International Journal of Scientific Research and Engineering Development-– Volume 6 Issue 3, May-June 2023

Available at www.ijsred.com

RESEARCH ARTICLE OPEN ACCESS

Biodiversity of Conservational Environmental Forms of Protozoa and Helminthes in the Drinking Water of Bamenda Municipality (North-West Region of Cameroon): Affiliation to Morphometric and Organoleptic Properties

Asakizi Nji Augustine[1,2] and Ajeagah Gideon Aghaindum[1]

1- Laboratory of Hydrobiology and Environment, faculty of science, university of Yaounde1

1,2Department of Microbiological Sciences, Faculty of Science, University of Bamenda

Corresponding email:asakizinji@gmail.com ;ajeagahg@yahoo.com

Abstract

Waterborne pathogens protozoa,helminthes are a direct threat to human health. Health-care costs attributed to some of the leading causes of waterborne diseases in the United States are estimated at more than $1 billion annually. The fate and transport of waterborne pathogens, into, and within waterbodies is influenced by factors that include precipitation and runoff; the type and location of sources on land; and the survival characteristics of individual organisms. Organism survival in environmental matrices in the drinking water in Bamenda(Up station, Nsongwa,Mankon, Nkwen) such as soil, water, and fecal waste is influenced by moisture, nutrient availability, temperature and sunlight.The organoleptic properties were analysed by Rodier, methodology with the aid of a pH meter and a spectrophotometer, while specialized techniques of katokatz, direct observation of water samples for infectious forms were applied in the identification of protozoan cysts and helminthic infectious forms. The organoleptic properties present the water pH varies from 5.0 to 6.0, the color is mostly clear , the water is odourless, *C parvum* is 5 um,*Entamoeba coli* was 12um, *Giardia lamblia* was 12um, *Ascarislumbricoides* was 152um, *Diphylobotumlatum* was 65, nematode larvae is 2mm, T cati(70um),Hymenolepis nana(45um),Taenasaginata(32um).

Key words: Biodiversity, protozoa, helminthes ,drinking water , morphometric organoleptic.

1. Introduction

.Biological properties of water are much related to gastroenteritis in many ways. For instance some

 ©IJSRED: All Rights are Reserved

organic content of water serves as excellent nutritional sources for the growth and multiplication of microorganisms. These pathogens are responsible for intestinal infections (gastroenteritis) such as bacillary dysentery, diarrhea, typhoid fever, cholera and paratyphoid fever, amoebiases, helminthiases, and viral water born diseases.

The origin of most common waterborne pathogens can be traced to the fecal wastes of animals and humans. More than 100 different types of pathogens can be found in contaminated water (ASM 1998).Waterborne pathogens spread to humans through ingestion of contaminated drinking water, exposure to contaminated water from recreational activities like swimming, or indirectly through contaminated food (Rose et al. 2001; Charron et al. 2004). Outbreaks of waterborne illness are most prevalent in economically disadvantaged communities where water supplies and sanitation are often inadequate. Human exposure can cause gastrointestinal, respiratory, eye, ear, nose, and throat irritation; skin diseases; impairment of cells of the digestive tract and organs; and life-threatening infections in people with depressed immune systems (USEPA 2001).Many waterborne pathogens are difficult to identify and isolate.The pathways between waterborne pathogens, their sources, and waterbodies is dependent on interactions between climate, hydrology, and land use.

Surface water and groundwater are susceptible to fecal contamination (which may contain pathogenic organisms) from agricultural runoff, sewage, and domesticated animals (Cho et al. 2010). Fecal waste can be discharged directly into waterbodies as point sources or transported to waterbodies in runoff from nonpoint sources (NPSs) or subsurface water flow (Schijven and Husman 2005; Arnone and Walling 2007). Major point source discharges originate from wastewater treatment plants (WWTPs) and aging/failing infrastructure, sanitary sewer overflows (SSOs) and combined sewer overflows (CSOs).Infrastructural impediments in favourof fecal contamination include septic tank leachate, runoff from land, urban litter, contaminated refuse, domestic pet and wildlife excrement (USEPA 2001; Benham et al. 2006).The persistence of pathogens in the environment depends on the organism and environmental conditions in upland sources (e.g., soils, manure) and water-bodies. Temperature and solar radiation are primary climate drivers that affect pathogen survival. Cooler temperatures generally enable longer survival times for many common waterborne pathogens (USEPA 2013). The effect of temperature on waterborne pathogens can differ, however, depending on the species/strain of organism (Herrador et al. 2015). Sunlight directly affects the survival of pathogens that are vulnerable to ultraviolet radiation and desiccation (Tyrrel and Quinton 2003). Precipitation and runoff transport fecal waste from upland sources to

