

## Microbial Hazards Associated With Floodwater

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### ABSTRACT

Flood is a natural event where an area that is usually dry land suddenly gets submerged by water. Floodwater is generally contaminated by various contaminants including human and animal feces, chemicals and sewage. Humans and other animals are at risks of exposure to microorganisms in floodwater which are threat to health. An investigation was conducted to assess microbial hazards associated with floodwater. A total of 21 floodwater samples was collected from three locations in Aba, Abia State and analyzed for total heterotrophic bacteria, fecal coliforms, fungi and some physicochemical properties using standard techniques. Bacterial counts for the locations ranged from  $3.3 \times 10^6$  to  $5.2 \times 10^6$  cfu/ml; Thermotolerant and faecal coliform bacteria ranged from 26 to 920 MPN index/100ml; while fungal counts ranged from  $2.0 \times 10^3$  sfu/ml to  $5.0 \times 10^3$  sfu/ml. The BOD was 9mg/L – 20mg/L and electric conductivity was 400 $\mu$ s/cm – 12,000 $\mu$ s/cm. Bacteria isolated and frequency were; The bacteria isolated and their frequency of occurrence in the floodwater during the study were, *Alcaligenes bronchisepticus* (2.4%), *Bacillus* (4.8%), *Campylobacter fetus* (4.8%), *Enterobacter aerogenes* (4.8%), *Escherichia coli* (21.4%), *Klebsiella* (7.1%), *Listeria monocytogenes* (2.4%), *Micrococcus luteus* (4.8%), *Proteus* sp (2.4%), *Serratia* sp (4.8%), *Shigella* sp (11.9%), *Staphylococcus* (7.1%), *Streptococcus pneumoniae* (11.9%), *Vibrio cholerae* (7.1%), and *Yersinia* sp (7.1%). While the fungi isolated and frequency of occurrence were *Aspergillus niger* (14.82%), *Aspergillus oryzae* 7.41%, *Epidermophyton floccosum* 11.11%, *Fusarium solani* (11.11%), *Microsporium canis* (3.7%), *Mucor* sp (18.51%), *Penicillium* sp (14.82%), *Rhizopus* sp (7.41%), *Rhizopus oligospora* (3.7%), and *Saccharomyces cerevisiae* (7.41%). The presence of these microorganisms especially *E. coli* is an indication that the floodwater is faecally contaminated and poses a health risk to the public.

**Keywords:** Floodwater, *E. coli*, *Shigella*, *Microsporium canis*, drinking water, health risk.

### Introduction

A flood is a natural event or occurrence where a piece of land or area that is usually dry land suddenly gets submerged by water. Some floods can occur suddenly and recede quickly, while others take days or even months to build and discharge (Boulder County, 2002; EA, 2015). Flood has many sources; rivers and streams, the sea, groundwater, overland flow, blocked or overloaded drainage system, erosion, rainfalls etc. Floodwaters devastates homes, buildings, public goods, agricultural lands, and industries, causes power failures which cause refrigerated foods to spoil fast (CDC, 2004). Floodwater renders people homeless and has been recorded to cause serious impoverishment where it has occurred. Human activities can increase the risk of flooding from rivers and streams in many areas (Doocy *et al.*, 2023).

Floodwater is generally contaminated by various pollutants and contaminants. It carries whatever it comes across upland, human and animal feces, industrials effluent, pesticides, insecticides, rusted building materials and sewage (Gerencher, 2005). During flood and aftermath there are threats to health and safety. Humans and other animals that get exposed to the water can get injured and are at risks of exposure to pathogenic microorganisms such as *Campylobacter*, *Giardia*, *Cryptosporidium*, and *E. coli* which are threat to health (Bich *et al.*, 2011).

Flooding impairs clean sources of drinking water with pollutants and devastates sanitary toilets, directly or indirectly. Whether through direct flood intakes, vector insects such as flies, unclean hands or dirty plates and utensils results in waterborne illnesses and life threatening infectious diseases.

Infection commonly results during bathing, washing, drinking, in preparation of food, or the consumption of food thus infected. Various forms of waterborne diarrhea disease probably are the most prominent examples, and affect mainly children in developing countries (Jonkman and Kelman, 2005). According to the World Health Organization (2005), such diseases account for an estimated 4.1% of the total daily global burden of disease, and cause about 1.8million human deaths annually. The World Health Organization estimates that 88% of that burden is attributable to unsafe water supply, sanitation and hygiene (WHO, 2005). A flood can cause both emotional and physical stress, emotional and mental stress. Also stress has adverse effect on immune system which can result to immune-deficiency disease caused by bacteria and microorganisms (WHO, 2014).

