Inorganic pollutants in drinking water of South Western Uganda: Implications on water safety and cancer risks for Ugandans

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REG Number: BPH/0013/143/DU

A FINAL DISSERTATION SUBMITTED TO THE SCHOOL OF PHARMACY
AS PARTIAL FULFILLMENT FOR THE REQUIREMENT OF
AWARD OF A BACHELORS DEGREE IN PHARMACY
OF KAMPALA INTERNATIONAL UNIVERSITY
WESTERN CAMPUS UGANDA

DECEMBER 2018

DECLARATION

I hereby declare that the work in this research has never been submitted to any academic institution both locally and internationally and it's a result of my hard work and my supervisor. All text has been referenced appropriately and all ethical concerns have been addressed within the report.

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Signature and Date

December, 10th, 2018.

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APPROVAL

The research student has been offered approval to submit the report for final examination after having fulfilled all terms and conditions set out in the Mentee and Mentorship agreement. The research student has also fulfilled all criteria for authorship as outlined by the International Committee of Medical Journal Editors (ICMJE) guidelines and has been added as a co-author on the manuscript which is under peer review at Hindawi (submitted November 2018).

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DEDICATION

I dedicate this work to my family and above all my mother who has been instrumental and guided me in my life to date.

ACKNOWLEDGEMENTS

I wish to acknowledge the mentorship and training offered by my supervisor from the day I signed the mentorship agreement with him. This has helped me appreciate research better and opened up a new world of knowledge that I had never known through theoretical class lessons. In particular, the introduction to international geographical resources, sampling, data collection, and analysis as well as data interpretation was important while working closely with him. I have learnt from the criticism, encouragement and mentorship which has helped me gain an optimistic outlook on key aspects of life that I would have ignored throughout my academic lifecycle.

This research project was conducted through inter-institutional and departmental collaborations, and I seek to acknowledge funding raised by my colleagues on the research from Kampala International University i.e. my supervisor, myself, Dr. Ejike Daniel Eze, Mr. Alfred Omachonu Mr. Okapanchi, Ms. Hellen Wambui Kinyi, Mr. Fred Ssempijja, Ms. Jovile Atusiimirwe, Dr. Joy Suubo, Dr. Edgar Mario Fernandez, colleagues from Makerere University i.e. Ms. Sarah Namubiru, Dr. Dickson Stuart Tayebwa, Dr. Andrew Tamale as well as Dr. Nathan Nshakira from Kabale University. Thank you very much for supporting ChemTox Research Group through your invaluable contributions, physically and intellectually.

Finally, I would like to thank my fellow colleagues who have supported me throughout my academic lifecycle since I joined university. Your company has been healthy and instrumental in helping me succeed in my work. Thank you very much.

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LIST OF ABBREVIATIONS

AAS	Absorbance Spectrophotometer
Ag	Silver
Ca	Calcium
Cd	Cadmium
Cr	Chromium
Cu	Copper
EC	European Commission
EPA	Environmental Protection Agency of the United States of America
EU	European Union
FDA	Food and Drug Authority
Fe	Iron
Hg	Mercury
Mn	Manganese
Ni	Nickle
Pb	Lead
ppb	Parts per billion
ppm	Parts per million
Se	Selenium
WHO	World Health Organization
Zn	Zinc

ABSTRACT

Background: There is a scarcity of information on the quality of safe drinking water for the attainment of Goal 6 of the SDGs. The study aimed to determine concentrations of inorganic compounds, estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI), incremental lifetime cancer risk (ILCR) and identify safe drinking water source points in southwestern Uganda.

Methods: This was an observational study in which 40 drinking water samples were collected from geo-referenced boreholes, springs, open wells, bottled and taps within Bushenyi district of southwestern Uganda. Water samples were analyzed for Copper (Cu), Iron (Fe), Zinc (Zn), Lead (Pb), Cadmium (Cd) and Chromium (Cr) levels using atomic absorption spectrometry (AAS). Water safety measures (EDI, HI, and ILCR) were established for each water source and compared with local and international water permissible standards for each analyte. A spatial map was drawn using qGIS® and analysis of quantitative data was done using MS Excel 2013 at 95% significance.

Results: Fe was the primary water pollutant in the order of open well > borehole > tap > spring > bottled water. This was followed by Zn levels in the order of tap > bottled > spring > borehole > open well. All compounds were safe by international standards except Pb showing a need to install Pb filters in drinking water in Uganda. The EDI was similar for water consumed from spring, bottled and tap sources for Fe and Zn levels and no differences were found in children and adults ($P > 0.05$). Furthermore, the HI showed an absence of non-carcinogenic risk associated although the ILCR was higher in adults than children ($P < 0.05$) due to the very high Zn concentrations.

Conclusion: Borehole water was the most recommended water source showing a need for increased water filtration in Uganda.

KEYWORDS: 'Heavy metals,' 'ecotoxicology,' 'cancer in Africa,' 'water quality,' 'Pb toxicity,' 'Zn toxicity.'

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Heavy metals such as lead (Pb), Mercury (Hg), Chromium (Cr), Copper (Cu), zinc (Zn), Managanese (Mn),Nickle (Ni) and Silver (Ag) are toxic to both man and animals, fishes and the environment (Govind, 2014). These compounds are highly reactive in nature and they exert their toxic effects through such mechanisms although while in trace amounts (for example, Fe, Cu, Zn) they exert some physiological benefit to the body (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Toxicity in the body arises as a result of bioaccumulation of these metals in higher amounts following consumption from the environment by either plants or animals (He & Chen, 2014). Primarily, humans get exposed to these compounds following consumption of contaminated food products including water (Sardar et al., 2013), thus demonstrating the role of environmental health on human health. Environmental contamination primarily arises as a result of human activities such asland-filling, mining, and transportation(Bradl, 2005). This metals are often washed off in running water which often drains the metals in the soil or water bodies that are often used by man thus raising major water safety concerns (Goretti, Pallottini, Ricciarini, Selvaggi, & Cappelletti, 2016; Sardar et al., 2013).

Drinking water by international standards should be clean and all humans have a right to clean water accessibility (Gorchev & Ozolins, 2011; World Health Organization, 2011). However, this continues to be a challenge especially with the contamination of drinking water with heavy metals beyond the recommended limits and the status is worse in developing countries than developed countries (Chowdhury, Mazumder, Al-Attas, & Husain, 2016a). This has subsequently made access to safe quality drinking water by a majority of households a dream (Mohod & Dhote, 2013). This is highly challenging in Africa were policies have not been streamlined to ensure that clean and safe water is provided to the general public especially in the rural communities which are often ignored (Adler, Claassen, Godfrey, & Turton, 2007). In Uganda, contamination of lakes and fish with heavy metals has been shown (Lwanga, Kansiime, Denny, & Scullion, 2003; Oyoo-Okoth et al., 2010), and this demonstrated a need for this study.

1.2 Problem Statement

Heavy metals are toxic in man and their presence in drinking water is a major public health concern (Govind, 2014). This is because these macromolecules are highly reactive and once consumed directly in food or indirectly in drinking water, they are bound to exert negative effects on the body (He & Chen, 2014; Kamran et al., 2013; Tchounwou et al., 2012). These compounds arise as a result of environmental contamination and in Africa, this has been shown to be a problem as a result of land-filling, mining, and transportation activities(Bradl, 2005; Goretti et al., 2016).

