Fish from a polluted lake (Lake Chivero, Zimbabwe): a food safety issue of concern

Francis Manjengwa*, Tamuka Nhiwatiwa*, Elijah Nyakudya** and Petronella Banda***

*Faculty of Science, Biosciences Department, University of Zimbabwe, PO Box MP167, Mt. Pleasant, Harare, Zimbabwe, **Faculty of Agriculture, Soil Sciences & Agricultural Engineering Department, University of Zimbabwe, PO Box MP167, Mt. Pleasant, Harare, Zimbabwe, and ***Faculty of Agriculture, Animal Sciences Department, University of Zimbabwe, PO Box MP167, Mt. Pleasant, Harare, Zimbabwe

Correspondence to: Tamuka Nhiwatiwa, Faculty of Science, Biosciences Department, University of Zimbabwe, PO Box MP167, Mt. Pleasant, Harare, Zimbabwe. E-mail: tnhiwatiwa@science.uz.ac.zw

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Abstract

Objectives: A study to determine food safety hazard status of fish products from Lake Chivero was conducted in selected high density suburbs of Harare. Lake water and fish were tested for E. coli O157:H7, total bacterial, fungal counts, mercury (Hg) and aflatoxin B1 (AFB1) to determine contamination levels and assessing human health hazard exposure.

Materials and Methods: Membrane filtration method was used to determine E. coli O157:H7 viable counts using CHROM agar. Plate count and Potato dextrose agar were used for determination of total viable bacterial and fungal counts. Concentrations of Hg and AFB1 in fish and water were determined by Atomic Absorption Spectrophotometer and High Performance Liquid Chromatography-Mass Spectrometry. A questionnaire survey was conducted on 136 adult fish consumers to determine the fish consumption patterns to assess hazard exposure against international standards.

Results: Significant levels of microbial contamination above international standards in both fish and water were recorded. Mean E. coli O157:H7 counts were 106 ± 10 (cfu/cm²) in fish and 52 ± 14 (cfu/100ml) in water. Mean Log₁₀ TBC were 8.98 ± 0.26 (cfu/cm²) in fish and 9.05 ± 0.05 (cfu/ml) in water. Mean Log₁₀ TFC were 4.83 ± 0.02 (cfu/cm²) in fish and 4.56 ± 0.03 (cfu/ml) in water. Hg and AFB1 were 0.018 (µg/kg) and 0.025 (µg/kg) in fish and 0.008 (µg/kg) and 0.005 (µg/kg) in water, both with a hazard quotient (HQ) <1 using CODEX STAN 193-1995 of 2015.

Conclusion: Fish and water are contaminated with E. coli O157:H7, contain bacterial and fungal loads above international food safety guidance levels. Fish were caught already contaminated with pathogens together with toxigenic Hg and carcinogenic AFB1 although their concentration levels are within international food safety toxicological levels. Levels of Hg and AFB1 in water were almost double that of fish, implying bio-accumulation occurred in fish. Thus, fish consumers are exposed to food safety hazards and are at risk of contracting foodborne illnesses from consuming contaminated fish.

Key words: food contamination; food safety hazards, E. coli O157:H7, mercury, aflatoxin B1

Introduction

Fish contamination is a measure of environmental health as well as a potential source of human illness. Consumption of contaminated fish can be a major food hazard exposure route for humans. Fish can accumulate toxic chemicals and dangerous pathogens through contaminated water, sediment, and prey which will pass on
along the food chain (Zheng et al., 2010). Toxic contaminants end up in waterbodies in different ways and contaminants can build up in consumer’s body over time and may result in health problems. Industrial, municipal discharges, agricultural practices, and storm water runoff can all put harmful substances into the water bodies. Rain can also wash chemicals from the land or air into streams or rivers which will be then deposited directly into the lakes.

Although fish products provide proteins, are low in saturated fat, rich in many micronutrients, provide certain omega-3 fatty acids not synthesized by the human body and are important for normal growth and development (EPA, 2017). However, as a result of natural processes and anthropogenic activities that pollute water bodies, fish may also contain chemical and biological food safety hazards residues which can be passed to consumers (WHO, 2008). The presence of biological, physical and chemical food hazards in the environment is a cause for major concern for food safety and human health (WHO, 2015a). These food hazards, even at minute concentration levels, they may result in food safety risks as a result of toxicity, food borne illnesses and their tendency to persist or bioaccumulate in the food chain. Chemical and microbiological toxicants usually accumulate in fish by concentrating in adipose tissues or through binding to muscle fibers, even very low levels of bioaccumulative contaminants detected in water or bottom sediments may biomagnifies enough along the food chain to cause health risks to fish consumers (Zheng, 2010). According to Chen et al., (2014) as cited in the Ohio EPA (2017), health problems that may result from contaminants in fish consumed from polluted water bodies range from minute, difficult see health effects to teratogenic, as well as physiological disorders and mental underdevelopment in children under the age of 5 years. Individuals from all age groups are affected by consumption of contaminated fish, but children, pregnant women, the elderly and those who are immune-compromised are most vulnerable, (Silbernagel et al., 2011). Their body defense systems are less capable to fight against the effect of toxic substances than adults (Sears & Genius, 2012). Many different age groups amongst fish consumers who consume more than average quantities of fish are more likely to be at higher risk of having bioaccumulative contaminant body burdens (USEPA, 2017).