Molds and mildews are fungi that grow in a short period of 24 to 48 hours in wet and damp areas of the buildings and homes that have not been cleaned after flooding. Such fungi when inhaled cause allergic reactions such as asthma and respiratory disease in immune-compromised individual and people with chronic lungs (CDC, 2006).

With the flood that occurred in 2012 in Nigeria, several persons were gasping for breath. Over 21,000 persons were rendered homeless and about 309 recorded deaths. There is no literature on the microorganisms associated with floodwater in Nigeria. There is therefore the need for such a study.

The aim of this study therefore is to investigate the water quality (microbiology and physicochemistry) of floodwater in Abia State with a view to ascertaining the health hazards associated with floodwater.

## Materials and Methods

### Study Area and Collection of Floodwater Samples

Floodwater samples were collected from different locations in the commercial City of Aba in Abia State, Nigeria. The locations were Faulks Road (Location 1), Ariaria International Market (Location 2), and Army Barrack Primary School, Okwukwemdi (Location 3). Flood water samples were collected from the locations at depth of about 1-2m, with sterile plastic bottles after each bottle was first rinsed with the floodwater.

The bottle was filled, capped tightly and properly labeled. Collected samples were placed in ice-packed cool box and immediately transported to the laboratory for analysis.

### Physicochemical Analysis

The methods of APHA (1985) were adopted for the physicochemical analysis of the floodwater samples.

### Microbiological Analysis

#### Cultivation and Enumeration of Total Heterotrophic Bacteria and Fungi

The common microbiological media used for the study were Nutrient agar, Sabouraud Dextrose Agar (SDA), MacConkey agar, and Lactose broth. Enumeration of the total viable count of bacteria and fungi in the flood water were estimated using the spread plate method.

Serial dilution was carried out on each flood water sample. The dilution factor for the isolation of bacteria was  $10^{-4}$  while the dilution factor for the isolation of fungi was  $10^{-2}$ . This was done so as to obtain discrete colonies when plated on the medium. One milliliter (1.0ml) of each flood water sample was added to separate 9.0ml of normal saline (diluent) and further dilution was made up to  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$ .

An aliquot (0.1ml) of the appropriately diluted sample was then inoculated onto nutrient agar plates for the isolation of bacteria and onto Sabouraud dextrose agar plates for the isolation of fungi. The spread plate method was done using sterile bent glass spreader to spread the sample evenly on the agar plates. Cultures were prepared in duplicates.

Cultured Nutrient agar plates were incubated at  $37^{\circ}\text{C}$  for 24 hours while the cultured SDA plates were incubated on the laboratory bench for 3 to 5 days. Discrete colonies that developed on the plates (overnight culture) were counted, the average taken and recorded as total heterotrophic counts of bacteria.

Discrete colonies were collected aseptically and streaked onto nutrient agar plates (for bacteria purification) and incubated at  $37^{\circ}\text{C}$  overnight. Pure colonies were later stored in MacCartney bottles containing nutrient agar slants and put into the fridge as stocks cultures for further biochemical tests.

A total of forty-two (42) pure cultures of bacteria isolates were stored. On the other hand, colonies which developed after 5 days on SDA plates were counted and the average count for the duplicate cultures were recorded as total viable fungi of each sample. The colour and colonial morphologies or characteristics were also recorded. Discrete colonies were subcultured onto freshly prepared SDA to obtain pure cultures and a total of twenty seven pure cultures of fungi were stored.

### Estimation of Coliforms and Enumeration of Faecal Coliform Test

Estimation of the coliform bacteria was done using the most probable number technique (MPN technique). Reaction to MPN technique and thermotolerant coliform bacteria MPN index 100ml of each floodwater sample was done using double strength MacConkey broth for 10ml of sample and single strength MacConkey broth for 0.1ml and 1ml of the sample. The test for the estimation of coliforms involves the following steps: presumptive, confirmatory and completed test. It was performed as described by Verma *et al.*, (1999). The test for coliform does not distinguish coliform of animal origin and from others (Doyle and Erickson, 2006). In this test, the test tube with the production of gas in the presumptive test were streaked with the aid of a sterile wire loop onto MacConkey agar plates and incubated at 37°C for 24hr.