 ©IJSRED: All Rights are Reserved

waterbodies.The lack of studies about potential future water quality changes can be a challenge to managers who must make specific decisions in the best interest of their programs and clients.

Waterborne pathogens can survive for long periods in different environmental matrices (e.g., soil, manure, and water) when conditions are favorable. Low temperatures, appropriate moisture levels, nutrient availability, and protection from external factors, such as ultraviolet radiation, can prolong survival for many common waterborne pathogens. Temperature, in particular, is an important factor affecting survival (Cho et al. 2016). Waterborne pathogens sourced to fecal waste are affected by urban and agricultural land use, pollutant discharges and water management infrastructure. The effects of climate change will interact with fecal sources on land (e.g., human, livestock and wildlife) in different regional and watershed settings. Combined effects of organoleptic properties and land use on pathogens, however, are yet to be fully integrated in most studies. More information on water management and the characterization of pathogens is still farfetched. The objective of this study is to assess the biodiversity of infectious forms of protozoan and helminths in drinking water in the municipality of Bamenda and to to correlate their availability with organoleptic properties of the ecosystems(well , tap,spring water).

2.Materials and Methods

The samples were taken in Bamenda municipality, at potable drinking water spots(at upstation,Nkwen, Nsongwa and Mankon).The water taken from the 1000 cc polyethylene bottles was brought back to the hydrobiology and environment laboratory of the University of Bamenda and the University of Yaoundé 1 for analysis.

a) Measurement of physico-chemical parameters

The physico-chemical analyzes took place both in the field and in the laboratory following the recommendations of Rodier et al. (2009).

b) Field measurements

In the field parameters such as temperature (°C), dissolved oxygen (% saturation), Ph (UC) respectively using an electronic thermometer, an oximeter

c) Experimental protocol

Observation of resistance forms of protozoa

The samples were collected using sterile 1 L polyethylene bottles and then transported to the laboratory. They will then be left to rest at ambient temperature for 24 hours for sedimentation, then the supernatant was poured out and the volume of the pellet was collected and assayed. Cysts and oocysts are observed under an optical microscope with a 40 X objective.

d) Sedimentation method

After homogenization of the pellet, 5ml of the sample (controls and tests) were taken and

 ©IJSRED: All Rights are Reserved

introduced into a test tube. The mixture obtained is brought to centrifugation at 1500 revolutions/min for 5 min using a centrifuge.

e) Zinc sulphate flotation method or modified Faust technique

This method allows the flotation of the cysts. After homogenization of the pellet, 5 ml of the sample are taken and introduced into a test tube. To this is added 3 ml of 33% zinc sulphate (specific density of 1.18) and the mixture obtained is brought to centrifugation at 500revolutions/min for 4 to 5 min using a centrifuge. Next, the surface layer of the supernatant was removed using a pipette and spread between slide and cover slip.

f) Modified Ziehl–Neelsen method

It is a method which makes it possible to highlight the oocysts of protozoa. It consists of staining the slides. Indeed, a 10% zinc sulphate solution (allowing the oocysts to float) is added to the samples taken and distributed in the test tubes. The contents of these test tubes are then centrifuged at 500 revolutions/min for 5 min using a MINOR35 brand centrifuge to float the oocysts. The supernatant is removed using a micropipette and distributed on slides which are then air-dried to promote the adhesion of the sample to the slides. The slides are fixed in methanol and stained with basic fuchsin for 1 and 5 minutes respectively, rinsed with distilled water and 2% sulfuric acid (acting as a decolorizer for organisms other than oocysts) for 2minutes. The slides are rinsed again and counterstained with 5% methylene blue (which stains other structures or organisms with the exception of oocysts. The preparation was placed on the stage of a YVIMEN brand microscope for observation.

