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Assessment of selected trace metals in fish feeds, pond water and edible muscles of *Oreochromis mossambicus* and the evaluation of human health risk associated with its consumption in Vhembe district of Limpopo Province, South Africa

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ABSTRACT

Fish is a rich source of proteins for humans and is widely consumed in various places in the world. This study assessed the levels of twenty trace metals (B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sn, Sb, Ba, Hg and Pb) in fish feeds (n = 2), water (n = 27), and edible muscles of *Oreochromis mossambicus* (n = 20 from 8 ponds) from fish farms and Luvuvhu River (n = 3 from 1 river site) in Vhembe district of Limpopo Province, South Africa. Physicochemical parameters of water in the study area were analysed. Temperature ranged between 21.4–30.47 °C, pH: 5.59–7.28, electrical conductivity: 608–1216 µS/cm, total dissolved solids: 156–675 mg/l, dissolved oxygen: 0.28 - 0.56 mg/l, turbidity: 3.92–356.7 NTU, respectively. Levels of most trace metals such as Cr (2 µg/l and 1000 µg/kg), Mn (100 µg/l and 500 µg/kg), Fe (10 µg/l and 300 µg/kg), Ni (20 µg/l and 100 µg/kg), As (50 µg/l and 3 µg/kg), Pb (10 µg/l and 300 µg/kg) and Cu (2 µg/l and 2250 µg/kg) in water and *O. mossambicus* muscles were mostly below the World Health Organization (WHO), Food and Agricultural Organization (FAO)/WHO and European Commission (EC) recommended limit in the collected samples (Tshifulalani site and Duthuni site). However, Cu and Fe recorded concentration above the recommended limit at the control site (Luvuvhu River) in water and *O. mossambicus* muscles, respectively. Results from the bioaccumulation factor (BF), suggests the presence of metals in the water which can bioaccumulate in the fish muscles. Most of the metals (As, Cd, Hg, Mo, Sr, Sb and Sn) that recorded no possible bioaccumulation also recorded levels that complied with their maximum permissible limit (MPL) of WHO, FAO/WHO and EC guideline values in the fish feeds except for Se and Co. Moderate bioaccumulation was recorded for Cr, Mn, Fe and Zn in some of the sampling sites. Pb and Ni showed extreme bioaccumulation (BF > 5000) in one of the sampling sites and also recorded elevated levels in the fish feeds. Average daily dose (ADD), hazard quotient (HQ) and total hazard quotient (THQ) computed were less than 1. The Cancer risk (CR) evaluated were all below 10^{-4} except in one site for children (Tshifulanani 2) but the overall average result showed no carcinogenic health risk to the consumers of the *O. mossambicus*. Therefore, *O. mossambicus* intake in the study area should be constantly monitored to prevent future health implications.

1. Introduction

Aquaculture entails fish farming in an economic, sustainable, selected and manageable environment mostly practiced for profit making [1]. Aquaculture system rears aquatic organisms and plants, such as fish, shellfish, mollusks, crustaceans, seaweeds and plankton [2]. However, the most common aquaculture practice is the breeding of fish. Traditional fisheries also benefit financially by supplying the local and global markets with fish, which sometimes results in overfishing and depletion of certain fish species [3].

Oreochromis mossambicus is a freshwater fish species from kingdom Animalia and family of cichlidae commonly known as Mozambique

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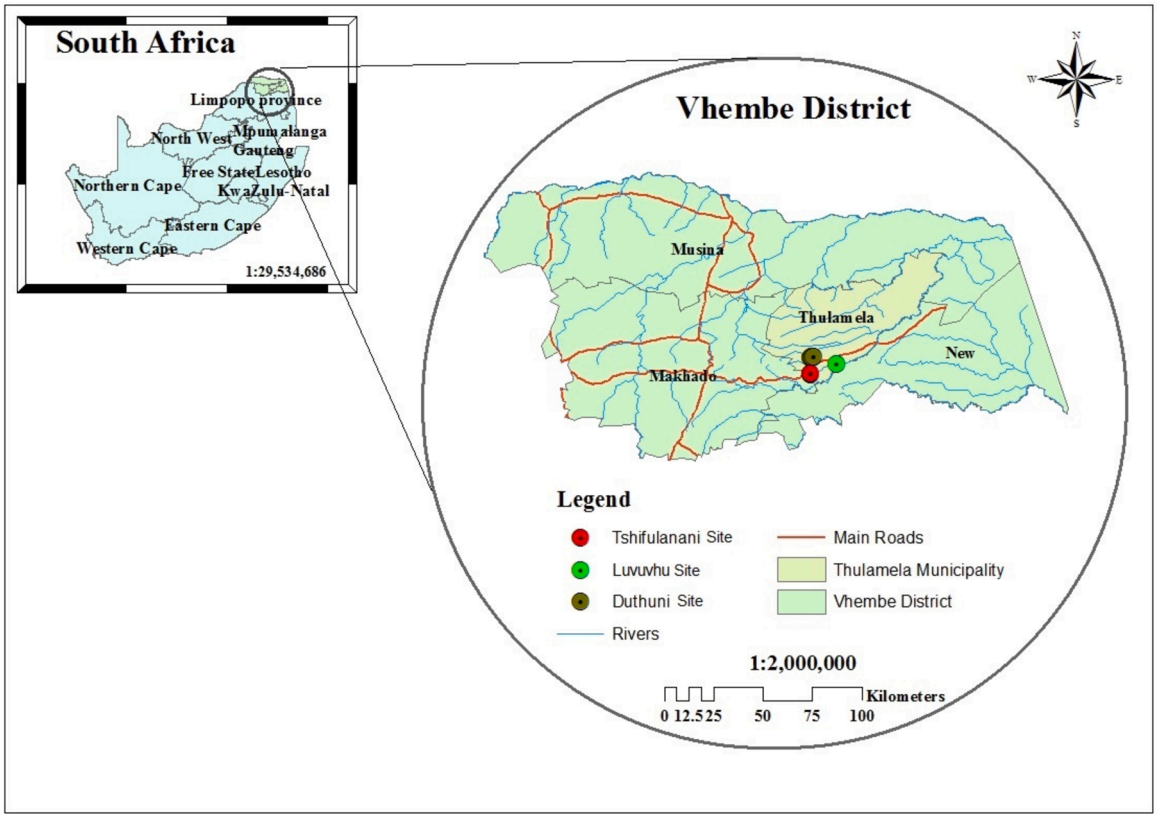

