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# Spatio-temporal Variations and Potential Health Risks of Heavy Metals in Water from River Manafwa, Uganda

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**Abstract:** The epicenter of flash flood inundations and landslides in Uganda have been areas around Mt. Elgon. By implication, it has led into loss of lives, food and water insecurity. This study assessed the seasonal variations in physiochemical parameters and heavy metals (HMs) content of water from River Manafwa (R. Manafwa) which is the major water source used around Mt. Elgon. Potential insidious human health risks associated with consumption and dermal contact with water from the river were assessed using target hazard quotient and incremental lifetime cancer risk methods. Results of atomic absorption spectrometry analysis showed that the concentrations of the HMs in the wet and dry seasons ranged from below detection limit to 1.407 ± 0.001 mg/L, which were below WHO limits. Health risk assessments indicated that there are discernable non-carcinogenic health risks from ingestion of water from R. Manafwa, as the total target hazard quotients were above 1 for some of the samples. Cancer risk values indicated that there are no potential cancer risks from ingestion of water from the river. This study recommends that regulatory authorities should intervene to mitigate pollution of R. Manafwa through strengthening restrictions on sand mining and dumping of wastes into the river.

**Keywords:** Water quality; Manafwa watershed; Bududa; carcinogenic risk; target hazard quotient; trace metals.

## 1. Introduction

Rapid economic growth, industrialization, and urbanization with inadequate regulatory structure has infringed on the pristine environment, raising many sustainability challenges [1]. A case in point is developing countries such as China and India, where pollution levels has surpassed regulatory limits, and has been a subject of obsessive research [2, 3]. Of immediate concern has been pollution of water resources with contaminants that make them unsafe for drinking, thereby impeding the realization of some Sustainable Development Goals such as SDG 6 and SDG 14 [4]. Over the years, the scope of water contaminants have widened to include heavy metals (HMs), endocrine disrupting chemicals, flame retardants, polycyclic aromatic hydrocarbons, current use pesticides, preservatives, personal care products [5-7], and other contaminants of emerging concern such as macro-, micro- and nanoplastics, algal toxins and active pharmaceutical ingredients [7]. Within this frame of reference, HMs remain the most ubiquitous contaminants in water resources due to their diverse occurrence and inclusion in appliances, consumer goods, industrial machinery and processes [8].

Heavy metals (HMs) are chemical elements with relatively high densities and are potentially toxic at concentrations above their established threshold limits [9]. They form part of the earth's crust in negligible concentrations but can continuously get enriched due to indiscriminate human activities (that alters their geochemical cycles and biochemical balance) and natural processes such as volcanic activity, metal corrosion, and metal evaporation from soil, water and sediments [8, 10]. Examples of HMs include typical metals and metalloids such as vanadium, tin, strontium, mercury, arsenic, lead, zinc, nickel, cadmium, chromium, cobalt, copper, iron, molybdenum, and titanium [9, 11]. Some of the HMs are important co-enzymes in reactions that drive living cells but are bioaccumulative and toxic to living organisms at elevated concentrations [12, 13]. For both terrestrial and aquatic organisms, exposure to HMs occur through direct ingestion in water, food, medicine, direct deposition from the atmosphere, dermal adsorption or inhalation from occupational sources [8, 14-16]. Ingestion of HMs in drinking water is the most common route of exposure, morbidities and mortalities from HMs [17]. Despite this, routine monitoring of HMs contamination and remediation in water resources of developing countries are limited.

There are various water resources in the East African community, a region constituted by seven developing countries in Eastern Africa. Nevertheless, there is still high incidences of water scarcity in the region [18, 19]. For example, Uganda, the area of focus on this study, has over 21 million (51%) of its population without access to safe drinking water. This is in part due to contamination of the available water resources by various anthropogenic activities. Around Mt. Elgon (Manafwa watershed) in Eastern Uganda, there has been several incidences of torrential rains, perennial (flash) floods and landslides which have led to an intolerable death toll, food and water insecurity [20, 21]. The floods has casted a spotlight on the country's climate change crisis, and emphasized the need for more research in this watershed [22].

To date, no study has examined the HMs content as well as health risks that could emanate from ingestion and dermal contact with contaminated water from River Manafwa (also known as Manafwa river; R. Manafwa henceforth) especially following torrential rains, and the attendant floods and landslides. This contribution therefore assessed the seasonal variations in some selected physiochemical parameters and assessed the health risks associated with ingestion and dermal contact with HMs in water from R. Manafwa which is the major water source used across Bududa, Manafwa, Butaleja and Mbale districts of Eastern Uganda.

## 2. Materials and Methods

### *2.1. Description of the study area*

The study was undertaken on water samples from R. Manafwa (0.9420°N 33.920°E), the longest river that flows through Mbale and Butaleja districts of Uganda. The river is fed by various tributaries (such as Sala, Liisi, Wukha, Tsutsu, Pasa, Kufu, Nambale, Makhuba) and small streams from the transboundary Mt. Elgon (an extinct volcanic agglomerate of Miocene age that rises up to 4321 m above sea level)[23]. River Manafwa is about 14.63 kilometers long and is the main source of water for the nearby communities but economic activities (such as sand mining, agriculture and welding works) and frequent floods have anecdotally been implicated in the deterioration of water quality of R. Manafwa that forms part of the Manafwa River Basin traversing Bududa, Manafwa, Butaleja and Mbale districts of Eastern Uganda (**Figure 1**)[24]. Rivers and streams from Mt. Elgon densely dissect the Manafwa River Basin, explaining the rugged topography characterizing the steep slopes in this area. Hydrologically, R. Manafwa joins the Mpologoma river and drains into Lake Kyoga, a mesotrophic lake connected to Lake Victoria and forms part of River Nile that flows up to Egypt and empties into the Mediterranean Sea [25].

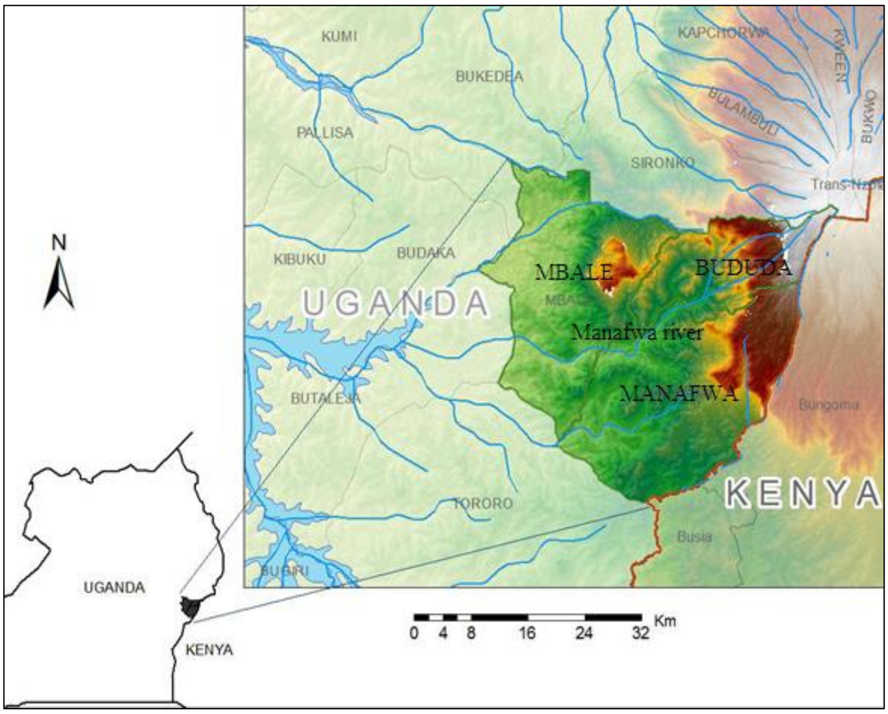

**Figure 1.** Map showing the location of River Manafwa in Eastern Uganda.

River Manafwa is the major river in the Elgon sub region. It is the major water source for Manafwa waterworks that supplies water for domestic use to Mbale municipality, Butaleja town and Kamonkoli town in Budaka district. Its water is also used for domestic use by the local community, agriculture along the river banks, fish farming and water for rice growing in Doho irrigation scheme in Butaleja district. This study considered R. Manafwa stretching from Bulucheke in Bududa district up to slightly beyond Manafwa Water Works, Mbale district (**Figure 1**). This covered a total distance of about 58 km. The river was divided into two sections namely; upper course and the lower course. The upper course of the river stretched from Bulucheke to Manafwa town. This part of the river was further subdivided into two segments A and B (**Table 1**). Segment A was from Bulucheke to Bududa and this was an area where landslides normally occur. Segment B was from Bududa Town up to Manafwa town, an area characterized by great human settlements and activity in the river catchment area. The lower course of the river was from Manafwa town up to slightly beyond Manafwa Water Works (**Figure 2**); with the greatest human activities including among others stone quarrying, sand mining, agriculture, automobile washing, vehicular movement and sewage treatment. Besides, this part of the river also receives road runoff from Mbale Municipality and Manafwa Town where many metal works activities and leachates from solid waste heaps occur.