g) Identification and counting of cysts and oocysts

Human parasitic intestinal cysts and oocysts were identified using the WHO charts (1994). The measurements of the dimensions will be made using a micrometer carried by one of the eyepieces of the microscope. The number (X) of parasitic cysts and oocysts in 1 L of samples was obtained using the formula of Ajeagah et al., 2014:

$$X= (y.Vx)/Vy$$

With: Vx= pellet volume in 1 L of samples, Vy= pellet volume used for observation, y= number of cysts observed in Vy.

h) Calculation of treatment efficiency

The performances of the STEP are expressed in terms of reduction rate on various parameters.Each abatement rate is calculated using the following form.With :

R (%) is the abatement rate for a given parameter (COD, BOD5, TSS, Ptotal, etc.),

Pe is the value of the parameter at the input.

Ps is the value of the same parameter at the output.

In order to assess the performance of the STEP, it is necessary to compare the abatement rates x%, y% and z% with the standards (Khammar et al., 2013).

i) Comparison test

 ©IJSRED: All Rights are Reserved

The spatial variation of the physicochemical and biological parameters measured was tested using direct comparison of the biological and physico chemical variables.

3.Results and Discusion

The resuls of the Organoleptic properties of water and qualitative diversity of protozoa and helminths in the different sampling stations are presented in table 1. Quantitative biodiversity of protozoan and helminthes in the different sampling points in table 2 and Morphometric characteristics of protozoan and helminthes in the different sampling points in table 3.

Table 1: Organoleptic properties of water and qualitative diversity of protozoa and helminths in the different sampling stations.

a) UP STATION

The pH varies from 5.0 to 6.0, the coloris mostly clear , the water is odourless, and the protozoan identified are *E. histolytica* cysts. Cryptosporidiumparvum cysts were not isolated(Table 1a).

S/N	Source	Physical properties	Protozoa/Helminths	*Cryptosporidoium pavum*
1.	Spring 1	pH: 5.8 Color : Clearwithdebris Ordour : Present	Negative	Negative
2.	Spring 2	pH: 6. Color : Clearwithdebris Ordour : Present	*E. histolytica*cysts	Negative
3.	Tap 1	pH: 6.0 Color : Clear Ordour : None	Negative	Negative
4.	Tap 2	pH: 5.8 Color : Clear Ordour : None	Negative	Negative
5.	Well 1	pH: 5.0 Color : Clear Ordour : None	*E. histolytica*cysts	Negative
6.	Well 2	pH: 5.6 Color : Clearwithdebris Ordour : None	*E. histolytica*cysts	Negative

 ©IJSRED: All Rights are Reserved

b) MANKON

The pH varies from 5.2 to 6.0, the coloris mostly clear, and the protozoan identified are *E. histolytica*cysts,Balantidium coli, G lambia,Ascarislumbricoides. Cryptosporidiumparvum cysts were not isolated(table 1b).

S/N	Source	Physical properties	Protozoa/Helminths	*Cryptosporidoium pavum*
1.	Spring 1	pH: 5.9 Color : Clear Ordour : Present	-*E. histolytica*cysts	Negative
2.	Spring 2	pH: 6.2 Color : Clearwithdebris Ordour : Present	- *E histolyticacysts*	Negative
3.	Tap 1	pH: 6.0 Color : Clear Ordour : None	- *B. coli cysts*	Negative
4.	Tap 2	pH: 6.0 Color : Clear Ordour : None	Negative	Negative
5.	Well 1	pH: 5.2 Color : Clear Ordour : None	*G. lambliacysts* *D. latumeggs*	Negative
6.	Well 2	pH: 5.7 Color : Cloudy Ordour : None	*B. coli cysts* *A. lumbricoideseggs*	Negative

 ©IJSRED: All Rights are Reserved

c) NKWEN

The pH varies from 5.5 to 6.2, the coloris mostly clear, and the protozoan identified are *E. histolytica* cysts. Cryptosporidiumparvum cysts were not isolated (table 1c).

