

## Microbial Freshwater Pollution and the Associated Risks

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**Abstract:** Microbial fresh water contamination occurs when faecal matters enter the water body. It is a global issue however; it is more severe in the developing countries due to; rapidly growing population and urbanization, land use and development, unhygienic and poor sanitation practices. Faecal matter pollution of fresh water is perhaps the most hazardous form of fresh water pollution since; it constitutes the greatest risk to the ecosystem and to public human health. Ecologically, it disrupts the nutrient load of the water body leading to eutrophication and the consequent production of toxins directly or indirectly affects man and animals especially, live of aquatic organisms. The public health risk is mainly due to the introduction of microbial pathogens whose diseases are associated with serious morbidity and mortality; and some, are capable of spreading rapidly leading to epidemics of varying proportions. In addition to causing diseases, it disseminates virulence and antibiotic resistance genes, which can be transferred to non-pathogenic and non-antibiotic resistant strains or species in the water body, resulting to diseases with less therapeutic options. The human health risk can be assessed by both the observed-adverse-effect-level approach (OAELA) and quantitative microbial risk assessment (QMRA). This paper thus, reviews risks associated with the use of microbiologically polluted freshwater and concludes that source water protection and point of use treatment measures is the ultimate means of mitigating such risks and should no longer be neglected.

**Keywords:** Pollution, Freshwater, Disease, Risk, Pathogen

### INTRODUCTION

Water is very essential for the survival and sustenance of life yet, 844 million people worldwide still lacked even a basic drinking water service (WHO/UNICEF, 2017). As a result, 159 million people still collect drinking water directly from surface water sources, of which 58% lived in sub-Saharan Africa (WHO/UNICEF, 2017). Such water bodies are susceptible to faecal contamination of human and animal origins thereby becoming of poor microbial quality and are often contaminated by pathogens, leading to outbreaks of water-borne infections and related diseases (Chigor, *et al.*, 2013; Pandey, *et al.*, 2014; King-Abia, *et al.*, 2017). An estimated 2.3 billion people lacked even a basic sanitation service and 892 million people worldwide still practised open defecation (WHO/UNICEF, 2017). According the reports from the Nigerian Federal Ministry of Water Resources and the

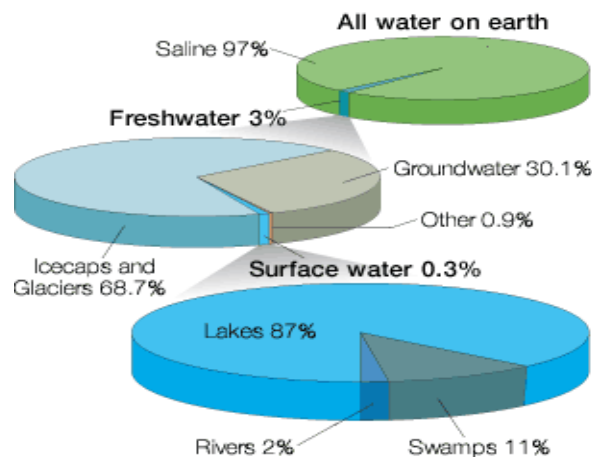
United Nations Children's Fund (UNICEF), Nigeria is among the nations with the highest number of open defecation cases where 46 million estimated people are involved (FGN/UNICEF, 2017).

The total volume of water on Earth is about 1.4 billion cubic kilometres. Of this, about 97.5% of the total volume is saltwater and only about 2.5% is freshwater. Freshwater consist of surface water (0.3%), groundwater (30.1%), ice caps and glaciers (68.7%) and others (0.9%) such as; soil moisture, atmosphere, ground ice and permafrost (Igor, 1993; National Geographic, 2010). Figure 1 indicated the global freshwater content with over 68% being ice and glaciers while 30% is stored in underground aquifers. The fresh surface water (water in rivers, streams, lakes, dams, ponds and similar bodies of water) constituted only 0.3% of the world's freshwater Figure 1 (Igor, 1993).

