasekara, C.L., Chandrajith, R. and Adikaram, N. K.B. (2014). *Pseudomonas aeruginosa* in bottled drinking water in Sri Lanka: a potential health hazard. *Water Supply* **14**, 1045-1050.

[17] WHO (2011). Guidelines for Drinking-Water Quality (4^{th} edn.) Microbiological Methods. World Health Organization, Geneva.

[18] Sri Lanka Standards 614: part 1: (1983). Specification for potable water – pt. 1: Physical and chemical requirement. Colombo. SLSI. 1–19.