Seasonal Variation of Microbial Quality of Irrigation Water in Different Sources in the Kathmandu valley, Nepal

S. Shrestha[1*], E. Haramoto[2], J. B. Sherchand[3], S. Rajbhandari[4], M. Prajapati[5], J. Shindo[2]

[1]Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi 400-8510 Japan
[2]Graduate Faculty of Interdisciplinary Research, University of Yamanashi Yamanashi 400-8510 Japan
[3]Public Health Research Laboratory, Institute of Medicine, Tribhuvan University, Kathmandu, Nepal
[4]The Small Earth Nepal, Kathmandu, Nepal
[5]Center of Research for Environment, Energy and Water, Kathmandu, Nepal
[*]Corresponding author: shankarshresthabandipur@gmail.com

***Abstract* – River water, sewage, and groundwater are commonly used for irrigation in the Kathmandu valley, Nepal. However, data on their microbial quality is limited. In this study, 24 water samples were collected during the mid-rainy season (August 2015) from rivers, sewage, groundwater, ponds, a canal, and from effluent from a wastewater treatment plant (WWTP) in the valley. All samples were tested for waterborne protozoa (*Giardia* and *Cryptosporidium*) by fluorescence microscopy and for detection of indicator bacteria (*Escherichia coli* and total coliforms) by the most probable number (MPN) method using Colilert reagent. Positive ratios of 96%, 79%, and 50% were obtained for indicator bacteria, *Giardia*, and *Cryptosporidium*, respectively. The contamination level in the river water was as high as in the sewage. Indicator bacteria and protozoan concentrations were the highest for sewage and river water samples, and the lowest for groundwater samples. The results obtained were compared to those from a previous study for late-rainy (September 2014) and dry (April 2015) seasons. The mean concentrations of indicator bacteria and protozoa obtained in this study (mid-rainy season) were significantly lower than those reported in the dry season (*t*-test, $p < 0.05$). This corresponded well with the highest precipitation amount in August, and the lowest amount in April, suggesting dilution as a cause for the decrease in concentrations. Despite having lower concentrations than those reported in the dry and late-rainy seasons, all water samples, except one from groundwater, exceeded the WHO standards for *E. coli* for use in irrigation, indicating strong contamination of irrigation water. Positive correlations and stability of the regression line between indicator bacteria and protozoa concentrations for all seasons suggested *E. coli* and total coliforms as rough indicators of protozoa contamination of irrigation water.**

Keywords – Indicator Bacteria, Irrigation Water, Kathmandu, Protozoa, Seasonal Variation.

1. INTRODUCTION

Nepal's average for total water withdrawal for use in agriculture (irrigation and livestock) is estimated to be 8,226 m^3/yr/ha (0.26 l/s/ha), where surface water, groundwater, and mixed water (surface water + groundwater) contribute to 80%, 19%, and 1% of the withdrawal amount, respectively [1]. The irrigated area in the Kathmandu valley is reported to be 32,000 ha [2]. Information on water use volume for agriculture in the Kathmandu valley is limited. However, we can consider Nepal's average as a general guideline to understand the situation of agricultural water use in the valley. Prior research estimated irrigation water requirements in the valley for winter wheat of 0.18 l/s/ha and 0.30 l/s/ha for potatoes [3]. Apart from a few studies on limited crop types, detailed information on irrigation water demand in the valley is lacking. A widespread practice in the valley for wastewater irrigation is pumping water from sewage, and from other polluted sources like rivers, ponds, and pools. However, this practice is largely unregulated with no proper documentation [4-7]. Similarly, information on emerging health consequences by using irrigation water in the valley is limited [7].

Diarrhea is the most common disease in the valley with high infection rates transmitted through the fecal-oral route from the intake of water or food predominantly contaminated with protozoa, such as *Giardia* and *Cryptosporidium*, and helminths [8,9]. Similarly, other fecal-oral diseases, such as typhoid fever, giardiasis, dysentery, and hepatitis, are also prevalent in the valley [10]. The presence of fecal indicator bacteria such as *Escherichia coli* in the water environment indicates sewage contamination of water sources and presence of other pathogens. Quantification of *E. coli* and protozoan concentrations in irrigation water can therefore help to evaluate the risk of infection to farmers and consumers via contaminated water or consumption of wastewater-irrigated crops, but such information is limited to a few samples and/or seasons [4,11].