Lake Chivero is a polluted lake which supports a thriving artisanal fishery, which has become a source of livelihood and business for many families and source of cheap protein for the Harare urban poor (Manjengwa et al., 2016). Fish from Lake Chivero have a big market in Harare and surrounding urban communities, yet the lake is ranked in the top 10 among the world's most polluted lakes. Harare, Chitungwiza and Ruwa were identified as the major polluters through the disposal of raw domestic sewage, raw industrial effluent and urban wastes into the river drainage systems that eventually feed into Chivero (Fig. 1) (Nhwiatiwa et al., 2011).

There are raising fears that if no immediate corrective measures taken, it would be too costly to recycle water for domestic use and there will be serious public health issues related to water pollution (EMA, 2014). Pollutants are entering the food chain system through consumption of fish from contaminated water body. Extensive legal frameworks and complicated food standards have been established internationally to monitor and control food safety hazards in an attempt to increase consumer safety, reducing exposure risk. To ensure

Figure 1. Catchment area of upper Lake Manyame and Lake Chivero.
an appropriate level of food safety is assured for consumers, food producers, processors and others in the food chain must be able to identify and describe potential food safety hazards that might be present in their food products and use best practice that control these potential hazards (CODEX-FAO/WHO, 2015). Pathogenic microorganisms and their toxins of public health importance as food safety hazards may be introduced through environmental contamination by domestic and/or industrial wastes at the point of fish capture.

The level of contamination of fish at the time of capture will depend on the environmental and the microbial quality of the water in which the fish are harvested. Heavy metals such as methylmercury are of particular concern for fish and can bioaccumulate in tissues (EPA, 2017). Many potential chemical food hazards do not cause acute toxicity, but rather may increase risk of cancer or other adverse effects through chronic exposures. The long-term risks of consuming contaminated fish could lead to higher rates of cancer, liver diseases and other afflictions (Genius & Kelln, 2015).

High concentrations of some heavy metals (notably, Lead, Cadmium, Arsenic and Copper), pathogenic microbes (salmonella and parasites), have been observed in water, sediment and fish tissue in the lake and within its catchment (Fig. 1), (Nhiwatiwa et al., 2011; Manjengwa et al., 2016), but little is known on the concentration levels of mercury (Hg), mycotoxins and E. coli O157:H7 load in fish products from Lake Chivero as potential food safety hazards. Pollutants add into the water resource large quantities of foreign toxic chemicals, heavy metals, microbial colonized debris and certain microbial species which arise from the human intestines, which result in water quality issues.

Municipal water supplied by the City of Harare and fish from Lake Chivero have been implicated in outbreaks of food and water borne diseases such as Typhoid and Cholera which occurred in Harare and Chitungwiza. In the past five decades, a body of knowledge has been gathered in regards to the pollution history of Lake Chivero, its aquatic creatures’ welfare, the catchment in general, and its impact on biotic diversity, with all scientists agreeing that the lake is polluted, (Nhiwatiwa et al., 2011; Brendonck et al., 2003; Rommens et al., 2003). The effects of parasites on birds and fish (Barson, 2004; Barson & Marshall, 2004), fish health hazard estimations, including the significance of microcystin presence and impact of algal blooms (Mhlanga et al., 2006; Ndebele & Magadza, 2006), were all investigated through research from the lake, and all the scientists agreeing that, Lake Chivero is posing severe risks to aquatic and human health if contamination-reduction solutions are not resolved.

Materials and Methods

Materials used for laboratory analysis included microbial culture media for fungi and bacteria, Potato dextrose agar, Malt extract agar, Nutrient agar, Plate count agar (Oxoid), CHROM agar (BIO-RAD France), aflatoxin B1 (AFB1) standard solution (Sigma Aldrich), Chromatographic-grade methanol, HPLC-grade acetonitrile, ammonium acetate, magnesium sulphate, sodium chloride, formic acid, iodine, mercric nitrate, sodium hydroxide, sodium tetrachloroborate, peptone water, distilled water, culture plates, hydrochloric acid, nitric acid, perchloric acid, ethanol, cotton wool, sterile cotton swabs, test tubes, electro-fisher, gill nets, beach seine net, cooler boxes, ice packs, hydride generator for Hg, atomic absorption spectrophotometer, HPLC-mass spectrometry system (Agilent 1260 HPLC equipped with a binary pump coupled to Agilent Q-TOF 6530 Mass Spectrometer), HPLC consumables and vials (Agilent Technologies), analytical column (Phenomenex-Luna Column), speed boat, incubator, balance, colon counter, oven, and dissecting kits.

Study area

The study was conducted at Lake Chivero, Zimbabwe (0308001°45′E; 17.8710.3°0′S). Lake Chivero was built in 1952 as the main source of municipal water supply for the city of Harare. It is located downstream from the city of Harare, Chitungwiza, and Ruwa which discharges industrial wastes, municipal effluent, agricultural and domestic sewage into Mkuvisi, Manyame, and Marimba Rivers (Figure 1). The population of the city of Harare and its dormitory towns, Chitungwiza which was developed in the 1970s, and Ruwa has grown dramatically in the last 30 years and the volume of industrial effluent and domestic sewage increased accordingly. The quantity of effluent was estimated to likely have doubled by 2010 (Moyo, 1997) and accumulation of dangerous pollutants and toxigenic chemical poisons continues to increase in the aquatic system. Some of the consequences of high pollution load in Lake Chivero were the shortages of clean water in the city of Harare, Chitungwiza, Ruwa, and Norton, open burst and flow of sewage, outbreaks of water- and food-borne diseases, and reports of fish deaths.