Environmental contamination of water sources and food products in Uganda has been demonstrated to be a realistic threat of public health concern (Lwanga et al., 2003; Oyoo-Okoth et al., 2010). This shows that there is a need to monitor the water is currently being distributed for human consumption to ensure that human right access to clean and safe drinking water is realized locally and in several developing countries (Gorchev & Ozolins, 2011; World Health Organization, 2011). There was a need to generate basic knowledge on the status of heavy metal load in drinking water of Uganda for improved policy in this regard.

1.3 General Objective

The study determined the heavy metal concentrations in drinking water of south western Uganda as markers on water safety.

1.4 Specific Objectives

These were,

1. To determine the heavy metal load in drinking water of South Western Uganda
2. To assess the safety of drinking water of Uganda.
3. To estimate the cancer risk posed by drinking water in the study area.
4. To identify areas in Bushenyi with safe drinking water.

1.5 Research Question

These were,

1. What was the heavy metal load in drinking water of South Western Uganda?
2. How safe was the drinking water in South Western Uganda?

3. What was the relationship between drinking and spring water of Bushenyi district, Uganda?

1.6 Justification

Globally safe water availability is a major public health concern and water in urban areas of Uganda has equally been shown to be contaminated (Fuhrimann et al., 2015). Bearing in mind that South Western Uganda was far from the capital city, Kampala, it was important to gain an insight on the status of water quality from a rural setting. Information gathered from this study guided policy and once recommendations implemented would lead to an improvement in safe water accessibility by Ugandans.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Heavy Metals in Water

Heavy metals in drinking water pose a threat to human health and can accumulate in the human body induce cancer and other risk (Chowdhury, Mazumder, Al-Attas, & Husain, 2016b). Heavy metal (Pb, Cd, Cr, Ni) have an appreciable increase in metal concentrations in going from the water to the sediment samples.The Heavy metal concentration, in water was in the order Pb> Cr > Cd >Ni , in sediments Pb> Cr > Cd > Ni . (Begum, Harikrishna, & Khan, 2009). Pb in soil, and Cd, Cr and Pb in the plants were above national thresholds were found in the Nakivubo wetland and the Lake Victoria ecosystem in Kampala (Fuhrimann et al., 2015).

2.2 Heavy Metals in Environment

Of the known elements, nearly 80% are either metals or metalloids. The highly reactive nature of most metals result in their forming complexes with other compounds such oxygen, sulfide and chloride.For some metals, there are no physiological concentrations that are beneficial; as such these metals only have the potential to cause toxicity (Tchounwou et al., 2012). Lake ecosystems are, in particular, vulnerable to heavy metal pollution (Rashed, 2001). Excessive levels of heavy metals can be introduced into the environment, for example, by industrial waste or fertilizers. Soil represents a major sink for heavy metals ions, which can then enter the food chain via plants or leaching into groundwater (Callender, 2003).

2.3 Toxic Effects of Heavy Metals to Man and Recommended Levels.

Toxic levels of heavy metal contamination pose a serious threat to the microorganisms, animal plants and human life.Soil microbial population is highly affected by the presence of heavy metals. In human and mammals, neurological damage, immune system suppression and fetal abnormalities are reported due to the toxic effects of heavy metals. Heavy metal contaminants in drinking water may affect kidney function and haemoglobin level of consumers(Badr, Agrama, & Badr, 2011) The presence of heavy metals in drinking water is of public health significance because of their toxicity at even low concentrations. Water should also be free of microorganisms.The quality of drinking water from tap, rain,river and bottled water should be evaluated by determining the minerals, heavy metals.(Kolawole & Obueh, 2015).

2.3.1 Arsenic in Drinking Water

Chronic arsenic toxicity due to drinking of arsenic-contaminated water has been a major environmental health .The prevalence of skin abnormalities such as pigmentation change and keratosis, chronic lung disease including pulmonary interstitial fibrosis. (Majumdar & Guha Mazumder, 2012)Ingestion of inorganic arsenic in drinking water causes cancer of the skin, bladder, lung, liver, and kidney and cause of chronic respiratory disease and mycardiac infaction (Smith & Steinmaus, 2011).

Aside from occurring naturally in the environment, arsenic can be released in larger quantities through volcanic activity, erosion of rocks, forest fires, and human activity. The wood preserving industry uses about 90% of the industrial arsenic in the U.S. Arsenic is also found in paints, dyes, metals, drugs, soaps and semi-conductors. Animal feeding operations and certain fertilizers and pesticides can release high amounts of arsenic to the environment as can industrial practices such as copper or lead smelting, mining, and coal burning.

2.3.1.1 Health effects of Arsenic

Arsenic is odorless and tasteless. Inorganic arsenic is a known carcinogen and can cause cancer of the skin, lungs, liver and bladder(Sabine & Wendy, 2009).

- Lower level exposure can cause nausea and vomiting, decreased production of red and white blood cells, abnormal heart rhythm, damage to blood vessels, and a sensation of "pins and needles" in hands and feet.

- Ingestion of very high levels can possibly result in death.

- Long-term low level exposure can cause a darkening of the skin and the appearance of small "corns" or "warts" on the palms, soles, and torso.

2.3.1.2 Regulatory limits

- Environmental Protection Agency (EPA) - 0.01 parts per million (ppm) in drinking water (EPA, 2007).

- Occupational Safety and Health Administration (OSHA) - 10 micrograms per cubic meter of workplace air (10 $\mu g/m^3$) for 8 hour shifts and 40 hour work weeks.

2.3.3 Chromium in Drinking Water

Drinking water supplies in many geographic areas contain chromium in the +3 and +6 oxidation states and presence of hexavalent Cr that is classified as a known human carcinogen via inhalation. Limited epidemiological evidence for Cr(VI) ingestion is suggestive of elevated risks for stomach cancers. The directly mutagenic mode of action and the incompleteness of gastric detoxification argue against a threshold in low-dose extrapolation of cancer risk for ingested Cr(VI) (Zhitkovich, 2011). A literature review revealed that coagulation-filtration (with and without prior redn. with iron(II)) is still the most commonly used and effective method of chromium removal from water while adsorptive filtration and ion exchange are suitable for small-scale applications. (Sharma, Petrusevski, & Amy, 2008).

Chromium is found in rocks, animals, plants, and soil and can be a liquid, solid, or gas. Chromium compounds bind to soil and are not likely to migrate to ground water but, they are very persistent in sediments in water. Chromium is used in metal alloys such as stainless steel; protective coatings on metal (electroplating); magnetic tapes; and pigments for paints, cement, paper, rubber, composition floor covering and other materials. Its soluble forms are used in wood preservatives.

2.3.3.1 Health effects of Chromium

Chromium (VI) compounds are toxins and known human carcinogens, whereas Chromium (III) is an essential nutrient(Sabine & Wendy, 2009).

- Breathing high levels can cause irritation to the lining of the nose; nose ulcers; runny nose; and breathing problems, such as asthma, cough, shortness of breath, or wheezing.
- Skin contact can cause skin ulcers. Allergic reactions consisting of severe redness and swelling of the skin have been noted.
- Long term exposure can cause damage to liver, kidney circulatory and nerve tissues, as well as skin irritation.