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**Figure 1: Distribution of earth's water. (Source: <http://ga.water.usgs.gov/edu/earthwherewater.html>)**

Fresh surface waters, including dams, rivers and streams, serve for drinking, domestic, agricultural, recreational, industrial and other purposes including transportation, and hydroelectricity (Chigor *et al.*, 2012). However, these waters are vulnerable to pollution (Azizullah, *et al.*, 2011; Chigor *et al.*, 2012), and are continually being impacted on, as a result of rapid population growths, land development along river basin, agriculture, urbanization and industrialization that place increased demand on surface waters both as sources of water for different uses and as disposal channels for treated/untreated wastewaters (Suthar *et al.*, 2010; Solaraj *et al.*, 2010).

Pathogenic microorganisms (bacteria, viruses, fungi, and protozoa) introduced via faecal pollution, render water contaminated and non-potable; and could result in the transmission of water-related diseases (Delgado-Gardea *et al.*, 2016; King-Abia *et al.*, 2017). Faecal pollution also affects the ecosystem and aquatic life, in addition to causing economic losses from closure of aquatic food harvesting areas, and bathing restrictions (Pruss *et al.*, 2002; Gourmelon *et al.*, 2007). Surface water sources are vulnerable to faecal contamination, from human and animal sources. This poses grave public health risks, as cholera outbreaks and diarrhoeal diseases are frequently reported in Nigeria, and every year, an estimated 124,000 Nigerian including children under the age of 5 die because of diarrhoea, mainly due to unsafe water, sanitation and hygiene (FGN/UNICEF, 2017).

Statistics has it that about 70 million people, out of Nigeria's population of 183 million (2015 projection), lacked access to safe drinking water, and over 110 million lacked access to improved sanitation (FGN/UNICEF, 2017). Although government is charged with the duty of providing potable water to its residence, access to potable water still remains a problem in every state of the Federation; and where it exists, it is grossly inadequate to meet the needs of the ever-growing population (Idu, 2015). As a result, greater percentage resort to the use surface water while the lesser percentage digs their own wells or rely on local water sellers (Idu, 2015).

Microbial fresh water pollution refers to the degradation of water quality that is intrinsically harmful in some ways, to some degree to the health of humans, animals and the environment (ecosystems), mainly due to contamination by pathogenic microorganisms and organic matter. This review considers the problem of water pollution caused by faecal contamination and microbial contaminants in surface waters.

#### **A Historical Perspective on Faecal Pollution**

Environmental pollution began with the earliest human societies, and the stress which man's activities exerts on the environment has been multiplied by the increase in human population due to urbanization and industrialization (Chigor *et al.*, 2013; Ubani *et al.*, 2014; Parmar *et al.*, 2016). The work of John Snow (Pandey *et al.*, 2014), who proved in 1855 that the cholera outbreaks in London were spread by drinking water contaminated with sewage, was crucial in developing public awareness on the importance of controlling water pollution.

Today, the pollution of water remains a global problem that weakens one of the resource bases on which human society is built (WHO, 2015), and engages a multitude of researchers. The problem is heightened by climate change and its attendant floods and droughts (Delpla *et al.*, 2009).

#### **Classes and Sources of Microbial Pollutants in Water**

There is a broad spectrum of microbial pathogens that have been documented to be found in the water environment including bacteria, protozoa, algae, viruses and helminths (WHO, 2011; Ramírez-Castillo *et al.*, 2015). The types, numbers and distribution of these pathogens in surface waters vary across different water bodies, parts of the world and times of year. These variations depend on the incidence of disease in the contributing population, and known seasonality in human infections (Skraber *et al.*, 2004).

It is known that contaminant loads to surface water bodies start off from point or non-point sources (Parveen *et al.*, 2001; Albek, 2003). Point-source pollution originates from discrete sources whose inputs into aquatic systems can often be defined in a spatially explicit manner (Ritter *et al.*, 2002). Examples of point-source pollution include; industrial effluents (food processing plants), municipal sewage treatment plants, combined sewage-storm-water overflows, septic tank leakage (Ritter *et al.*, 2002). In Nigeria, water resources consist of surface and groundwater components that are greatly impacted by human activities. Poor environmental practices including open-defecation, animal sources, including abattoirs, as well as dumping of sewage into surface waters introduce pathogens. It is almost a norm in Nigeria to dump untreated wastes (industrial, domestic, human, and animal) into surface water bodies (Chigor *et al.* 2012; Idu, 2015). In least developed countries, 22% of health care facilities have no water service, 21% no sanitation service, and 22% no waste management service (WHO, 2019a). This adds to the microbial waste burden on freshwater sources.