Collection of samples

Six sites were randomly selected in transect along Manyame basin to represent the whole lake and one site was a residential site (Figure 2). Fish were caught from Lake Chivero on three occasions in January, March, and May 2017 to represent the difference in water quality of different time periods, that is, peak, mid, and end of rainfall season. Fish were caught using gill nets, mesh size ranging from 1.2 to 12 cm, to represent all the sizes of fish that are caught and sold to consumers by fishermen or vendors. An electro-fisher and a beach seine net were also used to catch the fish. Caught fish were sorted according to species, measured to standard length where Oreochromis niloticus species of all sizes were randomly selected and weighed into 10 kg lots. Swab samples were taken from the 10 kg lots at every site aseptically in triplicates on fish skin surfaces, gills, and mouth and analysed in the laboratory to determine total bacterial, fungal, and E. coli O157:H7 load on the fish. Sorted 10 kg samples of O. niloticus were transported in sterile cooler boxes to the University of Zimbabwe Biological Sciences Department microbiology laboratory for further analysis. Total bacterial, fungal, E. coli O157:H7 tests were carried out on both fish swabs and Lake water samples from each site using standard methods, as described by Bartram and Balance (2001) and Navab-Daneshmand (2016). Total mercury and aflatoxin B1 were tested in both fish and water using AAS and LC/MS/MS (Trass et al., 2014). Fish for blank samples were bought from Lake Harvest Aquaculture Company in Kariba which were bred in fresh water ponds. Water samples from the harvest waters were collected as an integrated depth profile using the Rutnar sampler in triplicates 30-litre water bucket at every sampling point where 1-litre subsamples were taken in sterile, Hg-free and aflatoxin-free containers. The samples were quickly transported under chill conditions for storage and analysis to the University of Zimbabwe Biosciences Department Microbiology Laboratory.

Determination of total viable bacterial load

Viable bacterial counts were determined from the swabs and water samples using standard pour plate method (Bartram and Balance, 2001). Plate count agar was prepared by suspending 23 g of the
powder in 1 litre of distilled water and boiled whilst stirring until completely dissolved. The solution was sterilized by autoclaving at 121°C for 15 min. The agar solution was cooled to 45°C, well mixed and 1 ml of each sample was pipetted aseptically in a sterile plate in a biosafety cabinet and 20 ml of molten sterile agar, poured aseptically, was mixed and allowed to set. Serial dilutions were prepared where necessary, and plates were incubated at 37°C for 48 h. Colonies were counted using a colony counter and the dilution factor was used to calculate the final load.

Determination of total viable fungal load
Viable fungal counts were conducted from the swabs and water samples using standard pour plate method (Bartram and Balance, 2001). Thirty-nine grams of Potato dextrose agar were suspended in 1 litre of distilled water and boiled whilst stirring until completely dissolved. The solution were sterilized by autoclaving at 121°C for 15 min. The agar solution was cooled to 45°C, well mixed and 1 ml of each sample was pipetted aseptically in a sterile culture plate in a biosafety cabinet and 20 ml of molten sterile agar, poured aseptically, was mixed and allowed to set. Serial dilutions were prepared where necessary, and plates were incubated at 25°C for 72 h. Colonies were counted using a colony counter and the dilution factor was used to calculate the final load.

Determination of **E. coli** O157:H7
Fish contact surfaces on muscle skin, gills, and mouth were swabbed thoroughly with moistened sterile cotton swabs (Bartram and Balance, 2001). Each swab was placed in 9 ml of sterile Tryptone soy broth and transported on ice packs to the University of Zimbabwe Biological Sciences Microbiology Laboratory for further analysis. To determine **E. coli** O157:H7 viable counts load on fish, 1 ml from the swab sample was diluted using 99 ml of sterile quarter-strength ringer solution and filtered through a sterile Millipore 0.45 µm; white 47-mm gridded membrane filter (Navab-Daneshmand, 2016). Serial dilutions were prepared where necessary. The filter paper was placed on a previously made CHROM agar plate and incubated at 37°C for 24 h. After 24 h, the colonies were counted using a colony counter and the number of colony-forming units per sample was calculated from the dilution factor. To determine **E. coli** O157:H7 in lake water, 100 ml of lake water was filtered through a sterile Millipore 0.45 µm; white 47-mm gridded membrane filter and analysed as for swab samples. Dilutions were made where necessary.

Determination of Hg concentration
Preparation of subsamples and analysis were made according to CODEX STAN 193-1995 (Chen et al., 2014). The frozen fish were thawed, and each fish was dissected using stainless steel instruments. Muscles were taken out and combined to form composite samples which were oven-dried at 105°C for 6 h. Size was reduced into fine particles which were thoroughly mixed to achieve complete homogenization. Five grams were digested with ultra-pure nitric and perchloric acid (1:1) at 100°C in a water bath to obtain a clear solution. The solution was made up to 50 ml volume with deionized water and analysed for Hg using an atomic absorption spectrophotometer against (0.005–1000 ng/ml) calibration standards of Hg solutions. To determine Hg from lake water, 90 ml of water samples was acidified with 10 ml of nitric:perchloric (1:1) acid and analysed for Hg using an atomic absorption spectrophotometer against (0.005–1000 ng/ml) Hg calibration standards. The obtained results were expressed as (µg/kg) dry weight fish muscle. All the glassware
used were soaked overnight in 9% nitric acid and later rinsed with distilled water and dried in an oven prior to use to remove metal contamination.