**Table 1.** Summary of sampling sites and the potential sources of heavy metal contaminants for R. Manafwa.

| Sampling Site | Sample code | Activities/establishments |
|---|---|---|
| Bulucheke area | A1, A2 | Landslide affected site, residential, school science laboratories |
| Bukigai market area | B1 | Market activities, garages, residential cultivation |
| Bududa Town | B2 | Metal welding and fabrication, Bududa hospital, car washing bay |
| Manafwa Town downstream | M1 | Metal welding, garages, horticulture cultivation, slum and residential |
| Manafwa Bridge upstream | M2 | Sand mining, vegetable growing, motorcycle washing, stone quarrying |
| Manafwa Bridge Downstream | M3 | Vehicular traffic |
| Manafwa Water Works Area | M4 | Water treatment, sand mining, horticulture |

**Figure 2.** Overview of R. Manafwa (a) river water choked with high levels of sand and silt; (b) staff at Manafwa Water Works trying to clean water clarifiers to avoid them from silting up, (c) one of the sand mining site operations that is speeding up river bank erosion and sedimentation, and (d) section of the river where flooding swept away a bridge.

### *2.2. Sample collection*

The water samples were collected at a depth of 10-15 cm below the water surface, using clean 500 mL plastic bottles. Sampling was done at 8.00 am, 12.00 pm and 4.00 pm (East African Standard Time) to cater for variations that could have occurred in the river water due to temperature changes and the different human activities taking place during the day. Sampling was done thrice in each season (September, October and November 2018 for the wet season, and December 2018, January 2019 and February 2019 for the dry season).

All the three water samples collected from each sampling site were mixed to obtain a composite water sample. One liter of the composite water sample was measured and transferred into a cleaned plastic bottle, sealed and labelled for easy identification of the sampling site. Four composite water samples were taken from each of the two sections of the river during each sampling in the major seasons (dry and wet season) to account for seasonal variations. In the upper course of the river, two composite water samples were taken from each segment A and B and were labeled A1, A2 and B1, B2 respectively. In the lower course of the river, two water samples were taken from two selected sites within the area just before Manafwa water works and were labelled M1 and M2. Two other water samples were taken from two sites just after the bridge and beyond Manafwa water and these were labelled M3 and M4. The eight sampling points were chosen to capture the stretch where the landslides normally occur and the major anthropogenic activities carried out along the river banks (**Table 1**). Water samples were then transported to the laboratory in ice boxes within 24 hours from the time of collection.

### *2.3. Sample preparation and analyses*

During sampling of water, non-conservable parameters (electrical conductivity and pH) were measured on-site using calibrated Jenway pH/mV/Temperature and Conductivity meters (Jenway Gransmore Green, England). The other parameter (total hardness) was determined by EDTA complexometric titration method [26].

Each water sample was filtered through Whatmann No. 42 filter paper to remove suspended solids. They were then acidified with 1 ml of concentrated nitric acid to preserve ions in solution in preparation for elemental analyses. The acidified water samples (250 mL) were evaporated to 25 mL volumes. The concentrates were transferred into 50 mL volumetric flasks and diluted to the mark with diluents (0.1% of lanthanum chloride and 1% of concentrated nitric acid in distilled water). These were then transferred into clean sample bottles and analyzed for the HMs using atomic absorption spectrophotometer (AAS, Perkin Elmer Analyst 100) to determine the concentration of the eight selected HMs: copper (Cu), nickel (Ni), manganese (Mn), zinc (Zn), lead (Pb), cadmium (Cd), chromium (Cr) and iron (Fe).

Working standards prepared from dilution of 1000 ppm stock solution of the nitrate and chloride salts of the HMs were used to construct calibration curves. The concentration of the HMs in the sample digestates were determined from the calibration curves in mg/L. Quality control was performed through analysis of blanks and spiked samples according to the same procedure. Recoveries obtained ranged from 96% to 101%. Analytical precision (expressed as Relative Standard Deviation) varied between 3% and 4%. The method detection limits (LODs) were computed as Blank + 3 × Standard Deviations for four samples analyzed in triplicate.

### *2.4. Assessment of human health risks*

Health risk assessments establishes the link between the environment and human health that can be expressed quantitatively in terms of hazard degree. In this study, the carcinogenic and non-carcinogenic health risks were calculated separately for adults as the general population and children as a sensitive group.

#### 2.4.1. Non-carcinogenic health risks

The average daily doses were estimated to discern human exposure through direct ingestion ($ADD_{ingestion}$; mg/kg/day) and dermal contact ($ADD_{dermal\ contact}$; mg/kg/day) with water

(**Equations 1** and **2**)[15, 27, 28]. Dermal contact is expected to stem from sand mining in this river exposing the mining communities to HMs in the contaminated water.

$$\text{ADD}_{ingestion} = \frac{C \times W_{ir} \times E_f \times E_d}{W_{ab} \times T_{aet}} \tag{1}$$

$$\text{ADD}_{dermal\ contact} = \frac{C \times SAF \times DAF \times AF \times E_f \times E_d}{W_{ab} \times T_{aet}} \times 10^{-6} \tag{2}$$

From which C is the heavy metal concentration (mg/L), $W_{ir}$ is the water ingestion rate = 1.8 L/day and 21.0 L/day for children and adults, $E_f$ = exposure frequency (365 days/year), $E_d$ = exposure duration, the average lifetime (58.65 years for an adult Ugandan)[15, 29], $W_{ab}$ = average body weight (considered to be 15 kg for children and 60 kg for adults), $T_{aet}$ is the average exposure time for non-carcinogens = $E_f \times E_d$ [30], SAF is the exposed surface area = 2,800 $cm^2$ for children and 24,350 $cm^2$ for adults [28], DAF is the dermal absorption factor = 0.01 for carcinogenic HMs and 0.001 for non-carcinogenic HMs [31], AF is the skin adherence factor in $mg/cm^2/day$ = 0.2 and 0.7 for children and adults [32].