Non-point-source pollution, in contrast, arises from poorly defined, diffuse sources that typically occur over broad geographical scales (Kistemann *et al.*, 2002; Ritter *et al.*, 2002), with

examples that includes; agricultural runoff (pathogens and organic matter), storm-water and urban runoff and open-grazing. Worldwide, 2.0 billion people still do not have basic sanitation facilities such as toilets or latrines, in 2017 and of these, 673 million still defecate in the open, for example in street gutters, behind bushes or into open bodies of water (WHO, 2019b).

#### **Risks Associated with Faecal Contamination of Surface Water**

Faecal pollution of water sources comes with it a lot of risks both for aquatic ecosystems and for public health (Zhukinskii, 2003). The key public health problems arising from microbial contamination of freshwater include waterborne diseases (Igbiosa and Okoh, 2009; Chigor *et al.*, 2010a, b) and the dissemination of antimicrobial resistance (Chigor *et al.*, 2013; Sidrach-Cardona, 2014). The presence of microbial pathogens in aquatic systems also represents substantial economic losses mainly related to morbidity and mortality amongst the exposed populations or due to closure of recreational beaches (Gourmelon *et al.*, 2007).

#### **Ecological Risks**

When sewage, which contains microorganisms and decomposable organic matter, pollutes water, changes in the patterns of microbial activity occur due to increase nutrient composition (Albek, 2003). Decomposition of organic matter by microbes results in eutrophication of the polluted water and depletion of dissolved oxygen. Such anaerobiosis causes the death of fish and other oxygen-dependent water animals and permits the release of hydrogen sulphide and other malodorous substances by sulphate-reducing bacteria.

Eutrophic water bodies are prone to massive phytoplankton blooms and blooms of various planktonic species are directly or indirectly hazardous to human and animal health (Hitzfeld *et al.* 2000; Hunter 2003). Since the identification of *Nodularia spumigena* bloom in Lake Alexandria, Australia, numerous poisonings have been reported (Hitzfeld *et al.*, 2000). In 1988, a bloom of small flagellate algae, *Chrysochromolina polylepsia* damaged seaweeds, invertebrates and fish along a 200 km stretch of the coasts of Denmark, Norway and Sweden (McEldowney *et al.*, 1993).

Scum of Cyanobacteria accumulating along shores of ponds and lakes present a hazard to wild and domestic animals and the death of farm animals drinking scum of cyanobacterially-contaminated ponds and poisoning of dogs swimming in cyanobacterial scum have been described (Hitzfeld *et al.*, 2000).

### Public Health Risks

#### The Spread of antimicrobial resistance as public health risk

Resistance to antimicrobial agents began soon after the introduction antibiotics in the 1940s and has increased remarkably in the last three decades (Chigor *et al.*, 2010; CDC, 2013). The review, on trends in antimicrobial susceptibilities of Enterobacteriaceae isolated from hospitalized patients in the United States from 1998 to 2001, showed that decreased flouoroquinolone susceptibility was most pronounced for *E. coli* (Karlowsky *et al.* (2003). Other reports demonstrate, fresh water environment as a hot spot for the emergence and dissemination of antibiotic resistances (Sidrach-Cardona, 2014). Currently, microbial resistance to antibiotics spans all known classes of natural and synthetic drug agents (Zhang *et al.*, 2010), and bacterial resistance to antibiotics continues to pose a serious threat to human and animal health (Chigor *et al.*, 2010).