### Isolation, Characterization and Identification of Bacteria and Fungi in Floodwater

Pure cultures of bacteria were obtained by aseptically streaking representative colonies of different morphological types which appeared on the cultured plates onto freshly prepared nutrient agar plates which were incubated at 28°C for 24 hours. The isolates which developed were further sub cultured onto agar slopes/slants and incubated at 28°C for 24 hours.

These served as pure stock cultures used for subsequent characterization tests. The following characterization tests were performed in duplicates; Gram staining, catalase test, coagulase test, sugar fermentation test, methyl red test, indole test and acid gas test. The pure cultures were identified on the basis of their cultural, morphological and physiological characteristics in accordance with methods described by Cruikshank *et al.*, (1975) and with reference to Holt (1977). Pure cultures of fungi were obtained by sub culturing discrete colonies onto freshly prepared Sabouraud dextrose agar plates and incubated at 28°C for 5 to 7 days. The colonies which developed were further subcultured onto agar slopes or slants and incubated at 28°C for 5 to 7 days. The following standard characterization tests were performed in duplicate; macroscopic examination of fungal growth was carried out by observing the colony morphology-diameter, colour (pigmentation), texture and surface appearance. Microscopic examination was done by needle mount or wet mount method and observing sexual and asexual reproductive structures.

### Microscopic examination of fungi

A wet mount was carried out for the fungi isolated. A drop of sterile distilled water was aseptically dropped on a grease free clean slide. A piece of fungal hyphae under test was teased into it using two sterile needles. The teasing was done carefully and slowly so as to make good spread of the fungal hyphae. Each prepared slide was gently covered with a cover slip to avoid air bubble. The slides were observed under low and high power objective, and observation recorded as the cultural characteristics, sporangia, conidia, arthrospores, and vegetative mycelium, septate and non-septate hyphae according to Barnett and Hunter (1998).

### Results

The mean values of the physicochemical constituents of the flood water are shown in Table 1.

**Table 1: Physicochemical Constituents of the Floodwater in the Different Locations**

Parameter	Location 1 (Faulks Road)	Location 2 (Ariaria Int. Market)	Location 3 (Army Barrack Pri. Sch.)
Temperature (°C)	25	28.5	27.5
pH	7.45	7.1	7.15
Electrical conductivity (µs/cm)	11,697	11,771	11,756
Dissolved oxygen (DO) (mg/ml)	7.5	7.1	7.095
Biochemical oxygen demand (mg/ml)	15.05	14.49	14.74
Total suspended solids (mg/ml)	30.0	28.5	30.5