2.3.3.2 Regulatory limits of Chromium

- EPA– 0.1 ppm (parts per million) in drinking water (EPA, 2007).
- FDA – should not exceed 1 milligram per liter (1 ppm) in bottled water.
- OSHA – an average of between 0.0005 and 1.0 milligram per cubic meter of workplace air for an 8-hour workday, 40-hour workweek, depending on the compound.

2.3.4 Cadmium

Cadmium is a very toxic metal. All soils and rocks, including coal and mineral fertilizers, contain some cadmium. Cadmium has many uses, including batteries, pigments, metal coatings, and plastics. It is used extensively in electroplating(EPA, 2007).

2.3.4.1 Health effects of Cadmium

Cadmium and cadmium compounds are known human carcinogens. Smokers get exposed to significantly higher cadmium levels than non-smokers. Severe damage to the lungs may occur through breathing high levels of cadmium.

- Ingesting very high levels severely irritates the stomach, leading to vomiting and diarrhea.
- Long-term exposure to lower levels leads to a buildup in the kidneys and possible kidney disease, lung damage, and fragile bones.

2.3.4.2 Regulatory limits of Cadmium

- EPA – 5 parts per billion (ppb) or 0.005 parts per million (ppm) of cadmium in drinking water(EPA, 2007).
- Food and Drug Administration (FDA) – concentration in bottled drinking water should not exceed 0.005 ppm (5 ppb).
- OSHA – an average of 5 micrograms per cubic meter of workplace air for an 8-hour workday, 40-hour work week.

2.3.5 Lead

Lead used to be common in the environment due to its widespread historic use in petrol, paint and water pipes, as well as its natural occurrence in soils as a consequence of local geological conditions (HKSAR Government, 2015).As a result of human activities, such as fossil fuel burning, mining, and manufacturing, lead and lead compounds can be found in all parts of our environment. This includes air, soil, and water. Lead is used in many different ways. It is used to produce batteries, ammunition, metal products like solder and pipes, and X-ray shielding devices. Lead is a highly toxic metal and, as a result of related health concerns (see below), its use in several products like gasoline, paints, and pipe solder, has been drastically reduced in recent years. Today, the most common sources of lead exposure in the United States are lead-based paint and possibly water pipes in older homes, contaminated soil, household dust, drinking water, lead crystal, lead in certain cosmetics and toys, and lead-glazed pottery(EPA, 2007).

2.3.5.1 Health effects of Lead

EPA has determined that lead is a probable human carcinogen. Lead can affect every organ and system in the body. Long-term exposure of adults can result in decreased performance in some tests that measure functions of the nervous system; weakness in fingers, wrists, or ankles; small increases in blood pressure; and anemia.

- Exposure to high lead levels can severely damage the brain and kidneys and ultimately cause death.

- In pregnant women, high levels of exposure to lead may cause miscarriage.

- High level exposure in men can damage the organs responsible for sperm production.

2.3.5.2 Regulatory limits of Lead

- EPA – 15 parts per billion (ppb) in drinking water, 0.015 micrograms per cubic meter in air.

2.3.6 Selenium

Selenium is a trace mineral widely distributed in most rocks and soils. Processed selenium is used in the electronics industry; as a nutritional supplement; in the glass industry; in plastics, paints, enamels, inks, and rubber; in the preparation of pharmaceuticals; as a nutritional feed additive for poultry and livestock; in pesticide formulations; in rubber production; as an ingredient in antidandruff shampoos; and as a constituent of fungicides. Radioactive selenium is used in diagnostic medicine(Sabine & Wendy, 2009).

2.3.6.1 Health effects of Selenium

Selenium is toxic in large amounts, but trace amounts of it are necessary for cellular function in most, if not all, animals. For humans, selenium is an essential trace nutrient. For example, selenium plays a role in the element functioning of the thyroid gland. The Tolerable Upper Intake Level is 400 micrograms of selenium per day. Consumption above that level can lead to selenosis;

- Short-term oral exposure to high concentrations can cause nausea, vomiting, and diarrhea.
- Chronic oral exposure to high concentrations can produce selenosis. Major signs of selenosis are hair loss, nail brittleness, and neurological abnormalities.
- Brief exposures to high levels in air can result in respiratory tract irritation, bronchitis, difficulty breathing, and stomach pains. Longer-term exposure can cause respiratory irritation, bronchial spasms, and coughing.

2.3.6.2 Regulatory limits of Selenium

- EPA – 50 parts per billion of selenium (50 ppb) in drinking water(EPA, 2007).
- OSHA – 0.2 mg per cubic meter of workroom air for an 8-hour work shift.

2.3.7 Silver

Silver usually combines with other elements such as sulfide, chloride, and nitrate. Silver is used to make jewelry, silverware, electronic equipment, and dental fillings. Silver metal is also used in electrical contacts and conductors, in brazing alloys and solders, and in mirrors. Silver compounds are used in photographic film. Dilute solutions of silver nitrate and other silver compounds are used as disinfectants and as an antibacterial agent. Silver iodide has been used in attempts to seed clouds to produce rain(Sabine & Wendy, 2009).

2.3.7.1 Health effects of Silver

According to EPA, silver is not classifiable as a human carcinogen(EPA, 2007).

- Exposure to high levels for a long period may result in a condition called arygria, a blue-gray discoloration of the skin and other body tissues. Argyria appears to be a cosmetic problem that may not be otherwise harmful to health.
- Exposure to high levels of silver in the air has resulted in breathing problems, lung and throat irritation, and stomach pains.
- Skin contact with silver can cause mild allergic reactions such as rash, swelling, and inflammation in some people.

2.3.7.2 Regulatory limits of Silver

- EPA – recommends concentration in drinking water not to exceed 0.10 parts per billion (ppb). Requires that spills or accidental releases of 1,000 pounds or more be reported(EPA, 2007).
- OSHA – in workplace air, 0.01 milligrams per cubic meter (0.01 mg/m^3) for an 8-hour workday, 40-hour workweek.

2.3.8 Copper in Drinking water

Copper holds promise as a point-of-use solution for microbial purification of drinking-water, especially in developing countries (Sudha et al., 2012). Copper plumbing materials can be the source of copper ions in drinking water supplies (Dwidjosiswojo et al., 2011). Limits-1.3ppm.

2.4 Bioaccumulation of Heavy Metals in Food Chain

Bioaccumulation is the accumulation of substances or chemicals in an organism. There are a small number of plants that easily absorb high levels of metals from the surrounding soil. These are called hyperaccumulators. If these plants are harvested for human use, exposure to harmful levels of metals can happen. Normally, this is a concern only if plants are collected from areas with high concentrations of metals in the soil. Metals uptake by plants is dependent on soil acidity (pH). The higher the acidity, the more soluble and mobile the metals become, and the more likely they are to be taken up and accumulated in plants.In general, humans are more likely to be exposed to metal contamination from soil that sticks to plants than from bioaccumulation. This is because it is very difficult to wash all soil particles off of plant materials before preparing and ingesting them. Root crops (like potatoes and carrots), leafy vegetables (like spinach and lettuce), and parts of plants that grow near the soil (like strawberries) are a higher risk for exposure to metal contamination than the higher portions of plants, like fruits or berries(Sabine & Wendy, 2009).

Stressed plants may be a sign of metal contamination. Look for unusual changes in the coloring or growth pattern of plants as an indicator of a stressful growth environment (like drought) combined with high metals concentrations in the soil. These kinds of conditions make it more likely that the plants are bio accumulating (or up taking) metals. Deficiencies in the plant (like a low level of zinc) can also influence a plant's likelihood to accumulate metals(EPA, 2007; EU, 2002).