Similarly, the target hazard quotient (THQ) was calculated for both direct ingestion and dermal contact with water (**Equations 3** and **4**). Practically, THQ ≤ 1 is indicative that the exposure is unlikely to elicit adverse health effects on an individual. Otherwise, THQ > 1 attests to the potential of non-carcinogenic effects being experienced [32]. Because such effects are augmentative in the context of contaminants like HMs, the cumulative risk or total THQ was computed as the arithmetic sum of the THQ of the HMs. As per US EPA [33], the health risk calculations assumes that the ingested and adsorbed doses are equal to the dose absorbed into the body.

$$\text{THQ} = \frac{ADD_{ingestion}}{R_f D_{oral}} \tag{3}$$

$$\text{THQ} = \frac{ADD_{dermal\ contact}}{R_f D_{dermal}} \tag{4}$$

Where $R_fD_{oral}$ is the oral reference dose. Its values are $4.0\times 10^{-2}$, $3.0\times 10^{-4}$, $3.0\times 10^{-2}$, $3.0\times 10^{-2}$, $4.0 \times 10^{-3}$, $1.0 \times 10^{-3}$, $1.5 \times 10^{0}$ and $7 \times 10^{-1}$ mg/kg/day for Cu, Ni, Mn, Zn, Pb, Cd, Cr and Fe, respectively. The $R_fD_{dermal}$ is the dermal reference dose, with values of $1.0 \times 10^{-2}$, $5.40 \times 10^{-3}$, $9.6 \times 10^{-1}$, $6.0 \times 10^{-4}$, $5.25 \times 10^{-4}$, $6.0 \times 10^{-5}$, $6.0 \times 10^{-5}$, and $1.4\times 10^{2}$ mg/kg/day for Cu, Ni, Mn, Zn, Pb, Cd, Cr and Fe, respectively [33]. A reference dose is the maximum daily dose of a metal from a specific exposure pathway, that is believed not to lead to an appreciable risk of deleterious effects to sensitive individuals during a life time [34]. Thus, if the average daily dose ($\text{ADD}_{ingestion}$ or $\text{ADD}_{dermal\ contact}$) is lower than the respective reference dose, the THQ < 1 and adverse health effects are unlikely to appear. Otherwise, an average daily dose greater than the reference dose is indicative that THQ > 1 and adverse health effects are likely to appear.

### 2.4.2. Cancer risk assessment

The carcinogenic health risk (CR) estimated as the incremental lifetime cancer risk for the carcinogenic HMs (Pb, Cd, Cr and As) were calculated as the product of $\text{ADD}_{\text{ingestion}}$ and the ingestion cancer slope factor (CSF) using **Equation 5**. The total cancer risk (TCR) was calculated using **Equation 6** used in previous studies [31, 35].

$$\mathrm{CR} = \mathrm{ADD}_{\mathrm{ingestion}} \times \mathrm{CSF} \quad (5)$$

$$\mathrm{TCR} = \sum_{i=1}^{n} CR \quad (6)$$

The CSF for Pb, Cr, and Cd are $8.5 \times 10^{-6}$, $5.0 \times 10^{-4}$, and $3.8 \times 10^{-4}$ mg/kg/day, respectively. The CSF is defined as the risk generated by a lifetime average amount of one mg/kg/day of carcinogen chemical and is contaminant specific. The US EPA permissible limits lies between $10^{-6}$ and $< 10^{-4}$ for a single carcinogenic element and multi-element carcinogens [31].

### *2.5. Statistical analysis*

Quantitative data from triplicate analyses were entered into Excel where they were averaged. Significant differences of the spatial variations in water quality among the sampling sites along the river was evaluated using One Way Analysis of Variance (One Way ANOVA) with Tukey posthoc test. Pearson's bivariate correlation and Principal Component Analysis (PCA) were used to explore the inter-relationships between metal concentrations and the examined physicochemical parameters of R. Manafwa. The analyses were executed at 95% confidence interval employing GraphPad Prism for Windows (v9.3.1, GraphPad Software, San Diego, CA, USA).

## **3. Results and Discussion**

### *3.1. Variations in physicochemical parameters of R. Manafwa water*

Table 2 shows the results of pH, conductivity and total hardness of water from the different sections of R. Manafwa during the wet and dry seasons. The parameters did not differ significantly among the study stations ($p > 0.05$) and the seasons ($p > 0.05$). The pH (measure of hydrogen ions) ranged from 7.46±0.05 in the wet season to 8.19±0.06 in the dry season, and were within the acceptable limits of the World Health Organization (WHO) for drinking water [36]. These values were comparable to 7.96-8.22, 6.2-8.0, 6.6–7.5, 5.85-7.60 and 8.05-8.30 reported for water from Nyabugogo and Nyabarongo rivers, Rwanda [35], Mohokare River (Lesotho)[37], River Aturukuku [38], River Nyamugasani [39] and River Rwimi of Uganda [39, 40], but higher than 5.60-6.32 and 5.58-6.80 for River Nyamwamba and River Mubuku of Uganda [40]. Though the pH values obtained lie within the acceptable limits, it has been indicated that even slightly high or low pH of water is unpleasant. For example, high alkalinity confers a slippery feel to water, making it to taste like baking soda while at highly acidic pH, water possess a bitter or metallic taste and may lead to fixture corrosion [41]. At very low and high pH levels, solubility of toxic HMs in water tend to increase and can cause serious human health effects in humans and aquatic organisms [42, 43].

Electrical conductivity (EC) was measured to establish the total dissolved ions in the water samples. It ranged from 88.7±0.24 µS/cm in the dry season to 122.2±0.91 µS/cm in the wet season, which were within the WHO guidelines for drinking water. The values obtained in this study are comparable to 80.44, 63.15 and 12–119, 43–103 and 99.91 µS/cm for water in Ugandan rivers: Lhubiriha, Mobuku, Rwimi and Nyamwamba but lower than 460.51, 946.08, 118.57, 81–220 and 140.82 µS/cm in River Lubigi, River Nyamugasani, River Sio, River Rwimi and River Victoria Nile reported by Bwire et al. [44] and, Busulwa and Bailey [39]. Turinayo [45] reported EC of 108-1524 µS/cm for water from River Musamya in Uganda. In Nyabugogo and Nyabarongo rivers in Rwanda, EC of 74.3-102.0 were reported [35]. Another investigation in Mohokare River water (Lesotho)[37] reported EC of 2000-3800 µS/cm which are far higher than obtained in this study. In Nigeria, Butu et al. [46] found EC of River Rido to range from 79 to 146.3 µS/cm [46], which are close to the ones obtained in this study. The EC of water estimates the total amount of solids dissolved in water (its total dissolved solids) and is directly proportional to the water's temperature. It is directly related to the concentration of ions in the water, and this is supported by the low levels of HMs reported in this study. High EC of water samples indicate the presence of a higher content of different salts, organic and inorganic materials such as alkalis, chlorides, sulfides, and carbonates. As a measure of water quality, significant changes in EC are indicators of discharges or some other source of pollution entering the river.

**Table 2.** Physicochemical parameters of water samples from R. Manafwa, Eastern Uganda

| **Sampling site** | **Wet season** | | | **Dry season** | | |
|---|---|---|---|---|---|---|
| | **pH** | **Conductivity (µS/cm)** | **Total hardness (mg/L)** | **pH** | **Conductivity (µS/cm)** | **Total hardness (mg/L)** |
| $A_1$ | 7.95±0.02 | 107.6±0.12 | 104.2±0.14 | 8.10±0.01 | 100.3±0.05 | 100.5±0.19 |
| $A_2$ | 8.14±0.04 | 119.6±0.43 | 112.2±0.67 | 8.19±0.06 | 112.0±0.11 | 108.7±0.40 |
| $B_1$ | 7.84±0.01 | 98.3±0.23 | 103.2±0.28 | 7.99±0.01 | 88.7±0.24 | 101.5±0.01 |
| $B_2$ | 7.82±0.07 | 101.2±0.15 | 103.0±0.56 | 7.80±0.05 | 94.9±0.10 | 103.0±0.00 |
| $M_1$ | 7.74±0.01 | 101.9±0.45 | 115.9±0.76 | 7.79±0.01 | 89.5±0.22 | 106.1±0.52 |
| $M_2$ | 7.46±0.05 | 106.3±0.76 | 103.4±0.27 | 7.50±0.03 | 97.8±0.09 | 95.9±0.10 |
| $M_3$ | 7.73±0.04 | 122.2±0.91 | 101.8±0.11 | 7.77±0.00 | 115.0±0.14 | 93.6±0.24 |
| $M_4$ | 8.09±0.01 | 119.2±0.17 | 89.4±0.13 | 8.00±0.02 | 101.6±0.10 | 80.8±0.11 |
| WHO guidelines [36] | 6.5-8.5 | 250.0 | 200.0 | 6.5-8.5 | 250.0 | 200.0 |

Values are means ± standard deviations of analyses performed in triplicate.