Thus, the objectives of this research were to determine protozoan (*Giardia* and *Cryptosporidium*) and indicator bacteria (*E. coli* and total coliforms) concentrations in irrigation water samples [river water, sewage, groundwater, pond water, canal water, and effluent from a wastewater treatment plant (WWTP)] from the mid-rainy season in the Kathmandu valley in order to assess their microbial contamination level, and to compare the results to those reported for dry and late-rainy seasons in a previous study [4] in order to observe seasonal variation. Protozoa analysis is a costly experiment. Indicator bacteria analysis is simpler and cheaper. Therefore, the additional objective was to know suitability of indicator bacteria concentrations to estimate protozoa concentrations [4].

2. METHODOLOGY

2.1 Collection of Water Samples

Twenty four water samples were collected during the mid-rainy (August 2015) season in the Kathmandu valley. Different types of irrigation water were sampled as shown in Table 1.

Sterile polypropylene plastic bottles (1000 ml for groundwater and 100 ml for others) were used to collect water samples. The samples were kept in bags containing ice packs soon after sampling and were transported to the laboratory within 6 h of collection. Figure 1 demonstrates the location of sampling sites. The river sampling sites were RW01–RW05 (Hanumante River), RW06 (Kalacha River), RW07 (Kasan River), RW08 and RW09 (Manahara River), RW10 (Balkhu River), and RW11. All these rivers are tributaries of the Bagmati River, which is a major river flowing through the valley (figure 1). Similarly, other sampling sites are SW01–SW03 (sewage samples), PW01 (Khasi Dathu Pukhu pond), PW02 (Taudaha pond), WWTP (Sunga WWTP, a reed-bed type WWTP), irrigation canal, and GW01–GW06 (groundwater wells).

Table 1 Number of water samples collected in the Kathmandu valley, Nepal

Water Samples	Number of Samples
River water	11
Sewage	3
Groundwater	6
Pond water	2
WWTP effluent	1
Canal water	1
Total	24