Literature is replete with evidences that faecal pollution and discharge of antibiotic-resistant strains of pathogens into water can result in the transfer of resistance to previously susceptible strains or species (Ash *et al.*, 2002; Blake 2003; Zhang *et al.*, 2010; Chigor *et al.*, 2010; Sidrach-Cardona, 2014). Studies demonstrated the transfer of plasmid-borne resistance in aquatic organisms like in *E. coli*, Enterobacteriaceae and *Pseudomonas aeruginosa* (Veal *et al.*, 1992). The threat due to the transmissibility of resistance genes stands multiplied considering the vast potential hosts presented by microbial population in the gut and water environments (Woegerbauer *et al.*, 2002). This risk is further amplified were a river continuum passes through different communities and possibly disseminate antibiotic resistance determinants (Leff, 1994). In a review of antibiotic resistance genes (ARGs) in water environment, Zhang *et al.* (2010) revealed that the emergence of ARGs in the water environment is becoming an increasing worldwide concern. Hundreds of various ARGs encoding resistance to a broad range of antibiotics have been found in microorganisms distributed not only in hospital wastewaters and

animal production wastewaters, but also in sewage, wastewater treatment plants, surface water, groundwater, and even in drinking water (Guyomard-Rabenirina *et al.*, 2017; Ng and Gin, 2019).

### Waterborne Diseases

Human infectious diseases are among the most serious effects of faecal pollution of freshwater, especially in developing countries, where sanitation may be inadequate (WHO, 2011). Water-borne pathogens are responsible for several water-related diseases such as diarrhoea, dysentery, gastroenteritis, cholera, typhoid, polio and wound infections. Diarrhoea is usually a symptom of an infection in the intestinal tract, which is caused by variety of bacterial, viral, protozoa and parasitic organisms with more than 50% cases being bacterial intestinal infections (Cabral, 2010). Diarrhoea contributes about 4.8% to the global burden of disease and is the leading cause of death in children under 5 years of age (Pruss *et al.*, 2002; Hatami, 2013).

Globally, in 2017, at least 2 billion people used drinking water source contaminated with faeces, and contaminated water can transmit several diseases (see Table 1 to 3) that contribute significantly to the global disease burden (Pruss *et al.*, 2002; WHO, 2015). Enteric pathogens are transmitted mainly by the faecal-oral route, either directly from person-to-person or via consumption of contaminated food or water (Haramoto *et al.*, 2008). The transmission pathways are shown in Figure 2. The use of contaminated water in kitchen may also bring about the contamination of food and utensils. Poor sanitation is linked to transmission of diseases such as cholera, diarrhoea, dysentery, hepatitis A, typhoid and polio and exacerbates stunting. It is estimated to cause 432 000 diarrhoeal deaths annually and is a major factor in several neglected tropical diseases, including intestinal worms, schistosomiasis, and trachoma. Poor sanitation also contributes to malnutrition (WHO, 2019b).

**Table 1: Pathogenic protozoa, algae and helminths linked to drinking water or recreational water contact.**

Organism	Disease	Transmission	Clinical features
<b>Helminths</b>			
<i>Schistosoma spp.</i>	Schistosomiasis	Contact with surface water infected with free swimming cercariae	Urinary and intestinal damage, bladder cancer
<i>Dracunculus medinensis</i>	Dracunculiasis	Drinking water	Painful ulcers on lower limbs and
<b>Protozoa</b>			
<i>Giardia duodenalis</i>	Giardiasis	Faecal oral spread through drinking water or recreational water	Diarrhoea and abdominal pain, weight loss and failure to thrive
<i>Cryptosporidium parvum</i>	Cryptosporidiosis	Faecal oral spread through drinking water or recreational water	Diarrhoea, often prolonged
<i>Cyclospora cayetanensis</i>	Cyclosporiasis	Faecal oral spread through drinking water	Diarrhoea and abdominal pain, weight loss and failure to thrive
<i>Entamoeba histolytica</i>	Amoebiasis	Faecal oral spread through drinking water	Diarrhoea, may be severe dysentery
<i>Toxoplasma gondii</i>	Toxoplasmosis	Drinking water contaminated by feline animals	Glandular fever, foetal damage in pregnant women
Free-living amoebae	Amoebic meningoencephalitis	Aspiration of infected surface water into nose	Fatal encephalitis
<b>Algae</b>			
Cyanobacteria <i>Pfiesteria piscicida</i>	Various Estuary-associated syndrome	Toxins in drinking water or direct Toxins in water	Dermatitis, hepatitis, respiratory Respiratory and eye irritation, deficiencies in learning and

Source: Hunter (2003).