Fig. 1. Map of the study area showing the three sampling sites.

tilapia [5] but in Republic of South Africa it called *blou kurper* in Afrikaans dialect [4]. Wild *O. mossambicus* are known to feed on smaller fish, algae, reed beds, debris, and their diet changes according to the seasons and life span [5]. Farmed *O. mossambicus* has been reported to have higher protein content than wild *O. mossambicus* [6]. Fish are reported to be rich in proteins, micronutrients [7] and low in fatty acids such as omega-6 and omega-3, which are essential to human diet [8]. The benefits associated with the intake of fish with good quality include healthy development of human brain [9], reduction in the incidence of premature birth, heart diseases and stroke [10].

The quality of water used in fish farming as well as the fish feeds do have an impact on the quality of the reared fish. Water supply for fish farmers in the rural areas are usually from canals and rivers which are often contaminated with hazardous chemicals such as pesticides and trace metals [11]. Many fish farmers introduce trace metals into their fish ponds through commercial and formulated fish feeds made of raw materials containing high levels of trace metals [12]. The ability of the fish to digest the feed determines the amount of bioaccumulation of trace metals, therefore juveniles have less concentrations of trace metals than adult fish [13].

The consumption of fish has increased significantly due to its nutritional value [14]. The quality of fish consumed is of outmost importance because the ingestion of metal rich fish can outweigh its nutritive benefit [15]. Fish have the potential to bioaccumulate trace metals in their muscles [16]. Trace metals are accumulated into the fish muscles through ingestion, gills and skin [10]. The consumption of metal rich fish is of public health interest as it constitutes a potential health risk to humans. The high intake of metals contaminated fish above threshold limit have been reported to have detrimental effects causing non-carcinogenic hazards such as neurologic problems [17], liver, kidney diseases [87] and death [18]. Cases of diseases caused by the consumption of trace metals contaminated fish in rural areas and low-income countries are seldom reported [19]. However, Marouf, [20] reported on the occurrence of breast cancer linked to the consumption of metal (Cd, Hg, Pb, Cr and As) contaminated fish. Hazard quotient is often used to estimate the non-carcinogenic risk associated with chemical contaminants in food substances [21]. Carcinogenic risk due to the consumption of some metals such as As, Pb, Cd and Hg in fish species can also be computed .

A number of aquacultural studies, a few of which have investigated and monitored the occurrence of trace metals in fish, fish ponds and fish feeds using modern state of the art analytical methods, including inductively coupled plasma-mass spectrometer (ICP-MS) [22], are largely based on measuring accumulated trace metals in different tissues of the fish, such as liver, gills, muscle and heart . Because of the human health impacts associated with the bioaccumulation of trace metals from fish consumption it has become very important that the trace metals' concentrations in fish, the environment in which fish farming occurs and the fish feed be evaluated.

This study aims to therefore assess (i) the physicochemical and trace metals levels in fish ponds water and Luvuvhu River (ii) concentration of trace metals in farmed *O. mossambicus* muscle (iii) concentration of trace metals in fish feeds and (iv) estimation of the non-carcinogenic and carcinogenic risk associated with the consumption of *O. mossambicus* from the Vhembe district, Limpopo Province, South Africa.

2. Materials and methods

2.1. Study area and description of the fish pond

This study was carried out in the Vhembe District of South Africa. A total of 9 sampling points from 3 sampling sites were studied (Fig. 1). Two *O. mossambicus* farmers at Tshifulanani village (2 sampling points) and Duthuni village (6 sampling points) were visited during the wet season (August- November) of 2018. Luvuvhu River (1 sampling point) was used as a control for wild *O. mossambicus* to compare with the

Table 1
Agilent 8800 optimised instrument parameters for trace element analysis in water, fish and fish feed samples.