2.5 General Integration on Literature Search

Heavy metals pose a public health to human life (Chowdhury et al., 2016b) and their accumulation in drinking water to toxic levels would be a result of environmental contamination as a result of human activities(Begum et al., 2009; Fuhrimann et al., 2015). In the environment, contamination of water has been shown to be mainly a result of industrial waste or fertilizers as a result of human activities(Tchounwou et al., 2012). Following contamination of drinking water with heavy metals, bioaccumulation of these compounds in the food chain would lead to disastrous effects on human life (EPA, 2007; EU, 2002; Sabine & Wendy, 2009).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Design

This was a cross sectional study conducted over a span of 2 months in which quantitative data was collected.

3.2 Study Area

The study was conducted in 1 district of South Western Uganda.

3.3 Sampling Method

Simple random sampling technique was used. Sub county names were entered in MS Excel, assigned random numbers and auto-arranged and the first 4 sub counties were included in the study.

3.4 Sample Size Determination

This was calculated basing on the size of the district by simple cluster method. 10 samples of well/spring water, tap, and bottled water were collected from each sub county. A total of 10 samples were collected from each sub county. This implied that a total of 40 samples were collected for this study using this sampling strategy.

Table 1 Sample size determination by sampling strategy.

sub county	Well/bore-holes	Tap water	Spring Water	Bottled water (control)
Ishaka- Bushenyi	2	2	2	2
Keyizooba	2	2	2	2
Kyabugimbi	2	2	2	2
Kigoma	2	2	2	2
Nkanga	2	2	2	2
Total	**10**	**10**	**10**	**10**

3.4.1 Inclusion Criteria

All locally produced drinking water from boreholes, wells, taps and major brands were collected for sampling.

3.4.2 Exclusion Criteria

All drinking water internationally produced and water sources found in homesteads.

3.5 Data Collection

This was done according to the collection methods as described below;

Sampling bottle was used to obtain water from the midpoint of the water source.

Falcon sampling tubes (15ml) were used to obtain the sampled water which was later sent for analysis.

3.5.1 Laboratory analysis

This was done using an atomic absorption spectrometry (AAS) which had detection limits for Pb at 0.01 ppm while for Cu, Fe and Cr at 0.001 ppm. Working standards were prepared for each inorganic compound as previously described (Kasozi et al., 2018) from which linear equations for each metal were generated in the form y = mx; were y = absorbance, m = gradient, and x = concentration for each compound.

For Fe: $y = 0.0841x$, $R^2 = 0.8678$;

For Cu: $y = 0.184x$, $R^2 = 0.8748$;

For Zn: $y = 0.299x$, $R^2 = 0.9837$;

For Pb: $y = 0.0296$, $R^2 = 0.8637$;

For Cd: $y = 0.1025x$, $R^2 = 0.9552$.

These equations were used to determine the concentrations of the compounds in each sample as previously described (Kasozi et al., 2018).

3.5.2 Assessment of water safety against international reference standards

This was conducted by drawing parallels from the Uganda National Bureau of Standards (UNBS), the United States Environmental Protection Agency (US-EPA), European Union (EU) and the World Health Organization (WHO) using the Table drawn from Bamuwamye et al., (2017).

and Fe respectively. Exposure to multiple contaminants results in additive and interactive effects thus the hazard index ($HI = \sum THQ$) was used as an indication of risk.

3.5.5 Determination of the incremental lifetime cancer risk associated with drinking water amongst Ugandans

Following chronic exposure to inorganic pollutants in drinking water, the incremental lifetime cancer risk (ILCR) was used to measure the cancer risk in the Ugandan population. This was estimated using the equation below;

ILCR = CDI * CSF where CDI was the chronic daily intake of a particular metal and this was estimated over the 70 year lifespan for Ugandans (i.e. AT = 70 yrs. x 365 days = 25550 days) (Kasozi et al., 2018; World Bank, 2016). In addition the cancer slope factor (CSF) for Pb, Zn and Cd that were used are 0.0085, 0.0001 and 6.3 respectively (United States Environmental Protection Agency, 2014).

3.5.6 Spatial map on safe water sources in study area

Information acquired from the GPS readings were exported to qGIS® version 3.03 Cirona onto an administrative file for Uganda. A sentinel-2 satellite image number L1C_T3MRV_AO07540_20180816T082305 was acquired from the United States Geographical Surveys (USGS) system to show vegetation cover in the study area.

3.6 Statistical analysis

Data was entered and analyzed in MS Excel 2013 version after normality testing, after which parametric tests were conducted. Descriptive statistics were conducted and information was presented as mean ± SEM from which a One way ANOVA was conducted and significant differences were reported when $P < 0.05$. Information on safety was done using a one sample t-test and mean differences were used to define 'high' and 'low' after subtracting the sample mean from the hypothetical mean. These were used to define safety of drinking water at 95% significance. Furthermore, the EDI for children and adults for each metal was presented as mean ± SEM and a two sample t-test was conducted to determine differences in concentrations eaten and significance reported when $P < 0.05$. The HI was calculated to assess the presence of threat i.e. $HI > 1$ as indicative of a threat (Kasozi et al., 2018). Also, significant differences in the THQs for children and adults were determined at 95% significance. Finally, ILCR was presented descriptively and a two sample t-test for children and adults was conducted at 95% significance. ILCR greater than 1×10^{-4} was indicative of a cancer threat and presented with superscripts (Kasozi et al., 2018; United States Environmental Protection Agency, 2014).

3.7 Ethical considerations

This was acquired from the Scientific and Ethics review committee of Kampala International University Western Campus. Written consent was also acquired from Bushenyi district local government.

3.8 Availability of information

Information used in the study is presented in the report. Data files can be accessed at https://figshare.com/s/4b2da912a42de601e165 and a manuscript has been submitted to the Journal of Environmental Health and Hygiene at Hindawi publishing company and it's under peer review.

CHAPTER FOUR

4.0 RESULTS INTERPRETATION

4.1 Levels of Inorganic compounds from different water sources.

The study showed that Fe is the major water pollutant in the order of open well > borehole > tap > spring > bottled water. This was followed by high Zn levels in the order of tap > bottled > spring > borehole > open well. Cu and Pb concentrations were relatively comparable were Cu was found to be high in the order of tap > borehole > spring > open well > bottled. Furthermore Pb concentrations were found to be in the order of open well > borehole > tap > bottled > spring. Finally, Cd levels were found to be the lowest in all water samples, however, these were highest in the order of tap > open well, bottled water > spring > borehole. Cr was not detected in all water samples as shown in **Table 3**.

Table 3 Concentrations of different inorganic compounds from major water sources in the study area.

Water source	N	Fe	Cu	Zn	Pb	Cd	Cr
		Mean ± SEM concentration (ppm)					
Borehole	8	0.512±0.2839	0.01586±0.005784	0.0145±0.001739	0.02429±0.006494	0.003714±0.0004206	ND
Bottled	8	0.0290[a]	0.004833±0.001447	0.06523±0.04579	0.02286±0.004206	0.0040±0.0003086	ND
Open well	7	0.7841±0.4818	0.006143±0.000885	0.005338±0.0008608	0.02833±0.005426	0.004125±0.0002950	ND
Spring	9	0.0105±0.0045	0.007571±0.003791	0.02876±0.01629	0.01889±0.003514	0.0040±0.0002108	ND
Tap	8	0.2282±0.1719	0.05371±0.02876	0.4467±0.3008	0.02333±0.003333	0.00425±0.0003134	ND
P value		0.6387	0.0815	0.1283	0.7054	0.8092	

KEY: Fe = iron, Cu = copper, Zn = zinc, Pb = lead, Cd = cadmium, Cr = chromium. N = Number of samples submitted for analysis. ND = not detected during analysis. Superscript 'a' indicates one value included in the calculation. ANOVA conducted for all the compounds and respective p values are presented.