**Table 2: Bacterial pathogens linked to drinking water or recreational water contact**

Organism	Disease	Transmission	Clinical feature
<i>Vibrio cholerae</i>	Cholera	Drinking water	Watery diarrhoea, may be severe
<i>Salmonella</i> spp.	Salmonellosis	Occasional outbreaks with drinking water	Diarrhoea, colicky abdominal pain and fever
<i>Salmonella typhi</i>	Typhoid	Drinking water	Fever, malaise and abdominal pain with high mortality
<i>Shigella</i> spp.	Shigellosis (Bacillary dysentery)	Both drinking and recreational water	Diarrhoea frequently with blood loss
<i>Campylobacter</i> spp.	Campylobacteriosis	Both drinking and recreational water	Diarrhoea frequently with blood loss
Enterotoxigenic <i>Escherichia coli</i>		Drinking water	Watery diarrhoea
Enterohaemorrhagic <i>E. coli</i>		Drinking water and recreational water contact	Bloody diarrhoea and haemolytic uraemic syndrome in children
<i>Yersinia</i> spp.	Yersiniosis	Drinking water	Fever, diarrhoea and abdominal pain
<i>Francisella tularensis</i>	Tularaemia	Drinking water	Typhoid-like or mucocutaneous with suppurative skin lesions
<i>Helicobacter pylori</i>		Drinking water	Gastritis that can progress to gastric cancer
<i>Mycobacterium</i> spp. (Not <i>M. tuberculosis</i> )	Varies	Potable water systems in hospitals, some recreation	Varies, includes respiratory diseases, wound infections, skin disease

Source: Hunter (2003).

**Table 3: Human viruses documented to be found in the water environment.**

Genus (genome)	Popular name	Disease caused	Genus (genome)	Popular name	Disease caused
Enterovirus (ssRNA)	Poliovirus	Paralysis, meningitis, fever	Polyomavirus (dsDNA)	Polyomavirus	Progressive multifocal leucoencephalopathy, diseases of urinary tract
	Coxsackie A, B virus	Herpangina, meningitis, fever, respiratory disease, hand-foot-and-mouth disease, myocarditis, heart anomalies, rash, pleurodynia, diabetes			
	Echovirus	Meningitis, fever, respiratory disease, rash, gastroenteritis			
Hepatovirus (ssRNA)	Hepatitis A virus	Hepatitis	Mamastrovirus (ssRNA)	Human astrovirus	Gastroenteritis
Reovirus (segmented dsRNA)	Human reovirus	Unknown	Coronavirus (ssRNA)	Human coronavirus	Gastroenteritis, respiratory disease, SARS
Rotavirus (segmented dsRNA)	Human rotavirus	Gastroenteritis	Orthomyxovirus (segmented ssRNA)	Influenza virus	Influenza, respiratory disease
Norovirus (ssRNA)	Norovirus	Gastroenteritis	Parvovirus (ssDNA)	Human parvovirus	Gastroenteritis
Sapovirus (ssRNA)	Sapporo-like virus	Gastroenteritis	Mastadenovirus (dsDNA)	Human adenovirus	Gastroenteritis, respiratory disease, conjunctivitis
Hepevirus (ssRNA)	Hepatitis E virus	Hepatitis	Circovirus (ssDNA)	Torque Tenovirus	Unknown, hepatitis