RF Power (W)	1550
Robustness mode	General Purpose
Carrier gas (L/min)	1.03
Sample depth (mm)	8
Micromist nebulizer flow (ml/min)	0.2
Oxide ratio	< 0.3 %
ORS settings	
- He flow (ml/min)	4.8
- O_2 flow (ml/min)	0.6
ICP-MS Software	Mass Hunter V 4.1

farmed *O. mossambicus*.

Fish farming is practiced in an earthen fish ponds in the study area. The fish ponds ranged in depth between 1.3–1.5 m, width from 9 to 12 m and 18–20 m in length. Inlet and outlet polyvinyl chloride pipes is used to fill in and remove water in the pond during recharge and discharge.

2.2. Sample collection and analyses

2.2.1. Water samples

Water samples were collected in triplicate from each site in 100 ml sterilised polyethylene containers. Sampling were done in the morning (between 8:30 and 10:30 am) once monthly in a four month period of 2018. A sample grabber was used to access pond water 120 cm from pond edge and water sampled was in a range of 1.2 and 1.3 m with regard to the pond depth. Onsite measurements were carried out for physicochemical parameters (pH, temperature, electrical conductivity and total dissolved solids) using a multi-meter (ACCSEN PC 70, Italy). The dissolved oxygen (DO) was analysed using DO meter (BANTE instrument portable dissolved oxygen meter 821, China). The turbidity was measured with turbidity meter (Lovibond TB 20 W L, United States). The samples were transported on ice to the laboratory for further analysis. Water samples at room temperature were acidified with 3 ml of nitric acid (Sigma-Aldrich) and was sent to the accredited laboratory at Central Analytical Facilities, University of Stellenbosch for trace metal analysis.

The analysis of trace element was performed on an Agilent 8800 QQQ ICP-MS instrument. A 4th generation Octopole Reaction System (ORS), with He as collision gas, and O_2/H_2 as reaction gas is used to remove polyatomic interferences from the analytes of interest. Instrument conditions are summarized in Table 1. The instrument was optimized daily for sensitivity and low oxide ratios (CeO/Ce < 0.3 %). Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr, Mo, Cd, Sb, Ba, Hg and Pb were measured in helium collision mode, while oxygen reaction gas was used for B, As, Se and Sn.

NIST traceable multi-element stock solutions supplied by Inorganic Ventures (INORGANIC VENTURES – 300 Technology Drive, Christiansburg VA 24073) were used to prepare instrument calibration standards. Suprapur (65 %) double distilled nitric acid (HNO_3) and Suprapur (30 %) hydrochloric acid (HCl) were purchased from Merck KGaA, Darmstadt, Germany. Ultra-pure de-ionised water (18 MΩcm^{-1}) used for dilution was produced by a Milli-Q® IQ Element (Merck KGaA, Darmstadt, Germany). Measurement accuracy was verified with trace element standards from De Bruyn Spectroscopic Solutions, Bryanston, South Africa. Instrument drift and matrix effects were monitored and corrected by internal standard elements (^{45}Sc, ^{89}Y, ^{115}In, ^{72}Ge, ^{103}Rh) added automatically from a multi-element mixture in 2 % HNO_3 to each sample and standard before introduction into the ICP-MS instrument.

2.2.2. Fish samples

Oreochromis mossambicus samples (n = 20) were collected from the fish ponds using gill net and grab sampling nets. Three *O. mossambicus* samples of the desired size (adult fish) were selected from the sampling net and the remaining juveniles and adult fish were immediately returned into the fish pond. Polyethylene plastic bags were used to package each fish sample, which were subsequently stored in a cooler filled with ice for preservation during transportation to the laboratory. Three *O. mossambicus* samples from Luvuvhu River were bought at the fish market opposite Phangami shopping complex in Thulamela Municipality. A total of 23 *O. mossambicus* samples were used in this study. Removal of mud and grass on fish was done using distilled water and dried with a paper towel before weighing (kg) and measuring the length (cm) of the sample using Radwag balance scale WLC 6/A2 (Toruńska, Poland) and Penflex Shutterproof 30 cm measuring tape (Cape Town, South Africa), respectively [7].

Before dissection, *O. mossambicus* samples were sacrificed by severing behind the head on the spinal cord. *Oreochromis mossambicus* were dissected using steamed knife separating scales, skin, bones and intestines from the fillet [23]. Four grams of *O. mossambicus* fillet muscle was rinsed with distilled water and stored (at -70 °C) in a labelled sterile bag prior analysis. Using the modified method stated by Okoro et al. [24], *O. mossambicus* fillet muscle (2 *g*) was rinsed and dried before digesting it with nitric acid for 8 h. For extraction, methanol-distilled water (20:30 v/v) was added to the soaked fillet for lipid extraction over-night. The digested fillets were subsequently shaken and centrifuged at 2700 rpm for 20 min (Grant-bio laboratory centrifuge LMC-3000, United Kingdom). The filtered solutions of *O. mossambicus* muscles were analysed for trace metals using ICP-MS .