S/N	Source	Physical properties	Protozoa/Helminths	*Cryptosporidoium pavum*
1.	Spring 1	pH: 5.9 Color : Clearwithdebris Ordour : Present	*A. lumbricoides eggs*	Negative
2.	Spring 2	pH: 5.5 Color : Clear Ordour : None	*E. histolyticacyst*	Negative
3.	Tap 1	pH: 6.0 Color : Clear Ordour : None	Negative	Negative
4.	Tap 2	pH: 6.0 Color : Clear Ordour : None	Negative	Negative
5.	Well 1	pH: 6.2 Color : Clearwithdebris Ordour : None	*E. histolyticacyst*	Negative
6.	Well 2	pH: 6.0 Color : Clearwithdebris Ordour : None	Negative	Negative

 ©IJSRED: All Rights are Reserved

d) NSONGWA

- The pH varies from 5.7 to 6.5, the color is mostly clear, and the protozoan identified are *E. histolytica* cysts. *F F. hepatica eggs,.hepatica eggs, A. limbricoides*eggs,*Giardia*and *Cryptosporidium*(table 1d).

S/N	Source	Physical properties	Protozoa/Helminths	*Cryptosporidoiumpavum*
1.	Spring 1	pH: 5.7 Color : Clearwithdebris Ordour : Present	- *F. hepatica eggs* - *A. limbricoideseggs*	Positive
2.	Spring 2	pH: 5.8 Color : Clear Ordour : Present	- *A. limbricoideseggs* - *F. hepaticaeggs*	Negative
3.	Tap 1	pH: 6.0 Color : Clear Ordour : None	*E. histolyticacysts*	Negative
4.	Tap 2	pH: 6.5 Color : Clear Ordour : None	Negative	Negative
5.	Well 1	pH: 6.2 Color : Clearwithdebris Ordour : None	- *G. lambliacysts*	Negative
6.	Well 2	pH: 5.9 Color : Clearwithdebris Ordour : Present	- *G. lambliacysts*	Negative

Table 2: Quantitative biodiversity of protozoan and helminthes in the different sampling points

 ©IJSRED: All Rights are Reserved

a)UP STATION

Entamoebahistolytica was prominent in well 1 and spring 1(table 2a).

	Groundwater (wells and springs)				Surface water	
	Well 1	Well 2	Spring 1	Spring 2	Tap 1	Tap 2
E histolytica (cyst/L)	6	0	10	0	0	0

b)MANKON

Balantidium coli and Giardia lamndia were very prominent. Ascarislumbricoides was prominent in well 2.(table 2b).

	Groundwater (wells and springs)				Surface water	
	Well 1	Well 2	Spring 1	Spring 2	Tap 1	Tap 2
A lumbricoides(egg/L)	0	5	0	0	0	0
B coli (cyst/L)	0	9	0	5	0	0
E histolytica(cysts/L)	0	0	0	5	0	0
D latum(eggs/L)	2	0	0	0	0	0
G lamblia(cysts/L)	5	0	0	0	0	0

 ©IJSRED: All Rights are Reserved

c)NKWEN

A lumbricoides(egg/L) were present in spring 1,while*E histolytica* (cyst/L)was also present in the same spring..(table 2c)

	Groundwater (wells and springs)				Surface water	
	Well 1	Well 2	Spring 1	Spring 2	Tap 1	Tap 2
A lumbricoides(egg/L)	0	0	1	0	0	0
E histolytica (cyst/L)	0	0	6	0	0	0

d)NSONGWA

A lumbricoides(egg/L) was identified in spring 1, *Glamblia*(cysts/L) in well 1, *E histolytica*(cyst/L) *in tap water 1, F hepatica* (eggs/L) in spring 1 and *C pavum*(oocysts/L) in spring 1..(table 2d)

	Groundwater (wells and springs)				Surface water	
	Well 1	Well 2	Spring 1	Spring 2	Tap 1	Tap 2
A lumbricoides(egg/L)	0	0	2	0	0	0
G lamblia(cysts/L)	5	0	0	0	0	0
E histolytica(cyst/L)	0	0	0	0	2	0
F hepatica (eggs/L)	0	0	2	0	0	0
C pavum(oocysts/L)	0	0	2	0	0	0

Table 3:Morphometric characteristics of protozoan and helminthes in the different sampling points

a)Well

 ©IJSRED: All Rights are Reserved

C parvum was 5,2um,Entamoeba coli was 12um, giardia lamblia was 9um, Ascarislumbricoides was 52um, diphylobotumlatum was 70 umandFasciola hepatica was 145um(table 3a)