**Source:** Bosch *et al.* (2008).

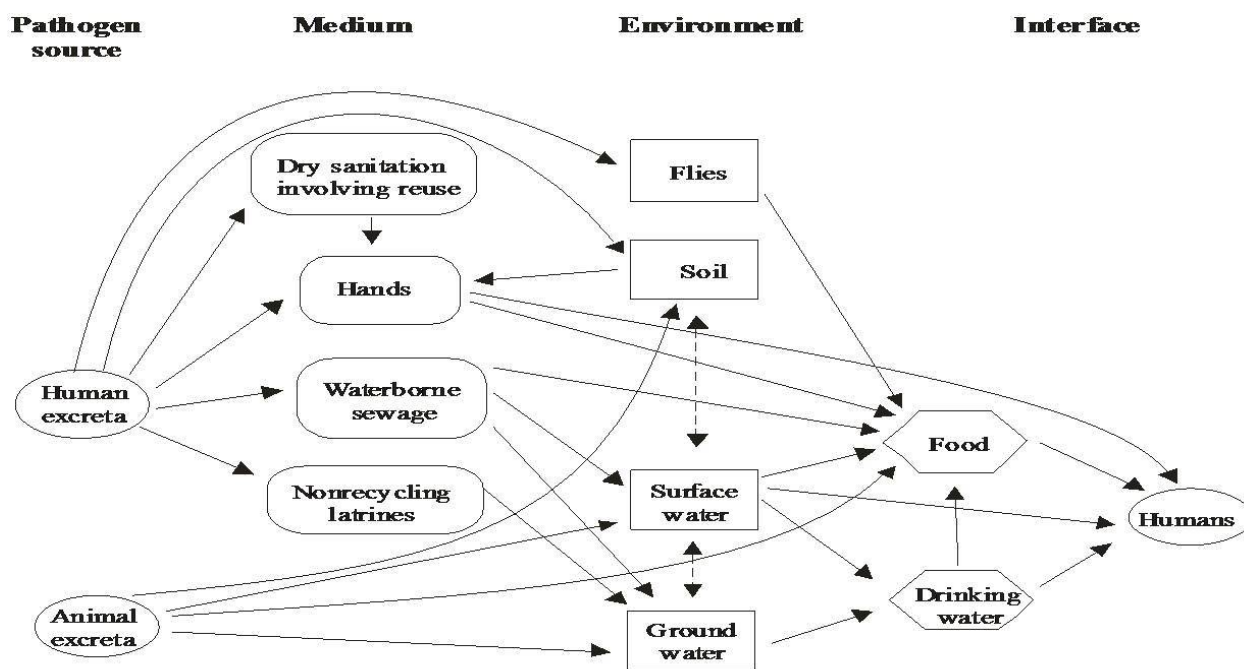


Figure 2: Transmission pathways of faecal-oral disease. (Pruss *et al.* (2002)).

Cholera, the classical waterborne disease has caused seven pandemics and still represents a serious problem, causing repeated epidemics especially in developing countries due to poor sanitation, water shortages, natural disasters (like, floods and wars) (Stewart-Tull 2001). Typhoid fever poses a serious health problem to the developing world. The annual incidence of this disease is estimated to be 20 million cases, resulting in more than 700,000 deaths (Mills-Robertson *et al.*, 2003). Most cases are the result of infection with *Salmonella typhi*, but colonization with *S. paratyphi* A, B or C, results in paratyphoid fever, a contagious condition similar to typhoid fever (WHO, 2011). Amongst other waterborne bacterial pathogens, *E. coli* O157:H7 has over the past 36 years emerged as a public health concern with worldwide distribution (Riley, *et al.*, 1983; Effler *et al.*, 2001; Chigor *et al.*, 2010). The first *E. coli* O157: H7 outbreak in Africa occurred in 1992 and was traced to river water contaminated by cattle faeces and carcasses in Swaziland and South Africa (Effler *et al.*, 2001).

According to the WHO (2011), when drinking water is contaminated with sewage, gastroenteritis and hepatitis could occur in epidemic proportions. It has been estimated that 30-90% of waterborne

disease outbreaks worldwide are caused by human enteric viruses (HEntVs) (Fong *et al.*, 2010). HEntVs have been detected in water sources globally (Bosch *et al.*, 2008; Iwai *et al.*, 2009; Fong *et al.*, 2010). HEntVs are capable of producing a wide variety of syndromes including rashes, fever, gastroenteritis, myocarditis, meningitis, respiratory disease and hepatitis (Bosch *et al.*, 2008; Buckwalter *et al.*, 2012). HEntVs include adenoviruses, rotaviruses, enteroviruses, hepatitis A virus, etc. (see, Table 3). Certain fungi are also known to cause infections in swimmers using recreational waters contaminated by faecal matter and organisms from the skin and nasopharynx. Such infections include “athlete’s foot” due to *Epidermophyton* and *Trichophyton* species (Ekowatia *et al.*, 2018). Phytoplankton blooms result in the release of cyanobacterial toxins that may present hazards for water supply safety. These toxins (microcystins, nodularins, saxitoxins, anatoxin-a, anatoxins-a(s), cyhndrospermopsin), when ingested or inhaled, are known to produce effects ranging from liver damage, including liver cancer, to neurotoxicity (Hitzfeld *et al.*, 2000; Carmichael *et al.*, 2001; Chia *et al.*, 2009).