2.2.3. Analysis of fish feeds

Commercial fish feed samples from the study areas were collected from the farmers and stored in a polyethylene plastic bags. The fish feeds were dried at 80 °C for 24 h [25]. The samples after cooling were grounded with mortar and pestle. Two grams of the samples were digested with 20 ml of nitric acid in a fume hood using a BUCHI heating bath (Switzerland) set at 70 °C for 2 h. A 10 ml aliquot of perchloric acid (Sigma-Aldrich) was subsequently added to the digestate which was then heated for additional 40 min until dense white fumes were observed [26]. Twenty five millilitres of distilled water was added to the filtered feed supernatant and transferred into a 15 ml centrifuge tube for trace metals analysis using ICP-MS .

2.2.4. Length-weight relationship and condition factor

The length and weight of the *O. mossambicus* are directly proportional and used for assessing the growth, maturity, mortality rate and life span of the fish [27]. Fulton's condition factor (K) (Eq. 1) hypothesised that fish length (L in cm) must be related to certain weight (W in g), if not the fish sample is suspected to have consumed toxic content preventing growth and thus confirming bad fish physiological condition [28]. However, fish sample with K = 1, K = 1.2 and K ≥ 1.40 are identified as of poor condition, moderate condition and good condition, respectively [29].

$$K = 100 \times \frac{w}{L^3} \quad (1)$$

2.2.5. Trace metal concentration

For this study, the concentrations of trace metals in *O. mossambicus* samples were determined by using the relation in Eq. 2.

$$C = \frac{D}{W} \times R \quad (2)$$

where C is the concentration of trace metals in wet fish sample in μg/kg, D is the dilution factor (0.1 L), W is the total weight of the wet fish sample (0.002 kg) and R is trace metal concentration (in μg/kg) from ICP-MS [25].

Table 2
Physico-chemical parameters of water samples.

Sampling point	Geographical Coordinates	Temp (°C)	pH	EC (μS/cm)	TDS (mg/l)	DO (mg/l)	Turbidity (NTU)
T1	23°02′29.75″S 30°24′01.43″E	30.47	7.14	1216.00	675.00	0.48	8.94
T2	23°02′30.01″S 30°24′02.05″E	29.70	7.28	1157.00	308.00	0.46	7.00
D3	22°57′56.98″S 30°23′44.96″E	22.43	5.97	650.00	188.00	0.52	30.23
D4	22°57′56.67″S 30°23′44.48″E	22.83	5.64	1119.00	175.00	0.36	69.25
D5	22°57′56.45″S 30°23′44.94″E	21.40	5.59	787.00	173.00	0.56	356.7
D6	22°57′57.43″S 30°23′44.23″E	23.20	6.38	748.00	156.00	0.41	290.57
D7	22°57′58.25″S 30°23′44.72″E	21.40	6.97	608.00	224.00	0.40	101.15
D8	22°57′58.64″S 30°23′45.03″E	22.50	6.54	1050.00	200.00	0.56	24.42
L9	22°59′31.04″S 30°33′26.30″E	24.20	6.77	623.00	190.00	0.28	3.92
Mean		23.43	6.39	842.75	201.75	0.44	110.41
SD		3.43	0.63	248.62	163.87	0.09	132.24
WHO [44,45]				1500	1000		
DWAF [37,52]		28.00 – 30.00	6.5-9.0			5.0 - 8.0	25.00
FAO [36]		25.00–30.00					

Food and Agriculture Organization, WHO- World Health Organization, DWAF-Department of Water Affairs and Forestry.

2.2.6. Human health risk assessment

Using Eq. 3 from Ahmed et al. [29], the average daily dose (ADD) in mg/kg/day was calculated.

$$\mathrm{ADD} = \frac{C_f \times DI}{\mathrm{BW}} \quad (3)$$

Where C_f is the average metal concentration (mg/kg) in the fish muscle, DI refers to the daily intake of fish consumed (kg/day) and BW is the mean body weight of the person (kg). For this study, DI is 7.9 g/day for children (age 6–11 old) [30] and 20.1 g/day for adults [31], respectively. While the average weight of children and adults used were 52.5 kg [30] and 70 kg [32], respectively.

The Hazard quotient (HQ) of each metal was calculated using Eq. 4. Please note that the RfD 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 lifetime [33].

$$HQ = \frac{ADD}{RfD} \quad (4)$$

HQ<1 implies that adverse health impact is unlikely to occur and HQ > 1 estimates high chances of adverse human health impacts [34].

The Cancer Risk (CR) was determined by multiplying the average daily dose and the cancer slope factor (CSF) using Eq. 5 [18]

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

CR values below 10^{-6} is usually considered as not having a potential risk to the consumers while the acceptable cancer risk limit ranges between 10^{-6} to 10^{-4} and cancer risk values above 10^{-4} displays high chances of cancer risk associated with the consumption of the fish [29].

2.2.7. Bioaccumulation of metals in O. Mossambicus tissues

Bioaccumulation (BF) of metals in fish muscles from water was determined using Eq. 6 [28].

$$\mathrm{BF} = \frac{C_m}{C_w} \quad (6)$$

Where C_m and C_w are metal concentration in fish muscles and metal concentration in water, respectively. BF are usually classified into various categories. BF < 1000 indicates no probability of bioaccumulation, BF in the range of 1000 > BF < 5000 indicates bioaccumulation and lastly BF > 5000 indicates high bioaccumulation [29].