**Determination of AFB1**

AFB1 was determined in both fish and water from Lake Chivero using HPLC-mass spectrometry using the QuEChERS method (Trass et al., 2014). The calibration standards were matrix matched by spiked known volumes of standards of AFB1 into blank fish samples obtained from Lake Harvest Aquaculture to target concentrations ranging from 0.005 to 1000 ng/ml. Preparation of subsamples was made according to CODEX STAN 193-1995 of 2015. Fish muscle samples were oven-dried at 105°C for 6 h, minced and pulverized into fine powder after which aflatoxins were extracted in polar protic solvent. The samples were homogenized using a blender and 5 g of ground fish muscle was transferred to a 50-mL extraction tube. Ten millilitres of deionized water and 10 mL of acetonitrile:formic acid solution (9:1) were added to the extraction tube. Four grams of Magnesium Sulphate (MgSO₄) and 1.0 g of NaCl were added into the 50 mL containing homogenized sample, shaken vigorously by hand for 1 min and centrifuged for 5 min at 4000 rpm, making sure that the supernatant formed on top of the solid material and that the solid material was at the bottom of the tube. A dispersive solid-phase extraction was done to clean-up the crude sample extract. The supernatant from the extraction process was transferred into a 15 mL centrifuge tube, containing 900 mg MgSO₄ and 150 mg NaCl, shaken vigorously by hand for 30 s and centrifuged for 5 min at 4000 rpm to separate solid materials from the liquid layer. The liquid portion was filtered through a 0.45 μm pore size and 25 μL were injected into the LC/MS/MS system for analysis. LC/MS/MS was performed using a Phenomenex 3 μm, Luna Column 50 × 2.1 mm HPLC column on an Agilent 1260 LC system (Agilent Technologies, Palo Alto, CA, USA) with an upper pressure limit of 600 bar, equipped with a binary pump, auto-sampler and interfaced with an Agilent Q-TOF 6530 Triple Quadrupole Time of Flight Mass Spectrometer detector (Agilent Technologies, USA). The ionization source was electrospray ionization (Trass et al., 2014). Data acquisition and analysis were performed using the Agilent Mass Hunter software, version B.06.01/B.07.01.

**Estimation of fish consumption pattern and human health exposure to Hg and AFB1**

To estimate human health exposure to Hg, and AFB1 from the consumption of fish from polluted water, PDI, APDI, and HQ values were calculated for the general adult population (18 years and above). A survey was carried out to determine the fish consumption trends using questionnaires and interviews. Adult fish consumers in the residences surrounding Lake Chivero and selected Harare high-density suburbs such as Kuwedzana Extension, White Cliff, and Kuwedzana were interviewed with the help of a questionnaire to obtain fish intake rate, consumer exposure frequency, consumer exposure duration, and adult consumer body weight. A body mass scale was used to obtain the body weight of each respondent. The levels of Hg and AFB1 and the fish consumption trends were used to calculate the exposure status. The values of PDI, APDI, and HQ were calculated using the following equations from the data obtained from the survey of each community and from combined data.

\[
PDI = \frac{C \times L \times (EF)}{(BW)}
\]

where PDI is the probable daily intake of Hg or AFB1, \(C\) the level of Hg or AFB1 (μg/kg) in the fish samples, \(L\) the average daily consumption of fish-based meals (kg), \(BW\) the average body weight (kg) of adult fish consumers.

The value of APDI of Hg or AFB1 was calculated from the following formula:

\[
APDI = \frac{C \times L \times (AEF)}{(ABW)}
\]

where APDI is the average probable daily intake, \(C\) the average concentration of Hg or AFB1 (μg/kg) in the fish samples, \(L\) the average daily consumption of fish-based meals (kg), and \(ABW\) is the average body weight (kg) of an adult fish consumer.

The value of the HQ of Hg or AFB1 was calculated using the following formula:

\[
HQ = \frac{APDI}{PTDI}
\]

where HQ is the hazard quotient, APDI the average probable daily intake, and PTDI is the provisional tolerable daily intake where no adverse effect is expected when a consumer is exposed to a food hazard.

**Results**

**Total Hg, AFB1, E. coli O157:H7, total bacterial count, and total fungal count concentration**

The mean concentration of Hg in fish was significantly higher (\(t\)-test; \(P < 0.05\)) than the mean concentration of Hg in water (Table 1). The AFB1 concentrations were significantly higher \(t\)-test; \(P < 0.05\) in fish than that in water (Table 1). Similarly, the mean concentration of \(E.\) coli O157:H7 in fish (2.008 log10 cfu/cm²) was significantly higher \(t\)-test; \(P < 0.05\) than that in water (1.71 log10 cfu/100 mL).

**Table 1. Summary of statistics of mercury (Hg), aflatoxin B1 (AFB1), Escherichia coli O157:H7, total bacterial count (TBC), and total fungal count (TFC) concentrations in fish and water samples**

| Parameter                      | Fish            | Water           | \(t\)-value | \(P\)-value |
|--------------------------------|-----------------|-----------------|-------------|-------------|
| Mean log₁₀ E. coli O157:H7 (cfu/100 ml) | 2.01 ± 0.03     | 1.71 ± 0.04     | 5.856       | 3.02 × 10⁻³⁺ |
| Mean log₁₀ TBC (cfu/ml)         | 8.98 ± 0.26     | 9.05 ± 0.05     | -1.271      | 0.212       |
| Mean log₁₀ TFC (cfu/ml)         | 4.83 ± 0.02     | 4.56 ± 0.03     | 7.954       | 2.68 × 10⁻³⁺ |
| Mean total mercury (μg/kg)      | 0.018 ± 0.001   | 0.008 ± 0.004   | 6.250       | 1.53 × 10⁻³⁺ |
| Mean aflatoxin B1 (μg/kg)       | 0.025 ± 0.012   | 0.005 ± 0.003   | 360.971     | 2.31 × 10⁻³⁺ |

The values are the mean ± SD.