Total hardness of the water samples ranged from 80.8±0.11 mg/L in the dry season to 115.9±0.76 mg/L in the wet season. The optimum range of hardness in drinking water is 80 to 100 mg/L. Water with more than 300 mg/L of hardness is generally considered to be hard, while more than 150 mg/L of hardness is noticed by most people, and water with less than 75 mg/L is considered to be soft. Water with hardness exceeding 200 mg/L is considered poor whereas those with hardness greater than 500 mg/L is unacceptable for domestic purposes [47]. Highly hard water is chemically rich in dissolved minerals, especially calcium and magnesium ions which for aesthetic reasons may have unpleasant taste [48]. Both low and high values of hardness may be harmful to the human body. Low levels of hardness may activate colon carcinogens or trigger rectal cancer and cardiovascular diseases [49, 50] because calcium and magnesium ions are capable of binding bile acids and fatty acids, thus affecting the creation of colon mucosa [51]. Higher hardness values may lead to development of kidney stones and dermal diseases [52]. In addition to these health risks, hard water is a nuisance as it causes mineral buildup on fixtures (hence corrosion) and poor soap or detergent performance due to scum formation.

### *3.2. Spatio-temporal variations in heavy metal content of River Manafwa water*

#### 3.2.1. Copper

The seasonal fluctuations in Cu content of R. Manafwa water is shown in **Figure 3**. The concentrations of Cu ranged from 0.023 ± 0.003 mg/L to 0.06± 0.01 mg/L during wet season and 0.0030 ± 0.01 mg/L to 0.0045 ± 0.01 mg/L in the dry season. The mean values were 0.0382 mg/L and 0.0034 mg/L for the wet and dry seasons, respectively. The concentration of copper generally increased from the upper course of the river downstream in both the wet and dry season ($p<0.05$) but none of the values exceeded the WHO guidelines of 0.5 mg/L in drinking water [36]. The increasing concentration of Cu downstream could be due to runoff from Manafwa town and Mbale municipal motor garages, metal fabrication works, road construction works, and leachates from dumped domestic biodegradable wastes. The concentration of Cu was higher during the wet season with 0.06 ± 0.01 mg/L at sampling point M4. This elevated concentration of Cu is probably due to road construction works that was going on during sampling at Manafwa bridge and leaching from Mbale municipal sewage pipes. Such Cu leaching is accelerated by water characteristics such as high acidity and temperature, and low hardness [53].

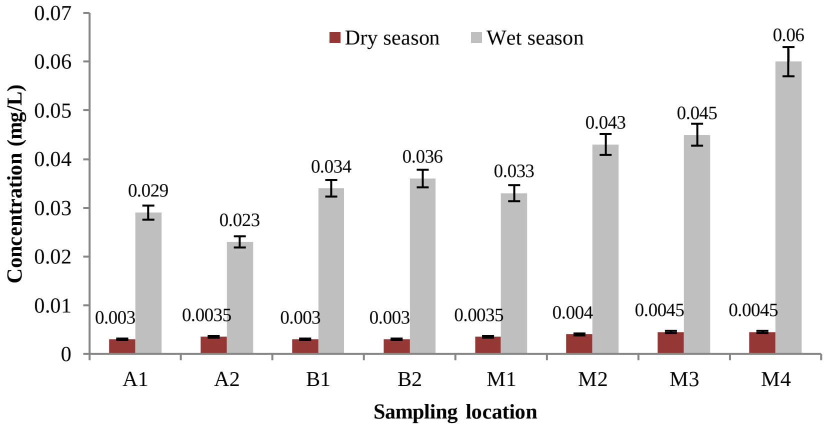

**Figure 3.** Concentration of copper in water from River Manafwa in the wet and dry seasons. Values are means of analyses performed in triplicate (n = 16 composite samples).

In comparison to previous studies (**Table 3**), the concentrations of Cu reported in this study are quite lower. Though it is an essential trace metal, ingestion of Cu at high concentrations leads to Cu poisoning, a condition characterized by vomiting, hematemesis (vomiting blood), and gastrointestinal distress. Similar effects and neurological disorders such as Wilson’s, Menkes’, Alzheimer’s and Parkinson’s diseases have been reported by individuals with dysregulation of the redox-active metal [53, 54].

**Table 3.** Comparison of the results of heavy metal contamination of water (mg/L) from R. Manafwa with previous studies

| River (Country) | Cu | Ni | Mn | Zn | Pb | Cd | Cr | Fe | References |
|---|---|---|---|---|---|---|---|---|---|
| River Manafwa (Uganda) | 0.003–0.060 | 0.0015–0.0095 | 0.011-0.262 | 0.0015-0.029 | 0.002-0.010 | 0.001-0.002 | 0.003-0.011 | 0.196- 1.407 | This study |
| Marimba River (Zimbabwe) | 0.13-0.14 | — | — | ND | 0.213-0.544 | — | — | 5.6-6.9 | [55] |
| Madanzhe, Dzindi and Mvudi rivers (South Africa) | 0.002–0.003 | — | — | 0.0021–0.0025 | 0.0105–0.0201 | 0.0016–0.0093 | — | — | [56] |
| River Sosiani (Kenya) | 0.001–0.275 | — | — | 0.07-0.57 | 0.02-1.89 | — | 0.003-0.050 | 0.011-3.789 | [57] |
| River Nyamwamba (Uganda) | 1.90–61.0 | 0.67-12.0 | 23.1-100.0 | ND [1] | 0.27-0.40 | — | — | 185.0-265.0 | [58] |
|  | 0.740 | — | — | 0.076 | 0.047 | — | — | — | [40] |
| River Rwimi (Uganda) | 0.010 | — | — | 0.010 | 0.067 | — | — | — |  |
| River Mubuku (Uganda) | 0.025 | — | — | 0.010 | 0.053 | — | — | — |  |
| Nyabarongo river (Rwanda) | BMDL-0.24 [1] | — | 0.02-0.53 | BMDL-0.09 | 0.05-0.75 | BMDL-0.106 | BDL-0.06 | 0.63-1.61 | [35, 59] |
| Rongna River (China) | 0.00189–0.806 | 0.00745–0.0601 | 0.0431–2.041 | 0.013–0.415 | 0.00049–0.00241 | 0.00012–0.00064 | 0.00156–0.00637 | — | [60] |
| Bolong river (China) | 0.00542–0.00737 | 0.0107–0.0182 | 0.0378–0.0519 | 0.0398–0.567 | 0.00034–0.00064 | 0.00011–0.00054 | 0.00174–0.00308 | — |  |

[1] BDML = Below method detection limit.

### 3.2.2. Nickel

The concentration of Ni ranged from 0.0015 ± 0.001 mg/L to 0.0095 ± 0.005 mg/L during the wet season and 0.001 ± 0.010 mg/L to 0.005 ± 0.010 mg/L in the dry season (**Figure 4**). Like in the case of Cu, the concentration of Ni was higher ($p>0.05$) during the wet season and samples taken within Bududa and Manafwa towns (sampling sites B2, M1, and M2). The major sources could be use of nickel-cadmium batteries, hydrogenated oils such as margarine, stainless steel, nickel-plated metallic items and combustion of petroleum fuels in Bududa and Manafwa towns. Sample A2 taken near Bukigai markets had higher concentration of Ni within the upper course of R. Manafwa. This could be due to wastes generated from the market and vehicular emissions due to heavy traffic flow during market days. In both the wet and dry seasons, the concentration of Ni in the water samples never surpassed the WHO maximum permissible limit of 0.05 mg/L for Ni in drinking water [36]. Previous studies in River Nyamwamba (Uganda) [58], Rongna and Bolong rivers (China) [60] detected Ni at concentrations that are higher than was obtained in this study.