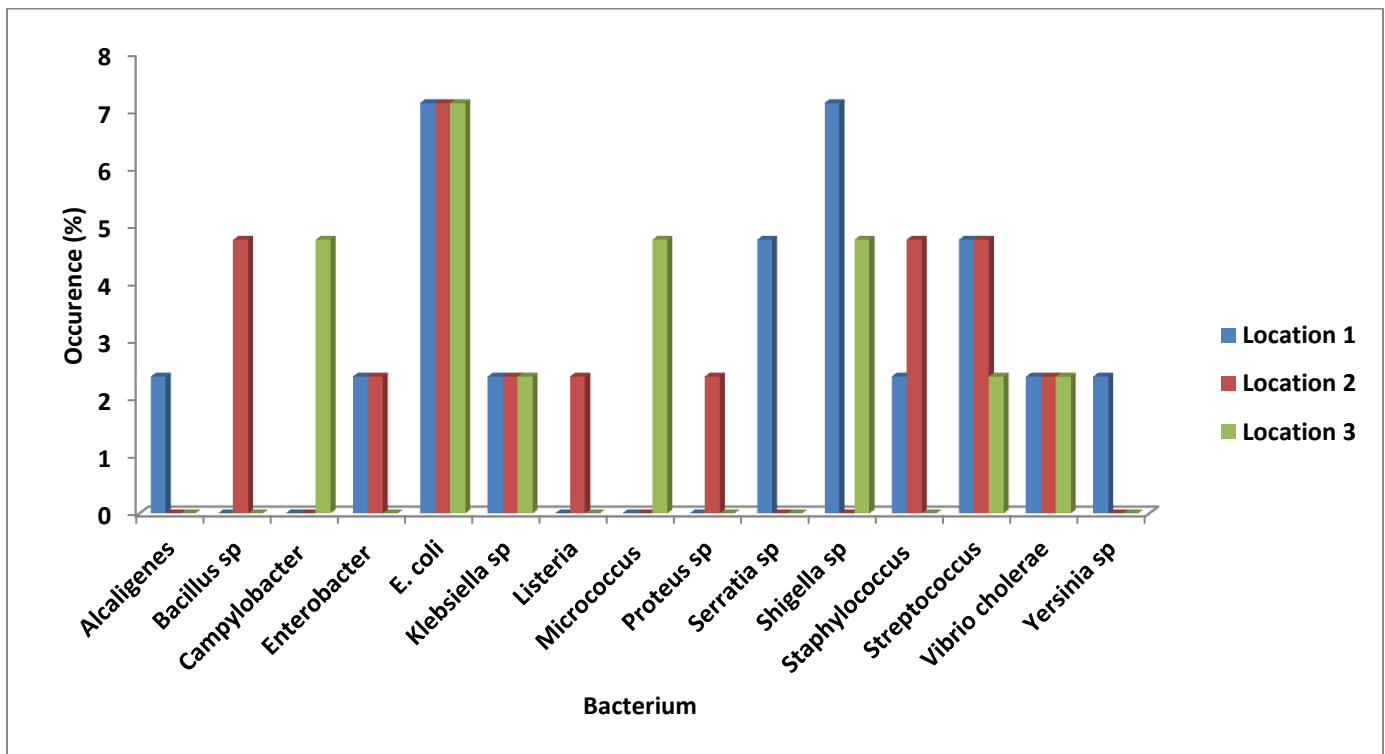
The mean of the bacteria count ranged from  $3.3 \times 10^6$  to  $5.2 \times 10^6$  CFU/ml with a mean value of  $4.0 \times 10^6$  CFU/ml in location 1, from  $3.9 \times 10^6$  CFU/ml to  $4.8 \times 10^6$  CFU/ml with a mean value of  $4.3 \times 10^6$  CFU/ml in location 2 and from  $4.2 \times 10^6$  CFU/ml to  $4.3 \times 10^6$  CFU/ml with a mean value of  $4.21 \times 10^6$  CFU/ml in location 3. The count was highest in location 2 and lowest in location 1. The mean value of fungi count ranged from  $4.0 \times 10^3$  SFU/ml to  $5.0 \times 10^3$  SFU/ml with a mean of  $4.67 \times 10^3$  SFU/ml in location 1, from  $3.0 \times 10^3$  SFU/ml to  $5.0 \times 10^3$  SFU/ml with a mean of  $4.0 \times 10^3$  sfu/ml in location 2 and from  $2.0 \times 10^3$  SFU/ml to  $5.0 \times 10^3$  SFU/ml with a mean of  $3.3 \times 10^3$  SFU/ml in location 3.

The thermotolerant coliform bacteria and faecal coliform bacteria ranged from 180 to 920 MPN index per 100ml in location 1, from 26 to 540 MPN index per 100ml in location 2, and from 26 to 350 MPN index per 100ml in location 3. Generally, the thermotolerant coliform bacteria and faecal coliform bacteria in the floodwater ranged from 26 to 920 MPN index per 100ml.

The bacteria isolated and their frequency of occurrence in the floodwater during the study were, *Alcaligenes bronchisepticus* (2.4%), *Bacillus* (4.8%), *Campylobacter fetus* (4.8%), *Enterobacter aerogenes* (4.8%), *Escherichia coli* (21.4%), *Klebsiella* (7.1%), *Listeria monocytogenes* (2.4%), *Micrococcus luteus* (4.8%), *Proteus* sp (2.4%), *Serratia* sp (4.8%), *Shigella* sp (11.9%), *Staphylococcus* (7.1%), *Streptococcus pneumoniae* (11.9%), *Vibrio cholerae* (7.1%), and *Yersinia* sp (7.1%).