2.2.8. Statistical analysis

Pearson's correlation was used to determine significant correlation (at p = 0.05) between trace metals in water, *O. mossambicus* muscle and fish feeds. Statistical analysis was performed using Microsoft Office Plus-Excel 2013 software.

2.2.9. Quality control

To ensure quality control of the experiments undertaken, limit of detection (LOD) and limit of quantification (LOQ) of trace metals in fish muscles, water and fish feed were obtained using 3x and 10x the standard deviation of the background equivalent from 7 blank measurements, respectively. Furthermore, the accuracy was calculated from the recovery of a spiked blank [35].

2.3. Ethical consideration

Ethical clearance was sought for and obtained from the University of Venda Research Ethical Committee with approval number SES/18/HWR/10/1903. Informed consent of *O. mossambicus* farmers were obtained as per standard protocol. Required and desired fish sizes from the fish ponds were selected during sampling and the remaining fish were returned to the pond immediately. Maximum of three fish samples per pond were used for analysis.

3. Results and discussion

3.1. Physicochemical water quality parameters of the fish ponds and Luvuvhu River

Physicochemical parameters such as temperature, pH, electrical conductivity, total dissolved solids, dissolved oxygen and turbidity levels were measured in triplicate and the mean of each of these parameters for each sampling point is presented in Table 2. The results obtained were compared with the guidelines of the South African Department of Water Affairs and Forestry for Agricultural Aquaculture Water Use (DWAF), World Health Organization (WHO) and Food and Agriculture Organization (FAO).

Temperature determined in this study ranged from 21.4 to 30.47 °C. The temperature of the Luvuvhu River as well as the fish ponds in Duthuni (21.4–22.5 °C) did not comply with the recommended limits of 25−30 °C [36] and 28−30 °C [37] for *O. mossambicus* farming. In a previous study, Khan et al. [38] reported a water temperature in the range of 27.50–31.10 °C for fish ponds at Gurgaon canal in India. *Oreochromis mossambicus* require moderate temperature for reproduction, growth, metabolism and physiology [39] and rapid change of temperature interferes with their reproduction and growth [40]. High temperature of fish pond water increases the solubility of trace metal content in the water. Moderate temperature of 24° C in fish pond is efficient for sufficient oxygen supply to the fish [41] while rapid changes in temperature usually leads to fish stress, diseases, growth impairment

Table 3
Fulton's Condition factor data of O. mossambicus samples.

Sampling point	Quantity (n)	Average Length (cm)	Average Weight (g)	K	Fish condition
T1	2	15.40	52.55	1.44	Good
T2	1	17.50	78.90	1.47	Good
D3	3	17.70	81.93	1.48	Good
D4	3	15.20	55.63	1.57	Good
D5	3	14.40	34.50	1.16	Poor
D6	3	13.80	32.80	1.25	Moderate
D7	3	13.03	27.90	1.26	Moderate
D8	2	13.55	41.95	1.69	Good
L9	3	22.37	183.00	1.63	Good
Mean		15.88	65.46	1.44	
SD		2.93	48.11	0.18	
SEM		0.98	16.04	0.06	

K- Fulton's condition factor; n-number; T- Tshifulanani; D- Duthuni; L- Luvuvhu River; SD- standard deviation; SEM- standard error of mean.

and alteration in feeding pattern [38].

The pH recorded in this study ranged between 5.59–7.28 (Table 2). A neutral to alkaline pH is usually recommended for water used in fish farming [42]. Fish mortality has been reported in fish ponds with pH < 4 and pH > 11 [38]. pH in the range of 6.5–9.0 is the threshold limit for water used in fish farming in South Africa [37] and 44.4 % of the samples in this study recorded pH lower than 6.5. Das et al. [25] reported a pH range of 7.1–8.1 from three fish farms which complied to acceptable standards for aquaculture.

Low numbers of ions are required in water solution in fish ponds for osmotic balance in fish species [43]. WHO [44] recommended the range of 200–1500 μS/cm of electrical conductivity (EC) for aquaculture purposes and the present study results were within this range (Table 2). The study by Şen & Aksoy [39] recorded EC of 779.6 μS/cm at Bulakbaşı which was also within the acceptable limit.

The total dissolved solids (TDS) of the samples also complied to WHO [45] standard of less than 1000 mg/l whereas the study by James [46] recommended 400−500 mg/l TDS limit. Water with high TDS values is usually not recommended for use in aquaculture, therefore frequent monitoring of TDS is required [42]. The use of commercialised fish feed has been also reported to contribute to EC and TDS levels in fish ponds [47].

Dissolved oxygen (DO) of fish ponds water is an important parameter that determines the health [48] and the production rate [49] of the fish. Hence, sufficient DO is required to prevent fish mortality [50] and slow growth which affect production and profit [51]. The desired dissolved oxygen (DO) for fish farming is 5–8 mg/l set by DWAF [52]. None of the ponds and river recorded a DO value greater than 1 mg/l (Table 2). Khan et al. [38] reported a DO level in the range of 4.7–5.6 mg/l in fish farming sites at Gurgaon canal in India. Similarly, Ng et al. [53] recorded DO which varied between 3.55–6.00 mg/l from three fish farms in Singapore.