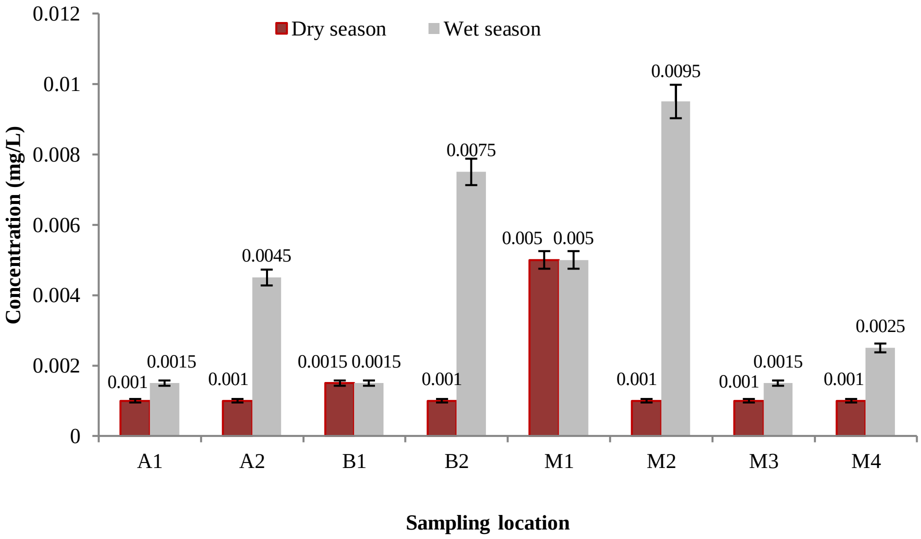

**Figure 4.** Concentration of nickel in water from R. Manafwa in the wet and dry seasons. Values are means of analyses performed in triplicate (n = 16 composite samples).

### 3.2.3 Manganese

In this study, Mn concentrations varied between 0.011± 0.05 mg/L and 0.164 ± 0.01 mg/L during the wet season and 0.053± 0.007 mg/L and 0.262 ± 0.03 mg/L during the dry season (**Figure 5**). In the wet season, the concentration of Mn was generally lower ($p<0.05$) and showed an increase downstream, which could be as a result of discharge from increasing human settlement at the banks of the river. Sample M4 taken downstream of Manafwa Water Works and Mbale-Tororo highway had elevated concentrations of Mn. Intense sand mining and construction repairs on Manafwa Bridge could be the cause. Evident metallic materials like spades and hoes used in sand mining, and the combustion of petrol in motor cars could be the sources of Mn to the river water.

In the dry season, the concentration of Mn increased from the upper course of the river downstream and was higher than in the wet season ($p<0.05$). The source of Mn could be metallic materials like spades and hoes used in sand mining. Samples from sites M3 and M4 taken downstream of Manafwa Water Works with more intense sand mining showed the highest Mn concentrations. The concentration of Mn in the wet season was below the WHO maximum acceptable limit of 0.1 mg/L at all the study sites except site M4 while in the dry season it was above the maximum acceptable limits in all the sites except A1 and B2. These values were comparable to 0.02 mg/L to 0.53 mg/L reported in Nyabarongo and Nyabugogo rivers of Rwanda [35, 59] but lower than 10.28 mg/L and 11.58 mg/L for water samples from Rwanzekuma and Ruganwa rivers of Rwanda [61].

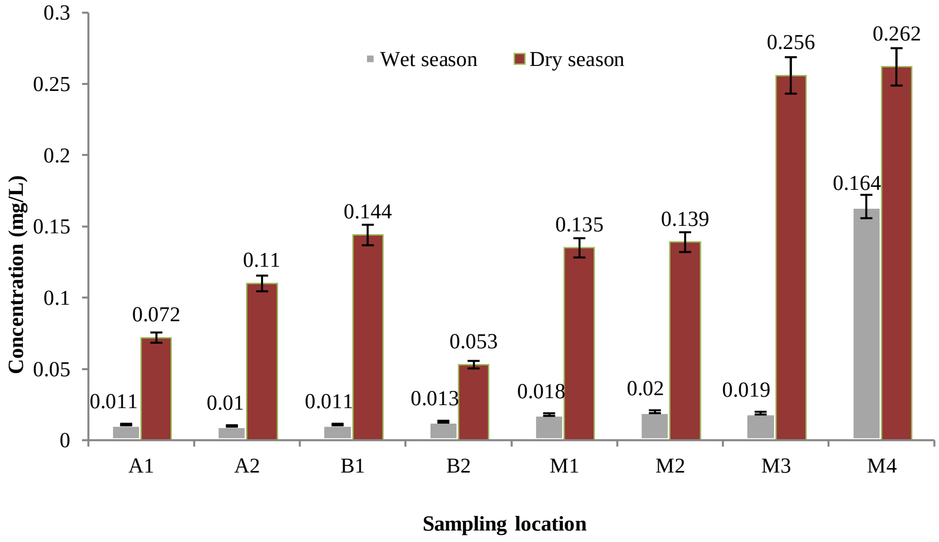

**Figure 5.** Concentration of manganese in water from R. Manafwa in the wet and dry seasons. Values are means of analyses performed in triplicate (n = 16 composite samples).

### 3.2.4. Zinc

On the other hand, Zn was found in levels of 0.008 ± 0.040 mg/L to 0.029± 0.001 mg/L in water samples collected during the wet season, and 0.0015 ± 0.010 mg/L to 0.0045 ± 0.000 mg/L during dry season (**Figure 6**). The highest concentration of 0.029 ± 0.001 mg/L was for samples from M4 in the wet season. The concentration of Zn in the wet season was higher than that in the dry season at all the sampling points ($p<0.05$). This could be due to mobilization of the soluble forms of the metal from anthropogenic activities by runoff rain water. In the wet season, water samples got from A2 near Bukigai market showed a higher concentration of Zn than those from sites A1, B1, B2 and M1. This could be attributed to the leachates from piles of biodegradable wastes generated from the market since Zn is one of the major micronutrients and vehicular emission. None of the samples however contained Zn in levels surpassing the WHO limit of 3 mg/L in drinking water.

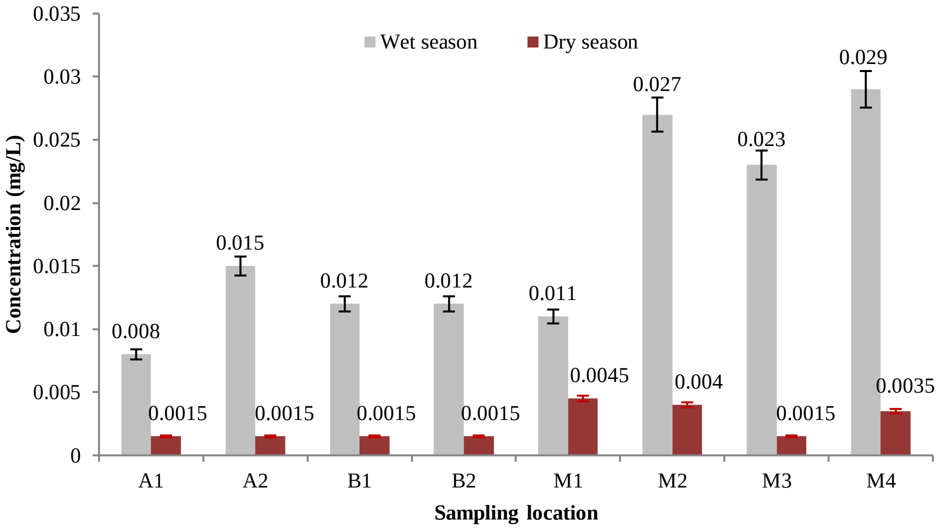

**Figure 6.** Concentration of Zinc in R. Manafwa water samples collected during the wet and dry seasons. Values are means of analyses performed in triplicate (n = 16 composite samples).