The symbol "⁺" indicates significant differences \((P < 0.05)\).
Fish (8.98 log 10 cfu/cm²), the differences were not significant (Table 1). The mean concentration of total fungal count (TFC) in fish was 4.83 ± 0.02 log 10 cfu/ml. Although the mean concentration of total bacterial count (TBC) in water (9.06 log 10 cfu/ml) was higher than the mean concentration in fish, the differences were not significant (t-test; P < 0.05) (Table 1).

The concentration of total fungal count (TFC) in fish was significantly higher (t-test; P < 0.05) than the mean TFC in water (Table 1).

### Exposure assessment results

*Escherichia coli* O157:H7 tested positive in all fish and water samples and high bacterial and fungal loads were recorded. All samples had microbial levels that were above the CODEX standards (Table 2). Hg and AFB1 tested positive in both fish and water and the concentrations were within the recommended limits of the CODEX standards for Hg. There are no available CODEX reference guidelines set for AFB1 in both fish and water (Table 2).

### Survey results

In the survey, 136 respondents who were 18 years and above participated and were both interviewed and responded to questionnaires: 97 (71%) were male and 39 (29%) were female (Table 3). Fifty-four percent of the respondents were aged between 18 and 34 years; 31% were 35–44 years; 11% were 45–54 years; and 4% were above 55 years. The mean fish intake rate (grams fish/meal), exposure frequency (fish meals/year), exposure duration (years), and body weight (kg) were 0.310 kg, 105.52 (fish meals/year), 13.74 years, and 68.98 kg, respectively (Table 4). There were no significant differences in the intake pattern for any of the four residential communities (P > 0.05).

The mean fish intake rate (grams fish/meal), exposure frequency (fish meals/year), exposure duration (years), and body weight (kg) for males were 0.301 kg, 115.21, 16.28 years, and 69.79 kg, respectively, and for females were 0.298 kg, 14.15 years, 98.12 fish meals/year, and 67.48 kg, respectively (Table 1). A total of 33 cases of bloody loose diarrhea and 17 cases of chronic illness were reported in male respondents and 22 cases of bloody loose diarrhea and 18 cases of chronic illnesses were reported in female respondents in the survey (Table 3). The consumption pattern between males and females was not significantly different (one-way ANOVA, P > 0.05) although males recorded 8.66% diarrhea cases compared to females 5.14% (Table 5). The mean age for males was 36.64 years whilst the mean age for females was 32.86 years. Again, the difference in the age was not significant (one-way ANOVA, P > 0.05).

All the respondents interviewed in Kuwadzana Extension and Kuwadzana (1–7) stated that all the fish they consume were bought from fishermen and fish vendors. In White Cliff, 91.34% of the respondents stated that they consumed bought fish from fish vendors and fishermen whilst 86.66% indicated that they do subsistence fishing from the lake or rivers. In Chivero Cottages 28.47% stated that they obtained their fish through subsistence fishing from the lake, while the other 49.16% obtain their fish from rations from National Parks or Fisheries Research Station. The latter were mostly Fisheries and National workers or their family members or relatives who occasionally receive rations from their employers, whilst 6.53% of the respondents obtain their fish through fishing from the lake. All the respondents in the survey indicated they consume fish in their meals at least once per week.

The mean PDI for Hg was 0.0097, 0.0086, 0.0076, and 0.0067 µg/kg bw/day, respectively, for Chivero Cottages, White Cliff, Kuwadzana Extension, and Kuwadzana (1–7), respectively. The mean PDI for Hg was highest at Chivero Cottages and lowest in Kuwadzana (1–7) (Figure 3).

There were no significant differences in the mean fish intake pattern among the four sites (one-way ANOVA, P > 0.05). The mean PDI for AFB1 was 0.0145, 0.0143, 0.0134, and 0.0104 µg/kg bw/day, respectively, for Chivero Cottages, White Cliff, Kuwadzana Extension, and Kuwadzana (1–7), respectively. The value of PDI for AFB1 was highest in Chivero Cottages and lowest in Kuwadzana (1–7) (Figure 4). There were no significant differences in the intake pattern for any of the four residential communities (P > 0.05).

The value of APDI for Hg in fish was 0.0082 µg/kg bw/day and there were no significant differences in the intake pattern for the four residential sites. The APDI for AFB1 in fish was 0.0125 µg/kg bw/day and there were no significant differences in the intake pattern (P > 0.05) (Table 6). The value of APDI of Hg for males and females was 0.0089 and 0.0077 µg/kg bw/day, respectively. The value of APDI of Hg for females was 0.0147 and 0.0135 µg/kg bw/day, respectively. The differences between males and females in APDIs for Hg and AFB1 were not significant (P > 0.05).

The APDI for Hg and AFB1 for the four residential sites were 0.0073 and 0.0141 µg/kg bw/day. There were no significant differences in the APDIs for any of the four residential communities (P > 0.05).