About 55.56 % of the sampling points recorded turbidity values which exceeded the acceptable standard of DWAF (25 NTU). The lowest value of turbidity was determined in Luvuvhu River (3.92 NTU) and ranged between 7.00–356.7 NTU in the fish ponds. In Bangladesh, Das et al. [25] reported fish ponds turbidity ranging from 80 to 402 NTU.

3.2. Fish physiology

The weight and length of the *O. mossambicus* samples recorded were in the range of 27.90–183.00 g and 13.03–22.37 cm, respectively (Table 3). Fulton's condition factor (K) computed in this study were greater than 1.2 implying that the fish samples had acceptable physiological traits except for fish samples from D5 (K = 1.16). The fish sample from the Luvuvhu River (L9) recorded the highest average length and weight. The study by Gesto et al. (2017) recorded K ranging from 1.52 to 1.81 from their *Oreochromis niloticus* samples displaying good physical condition.

3.3. Concentration of metals

The concentrations of trace metals in O. *mossambicus* muscles, water samples and fish feeds are presented in Tables 4–6, respectively. The LOD, LOQ and the measurement accuracy of the trace metals in the samples are presented in Table 4. Several scholars have shown that

Table 4
Trace metals concentrations of O. mossambicus muscle (μg/kg).

Trace metals	Sampling points										Refs.	LOD	**LOQ**	Measurement
	T1	T2	D3	D4	D5	D6	D7	D8	L9	ML		(μg/L)	(μg/L)	accuracy (%)
B	546.17	331.23	BDL	BDL	BDL	BDL	BDL	BDL	BDL	NA		8.0	26.67	108.6
Al	607.64	437.81	1371.86	650.09	565.18	14703.90	437.81	437.81	395.35	1000	FAO [60]	0.10	0.33	107.1
V	63.29	327.34	100.51	55.14	65.61	64.45	47.00	83.06	53.98	500	FAO/WHO [61]	0.10	0.33	105.3
Cr	BDL	1075.64	382.86	345.08	393.78	91.52	17.63	26.87	6.72	1000	WHO [62]	0.10	0.33	100.6
Mn	116.06	4562.31	693.37	804.93	431.09	73.86	64.81	3823.35	58.78	500	FAO [60]	0.10	0.17	101.4
Fe	398.50	3168.38	1574.14	1814.59	5026.05	624.86	1561.74	1551.83	1068.48	300	WHO [63]	0.10	2.67	101.0
Co	22.25	3610.88	429.50	62.63	542.18	96.90	71.11	637.91	99.96	NA		0.05	0.03	104.3
Ni	128.63	3573.04	326.93	141.30	246.07	34.88	40.76	152.18	49.39	100	WHO [63]	0.80	0.33	99.8
Cu	978.91	1781.32	1834.94	98.37	1550.29	1044.72	280.03	962.35	402.01	2250	WHO [63]	0.01	0.33	99.3
Zn	652.06	1253.25	1118.05	537.01	1736.51	545.64	335.66	1051.89	657.81	40 000	[55,56]	0.10	0.67	102.6
As	13.92	106.38	25.40	BDL	16.27	BDL	BDL	14.05	BDL	3	FAO [60]	0.10	0.33	100.8
Se	132.17	551.22	184.77	70.80	209.32	46.26	139.18	256.66	275.94	300	ANVISA [64]	0.20	0.03	98.0
Sr	112.87	120.60	132.74	0.26	23.44	BDL	BDL	7.99	BDL	NA		0.10	0.33	104.2
Mo	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	NA		0.01	0.33	107.3
Cd	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	1000	FAO [60]	0.10	0.03	89.8
Sn	BDL	BDL	BDL	0.36	BDL	BDL	1.78	BDL	BDL	2000	WHO [65]	0.10	0.03	98.2
Sb	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	NA		0.01	0.03	108.7
Ba	88.57	39.37	34.45	7.38	81.19	19.68	31.98	29.52	17.22	NA		0.01	0.03	104.6
Hg	4.96	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	500	[55,56]	0.01	0.03	104.4
Pb	BDL	BDL	7281.43	BDL	BDL	BDL	BDL	BDL	BDL	300	EC [66]	0.01	0.33	101.5

BDL- Below Detectable Limit; NA- Not Available; ML- Maximum limit; T- Tshifulanani pond; D- Duthuni pond; L- Luvuvhu River; WHO- World Health Organization; EC- European Community, DWAF-Department of Water Affairs and Forestry, Food and Agriculture Organization, ANVISA- Agência Nacional de Vigilância Sanitária, LOD: limit of detection, LOQ: limit of quantification.

Table 5
Trace metals concentrations in water samples (μg/l).