The tragic death of 60 dialysis patients in Caruaru, Brazil in 1996 was attributed to the presence of cyanobacterial toxins in water supply used in a haemodialysis unit (Pouria *et al.*, 1998). Subsequent examination by Carmichael *et al.* (2001) of phytoplankton from the dialysis clinic's water source, analyses of the clinic's water treatment system, plus serum and liver tissue of clinic patients led to the identification of two groups of cyanobacterial toxins, the hepatotoxic cyclic peptide microcystins and the hepatotoxic alkaloid cylindrospermopsin.

Apart from the pathogen-mediated waterborne diseases discussed and/or listed above, public health concern over faecally polluted water exists regarding diseases associated with excessive levels of nitrate in aquatic systems (WHO, 2007). Nitrate itself does not pose any health threat; however, nitrate ( $\text{NO}_3^-$ ) is readily reduced to nitrite ( $\text{NO}_2^-$ ) by the enzyme nitrate reductase found in microorganisms (McEldowney *et al.*, 1993). Nitrite poses two distinct health risks, being potentially carcinogenic and causing methaemoglobinaemia (blue-baby syndrome).

#### **Risk Assessment**

Microbial risk assessment is a process that evaluates the likelihood of adverse human health effects following exposure to a medium in which pathogens are present (Soller and Eisenberg, 2008). The risk for human health associated with the presence of pathogens in water bodies can be evaluated using both the observed-adverse-effect-level approach (OAELA) and quantitative microbial risk assessment (QMRA). The OAELA is based on the occurrence of microbiological indicator organisms instead of actual pathogens (Steyn *et al.*, 2004), and the basic assumption in this indicator concept is that their presence indicates the presence of faecal pollution and the potential for the presence of pathogens (Savichtcheva and Okabe, 2006; Abdelzaher *et al.*, 2010). The QMRA process involves four steps: hazard identification, exposure assessment, dose-response assessment and risk characterization (Toze *et al.*, 2010). The quantitative methods to characterize human health risks associated with exposure to pathogens have been reported in the last four decades (Haas *et al.*, 1993; Ahmed *et al.*, 2010; Chigor *et al.* 2014) and guidelines have, accordingly, been established (FAO/WHO, 2003; USEPA/USDA/FSIS, 2012).

#### **Protecting Water Sources**

Source Water Protection (SWP) is recognized as the first barrier and the most effective means of reducing the risk of water contamination (Ibrahim and Patrick, 2017; Bruni, 2019). It involves protecting water sources from contamination and overuse especially, at the source. By so doing, public health risks associated with contaminated water, as well as the cost of water treatment/purification are reduced. According to UNEP (2010), three basic strategies exist for protection of surface water sources. They include preventing (waste, pollutants or untreated water from domestic, industrial or agricultural use from been discharged into the water body), treatment (of polluted water, including stormwater management before discharge into water bodies) and a mind set to restore the ecosystem.

For the protection of water sources to be achieved, a legal framework (which has to involve a protection plan, formulated responsibilities, specific protection measures and basic rules that apply to all community members and water source users) needs to be implemented (Bruni, 2019). Above all, water education and public enlightenment on the dangers of water contamination and the need for water protection will go a long way to seeing that all and sundry gets involved in this urgent and vital need to protect our ecosystem.

#### **CONCLUSION**

Freshwater sources are vulnerable to contamination. Poor environmental practices including open defecation, untreated wastewater disposal, and open grazing, with the attendant *in-situ* herd watering introduce organic matter and pathogens into the water resources; which might lead to diseases like diarrhoea, cholera, typhoid fever, to mention but a few. Disease with multidrug resistance strains reduces therapeutic options, increases hospital stay, causes more economic losses, as well as increased fatal cases. The best, safest and most assured way of reducing these risks is by protecting our water sources from pollution and employing point of use treatment measures. Protection and conservation of surface freshwater resources should no longer be neglected. It requires a clear legal framework that outlines responsibilities, specific protection measures and basic rules that apply to all community members and water source users.

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