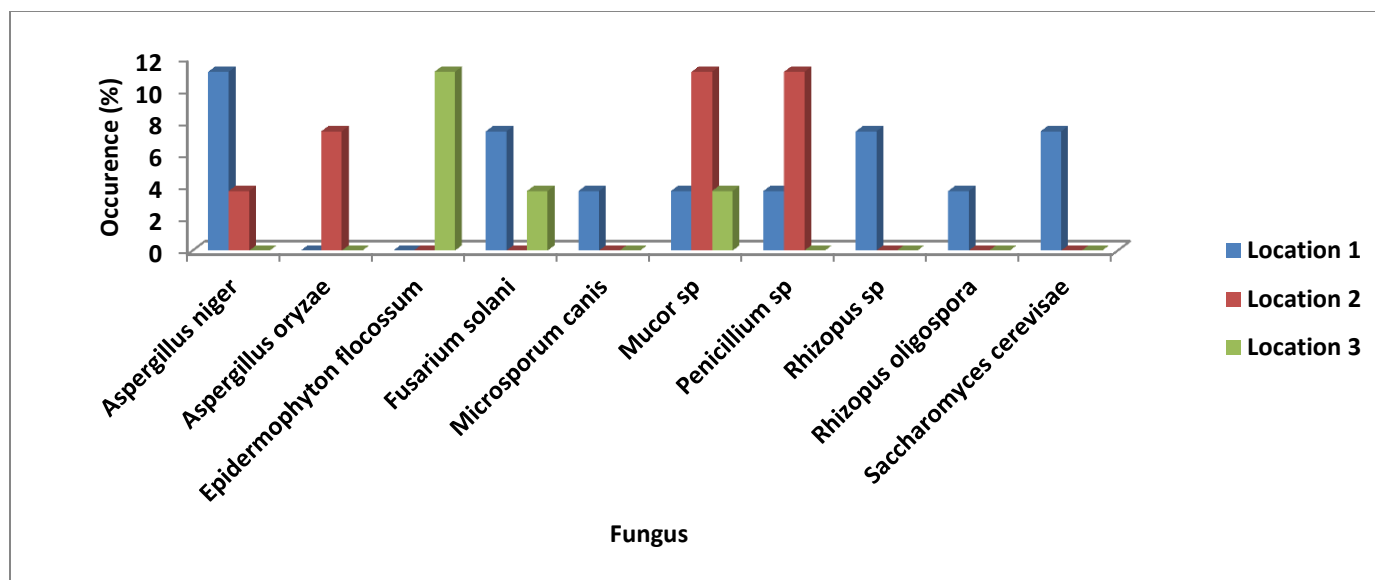
While the fungi isolated and frequency of occurrence were *Aspergillus niger* (14.82%), *Aspergillus oryzae* 7.41%, *Epidermophyton floccosum* 11.11%, *Fusarium solani* (11.11%), *Microsporium canis* (3.7%), *Mucor* sp (18.51%), *Penicillium* sp (14.82%), *Rhizopus* sp (7.41%), *Rhizopus oligospora* (3.7%), and *Saccharomyces cerevisiae* (7.41%).

The occurrence (%) of bacteria in the floodwater of the locations is shown in Figure 1; while the occurrence (%) of fungi in the floodwater of the locations is shown in Figure 2.



**Fig. 1: Occurrence (%) of Bacteria in Floodwater in the Different Locations**

**Key:** Location 1 = Faulks Road; Location 2 = Ariaria International Market; Location 3 = Army Barrack Primary School



**Fig. 2: Occurrence (%) of Fungi in Floodwater in the Different Locations**

**Key:** Location 1 = Faulks Road; Location 2 = Ariaria International Market; Location 3 = Army Barrack Primary School

## Discussion

The present study has revealed some physicochemical constituents of floodwater in different locations in Aba, Abia State, Nigeria. The temperature and pH values obtained are such that would enhance the growth of heterotrophic bacteria and saprophytic fungi. The pH values of the floodwater are within the neutral and slightly alkaline range with Location 1 being more alkaline than the other Locations.

The values of the total suspended solids are quite high and the biochemical oxygen demand (BOD) values are far higher than the dissolved oxygen values. This is an indication that the floodwater is loaded with degradable organic matter and that the floodwater is highly polluted.

The present study has also revealed the population and types of bacteria and fungi in floodwater in Aba. The results showed that, the bacterial population was highest in location 2 which is the Ariaria International Market and lowest in Location 1 which is Faulks Road. On the other hand, the thermotolerant and fecal coliform MPN index, and fungal populations were highest in Faulks Road and lowest in Army Barrack Primary School.