### 3.2.5. Lead

The seasonal fluctuations in Pb concentrations were more pronounced. The concentrations were from 0.002 ± 0.040 mg/L to 0.01 ± 0.005 mg/L in the wet season and 0.005 ± 0.02 to 0.0085 ± 0.010 mg/L in the dry season (**Figure 7**). The concentration of Pb decreased from the upper course of the river downstream to Bududa town in both the wet and dry seasons. This indicates existence of a mild point source of Pb in the upper course of the river. Since there is hardly any settlements or economic activity evidenced at the upper most course of R. Manafwa, the higher concentration of Pb at this section of the river could be due to natural sources such as landslides or hydro-geochemical reactions occurring rather than anthropogenic sources. The decrease in concentration of Pb towards Bududa town is attributed to increase in distance away from the source of pollution as a result of possible sedimentation and precipitation. From Bududa town (site B2) downstream up to slightly beyond Manafwa Bridge along Mbale-Tororo highway (site M4), the concentration of Pb increased. This could be due to vehicular emissions, metal welding and fabrication works, disposal of Pb acid battery contents, use of leaded gasoline [62, 63] and sewage leakages from Manafwa and Mbale towns.

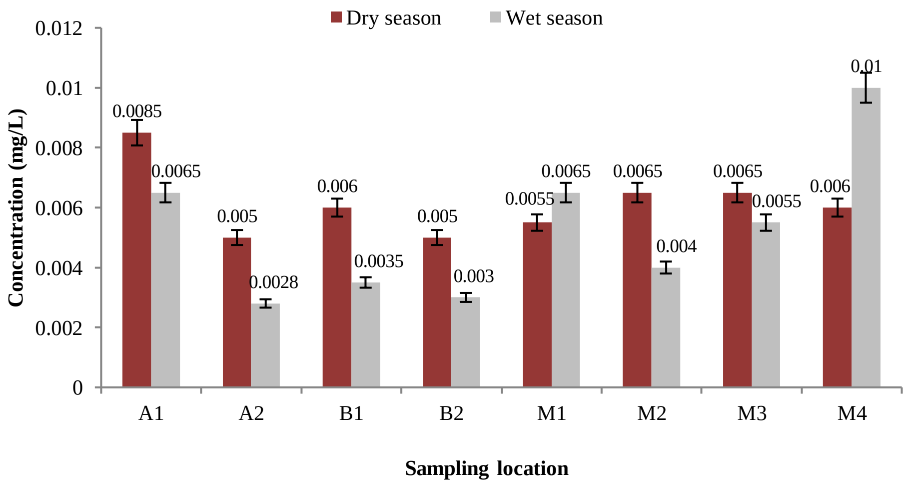

**Figure 7.** Concentration of lead in R. Manafwa water samples. Values are means of analyses performed in triplicate (n = 16 composite samples).

The concentration of Pb reported in this study did not exceed WHO maximum limit of 0.01 mg/L. Similar results have been documented for other rivers on the African continent. For example, Okonkwo and Mothiba [56] reported concentrations of 0.010 mg/L to 0.012 mg/L for Pb in water from Dzindi, Madanzhe and Mvudi rivers, South Africa. Other studies such as Amadi [57] in Sosiani River, Kenya and Mvungi et al. [55] in Marimba River (Zimbabwe) reported higher Pb contents than reported in this study (**Table 3**). Pb is toxic and a non-essential trace metal. Its chronic ingestion or occupational exposure has been associated with renal failure and liver degradation [64]. In infants, Pb retards interactive, survival, growth, development and metabolic processes in addition to increasing mucus synthesis and triggering nervous system disorders [65].

### 3.2.6. Cadmium

Cadmium was detected at relatively lower concentrations (0.001 ± 0.04 to 0.002 ± 0.00 mg/L) during wet season but was not detected in the dry season (**Figure 8**). The highest concentration of 0.002 mg/L was recorded in water sample from M4. These results suggest that the Cd found in water samples for the wet season could have been mobilized by runoff water from metal fabrication works, disposed nickel cadmium batteries, sewage and other sources related to man's use of metals. The absence of Cd in the dry season could also be due to sedimentation/precipitation of the insoluble forms of Cd into sediments. In all the water samples, the concentration of cadmium was below WHO permissible limit of 0.03 mg/L.

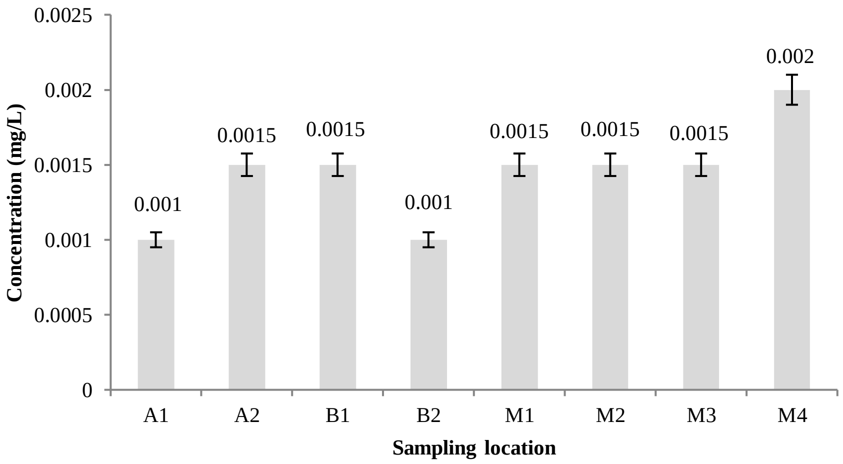

**Figure 8.** Concentration of cadmium in water samples from R. Manafwa, Uganda during the wet season. Values are means of analyses performed in triplicate (n = 8 composite samples). Dry season samples had no detectable Cd.

### 3.2.7. Chromium

Unlike Cd, Cr was detected during the dry season at site $M_4$ at a concentration of 0.00093 ± 0.01 mg/L (**Figure 9**). For wet season samples, the concentration of Cr ranged from 0.003 ± 0.05 mg/L to 0.011 ± 0.01 mg/L for samples obtained near Manafwa water works (site M4). The relatively higher concentration of Cr at this sampling point could be due to releases from the heavy traffic flow along the Mbale-Tororo highway and leaching from the sewer pipes of Manafwa Water Works. By implication, the observed concentration of Cr during the wet season should be due to its mobilization from anthropogenic and geologic sources by rain water.

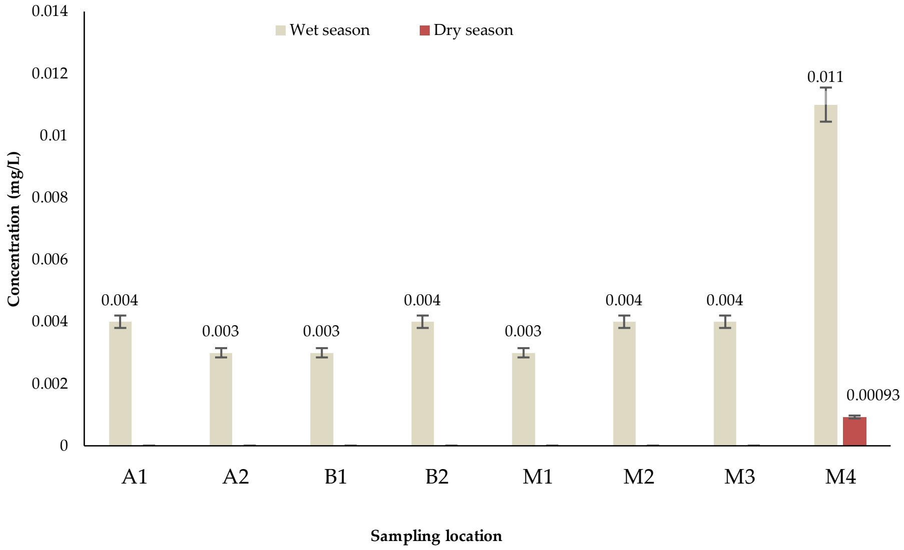

**Figure 9.** Concentration of chromium in R. Manafwa water samples. Values are means of analyses performed in triplicate (n = 16 composite samples)

### 3.2.8. Iron

In comparison to other HMs, Fe was found to be higher in the river water during both seasons, ranging from 0.967 ± 0.06 and 1.407 ± 0.001 mg/L in the dry season and between 0.196 ± 0.01 and 0.909 ± 0.03 mg/L in the wet season (**Figure 10**). The dry season had higher concentrations of Fe, possibly due to increase in sand mining within the river when the water levels have reduced as a result of using metallic spades and hoes.