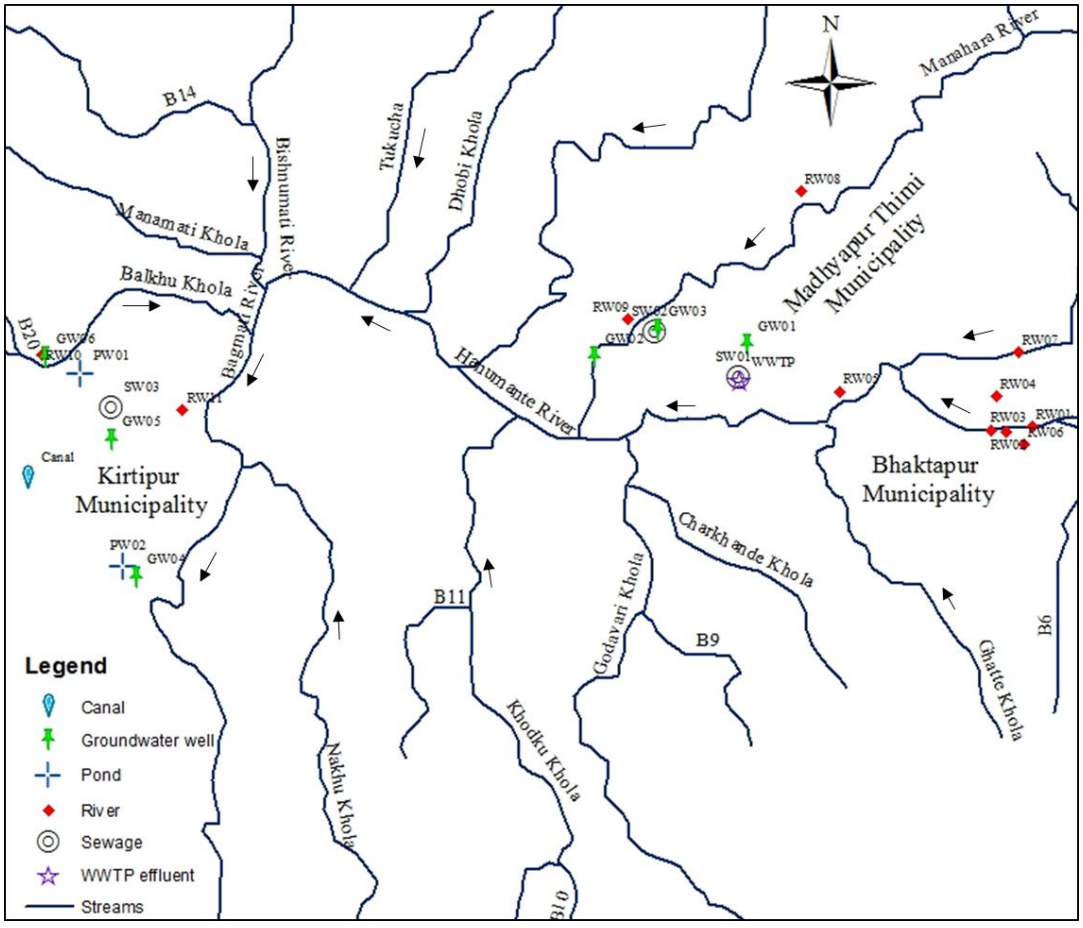

Figure 1 Location of sampling sites in the Kathmandu valley, Nepal

2.2 Detection of Cryptosporidium, Giardia, E. coli, and Total Coliforms

Upon arrival to the laboratory, the water samples were subjected to a protocol for simultaneous concentration of protozoa and viruses in water [12], which consisted of a series of steps including filtration, vortexing in the presence of elution buffer and centrifuging. The volume of each water sample subjected to protozoa detection was 1L for groundwater, and 50 ml for the others. After centrifuging, the resultant pellet was used for protozoa detection, which consisted vortexing in the presence of phosphate buffered saline (PBS (-)) solution, immunomagnetic separation (IMS) using a Dynabeads GC-Combo (Invitrogen, CA, USA), direct fluorescent antibody staining of protozoa on a membrane using an EasyStain (BTF, North Ryde, Australia), and fluorescence microscopy using a fluorescence microscope BX60 (Olympus, Tokyo, Japan) [4].

All the samples were subjected to the protocols for detection of *E. coli* and total coliforms. *E. coli* and total coliforms present in 100 ml of original and diluted water samples (dilution ratio: 10^2, 10^4, and 10^6) were determined by the most probable number (MPN) method using Colilert reagent (IDEXX Laboratories, ME, USA) according to the protocol of the manufacturer.

2.3 Statistical Analyses

Statistical analyses were performed to evaluate the variation of microbial concentrations in water samples taken in different seasons (e.g. mid-rainy vs. dry seasons), different sources (e.g. river vs. sewage), and different pathogens (e.g. *Giardia* vs. *Cryptosporidium*). The log values of indicator bacteria and protozoa concentrations were used for representations and statistical analyses. The limit of detection (LOD) for protozoa analysis was 20 (oo)cysts/L (1.3 log (oo)cysts/L) for all samples except for groundwater, where LOD was 1 (oo)cyst/L (0 log (oo)cyst/L). Similarly, LOD for indicator bacteria analysis was 1 MPN/100 ml (0 log MPN/100 ml). For samples with no indicator bacteria or protozoa detection, one-tenth value of LOD was used for statistical analysis as done in some studies [4,13]. Indicator bacteria–protozoa relations were evaluated based on regression analysis, analysis of variance (ANOVA) and analysis of covariance (ANCOVA). The Tukey's *q*-test or Scheffe test was done for multiple-data comparisons, whereas the *t*-test or Welch test was performed for unpaired two-data comparisons, and the *t*-test for paired data comparison by using Excel statistics software 2015 for Windows (Social Survey Research Information, Tokyo, Japan).