Generally, the bacterial and fungal populations were very high. This is attributed to the presence of high load of organic matter as evidenced in the high values of the BOD. The bacteria in the floodwater were *Alcaligenes bronchisepticus*, *Bacillus*, *Campylobacter fetus*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella*, *Listeria monocytogenes*, *Micrococcus luteus*, *Proteus sp*, *Serratia sp*, *Shigella sp*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Vibrio cholerae*, *Yersinia sp*. Of these bacteria, *Escherichia coli* which is an indicator of fecal contamination had the highest frequency of occurrence (21.4%), and was present in all the Locations. While the fungi in the floodwater were *Aspergillus niger*, *Aspergillus oryzae*, *Epidermophyton floccosum*, *Fusarium solani*, *Microsporium canis*, *Mucor sp*, *Penicillium sp*, *Rhizopus sp*, *Rhizopus oligospora*, and *Saccharomyces cerevisiae*. Of these fungi, *Aspergillus* species had the highest frequency of occurrence (22.23%) while *Microsporium canis* which causes mycoses of cats and dogs had the lowest frequency of 3.7%.

The diseases associated with the bacteria isolated from the floodwater during this study are; *Bacillus sp* causes Diarrhea and food borne illness; *E. coli* and *Campylobacter fetus* causes Gastroenteritis.

*Enterobacter* spp causes sepsis and septic shock, *Klebsiella* causes pneumonia and urinary tract infection (Humphries and Linscott, 2015). *Proteus* spp. causes urinary tract infection *Salmonella* species causes typhoid fever, salmonellosis (*Salmonella* gastro-enteritis), *Serratia* is an opportunist pathogen causing nosocomial infection; *Shigella* causes shigellosis (bacillary or bacterial dysentery); *Staphylococcus* spp. various staphylococcal disease e.g. boils (Szabó, 2014). *Streptococcus pneumoniae* causes pneumonia, sore throat, otitis media, bacteremia, meningitis and sinusitis; *Vibrio cholera* causes cholera; *Yersinia sp* causes Yersiniosis (Singleton and Sainsbury, 2001; ADHS, 2017). Diseases associated with the fungi isolated are; *Aspergillus* species causes aspergillosis and onychomycosis, *Aspergillus niger* causes a disease called black mould on certain fruits and vegetables; *Epidermophyton floccosum* causes a severe case of tinea pedis, athlete's foot (Person *et al.*, 2010). *Fusarium solani* causes keratitis, endophthalmitis and onychomycosis; *Microsporum canis* causes mycoses of cats and dogs; *Mucor* causes Mucor mycosis (ISHAM, 2018). Some *Penicillium* species produce mycotoxins that causes extrinsic asthma *Rhizopus* species causes tellutis, *Fusarium solani* causes pneumonia and onychomycosis (Singleton and Sainsbury, 2001; Egbuta *et al.*, 2017).

Generally, the bacteria and fungi populations of the floodwaters are very high. This is attributed to the presence of high load organic matter as evidenced in the high values of the BOD. The presence of bacteria and fungi which are potential pathogens is attributed to the low standard of living and poor sanitary hygiene of the inhabitants of the City of Aba. Flood water in many cases is generally contaminated. This is the source of microorganisms and health risks it poses. During flood and aftermath there are threats to health and safety (CDC, 2005; USEPA, 2017). Humans and other animals that get exposed to the water can get injured and are at risks of exposure to pathogenic microorganisms such as *Campylobacter*, *Escherichia coli* and other pathogenic microorganisms that are associated with drinking contaminated water. Floodwater contaminates water for other purposes and also contaminates foods directly or indirectly. Human and animal contact with spores of molds and mildews, mosquitoes and animals after flood becomes an easy and simple way to contract infectious diseases which are threat to health (Gerencher, 2005; CDC, 2006).

It is recommended that floodwater should not be used for cooking or drinking or for other purposes such as bathing or swimming in the floodwater as to avoid exposure to microorganisms in floodwater and their hazards to health.

The following should be adopted in areas where floods normally occur; Retaining walls, levees, lakes, dams, reservoirs or retention ponds should be constructed to hold extra water during flooding. It is important that town planning be strictly adhered to and builders should acquire building permits before buildings are erected. This will ensure that waterways are not blocked. All drainage systems should be covered to prevent litter from getting into them and should be checked regularly and cleaned when necessary.

It is important to regularly conduct mobilization and outreach campaigns in educating the public on what causes floods, and what can be done to minimize its impact on the environment and the public; on the dangers of floods and the associated health risk if such water is consumed or used for other purposes. These will help to minimize or prevent the health risks that are associated with floodwaters.

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