4.2 Safety of drinking water in south western Uganda

Concentrations of Fe, Zn and Cu were permissible by local and international regulatory agencies, while levels of Pb were found to be unacceptable in all water samples except borehole water using UNBS, EU and WHO cut off limits. In addition, concentrations of Cd were only acceptable using US-EPA and EU cut offs, however, these were only permissible in borehole water using UNBS and WHO cut offs as shown in **Table 4**. Findings in the study show that borehole is the major clean source for drinking water in Uganda.

Table 4 Drinking water safety assessment using cut offs from local and international regulatory agencies

N =24)	BHW (N =7)	BotW (N =1)	OW (N =8)	SW (N =2)	TW (N =6)	95% significance
;ulatory :ies	P values (conclusions based on mean differences)					
BS	0.4834(High)	NA(Low)	0.3484(High)	0.0099(Low)	0.6934(Low)	All safe
-EPA	0.4834(High)	NA(Low)	0.3484(High)	0.0099(Low)	0.6934(Low)	All safe
	0.3139(High)	NA(Low)	0.2647(High)	0.0151(Low)	0.8763(High)	All safe
IO	0.4834 High)	NA(Low)	0.3484(High)	0.0099(Low)	0.6934(Low)	All safe
N =35)	(N =7)	(N = 7)	(N = 6)	(N =9)	(N =6)	
BS	0.0701(High)	0.0223(High)	0.0197(High)	0.0353(High)	0.0103(High)	Accept BHW
-EPA	0.2027(High)	0.1109(High)	0.0574(High)	0.3005(High)	0.0545(High)	All safe
	0.0701(High)	0.0223(High)	0.0197(High)	0.0353(High)	0.0103(High)	Accept BHW
IO	0.0701(High)	0.0223(High)	0.0197(High)	0.0353(High)	0.0103(High)	Accept BHW
(N=35)	(N =6)	(N = 6)	(N = 7)	(N =9)	(N =7)	
BS	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	All safe
-EPA	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	All safe
	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	0.0002(Low)	All safe
IO	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	0.0002(Low)	All safe
(N=39)	(N =6)	(N = 7)	(N = 8)	(N =10)	(N =8)	
BS	0.1403(High)	0.0177(High)	0.0066 (High)	0.0011(High)	0.0053(High)	Accept BHW
-EPA	0.0223(Low)	0.0117(Low)	0.0209(Low)	0.0011(Low)	0.479(Low)	All safe
	0.0223(Low)	0.0117(Low)	0.0209(Low)	0.0011(Low)	0.479(Low)	All safe
IO	0.1403(High)	0.0177(High)	0.0066 (High)	0.0011(High)	0.0053(High)	Accept BHW

(N=34)	(N =7)	(N = 6)	(N = 7)	(N =7)	(N =7)	
BS	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	All safe
-EPA	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	All safe
	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	All safe
IO	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	<0.0001(Low)	All safe

KEY: BHW = Borehole water; BotW = Bottled water; OW = open well; SW = spring water and TW = Tap water. . Fe = iron, Pb = Lead, Zn = Zinc, Cd = cadmium and Cu = Copper. N = number of samples detected by atomic absorption spectrometry (AAS). NA = not applicable since mean wasn't calculated. Regulatory monitoring agencies included UNBS = Uganda National Bureau of Standards, US-EPA = United States Environmental Protection Agency, EU = European Union, WHO = World Health Organization. One sample t-test conducted against respective metals with hypothetical means set by different international regulatory agencies and p values are included from which conclusions on safety were made.

4.3 Levels of inorganic pollutants consumed daily in drinking water by Ugandans

Drinking water from borehole consumption of Zn and Cd was found to be significantly different amongst children and adults ($P < 0.05$). Also, bottled water consumption of Pb and Cd were different amongst children and adults ($P < 0.05$). In open well drinking water, daily consumption of Cu, Pb and Cd were found to be different amongst children and adults while significant differences in spring and tap water were only limited to Pb and Cd ($P < 0.05$) as shown in **Table 5**.

Table 5 Estimated daily intake of inorganic compounds in drinking water amongst Ugandans.

Pollutants in drinking water	N	Children	Adults	P values
		Mean ± SEM ppm/day		
		Borehole water		
Fe	7	0.0341300±0.0189300	0.0146300±0.0081110	0.3708
Cu	7	0.0010570±0.0003856	0.0004531±0.0001652	0.1872
Zn	7	0.0009667±0.0001159	0.0004143±0.0000497	0.002259
Pb	7	0.0016190±0.0004330	0.0006939±0.0001856	0.084533
Cd	7	0.0002476±0.0000280	0.0001061±0.0000120	0.00159827
		Bottled water		
Fe	1	NC	NC	NC
Cu	6	3.221E-04±9.651E-05	1.381E-04±4.137E-05	0.4137
Zn	7	4.349E-03±3.052E-03	1.864E-03±1.308E-03	0.4754
Pb	7	1.524E-03±2.804E-04	6.530E-04±1.201E-04	0.020975
Cd	7	2.667E-04±2.057E-05	1.143E-04±8.817E-06	0.00012669
		Open well		
Fe	8	0.05228±0.03212	0.0224±0.01377	0.7155
Cu	7	0.00041±0.000059	0.000176±0.0000253	0.006336
Zn	8	0.000356±0.0000575	0.000153±0.0000246	0.009231

Pb	6	0.001889±0.000362	0.00081±0.000155	0.029749
Cd	8	0.000275±0.0000197	0.000118±0.00000844	0.00003289
		Spring water		
Fe	2	0.0007±0.0003	0.0003±0.000129	0.3918
Cu	7	0.000505±0.000253	0.000216±0.000108	0.3244
Zn	10	0.001917±0.001086	0.000822±0.000465	0.3718
Pb	9	0.001259±0.000234	0.00054±0.0001	0.016775
Cd	10	0.000267±1.4E-05	0.000114±6.04E-06	0.0000003236
		Tap water		
Fe	6	0.01521±0.01146	0.006519±0.004912	0.5090
Cu	7	0.003581±0.001917	0.001535±0.000822	0.3549
Zn	8	0.02978±0.02005	0.01276±0.008594	0.454371
Pb	6	0.001556±0.000222	0.000667±9.52E-05	0.00836
Cd	8	0.000283±2.09E-05	0.000121±8.95E-06	0.000042170

The study also showed that Fe consumption was highest in children than adults from both borehole and open wells. Fe consumption was in the order of open well > borehole > tap > spring > bottled. Zn consumption was also found to be highest in tap > bottled > spring water as shown in **Figure 1**.