3. Results and Discussion

3.1 Variation of Cryptosporidium, Giardia, Total Coliforms, and E. coli Concentrations in Water Samples from Various Irrigation Sources

Table 2 shows the positive ratios and concentrations of *Cryptosporidium*, *Giardia*, total coliforms, and *E. coli* in water samples from various irrigation sources in the mid-rainy (August 2015) season. The highest positive ratio was obtained for *E. coli* and total coliforms (96%), followed by *Giardia* (79%) and *Cryptosporidium* (50%). *Cryptosporidium* was not detected in the samples from the Manahara River upstream (RW08), the Kasan River (RW07), groundwater wells, ponds, WWTP effluent, and the canal.

As shown in Table 2, the average concentrations of protozoa and indicator bacteria were highest for sewage and river water samples, followed by the pond, WWTP effluent, and canal water samples, and the lowest was for the groundwater samples. The differences were statistically significant between sewage and groundwater samples, between sewage and other samples, between river water and groundwater samples, and between river water and other samples for *Giardia* (Scheffe test, $p < 0.05$), and also between sewage and groundwater samples, and between river water and groundwater samples for *E. coli* (Scheffe test, $p < 0.05$). However, no significant difference was obtained among all sample types for total coliforms (Scheffe test, $p > 0.05$). The river water and sewage samples had no significant difference for all the pathogens tested (*t*-test for *Cryptosporidium*, and Scheffe test for *Giardia*, *E. coli* and total coliforms, $p > 0.05$).

According to World Health Organization (WHO), *E. coli* should be less than or equal to 3 log counts per 100 ml, for wastewater used under unrestricted irrigation for root crops [14]. However, except for one groundwater sample, the WHO standards were exceeded in all the samples (Table 2), suggesting strong contamination of all types of irrigation water. High *E. coli* concentration suggests contamination of a waterway by sewage and the possible presence of other pathogens [4]. Similarly, high concentrations of protozoa obtained in the current study, especially for river water and sewage, indicate increased health risk for farmers and consumers in the region by using river water and sewage for irrigation. Much higher concentrations of *Giardia* than *Cryptosporidium* (*t*-test, $p < 0.05$) indicates high risks of *Giardia* infection.

Among the river water samples, protozoa and indicator bacteria concentrations were generally higher for those from the Hanumante River, the Kalacha River, the river RW11, and the Manahara River downstream than those for the Balkhu River, the Kasan River, and the Manahara River upstream. The reason for this difference in concentrations among rivers might be due to the difference in population distribution near these river areas. Hanumante River, Kalacha River, and the river RW11 are located near city areas which may have resulted in high pollution from human activity. On the other hand, Manahara River upstream, Balkhu River, and Kasan River are located farther from the city. However, all samples exceeded the water quality criteria and standards proposed for the Bagmati River and its tributaries, where total coliforms should be less than 3 log MPN/100 ml for use in agriculture [15], indicating strong contamination of rivers. Our previous study showed the standards for total coliforms were also exceeded in these rivers in the dry (April 2015) and late-rainy (September 2014) seasons [4], suggesting contamination of these rivers throughout the year.

Table 2 Positive ratio and concentrations of *Cryptosporidium*, *Giardia*, total coliforms, and *E. coli* in water samples from various irrigation sources in the mid-rainy (August 2015) season