### Table 2. Concentrations of mercury (Hg), aflatoxin B1 (AFB1), *Escherichia coli* O157:H7, total bacterial count (TBC), and total fungal count (TFC) compared to CODEX Alimentarius international food safety standards (2015)

| Parameter | Mean fish concentration | Mean water concentration | CODEX Alimentarius guideline standards (mg/kg) |
|-----------|-------------------------|--------------------------|-----------------------------------------------|
| Mean log₁₀ *E. coli* O157:H7 (cfu/100 ml) | 2.008 ± 0.03 | 1.706 ± 0.04 | 0.0 |
| Mean log₁₀ TBC (cfu/ml) | 8.98 ± 0.26 | 9.05 ± 0.05 | <5.7 log₁₀ cfu/100 ml |
| Mean log₁₀ TFC (cfu/ml) | 4.83 ± 0.02 | 4.56 ± 0.03 | <2.7 log₁₀ cfu/100 ml |
| Mean total mercury (µg/kg) | 0.018 ± 0.001 | 0.008 ± 0.004 | <0.001 |
| Mean aflatoxin B1 (µg/kg) | 0.025 ± 0.012 | 0.005 ± 0.003 | No guidance set |

Values are mean ± SD.

### Table 3. Demographic composition of respondents who participated in the survey

| Demographic characterization | Number | Percentage (%) | Cases of loose or bloody diarrhoea illness reported | Cases of chronic illness reported |
|-----------------------------|--------|----------------|-----------------------------------------------|----------------------------------|
| 18–34 years                 | 74     | 54             | 28                                             | 13                               |
| 35–44 years                 | 43     | 31             | 23                                             | 7                                |
| 45–54 years                 | 13     | 11             | 5                                              | 3                                |
| 55 years and above          | 5      | 4              | 2                                              | 2                                |
| Male                        | 97     | 71             | 33                                            | 17                               |
| Female                      | 39     | 29             | 22                                            | 8                                |

Although the mean concentration of total bacterial count (TBC) in water (9.06 log₁₀ cfu/ml) was higher than the mean concentration in fish (8.98 log₁₀ cfu/cm²), the differences were not significant (Table 1). The mean concentration of total fungal count (TFC) in fish was significantly higher (t-test; P < 0.05) than the mean TFC in water (Table 1).
Table 4. Comparison of mean fish consumption pattern for the four urban communities

| Parameter               | Chivero Cottages | White Cliff | Kuwadzana Extension | Kuwadzana (1–7) | Mean | P-value |
|-------------------------|------------------|-------------|----------------------|-----------------|------|---------|
| Exposure frequency      | 115.44           | 117.0       | 104.00               | 81.12           | 104.52 | 0.046*  |
| Exposure duration       | 13.00            | 10.84       | 14.73                | 16.28           | 13.74 | 0.071   |
| Fish intake rate        | 0.301            | 0.297       | 0.303                | 0.341           | 0.330 | 0.741   |
| Body weight             | 67.78            | 67.48       | 68.78                | 70.80           | 68.98 | 0.668   |

The symbol * indicates significant differences (P < 0.05).

Table 5. Comparison of mean consumption parameters and mean body weight for males and females

| Parameter                  | Males       | Females    | P-value |
|----------------------------|-------------|------------|---------|
| Fish intake rate (kg)      | 0.301 ± 0.12| 0.298 ± 0.12| 0.0934  |
| Exposure frequency (kg)    | 115.21 ± 15.31| 98.12 ± 21.56| 0.0536  |
| Exposure duration (years)  | 16.28 ± 6.2  | 14.14 ± 4.3  | 0.0744  |
| Body weight (kg)           | 69.79 ± 10.14| 67.48 ± 10.13| 0.773   |

The values are mean ± SD.
The symbol * indicates statistical difference (P < 0.05).

differences between APDI in males and APDI in females among the four sites (P > 0.05). The HQ for both male and female fish consumers had an HQ of <1 (Table 7).

Discussion

Although fish is an important source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are essential nutrients (omega-3 fatty acids) keeping our heart, central nervous system (CNS), and brain healthy because our body is not able to produce them. Consumption of fish from contaminated sources such as Lake Chivero must be considered as a food safety issue that requires immediate action, because the fish becomes a source of food hazards to the consumers and the public at large as pollutants enter the food chain (FAO/WHO, 2005). The presence of E. coli O157:H7 in both water and fish was of great concern because this bacterium is a pathogen which causes food-borne illness (Table 1). Escherichia coli O157:H7 occurrence in high counts in water and in fish as observed in this study is often an indicator of recent contamination with pollutants of faecal origin. Raw sewage effluent is being continuously discharged into the water body because this organism do not survive for long under normal circumstances at lake water conditions. According to the New York State Department of Health (2004), the presence of high bacterial and fungal counts in water or food is a strong indication of spoilage and contamination, which may be in association with many other types of pathogenic microorganisms (Cabral, 2010). The high microbial concentration levels recorded in this study confirmed the uncontrolled pollution of Lake Chivero (Table 2). Consumers are at high risks of exposure to many other food safety hazards together with food-borne pathogens, because the fish are caught already contaminated (Table 1).

The cases of diarrhoea reported in the survey in this study can also be attributed to the presence of E. coli O157:H7 in the consumed fish (Table 6). In the survey conducted in the four communities, 42.65% of respondents reported cases of bloody diarrhoea and loose stool after consuming fish from Lake Chivero which lasted an average of 3–8 days and this simulate the symptoms of E. coli O157:H7 (Kaper and Karmali, 2008) (Table 6). Some respondents reported instant diarrhoea with severe pain. Escherichia coli O157:H7 is contagious and it can cause cross-contamination with other food stuffs and results in food-borne disease outbreaks (Lynn et al., 2005) (Table 6). The infection of E. coli O157:H7, in some individuals, particularly the elderly, immune-compromised, and children under 5 years of age, can result in a complication called haemolytic uraemic syndrome (HUS), in which erythrocytes are damaged and the renal system fail to function (Kuehne et al., 2016; Launders et al., 2016). About 2%–7% of infections lead to this complication in the USA (CDC, 2016b). In most cases, people recover without the use of antibiotics or other special medication within 5–10 days and there is no proof that medication reduce the course of disease (Hwang and Huang, 2010) and it is thought that treatment with some antibiotics may precipitate kidney complications (Ejrnæs, 2011).