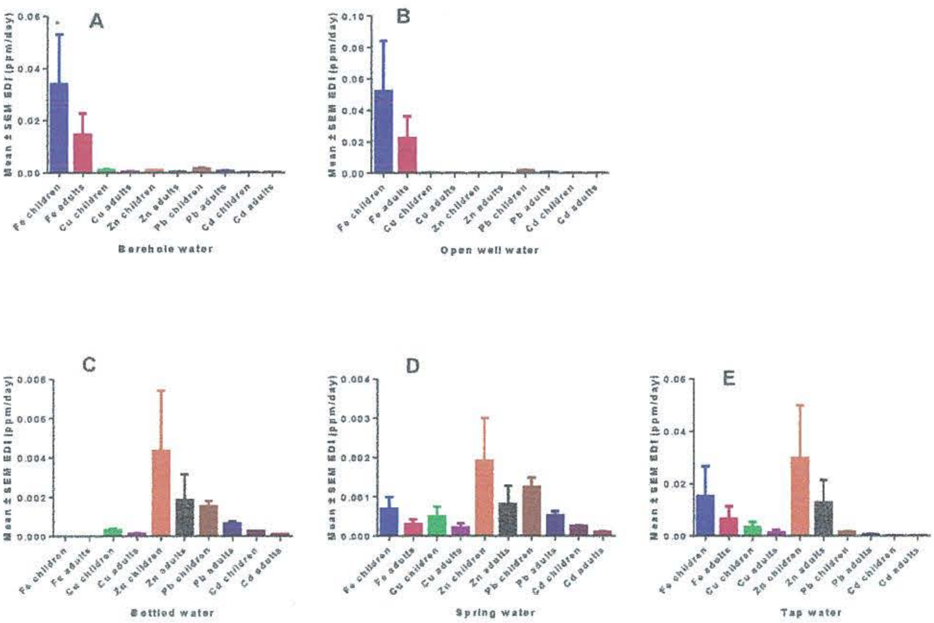

Figure 1 Heavy metal consumption amongst children and adults in Uganda.

		Tap water		
Fe	6	0.02173±0.01637	0.009313±0.007018	0.508994644
Cu	7	0.08952±0.04794	0.03837±0.02054	0.354933363
Zn	8	0.09928±0.06684	0.04255±0.02865	0.454371281
Pb	6	0.3889±0.05556	0.1667±0.02381	0.00835973
Cd	8	0.2833±0.02089	0.1214±0.008954	4.21699E-05
$\sum THQ = HI$	39	0.88273±0.2076	0.378333±0.088972	-

Increased consumption of drinking water from borehole, bottled, open well, spring and tap water was associated with an increased threat in children than adults due to high Pb levels. This was followed by Cd showing the relevance of Pb and Cd toxicities amongst children as shown in **Figure 2**.

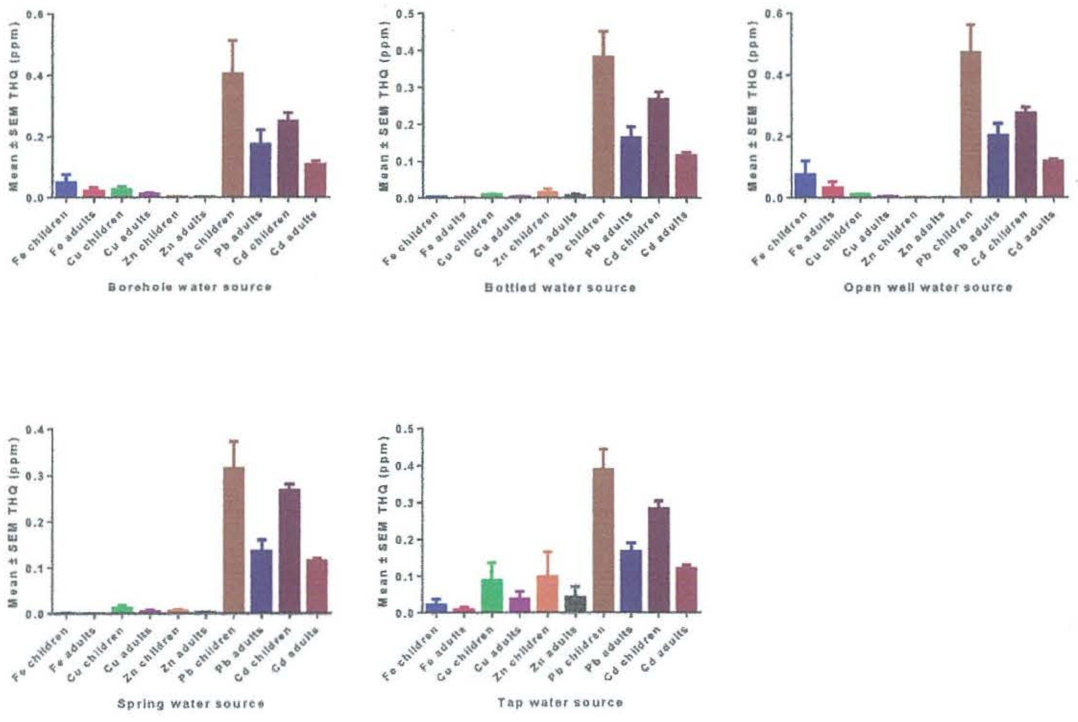

Figure 2 Target quotients for children and adults in Uganda following consumption of drinking water from different sources.

4.5 Incremental lifetime Cancer risk associated with drinking water consumed by Ugandans

Consumption of drinking water from all the water sources was associated with a very high threat of cancer in the Ugandan population. In particular significant differences were shown to exist in the incremental lifetime cancer risk (ILCR) in borehole, bottled, open well drinking water in which adults had higher ILCR than children i.e. Zn > Cd > Pb. In addition, the ILCR for spring and tap water was only significantly higher in adults than children for Cd > Pb as shown in **Table 7**.

Table 7 Cancer risk amongst Ugandans consuming drinking water from different sources.

Pollutants in drinking water	Number of values	Children	Adults	P values
		Mean ± SEM		
		Borehole water		
Zn	7	5.409±0.6487[a]	11.59±1.39[a]	0.003346965
Pb	7	1.18E-06±3.15E-07[b]	0.000297±7.95E-05[b]	0.00979795
Cd	7	5.73E-05±6.49E-06[b]	0.000287±3.24E-05[b]	0.000319574
∑ILCR	21	5.4091±0.6487[a]	11.5906±1.3901[a]	-
		Bottled water		
Zn	7	24.33±17.08[a]	52.14±36.6[a]	0.003346965
Pb	7	1.11E-06±2.04E-07[b]	0.00028±5.16E-05[b]	0.001642926
Cd	7	6.17E-05±4.76E-06[b]	0.0003086±2.381E-05[b]	3.21335E-05
∑ILCR	21	24.3301±17.08[a]	52.1406±36.601[a]	-
		Open well water		
Zn	8	1.991±0.3211[a]	4.267±0.6881[a]	0.013549601
Pb	6	1.38E-06±2.64E-07[b]	0.000347±6.65E-05[b]	0.003464546
Cd	8	6.36E-05±4.55E-06[b]	5.05E-05±3.61E-06[b]	6.55731E-06
∑ILCR	22	1.9911±0.3211[a]	4.2674±0.6882[a]	-
		Spring water		
Zn	10	10.73±6.077[a]	22.99±13.02[a]	0.409303867
Pb	9	9.17E-07±1.71E-07[b]	0.000231±4.31E-05[b]	0.000682081
Cd	10	6.17E-05±3.25E-06[b]	0.000309±1.63E-05[b]	4.01931E-06
∑ILCR	29	10.7301±6.0770[a]	22.9905±13.0201[a]	-
		Tap water		
Zn	8	166.6±112.2[a]	357.1±240.4[a]	0.489456588
Pb	6	1.13E-06±1.62E-07[b]	0.000286±4.08E-05[b]	0.000933579
Cd	8	6.56E-05±4.84E-06[b]	0.000328±2.42E-05[b]	8.15212E-06
∑ILCR	22	166.6001±112.2000[a]	357.1006±240.4001[a]	-

KEY: Different superscripts indicate ILCR comparisons against US EPA limits. Superscripts a = threat of cancer; b = no threat.