Protozoa/indicator bacteria	Concentration (log (oo)cysts/L or log MPN/100 ml) and % positive						
	River water	Sewage	Ground-water	Pond water	WWTP effluent	Irrigation canal	Total
Cryptosporidium							
Positive/total samples	9/11	3/3	0/6	0/2	0/1	0/1	12/24
% positive	82	100	0	0	0	0	50
***Min. concentration**	1.3	1.3	-	-	-	-	
Max. concentration	2.3	2.9	-	-	-	-	
****Mean ± sd**	1.5 ± 0.7	2.3 ± 0.9	-	-			
Giardia							
Positive/total samples	11/11	3/3	3/6	1/2	1/1	0/1	19/24
% positive	100	100	50	50	100	0	79
***Min. concentration**	1.6	2.5	0	1.6	2	-	
Max. concentration	3.9	4.5	1.4	1.6	2	-	
****Mean ± sd**	3.1 ± 0.6	3.7 ± 1.1	-0.1 ± 1.1	1.0 ± 1.0			
Total coliforms							
Positive/total samples	11/11	3/3	5/6	2/2	1/1	1/1	23/24
% positive	100	100	83	100	100	100	96
***Min. concentration**	5.1	6.1	4.3	4.9	5.2	4.7	
Max. concentration	7.4	7.9	7.0	5.4	5.2	4.7	
****Mean ± sd**	6.6 ± 0.8	7.2 ± 1.0	4.7 ± 3.0	5.0 ± 0.3			
Escherichia coli							
Positive/total samples	11/11	3/3	5/6	2/2	1/1	1/1	23/24
% positive	100	100	83	100	100	100	96
***Min. concentration**	3.9	5.4	3.0	3.4	4.2	4.0	
Max. concentration	7.0	7.5	5.0	4.2	4.2	4.0	
****Mean ± sd**	6.1 ± 0.9	6.7 ± 1.1	3.2 ± 2.2	3.9 ± 0.4			

*Remark: *minimum among detected values,*
***undetected values (10% of LOD) considered for calculating average and sd,*
Sd = standard deviation

3.2 Seasonal Variation of Cryptosporidium, Giardia, Total Coliforms, and E. coli Concentrations in Irrigation Water Samples

Indicator bacteria and protozoan concentrations obtained in this study (mid-rainy season) for river water, sewage and others (ponds, WWTP effluent, and canal) were compared to those reported in our previous study for dry and late-rainy seasons [4] to observe seasonal variation. Groundwater samples used for irrigation were analyzed only in this study so the comparison with previous studies could not be done. Table 3 shows variation of *Cryptosporidium*, *Giardia*, total coliforms, and *E. coli* concentrations in water samples from the paired datasets of the three seasons. Numbers for paired samples between the mid-rainy and dry seasons were different than those between the mid-rainy and late-rainy seasons (Table 3) because of the different sample size for each season.

The indicator bacteria and protozoan concentrations were highest for the dry season (April 2015) samples, followed by the late-rainy season (September 2014) samples, and the lowest for the mid-rainy season (August 2015) samples. Interestingly, comparing the 3 months, the average precipitation amount recorded by the Department of Hydrology and Meteorology, Kathmandu, at 19 precipitation stations in the valley (1967–2010) was the highest for August (410 mm), followed by September (226 mm), and the lowest for April (59 mm). This suggests that dilution might be a reason for decrease in the concentration in the rainy-seasons. The differences were statistically significant between the dry and mid-rainy seasons (*t*-test, $p < 0.05$). However, the differences between the mid-rainy and late-rainy seasons were not significant (*t*-test, $p > 0.05$). During the dry season, the frequency of water use from these polluted sources for irrigation is expected to increase due to limited rainfall. Therefore, health risks might be higher for farmers and consumers during the dry season as a result of wastewater irrigation in the Kathmandu valley. However, specific conclusions can be drawn only after performing detailed health risk analysis for each season. Further studies will focus on seasonal health risk analysis for farmers and consumers as a result of wastewater irrigation in the valley.

3.3 Analysis of Indicator Bacteria–Protozoa Correlations in Water Samples

Regression analysis, ANOVA and ANCOVA for each indicator bacteria–protozoa correlation showed no significant difference of slope and intercept among the

regression lines for the three seasons (results are not shown), suggesting strong stability of the regression lines for each indicator bacteria–protozoa correlation. Therefore, a single regression line was used representing all seasons for each indicator bacteria–protozoa correlation. Data of indicator bacteria–protozoa relations for different seasons are shown with different colors (figure 2).

Coefficient of determination R^2 values of 0.8, 0.7, 0.6, and 0.5 with significant F (< 0.05) values, were obtained for *E. coli–Giardia*, *E. coli–Cryptosporidium*, total coliforms–*Giardia*, and total coliforms–*Cryptosporidium* correlations, as shown in figure 2 (total coliforms–protozoa correlations are not shown), suggesting *E. coli* as a better indicator than total coliforms to show the extent of contamination of protozoa. Thus, *E. coli* concentration can be used for estimation of protozoa concentrations in irrigation water which can help to alleviate the high cost of protozoa analysis.