Metals	Sampling points										
	T1	T2	D3	D4	D5	D6	D7	D8	L9	ML	Refs.
B	14.44	16.76	12.78	9.59	5.80	9.05	10.98	8.52	14.38	1200	CCME [71]
Al	2.95	0.56	3.26	3.68	1.11	0.31	0.97	4.44	0.45	30	DWAF [52]
V	1.20	0.32	0.34	0.14	0.09	BDL	0.31	0.82	0.87	NA	
Cr	0.17	0.15	0.17	0.19	0.17	0.15	0.20	0.23	0.15	2	DWAF [52]
Mn	48.57	16.14	0.23	0.22	0.56	0.47	0.35	0.27	5.57	100	DWAF [37]
Fe	1.65	1.74	9.00	24.55	1.93	2.69	27.40	9.61	1.48	10	DWAF [37]
Co	0.20	0.14	0.06	0.05	0.08	0.06	0.04	0.03	0.03	NA	
Ni	0.40	0.38	0.14	0.12	0.13	0.19	0.18	0.12	0.64	20	WHO [[63]]
Cu	1.36	1.40	0.98	0.89	1.02	0.62	1.91	1.33	10.84	5	DWAF [52]
Zn	2.37	2.22	1.11	0.63	0.71	2.59	1.24	2.42	8.22	30	DWAF [52]
As	0.15	0.13	0.16	0.15	0.10	0.12	0.15	0.10	0.19	50	DWAF [52]
Se	0.11	0.09	0.04	0.09	0.07	0.05	0.07	0.08	0.07	300	DWAF [37]
Sr	31.14	91.76	7.51	9.57	13.91	17.38	8.88	7.45	102.6	NA	
Mo	0.31	0.24	0.16	0.18	0.16	0.15	0.15	0.13	0.25	NA	
Cd	0.01	BDL	BDL	BDL	0.01	0.01	BDL	BDL	0.01	5	EC [72]
Sn	0.06	BDL	0.06	0.02	0.01	BDL	0.01	BDL	0.06	NA	
Sb	0.07	0.06	0.06	0.04	0.03	0.05	0.06	0.05	0.05	5.00	EC [72]
Ba	34.72	98.42	4.54	3.98	2.97	1.55	7.89	4.03	90.16	NA	
Hg	0.01	BDL	BDL	BDL	BDL	BDL	0.01	BDL	BDL	1	DWAF [52]
Pb	0.09	0.10	0.10	0.11	0.10	0.10	0.13	0.12	0.10	10	DWAF [37]

BDL- Below Detected Limit (<0.01 μg/l); NA- Not Available; ML- Maximum limit; T- Tshifulanani pond; D- Duthuni pond; L- Luvuvhu River; WHO- World Health Organization; EC- European Community; DWAF-Department of Water Affairs and Forestry; CCME- Canadian Council of Ministers of the Environment.

Table 6
Trace metals concentrations in pellets fish feed (mg/kg dry mass).

Trace metals	Sample 1	Sample 2	MPL	Refs.
B	9.82	13.87	NA	
Al	194.95	220.70	NA	
V	0.38	0.45	NA	
Cr	6.27	6.51	1.00	EC [66]
Mn	125.91	122.58	100	EC [67]
Fe	276.23	228.97	100	FAO [60]
Co	0.56	0.60	1.5	EC [66]
Ni	7.77	6.90	1.00	EC [66]
Cu	28.95	31.00	100	EC [68]
Zn	214.84	216.30	150	FAO [60]
As	0.07	0.05	4.00	EC [69]
Se	0.29	0.18	0.5	EC [70]
Sr	21.98	21.25	NA	
Mo	0.60	0.75	NA	
Cd	0.02	0.02	2.00	EC [66]
Sn	0.05	0.01	NA	
Sb	1.24	1.53	NA	
Ba	14.00	14.11	NA	
Hg	BDL	0.01	0.02	EC [69]
Pb	6.38	55.67	5.00	EC [69]

BDL- Below Detectable Limit; MPL- Maximum Permissible Level; EC-European Commission; WHO-World Health Organization; NA- Not available, Food and Agriculture Organization.

bioaccumulation of Hg in fish muscles is high when compared to other metals and it is carcinogenic to humans [54]. The permissible limit of mercury in fish muscle is 500 μg/kg [55,56] and in fish pond water is 1 μg/l [52]. Most of the samples recorded no detectable levels of Hg in the fish muscles and a low level of 4.96 μg/kg was recorded for one of the samples (Table 4). Similarly, the water samples recorded Hg levels that complied to regulatory standards (Table 5). Zhang et al. [57] determined Hg in fish muscles which ranged between 10–50 μg/kg which is higher than those recorded in this study. Similarly, low levels of Hg were determined in the fish feed samples below regulatory threshold limit (Table 6). Hg is also known to cause human health effects such as impaired foetus development during pregnancy [14], blurred vision, hair loss, depression, lung damage [58], nervous system damaged and sleeping disorder [59].