	Average size
A lumbricoides	≈52µm in diameter
B coli	≈68µm in diameter
C pavum	≈5.2µm in diameter
D latum	≈70µm in diameter
E histolytica	≈12µm in diameter
F hepatica	≈145µm in length
G lamblia	≈9µm in diameter

b) Spring and tap

C parvum is 5 um,Entamoeba coli was 12um, Giardia lamblia was 12um, Ascarislumbricoides was 152um, diphylobotumlatum was 65, nematode larvae is 2mm, T cati(70um),Hymenolepis nana(45um),Taenasaginata(32um)(table 3b).

	Average size
A lumbricoides	≈48µm in diameter (fertilised)
B. coli	≈152µm in diameter
C. pavum	≈5µm in diameter
D. latum	≈65µm in diameter
E.histolytica	≈14µm in diameter

 ©IJSRED: All Rights are Reserved

S. hematobium	≈125µm in length
G lamblia	≈12µm in diameter
Nematode lavae	≈2mm long
T cati	≈70µm in diameter
H nana	≈45µm in diameter
T saginata	≈32µm in diameter

4.Discussion

Water can harbor germs that threaten the safety of patients and spread antibiotic resistant pathogens or healthcare-associated infections. The contamination of drinking water by pathogens causing diarrhoeal disease is the most important aspect of drinking water quality. The problem arises as a consequence of contamination of water by faecal matter, particularly human faecal matter, containing pathogenic organisms. One of the great scourges of cities in Europe and North America in the 19th century was outbreaks of waterborne diseases such as cholera and typhoid. In many parts of the developing world it remains a major cause of disease as Bamenda municipality is not spared out from recurrent water borne diseases (Tabls1-4). It is therefore essential to break the faecal–oral cycle by preventing faecal matter from entering water sources and/or by treating drinking water to kill the pathogens. However, these approaches need to operate alongside hygiene practices such as hand washing, which reduce the level of person-to-person infection,cleaning the wells and springs and making sure that the sanitary infrastructures for the transmission of potable water quality are up to date..

Detection and enumeration of pathogens in water are not appropriate under most circumstances in view of the difficulties and resources required. The assumption is that if the indicators are detected, pathogens, including viruses, could also be present and therefore appropriate action is required. However, the time taken to carry out the analysis means that if contamination is detected, the contaminated water will be well on the way to the consumer and probably drunk by the time the result has been obtained. It is also essential to ensure that the multiple barriers are not only in place but working efficiently at all times, whatever the size of the supply. The organisms C parvum is 5

 ©IJSRED: All Rights are Reserved

um,Entamoeba coli was 12um, Giardia lamblia was 12um, Ascarislumbricoides was 152um, diphylobotumlatum was 65, nematode larvae is 2mm, T cati(70um),Hymenolepis nana(45um),Taenasaginata(32um)identified in our samples are responsible for many waterborne, gastrointestinal disease in the municipality, as noted by medical sources.The potential for increased pathogen loads in source waters used as a drinking water supply presents a challenge in designing and operating drinking water treatmentto efficiently remove a high number of the pathogens due to their organoleptic and morphometric characteristics.Shallow groundwater wells are likely to be more vulnerable to fecal contamination as they are most influenced by surface runoff (Levin et al. 2002). Many previous waterborne disease outbreaks have been linked to drinking water supplies sourced from groundwater (Levin et al. 2002). al. 2014). The development of simple, fast, and affordable detection methods would permit streamlined analysis and assessment (Jung et al. 2014). In addition, new technologies should be explored to expand the use of continuous in-situ monitoring, regular remotely sensed monitoring, or other approaches that can provide efficient, accurate, and reliable detection and tracking of observed changes. Future changes in waterborne pathogens could result in significant impacts to water quality and aquatic ecosystems (for drinking water, recreation, ecosystem services).