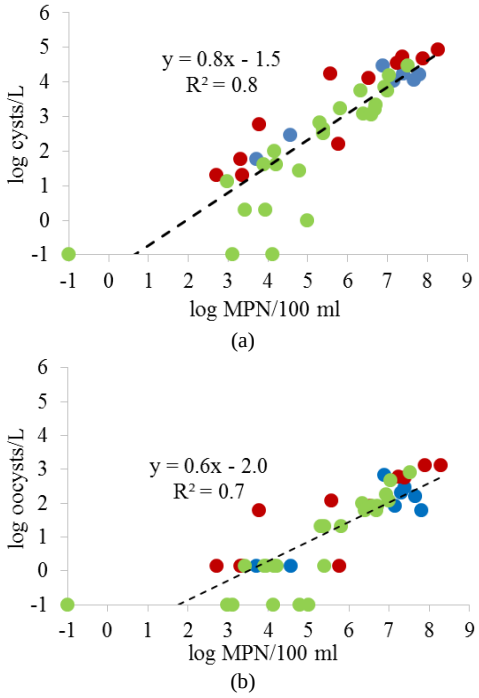

Figure 2 Relationships between indicator bacteria and protozoan concentrations in water samples
(a) *E. coli–Giardia* (b) *E. coli–Cryptosporidium*
(Green: August 2015, Red: April 2015, and Blue: September 2014)

Table 3 Seasonal variation of *Cryptosporidium*, *Giardia*, total coliforms, and *E. coli* concentrations in irrigation water samples from paired datasets

Protozoa/indicator bacteria	**Concentration (log (oo)cysts/L or log MPN/100 ml)**			
	August 2015 (mid-rainy)	April 2015 (dry)	September 2014 (late-rainy)	August 2015 (mid-rainy)
Cryptosporidium	*			
Total paired samples	11	11	6	6
Mean	1.2	1.6	2.2	2.1
sd	± 0.9	± 1.3	± 0.4	± 0.3
Giardia	*			
Total paired samples	11	11	8	8
Mean	2.5	3.3	3.7	3.4
sd	± 1.3	± 1.5	± 1.0	± 0.9
Total coliforms	*			
Total paired samples	15	15	13	13
Mean	6.4	6.9	6.7	6.4
sd	± 1.0	± 1.6	± 1.4	± 1.1
Escherichia coli	*			
Total paired samples	15	15	13	13
Mean	5.7	6.2	6.0	5.8
sd	± 1.3	± 2.0	± 1.8	± 1.4

*Remark: *statistically significant difference (t-test, $p < 0.05$), Sd = standard deviation*

4. CONCLUSIONS

- Concentrations of indicator bacteria and protozoa present in the water samples from the mid-rainy season were significantly lower than those reported for the dry season, suggesting that dilution as a result of high precipitation in the mid-rainy season might be responsible for the decrease in microbial concentrations.
- Although lower than that reported for the dry season, concentrations of *E. coli* obtained in this study exceeded the WHO standards for use of wastewater in irrigation (3 log counts per 100 ml) in 23 out of 24 water samples, indicating strong contamination and public health concerns all the year round.
- The microbial concentrations in river water in the mid-rainy season were not significantly different from those in sewage samples. Similar findings were reported for the dry and late-rainy seasons, suggesting strong contamination of river water throughout the year.
- *E. coli* can be regarded as a good indicator of protozoa contamination of irrigation water sources.

5. ACKNOWLEDGEMENTS

This study was supported by the Science and Technology Research Partnership for Sustainable Development (SATREPS) program of JICA and JST, entitled 'Hydro-microbiological Approach for Water Security in Kathmandu valley, Nepal'. Many thanks to Mr. Roy Morien of the Naresuan University Language Centre for his editing assistance and advice on English expression in this manuscript.