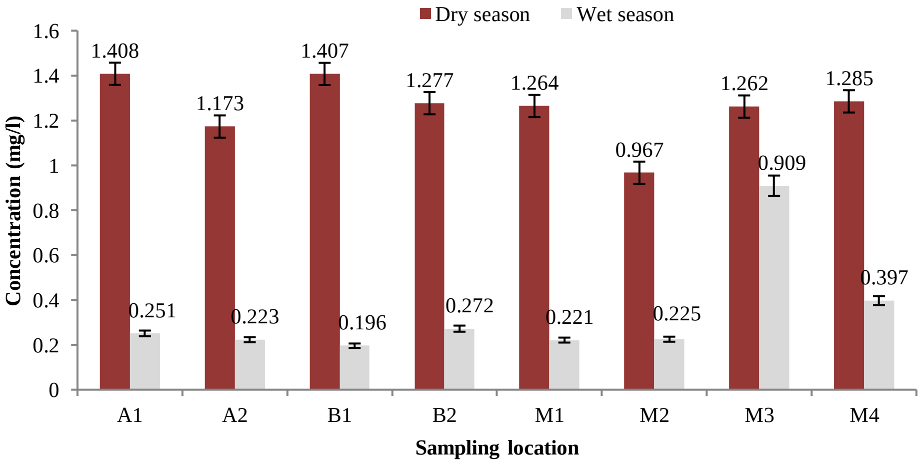

**Figure 10.** Concentration of iron in R. Manafwa water samples. Values are means of analyses performed in triplicate (n = 16 composite samples).

Inorganic Fe (principally $Fe^{2+}$) is naturally present in water resources at concentrations upto 50 mg/L [66]. For example, Amadi [57], Eliku and Leta [67] found Fe (0.011 to 2.897 mg/L, and 1.11 to 4.12 mg/L) in water from River Sosiani (Kenya) and Awash river (Ethiopia) which are comparable to the values obtained in this study. The levels found in water by this study is however lower than previously detected in some rivers. Nhapi et al. [61] reported elevated Fe levels (8.76 and 6.85 mg/L) in water from Rusine and Marenge rivers of Rwanda whereas Kihampa and Wenaty [68] detected Fe in Mara river water (Tanzania) at levels (12.6 to 15.51 mg/L) which are several folds higher than found in this study.

Taken together, the differences in HMs concentration reported in water from rivers across the globe and the present study could plausibly be due to disparities in geological formation of the rivers, their physicochemical conditions and the sources of contamination in their vicinities [60, 61].

### *3.3. Multivariate statistical analysis results*

To discern the pollution sources for the HMs, Pearson’s correlation analysis and PCA were performed. In the wet season, there were positive correlations between Cu and Mn ($p = 0.013$), Cu and Zn ($p = 0.012$), Cr and Cu ($p = 0.009$), Pb and Mn ($p = 0.015$), Zn and Mn ($p = 0.088$), Cd and Mn ($p = 0.040$), and Cr and Mn ($p = 0.000$). Total hardness had strong and significant negative correlations with Cu ($p = 0.011$), Mn ($p = 0.028$) and Cr ($p = 0.010$) (Table 4). These observations agreed with PCA results (Figure 11). The strong correlation between the metal pairs are suggestive that they entered into the environment through anthropogenic contributions, or may be due to the similarities in their retention phenomena in solid matrices [16, 69, 70]. For the negative correlations, this is indicative that the presence of these ions in water have negative effect on hardness values [71].

**Table 4.** Pearson’s correlation matrix for the physicochemical parameters and HMs content in water from R. Manafwa during the wet season.

| | **Cu** | **Ni** | **Mn** | **Zn** | **Pb** | **Cd** | **Cr** | **Fe** | **pH** | **Conductivity** | **Total hardness** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Ni** | -0.041 | 1 | | | | | | | | | |
| **Mn** | 0.820* | -0.195 | 1 | | | | | | | | |
| **Zn** | 0.823* | 0.190 | 0.639 | 1 | | | | | | | |
| **Pb** | 0.674 | -0.410 | 0.808* | 0.382 | 1 | | | | | | |
| **Cd** | 0.643 | -0.170 | 0.729* | 0.726* | 0.525 | 1 | | | | | |
| **Cr** | 0.837* | -0.181 | 0.985* | 0.643 | 0.798* | 0.626 | 1 | | | | |
| **Fe** | 0.446 | -0.387 | 0.135 | 0.428 | 0.242 | 0.196 | 0.177 | 1 | | | |
| **pH** | -0.122 | -0.540 | 0.394 | -0.193 | 0.259 | 0.170 | 0.375 | -0.099 | 1 | | |
| **Conductivity** | 0.334 | -0.313 | 0.420 | 0.567 | 0.342 | 0.471 | 0.433 | 0.646 | 0.417 | 1 | |
| **Total hardness** | -0.831* | 0.228 | -0.761* | -0.626 | -0.528 | -0.389 | -0.834 | -0.322 | -0.157 | -0.306 | 1 |

* Significant at the 0.05 level (2-tailed).

For the dry season, the Pearson’s bivariate correlation coefficients for the physicochemical parameters and the HMs in the water samples (Table 5) indicated a positive correlation between Cu and Mn ($p = 0.03$), Fe and pH ($p = 0.10$) and significant negative correlations between Cu and total hardness ($p = 0.038$), and Mn and total hardness ($p = 0.027$). The positive correlation indicates that Cu and Mn are indicates that they have entered into the environment through the same anthropogenic contributions, and their concentrations would therefore increase or decrease proportionately [16, 72]. The association of Fe with pH is because its solubility in aqueous matrices is pH-dependent [73]. These results were also in agreement with PCA (Figure 12). Overall, multivariate statistical analyses indicate that the comparatively higher levels of pollution experienced in R. Manafwa during the wet season are due to anthropogenic contributions.

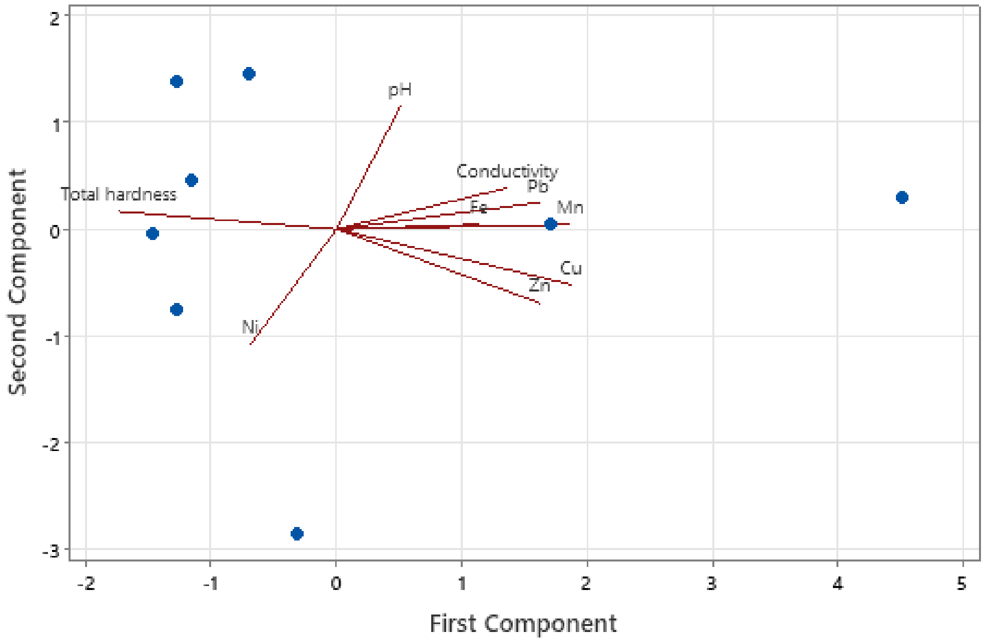

**Figure 11.** Principal Component Analysis plot showing the effect of three components influencing the variation of HMs in water from R. Manafwa during the wet season.