The risk of cancer was highest in the order of tap > bottled > spring > borehole > open well water especially amongst adults than children. This was shown to be primarily associated with the very high Zn levels in drinking water from these different sources as shown in **Figure 3.**

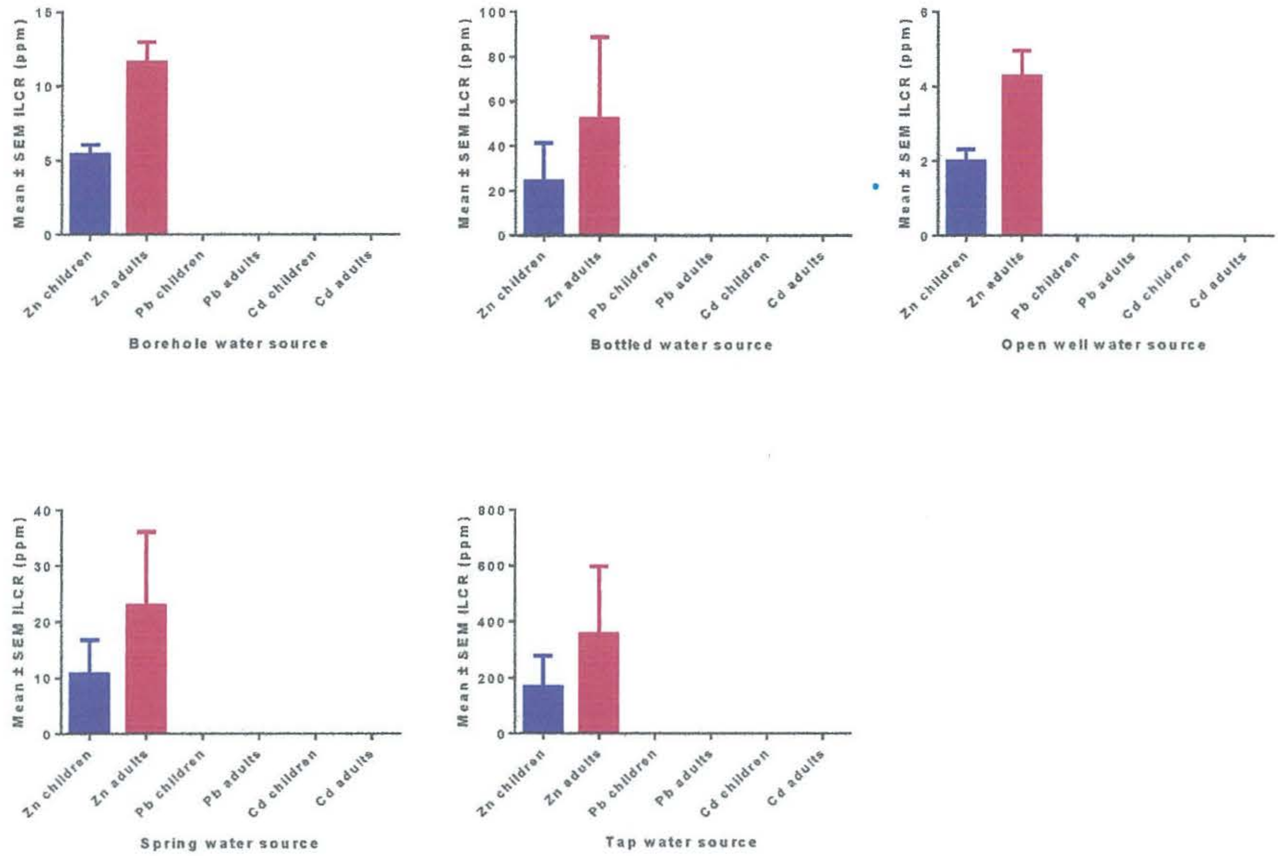

Figure 3 Incremental lifetime cancer risk in children and adults consuming drinking water from different sources in Uganda.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

The study showed that all water sources had high Fe concentrations although Fe contamination was highest in open water sources (**Table 3**). This was because Open water sources have a high concentration of soil in within which it has a largest component of all heavy metals in the earth crust (Puri & Kumar, 2012). In addition, increased water wash off and drainage of heavy metals including Fe in open water sources would be higher concentrations than in other water sources as observed in this study (Goretti et al., 2016; Sardar et al., 2013). In addition, high Zn levels in Tap water (**Table 3**) were found to be over 100 times higher than those shown by a study in Central Uganda (Bamuwamye et al., 2017). In China, high Zn levels in drinking water have been associated with mining activities (Wu, Man, Sun, & Shang, 2018), however, there was no strong evidence for this in Bushenyi district of southwestern Uganda were the samples were collected showing that environmental contamination with Zn was related to the heavy pesticide usage which is common in many agricultural ecosystems (Kasozi et al., 2018; Wuana & Okieimen, 2011). Furthermore, Cu concentrations were highest in Tap water (**Table 3**) due to the old practice of using copper pipes in the transportation of water by the Uganda National Water and Sewerage Cooperation (UNWSC), however, new developments by the UNWSC have seen an introduction of Polyvinyl chloride (PVC) pipes especially in major towns within Uganda (Bamuwamye et al., 2017). At the time of the study, the water pipes in Bushenyi were outdated and were in the process of being replaced with PVC pipes which have been found to be more stable and durable than Cu pipes (personal observation in the community). Information in the study, also showed no significant differences (ANOVA, $P > 0.05$) in heavy metal concentrations for Fe, Cu, Zn, Pb, Cd showing their importance in humans due to the threat of bioaccumulation once consumed over a large period of time (He & Chen, 2014).

Heavy metal concentrations for Fe, Zn and Cu were found to be acceptable for human consumption with the exception of Pb (**Table 4** and **Table 2**). In Central Uganda, high Pb toxicities in drinking water had been reported (Bamuwamye et al., 2017; Semuyaba, Segawa, & Wamala, 2014), however, this is the first study in which Pb concentrations in drinking water from rural communities in Uganda is being presented. Information the study supports the hypothesis that heavy metal concentration in Uganda is a national crisis since Pb has continuously been presented as a primary pollutant (Bamuwamye et al., 2017; Kasozi et al., 2018). Amongst all the water sources examined in the study, borehole drinking water was found to score best for each heavy metal in reference to international standards demonstrating the wisdom in promoting ecosystem health in Uganda. This would be important since borehole water is often supplied by rain water which collects within the environment and is subject to ultrafiltration at different levels within the water table. In addition, in the construction of boreholes especially in southern Uganda (Rakai district), the use of filters and large aeration tanks has been associated with less heavy metal load (Hanna & Johansson, 2002) demonstrating the wisdom in treating drinking water for heavy metals in Uganda since this would lead to an improvement in water quality (Chubaka, Whiley, Edwards, & Ross, 2018). In addition, consistent observations in spring, bottled and tap water show that current water treatment practices in Uganda for bottled water which is the current national standard (Bamuwamye et al., 2017) are low and need serious revisions for improved human protection.