6. REFERENCES

[1] FAO. Aquastat. (2011). "Irrigation in southern and eastern Asia in figures – AQUASTAT survey – 2011," Available: http://www.fao.org/nr/water/aquastat/countries_regions/npl/NPL-CP_eng.pdf

[2] CRAD/GON, "Regional statistical book of agriculture," Regional Directorate of Agriculture (Central-Region), Government of Nepal, Lalitpur, Nepal, 2013.

[3] P. Pradhan, "Water demand forecast and management modelling in Kathmandu valley, Nepal," M.Sc. Thesis (Ref. No. WM-02-11), Asian Institute of Technology, Bangkok, Thailand, Apr. 2003.

[4] S. Shrestha, E. Haramoto, J. B. Sherchand, S. Hada, S. Rajbhandari, and J. Shindo, "Prevalence of protozoa and indicator bacteria in wastewater irrigation sources in Kathmandu valley, Nepal: cases from Kirtipur, Bhaktapur, and Madhyapur Thimi municipalities," *Journal of Water and Environment Technology*, in press.

[5] T. L. Rutkowski, L. Raschid-Sally, and S. Buechler, "Wastewater irrigation in the developing world—two case studies from the Kathmandu valley in Nepal," *Agric. Water Manage.*, *88(1)*, pp. 83-91, 2007.

[6] S. Shrestha, E. Haramoto, J. B. Sherchand, and J. Shindo, "Detection of coliform bacteria in irrigation water and on vegetable surfaces in the Kathmandu valley of Nepal," *J. Inst. Med.*, in press.

[7] A. Shukla, U. R. Timilsina, and B. C. Jha. (2012). "Wastewater Production, Treatment and Use in Nepal," Available: http://www.ais.unwater.org/ais/pluginfile.php/232/mod_page/content/120/Nepal_CountryPaper.pdf

[8] K. Ono, S. K. Rai, M. Chikahira, T. Fujimoto, H. Shibata, Y. Wada, H. Tsuji, Y. Oda, G. Rai, C. D. Shrestha, K. Masuda, H. G. Shrestha, T. Matsumura, H. Hotta, T. Kawamura, and S. Uga, "Seasonal distribution of enteropathogens detected from diarrheal stool and water samples collected in Kathmandu, Nepal," *Southeast Asian J. Trop. Med. Public Health*, *32(3)*, pp. 520-526, 2001.

[9] Y. Oda, J. B. Sherchand, and C. K. Gurung, "Cryptosporidium and Cyclospora studies in two seasons at Kanti-Children Hospital Kathmandu Nepal," *Parasitol. Int.*, vol. 47, pp. 166, 1998.

[10] A. Neupane. (2013, May 6). "Summer scares water and food-borne diseases," *The Kathmandu Post* [Online]. Available: http://kathmandupost.ekantipur.com/printedition/news/2013-05-05/summer-scares-water-and-food-borne-diseases.html

[11] E. Haramoto, K. Yamada, and K. Nishida, "Prevalence of protozoa, viruses, coliphages and indicator bacteria in groundwater and river water in the Kathmandu valley, Nepal," *Trans. R. Soc. Trop. Med. Hyg.*, *105(12)*, pp. 711-716, 2011.

[12] E. Haramoto, H. Katayama, M. Asami, and M. Akiba, "Development of a novel method for simultaneous concentration of viruses and protozoa from a single water sample," *J. Virol. Methods*, *182(1-2)*, pp. 62-69, 2012.

[13] N. Kishida, H. Morita, E. Haramoto, M. Asami, and M. Akiba, "One-year weekly survey of noroviruses and enteric adenoviruses in the Tone River water in Tokyo metropolitan area, Japan," *Water Res.*, *46(9)*, pp. 2905-2910, 2012.

[14] WHO, "WHO guidelines for the safe use of wastewater, excreta and greywater. Vol II: Wastewater use in agriculture," World Health Organization, Geneva, Switzerland, 2006.

[15] S. Sharma, R. M. Bajracharya, B. K. Sitaula, and J. Merz, "Water quality in the Central Himalaya," *Curr. Sci.*, *89(5)*, pp. 774-786, 2005.