**Table 5.** Pearson's correlation matrix for the physicochemical parameters and HMs content in water from R. Manafwa during the dry season.

| | Cu | Ni | Mn | Zn | Pb | Fe | pH | Conductivity | Total hardness |
|---|---|---|---|---|---|---|---|---|---|
| **Cu** | 1 | | | | | | | | |
| **Ni** | -0.129 | 1 | | | | | | | |
| **Mn** | 0.891$^*$ | -0.062 | 1 | | | | | | |
| **Zn** | 0.390 | 0.601 | 0.252 | 1 | | | | | |
| **Pb** | -0.025 | -0.232 | 0.001 | -0.114 | 1 | | | | |
| **Fe** | -0.431 | 0.080 | -0.061 | -0.490 | 0.269 | 1 | | | |
| **pH** | -0.321 | -0.167 | -0.125 | -0.526 | 0.062 | 0.622 | 1 | | |
| **Conductivity** | 0.549 | -0.507 | 0.391 | -0.380 | 0.073 | -0.221 | 0.222 | 1 | |
| **Total hardness** | -0.735$^1$ | 0.357 | -0.764$^*$ | -0.260 | -0.253 | 0.032 | 0.167 | -0.187 | 1 |

$^*$ Significant at the 0.05 level (2-tailed). Cd and Cr were below detection limits in the dry season.

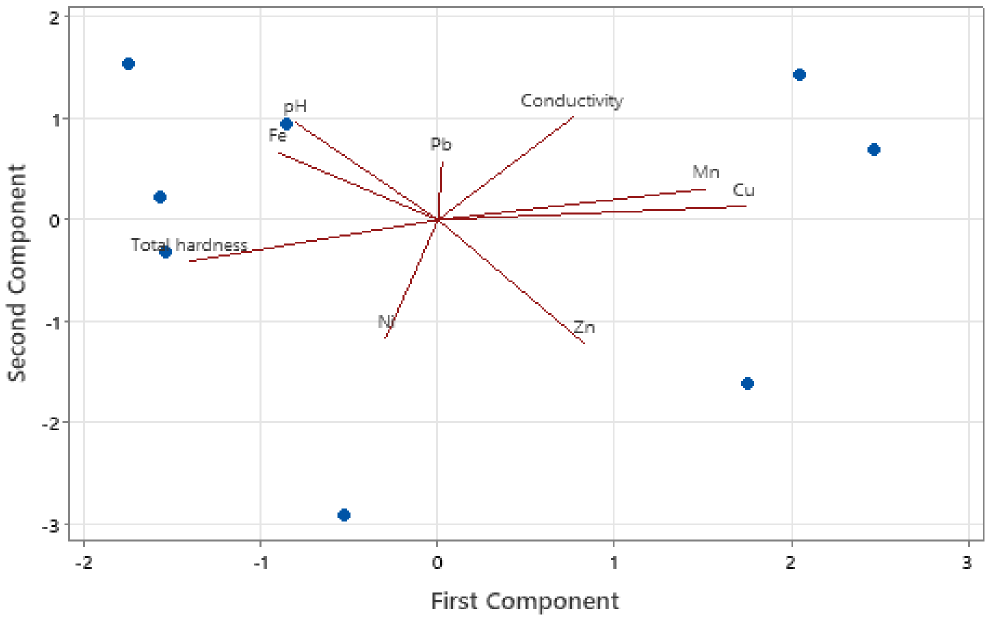

**Figure 12.** Principal Component Analysis plot showing the effect of three components influencing the variation of HMs in water from R. Manafwa during the dry season.

### *3.4. Health Risks Assessment Results*

In the wet season, the average daily doses through ingestion of contaminated water ranged from 0.120 × $10^{-3}$ mg/kg/day (for Cd ingested by children at $A_1$, $A_2$, $B_1$ and $B_2$) to 138.95 × $10^{-3}$ mg/kg/day for Fe ingested in water from site M4 by adults (**Supplementary materials**: **Table S1**). The daily doses through dermal contact ranged from 0.0010 × $10^{-8}$ mg/kg/day for adult's exposure to Cd at $B_2$ to 258.2318 × $10^{-8}$ mg/kg/day for adult's exposure to Fe at $M_4$. In the dry season, the average daily doses through ingestion of contaminated water span from 0.006 × $10^{-3}$ mg/kg/day (for Zn ingested by children at $A_1$, $A_2$ and $B_1$) to 154.200 × $10^{-3}$ mg/kg/day (for Cu ingested in water from site M4 by children). On the other hand, the estimated daily doses through dermal contact varied between 0.0037 × $10^{-8}$ mg/kg/day (for Cr adsorbed by the skin of children at $M_4$) to 399.99 × $10^{-8}$ mg/kg/day for Fe adsorbed onto adult's skin at $A_1$. The THQ through ingestion of the HMs, and hence the total THQ for the foregoing HMs exceeded 1 during the wet season at $B_2$ and $M_1$ for Ni ingested by children, and at all sites for Ni ingested by adults. Similarly, the THQ for ingestion of the HMs during the dry season, and hence the total THQ for the HMs exceeded 1 for Ni and Cu ingested by children at $A_2$, $M_1$ and $M_4$, respectively (**Supplementary materials**: **Table S1**). All the THQ from dermal contact were less than 1, and varied between 0.0005 × $10^{-6}$ (for Fe at $B_1$ adsorbed by the skin of children) and 137.3067 × $10^{-6}$ (for Zn at $M_4$ adsorbed by the skin of adults) for the wet season, and 0.0003 × $10^{-6}$ (for Mn at $A_1$ adsorbed by the skin of children) to 21.3067 × $10^{-6}$ (for Zn at $M_1$ adsorbed by the skin of adults) for the dry season. Since some of the average daily doses for Ni and Cu were higher than the corresponding reference doses for ingestion of contaminated water (THQ >1), non-carcinogenic health risks may result from consumption of water from the sampled stations of R. Manafwa.

For carcinogenic health risks, the cancer risk values ranged from 4.4625 ×$10^{-10}$ to 2.22075 ×$10^{-6}$, with lower cancer risk values in the dry season than wet season (**Supplementary materials**: **Table S2**). For carcinogenic health risks, the borderline given by US EPA is 1 × $10^{-4}$ to 1 × $10^{-6}$ and is considered unacceptable where the risk surpasses 1 × $10^{-4}$. The cancer risk values (and the total cancer risk values) obtained for the HMs per sampling site in this study did not surpass 1 × $10^{-4}$, suggesting that there are no potential cancer risks that could arise from consumption of water from R. Manafwa, Uganda.

Overall, the health risks assessment performed in this study were based on the HMs analyzed. It is recommended that risk characterizations should be cumulative to account for aggregate exposures to multiple compounds or mixtures causing similar toxicological effects [74]. This is achieved using Adversity Specific Hazard Index for Cumulative risk assessment suitable for toxicants with multiple residues that exert similar toxicological effects such as pesticides and polychlorinated biphenyls.

## 4. Conclusions

This study showed that R. Manafwa in Eastern Uganda is contaminated with HMs at concentrations well below the maximum WHO guidelines for drinking water. However, there are discernable non-carcinogenic health risks that may rise from ingestion of water from the river. Children are at higher cancer risks than adults, and in all cases, the contribution of Ni (in both seasons) and Cu (during the dry season) towards the target hazard quotient were significant. The heavy metal Cr is the main driver of potential carcinogenicity in the incremental lifetime cancer risk assessment results for the wet season while Pb is the sole driver in the dry season. Thus, regulatory authorities should intervene and reduce pollution of R. Manafwa through strengthening restrictions on sand mining and dumping of wastes into the river. Further studies should examine the spatial variations in the concentrations of the HMs in sediments and biota.

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## Conflicts of Interest

The authors declare no conflict of interest.

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