5- Conclusion

The origin of most common waterborne pathogens can be traced to the fecal wastes of animals and humans. The organoleptic properties present the water include the pH which varies from 5.0 to 6.0, the color is mostly clear , the water is odourless, C parvum is 5 um,*Entamoeba coli* was 12um, *Giardia lamblia* was 12um, *Ascarislumbricoides* was 152um, *Diphylobotumlatum* was 65, nematode larvae is 2mm, T cati(70um),*Hymenolepis nana(*45um),*Taenasaginata*(32um). The potential for increased pathogen loads in source waters used as a drinking water supply presents a challenge in designing and operating drinking water treatment to efficiently remove a high number of the pathogens due to their organoleptic and morphometric characteristics.Shallow groundwater wells are likely to be more vulnerable to fecal contamination as they are most influenced by surface runoff. This study is a premium in the investigation of pathogen load in drinking water and is suitable for ameliorating the health and drinking water quality situation of the municipality.

6. References

(ASM 1998).Ingerson-Mahar M., Reid A. *Microbes in Pipes: The Microbiology of the Water Distribution System A Report on an American Academy of Microbiology Colloquium.* ASM Academy; Boulder, CO, USA: 2012. p. 26.

 ©IJSRED: All Rights are Reserved

Schijven and Husman 2005; Arnone and Walling 2007). Arnone RD, Walling JP. Waterborne pathogens in urban watersheds. *J Water Health.* 2007;4(1):149–162.

Tyrrel and Quinton 2003. The effect of incorporating slurries on the transport of faecal coliforms in overland flow; Soil Use and Management 19(2):185 - 186 DOI:10.1111/j.1475-2743.2003.tb00302.x

Cho et al. 2016; Cho KH, Pachepsky YA, Kim JH, Guber AK, Shelton DR, Rowland R. Release of Escherichia coli from the bottom sediment in a first-order creek: Experiment and reach-specific modeling. *J Hydrol.* 2010;4(3–4):322–332.

World Health Organization. *Guidelines for Drinking-Water Quality, 3rd edn* . www.who.int/water_sanitation_health/GDWQ/draftchemicals/list.htm. Last accessed June 2003. Geneva: WHO,
IPCS. *Arsenic and Arsenic Compound, 2nd edn*. Environmental Health Criteria 224. Geneva: World Health Organization, 2001

Schijven, J.F., and A.M.D. Husman. 2005. Effect of climate changes on waterborne disease in The Netherlands. *Water Science and Technology* 51(5):79-87.

USEPA (U.S. Environmental Protection Agency). 2001. *Protocol for Developing Pathogen TMDLs*. EPA 841-R-00-002. U.S. Environmental Protection Agency, Office of Water. Washington, DC.

USEPA (U.S. Environmental Protection Agency). 2013. *Literature Review of Contaminants in Livestock and Poultry Manure and Implications for Water Quality*. EPA 820-R-13-002. U.S. Environmental Protection Agency, Office of Water. Washington, DC.

Benham, B., C. Baffaut, R. W. Zeckoski, Y. A. Pachepsky, K. R. Mankin, A. M. Sadeghi, K. M. Brannan, M. L. Soupir, and M. J. Habersack. 2006a. Modeling bacteria fate and transport in watersheds to support TMDLs. Trans. ASABE. 49(4): 987–1002.

Guzman Herrador B, de Blasio BF, Carlander A, Ethelberg S, Hygen HO, Kuusi M, Lund V, Löfdahl M, MacDonald E, Martinez-Urtaza J, Nichols G, Schönning C, Sudre B, Trönnberg L, Vold L, Semenza JC, Nygård K. Guzman Herrador B, et al. J Water Health. 2016 .Association between heavy precipitation events and waterborne outbreaks in four Nordic countries, 1992-2012.Dec;14(6):1019-1027. doi: 10.2166/wh.2016.071. J Water Health. 2016. PMID: 27959880

Charron, D.F., M.K. Thomas, D. Waltner-Toews, J.J. Aramini, T. Edge, R.A. Kent, A.R. Maarouf, and J. Wilson. 2004. Vulnerability of waterborne diseases to climate change in Canada: A

 ©IJSRED: All Rights are Reserved

review. *Journal of Toxicology and Environmental Health, Part A: Current Issues* 67(20-22):1667-1677. doi: 10.1080/15287390490492313.

Rose, J.B., S. Daeschner, D.R. Easterling, F.C. Curriero, S. Lele, and J.A. Patz. 2000. Climate and waterborne disease outbreaks. *Journal American Water Works Association* 92(9):77-87.

 ©IJSRED: All Rights are Reserved