The diagnosis E. coli O157:H7 is done by identifying it in the patient’s stool through laboratory analysis (Kostyla et al., 2015); however, in Zimbabwe, there are very few studies or reports for testing for E. coli O157:H7 in available literature, which makes it difficult to understand its behaviour and incidences amongst local consumers. This study, therefore, urges all individuals who suddenly suffer from diarrhoea with blood to report to the nearest health centre and get their stools examined for E. coli O157:H7 so as to have records and incidence data records. Individuals who suffer from long-term illnesses such as cancer, HIV/AIDS, or other chronic diseases are at greater risk of severe symptoms and illness, especially, the elderly, children under the age of 5 years, and people whose health is weakened (Adams et al., 2016; Launders et al., 2016).

Although microbial levels in fish from Lake Chivero can be reduced during cooking, improper handling of the fish can result in cross-contamination with other household food products and this can trigger spreading of food pathogens and outbreak of food-borne illness amongst consumers (Manjengwa et al., 2016). The presence of E. coli O157:H7 in all samples highlights the seriousness of the problem (Table 1).

Harare municipal water is supplied from the same source (Lake Chivero), shortages of water supply in the city of Harare and bursts of sewer pipes were implicated in the outbreaks of typhoid and cholera in many parts of Harare’s high-density suburbs (Manjengwa et al., 2016). It may be very difficult to get rid of these microorganisms from fish especially when they originate from the polluted water sources from capture and along the handling chain to the point of consumption. For a food processor to maintain the number below or within specified limits safe for consumers, a good manufacturing practice (GMP/GHP) needs to be adopted or applied properly along the whole handling chain (Table 1).

The high microbial levels of bacteria and fungi in both fish and water which were above food safety international standards indicate potential risk and health hazard exposure to consumers (Table 1).
In comparing results from this study to CODEX Alimentarius International Food Standards (FAO/WHO, 2015), guidelines for general standard for contaminants and toxins in food and feed (CODEX STAN 193-1995). The microbial load results clearly showed that consumers of fish from Lake Chivero are exposed to pathogenic microorganisms and are at risk of contracting foodborne illnesses due to consumption of contaminated fish. The fish were caught already contaminated.

Although fish is an important source of nutrition in Zimbabwe, Hg and AFB1 were also detected in both fish and water. These chemicals are very dangerous which exposes consumers to health risks even if their concentrations were very low (WHO, 2006) (Table 1). The naturally occurring mycotoxins (aflatoxins) are considered to be carcinogenic, mutagenic, and teratogenic to humans (Hussein and Brasel, 2001). The initial risk assessment by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) resulted in the recommendation to take management actions to reduce aflatoxin intake amongst populations to the lowest levels possible (CODEX, 2015).

Work done by Doke and Gohlke in Ghana (2014) in fish also shows the presence of Hg in fish in levels above the international standards. This is of concern because Hg is a dangerous heavy metal which is toxigenic and carcinogenic, especially to the CNS (FDA, 2017). The results stated no significant difference to hazard exposure or consumption patterns between males and females due to gender or age \( P > 0.05 \) (Table 7). This suggests that age and gender have no influence in the consumption patterns.

Although there is no international standard on the permissible levels of AFB1 in fish products, this study shows that AFB1 is present in fish at levels that were almost double that in water. This is of a serious consumer health concern because of possible bioaccumulation and
Table 6. Average probable daily intake (APDI) of mercury (Hg) and aflatoxin B1 (AFB1) in fish for the four urban communities

| Parameter       | Chivero Cottages | Kuwadzana Extension | Kuwadzana (1–7) | White Cliff | P-value |
|-----------------|------------------|----------------------|-----------------|-------------|---------|
| APDI (Hg μg/kg bw/day) | 0.0099           | 0.0078               | 0.0068          | 0.0104      | 0.2310  |
| APDI (AFB1 μg/kg bw/day) | 0.0145           | 0.0143               | 0.0104          | 0.0134      | 0.3182  |

The values are mean ± SD.

Table 7. Hazard exposure assessment of mercury (Hg) and aflatoxin B1 (AFB1) for both male and female respondents

| Parameter               | Mean APDI Hg in fish (μg/kg bw/day) | Mean APDI AFB1 in fish (μg/kg bw/day) | Hazard quotient (HQ) |
|-------------------------|------------------------------------|--------------------------------------|----------------------|
| Male                    | 0.0089 ± 0.0036                    | 0.0147 ± 0.0011                      | <1                   |
| Female                  | 0.0077 ± 0.0032                    | 0.0135 ± 0.0010                      | <1                   |
| Standard (CODEX STAN 193-1995 of 2015) | 0.0011 (mg/kg) | No standard set in fish              | <1                   |
| PTWI (JECFA 2003)       | 0.0016 (mg/kg bw/week)             | No standard set in fish              | <1                   |
| Toxicological guidance  | 0.0002 (mg/kg bw/day)              | No guidance set in fish              | <1                   |

The values are mean ± SD. APDI, average probable daily intake.

refinement doses (RfD) of 0.0001 mg/kg bw/day (IRIS, 2001), but their presence is of concern since these substances exist in several forms. The adverse health exposure effects start to be certain from a lifetime consumption rate of polluted fish at the approximated rate of 0.068 kg bw/day-Hg, as reported by Doke and Gohlke in Ghana 2014.