Daily consumption of heavy metals from boreholes was only found to be significantly different for Zn and Cd (**Table 5**) amongst children > adults demonstrating the wisdom in strengthening water quality systems for these two compounds within borehole water sources in Bushenyi district. This would be effected through a revision of the current borehole construction guidelines to ensure that all essential filters are in-cooperated into each rural structure (Chubaka et al., 2018; Hanna & Johansson, 2002). In addition, the estimated daily intake (EDI) was higher in children than adults in bottled water in which Pb > Cd (**Table 5**). These observations suggest that bottled water is not necessarily recommended for children in Uganda and this was contrary to a previous recommendation from a study conducted in central Uganda (Bamuwamye et al., 2017). The lack of consistency in findings with bottled water within Uganda imply that the national regulatory authorizes such as the Uganda National Bureau of Standards (UNBS) have to either revise their priorities in the sector or seek for practical mechanisms to ensure that quality drinking water is supplied on the Ugandan market. Furthermore, open well drinking water showed significant variations in Cu, Pb and Cd levels amongst children > adults (**Table 5**). These findings show that open water drinking water is heavily contaminated by heavy metals in comparison to closed water sources due to the heavy wash off following a heavy rain down pour (Goretti et al., 2016; Puri & Kumar, 2012; Sardar et al., 2013). The higher consumption of Fe in children than adults in drinking water from boreholes and open wells (**Figure 1**) would be related to the poor maintenance of these facilities (Hanna & Johansson, 2002), demonstrating a need to prioritize these structures in prospective water quality extension activities in rural communities of Uganda.

Estimation of the non-carcinogenic health effects was done by using the hazard index (HI <1) and all water samples were found to be acceptable, however, significant differences in borehole water were in Pb > Cd > Zn on borehole water higher in children > adults (**Table 6**). These findings are in agreement by recent studies in Bushenyi which have shown children to be at a higher risk than adults to heavy metal toxicity (Kasozi et al., 2018). Furthermore, bottled, spring and tap water all had significantly higher THQs in children for Pb > Cd than in adults (**Table 6**). This re-emphasized previous finding in central Uganda that drinking water in Uganda is contaminated with Pb (Bamuwamye et al., 2017; Semuyaba et al., 2014). Bearing in mind that access to safe drinking water is a universal human right, there is need for the local authorities to promote routine monitoring of heavy metals from various water sources to promote human health in Uganda (Gorchev & Ozolins, 2011; World Health Organization, 2011). This would help reduce on the health burden associated with heavy metal ingestion, thus promoting public and ecosystem health (Goretti et al., 2016; Govind, 2014). The study also showed that Pb and Cd toxicities are important in children (**Figure 2**) probably due to their smaller body weights relative to adults. In humans Pb has been associated with gastrointestinal irritation which would lead to vomiting and diarrhea while Cd is often associated with brain and kidney damage (Jaishankar, Tseten, Anbalagan, Mathew, & Beeregowda, 2014; Singh, Sharma, Agrawal, & Marshall, 2010).

Chronic consumption of heavy metals from drinking water would subsequently predispose the local population to cancer. The incremental lifetime cancer risk showed the threat to be propagated mainly by the very high Zn levels (**Table 7**). In Bushenyi district, as is common with most rural communities in developing countries, agricultural usage of pesticides was the major mechanism of toxicity. This has been propagated by the poor implementation of the drug liberalization policy leading to poor application of herbicides and pesticides, leading to a heavy wash off of these chemicals into the ecosystem (Goretti et al., 2016; Puri & Kumar, 2012; Sardar et al., 2013). These findings are in agreement with the very high heavy metal concentrations in milk and beef in southwestern Uganda (Kasozi et al., 2018), an area without any active mining nor industrial plants which would be associated with these high Zn contaminants. The risk of cancer was found to be highest in tap water and this was lowest in open well water (**Figure 3**). Findings in the study show that Tap water is not a safe source for drinking water, in comparison to borehole water in these communities. Finally, the study showed that 2 safe borehole water sources exist in Nyabubaare, Ward 1 and Kitwe as well as Nyamiyaga of Sheema within the study area (**Figure 4**). Heavy metals enter the soils and groundwater, bioaccumulate in food webs, and adversely affect the ecosystem (Gall, Boyd, & Rajakaruna, 2015) demonstrating the importance in allocating resources to improve on the quality and quantity of available boreholes in rural communities of Uganda.

5.2 CONCLUSION

The study showed that,

5.2.1 Drinking water of Uganda is highly contaminated with Zn.

5.2.2 The most safe water source was borehole water followed by spring water in the study area.

5.2.3 Cancer risk was highest in adults than children due to the very high Zn levels although the threat posed by other non-cancer ailments was low.

5.2.4 Centers with boreholes had the safest water, although these are were few in the area.

5.3 RECOMMENDATION

Heavy metal contamination of drinking water in the ecosystem by Zn is a major public health concern. Information from the study shows that borehole water is relatively safer than bottled water, thus conveying key concerns to the national regulatory agencies with a goal on establishing practical guidelines which would lead to increased consumer protection. This would be important since access to safe drinking water is a universal human right, and yet heavy metal concentrations from the study have been shown not to be significantly different from all the five water sources sampled. It appears a lot of emphasis has been placed on microbial load and little progress has been made to ensure that drinking water in Uganda is adequately filtered. A revision in the national water processing guidelines to increase on supervision of private and government water agencies would lead to an improvement in water quality for the promotion of both human and ecosystem health in Uganda.

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ır Ref: Our Ref: Date: 19th July, 2018

Chief Administrative Officer
Bushenyi District Local Government
Bushenyi, Uganda

ɹ: Office of the Associate Dean
Faculty of Biomedical Sciences
Kampala International University Western Campus
Bushenyi, Uganda

19 JUL 2018

ır M/s,

ɥuest for your consent to conduct a water quality study in Bushenyi district in collaboration with ır office

ite this letter to introduce to you a study on water quality entitled: ***'Heavy metal load and safety of ıking water in Bushenyi district.'*** This study is being conducted by the ChemTox research group at npala International University Western Campus, under the department of Physiology.

ɘ objective of the study is to establish the presence of environmental pollutants in drinking water by using ɛer testing kits, and atomic absorbance spectrometry. We have had two meetings to date with the District ıter officer, from whom we have gained a mutual understanding on our research project since work ıducted by our team, would be synergistic to local government water priority objectives

ɛ kindly request for assistance with a <u>GPS device</u>, which would help us pinpoint the water sources which will visited and <u>a water testing kit</u>, which are all available at the water offices. The GPS will be returned within ıeriod of 7 working day, and a complete report on our findings will be shared with your office, and the water ɔartment. The study has been submitted to the university as per attached proposal.

ur support for this activity is very much appreciated as it will help continue to the national efforts of putting anda on the path of attaining the Sustainable Developmental Goals in this sector.

ıcerely,

. KENETH ICELAND KASOZI

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emical Hazard Control Expert, Japan
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ow at the British Parasitology Society, United Kingdom
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