The average concentrations of Hg in fish and water reveal that Hg concentration was higher in fish than in water and the difference is significant (t-test, P < 0.05) (Table 1). The mean concentration of AFB1 in water also showed that the concentration of AFB1 in fish was almost double than that in water and the difference is significant (P < 0.05) (Table 1). The high concentration of AFB1 and Hg in fish compared with water suggests bioaccumulation of Hg and AFB1 in fish tissues due to excessive pollution of the lake. Fish can feed on sediments and there is a lot of deposited sediments at the bottom of Lake Chivero due to deposition of dumped raw sewage and industrial wastes (Nhiwatiwa et al., 2011). Heavy metals accumulate in the sediments which act as sinks of toxicants (Nhiwatiwa et al., 2011). These together with other fungal colonized debris are eventually ingested by fish as food and bioaccumulate or biomagnifies along the food chain and as a result fish consumers are exposed to these pollutants.

Although there are no toxicological guidance levels for AFB1 in fish and water in the CODEX standards, other ready to eat foods such as grains, cereals, nuts, and some fruits have the carcinogenic potency estimates for total aflatoxins in place ranging from 4 to 15 pg/kg (CODEX, 2015). This study being the first to come up with available data on hazard exposure assessment on E. coli O157:H7, Hg, and AFB1 via consumption of contaminated fish products in Zimbabwe, the CODEX Alimentarius international standards were used to evaluate the exposure assessment (Table 4). However, there were limitations of publications or data in literature in Zimbabwe which have information on the estimation of AFB1 and E. coli O157:H7. Some other types of fish consumed from Lake Chivero were not represented in this study, focus was only on O. niloticus; of which it is not the only fish caught for consumption from this lake, hence further investigations are needed. Evaluation of the levels of E. coli O157:H7, Hg, and mycotoxins in sediments is vital to assess the status of the sediments in the lake. Mycotoxins usually exist in association with each other; so, there is also need for investigating other types of mycotoxins in the fish and water bodies.

According to Doke and Gohlke 2014 in Ghana, it is very important to take into consideration that both JECFA provisional daily intake and reference dose for USEPA include uncertainty factors, so the use of HQ in this study is defined conservatively as an estimate of the probable or exposure potential for consumer health effects and not directly measuring the risk. In Zimbabwe, there are no regulatory information concerning the occurrence of these mycotoxins in fish products from water sources such as Lake Chivero. Such regulatory information is necessary in help providing knowledge and toxicological guidance to fish consumers in order to control unnecessary hazard exposures. This study assessed health exposure to adult
consumers only; however, different age groups have different dietary requirements, but fish consumption rates may vary in children, pregnant women, elderly, and those who are immune-compromised. So, there is a need to perform exposure assessments in relation to distinct age groups so as to see how they vary or related to each other.

Conclusions

Fish and water from Lake Chivero are contaminated with high levels of pathogenic strains of *E. coli* (*E. coli* O157:H7), and contain high bacterial and fungal loads which are all above the international food safety guidance levels. The lake is contaminated with toxicogenic Hg and carcinoagenic AFB1 even although their concentration levels are within the international food safety toxicological guidance levels. Thus, fish consumers are exposed to dangerous food safety hazards and are at risks of contracting foodborne illness due to consumption of contaminated fish. Water pollution greatly affects food chains and food webs that are connected to water. If one species is affected, it creates a chain reaction and the entire food chain is affected. Consumers and vendors of fish products from Lake Chivero must consider the dangers of health effects associated with consuming contaminated food. They may need to consider stopping the consumption and vending of fish from Lake Chivero because they contain food safety hazards and should obtain fish from alternative clean or fresh water sources.

Recommendations

Consumers and vendors of fish products from Lake Chivero must consider the health effects associated with consuming contaminated food. They may need to stop the consumption and vending of fish from Lake Chivero because they contain food safety hazards and must consider obtaining fish from clean water sources. Regulators, city of Harare authorities, and advisory bodies must put in place effective measures to stop pollution of Lake Chivero and consider development of toxicological consumption guidance for fish consumption advisories when warranted, in order to maximize the health benefits of fish consumption and to protect the environment. It was noted that fish in Zimbabwe are usually cooked before consumption; therefore, further investigations and assessments into the method of cooking fish of Zimbabwean origin and concentrations of Hg and mycotoxins in the cooked fish are needed to come up with more accurate exposure concentration levels. Mycotoxins and Hg usually exist in several types or forms and in association, also, further investigations are needed for the other types of mycotoxins in fish and other forms of Hg in fish such as the deadly poisonous methylmercury.

Zimbabwean scientists should come together, using brain storming or collaborative research, to examine the links between food safety hazard exposures and human health, with researchers filling in missing pieces of the puzzle on hazard exposure. One approach is the development of adverse outcome pathways. Adverse outcome pathways are a way of assembling all the existing knowledge and data about small biological changes—to a cell, tissue, or organ—resulting from exposure to health hazards, and their connection to more serious harmful health effects detected in human beings and ecosystems. However, like all science, they must be tested to determine how they can best assist regulators in their efforts to protect the public and the natural habitats from exposures to dangerous pathogenic microorganisms and toxigenic substances. It is important to evaluate and develop risk communication and risk management strategies which minimize exposure risks and maximizes benefits of fish consumption, data generation and analysis of evaluating or monitoring data on fish and water contamination levels chemical hazards is very critical (Doke and Gohike, 2014).

Conflict of interest statement

No conflict of interest declared.

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