Like mercury, lead (Pb) is a very toxic metal with no nutritive value to humans. The impact of Pb on fish health includes gills damage and fish suffocation [73]. EC [69] recommended a maximum level of 300 μg/kg for Pb in fish muscles, although below detectable limit of Pb was established in most of the fish samples, one of the samples recorded a very high level (7281.43 μg/kg) which exceeded the threshold limit of EC which might be caused by the high concentration of lead in the sediment (Table 4). Addo-Bediako et al. [32] also reported Pb concentration in fish in the range of 1000–1200 μg/kg which exceeded the regulatory standard limit. Gwimbi et al. [74] reported that Pb in fish is absorbed in the blood which distribute the metal to the bone, tissue muscles and liver. The consumption of such fish will have detrimental effect on the consumer. The levels of Pb determined in the water samples ranged between 0.09-0.13 μg/l and complied to regulatory standard for aquaculture systems (Table 5). However, higher levels of Pb were recorded in the fish feeds (6.38 and 55.67 mg/kg) which exceeded the permissible limit of 5.00 mg/kg recommended by EC [69]. The levels of Pb in the fish feeds, however, does not contribute to an increasing trend in water or the fish muscles. This could be due to the pH of the water that limits the dissolution of metals and their absorption into the fish tissue. Results from this study clearly show that other environmental drivers are necessary for the dissolution of metals from fish feeds and their eventual uptake and bioaccumulation in fish.

Arsenic (As) is abundant [58] and carcinogenic in nature [74], and its chemical form often determines the toxicity level [18]. Fish has been reported to contain organic As in the food chain [75]. The acceptable limit of As in fish muscle is 3 μg/kg [60] and results from this study show As levels ranging from below detectable limit to a maximum of 106.38 μg/kg (Table 4). Thakur & Mhatre [76] reported 670 μg/kg of As which is above the guideline value and results of this study. However, the water samples from all the sampling points were within the acceptable limit of 50 μg/l for aquaculture water as recommended by DWAF [52] (Table 5). The levels of As in Fish feed samples were all below the European Commission [68] guideline of 4 mg/kg (Table 6). Therefore, there is a weak relationship between fish feed, water and fish muscles.

Fish gills are known to bioaccumulate high concentration of Cr which often affect their movement in water [77]. Cr concentration in *O. mossambicus* muscles were within the limit set by WHO [62] of 1000 μg/kg except in T2 sample (1075.64 μg/kg) (Table 4). The levels of Cr (0.15 - 0.23 μg/l) determined in the pond water were also below DWAF [52] standard of 2 μg/l (Table 5). However, Cr concentration in fish feed samples were both above the permissible limit of 1 mg/kg recommended by EC [66] (Table 6). Sabbir et al. [78] reported a range of 2.83–15.45

Table 7
ADD (mg/kg/day) for trace metals in *O. mossambicus* muscles.

Site	Age Group	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Ba	Pb
T1	Children	9.14E-05	9.52E-06	NA	1.75E-05	6.00E-05	3.35E-06	1.94E-05	1.47E-04	9.81E-05	2.09E-06	1.99E-05	1.70E-05	1.33E-05	NA
	Adults	1.74E-04	1.82E-05	NA	3.33E-05	1.14E-04	6.39E-06	3.69E-05	2.81E-04	1.87E-04	4.00E-06	3.80E-05	3.24E-05	2.54E-05	NA
T2	Children	6.59E-05	4.93E-05	1.62E-04	6.87E-04	4.77E-04	5.43E-04	5.38E-04	2.68E-04	1.89E-04	1.60E-05	8.29E-05	1.81E-05	1.81E-05	NA
	Adults	1.26E-04	9.40E-05	3.09E-04	1.31E-03	9.10E-04	1.04E-03	1.03E-03	5.11E-04	3.60E-04	3.05E-05	1.58E-04	3.46E-05	1.13E-05	NA
D3	Children	2.06E-04	1.51E-05	5.76E-05	1.04E-04	2.37E-04	6.46E-05	4.92E-05	2.76E-04	1.68E-04	3.82E-06	2.78E-05	2.00E-05	5.18E-06	1.10E-03
	Adults	3.94E-04	2.89E-05	1.10E-04	1.99E-04	4.52E-04	1.23E-04	9.39E-05	5.27E-04	3.21E-04	7.29E-06	5.31E-05	3.81E-05	9.89E-06	2.09E-03
D4	Children	9.78E-05	8.30E-06	5.19E-05	1.21E-04	2.73E-04	9.42E-06	2.13E-05	1.48E-05	8.08E-05	NA	1.07E-05	3.87E-08	1.11E-06	NA
	Adults	1.87E-04	1.58E-05	9.91E-05	2.31E-04	5.21E-04	1.80E-05	4.06E-05	2.82E-05	1.54E-04	NA	2.03E-05	7.38E-08	2.12E-06	NA
D5	Children	8.50E-05	9.87E-06	5.93E-05	6.49E-05	7.56E-04	8.16E-05	3.70E-05	2.33E-04	2.61E-04	2.45E-06	3.15E-05	3.53E-06	1.22E-05	NA
	Adults	1.62E-04	1.88E-05	1.13E-04	1.24E-04	1.44E-03	1.56E-04	7.07E-05	4.45E-04	4.99E-04	4.67E-06	6.01E-05	6.73E-06	2.33E-05	NA
D6	Children	2.21E-03	9.70E-06	1.38E-05	1.11E-05	9.40E-05	1.46E-05	5.25E-06	1.57E-04	8.21E-05	NA	6.96E-06	NA	2.96E-06	NA
	Adults	4.22E-03	1.85E-05	2.63E-05	2.12E-05	1.79E-04	2.78E-05	1.00E-05	3.00E-04	1.57E-04	NA	1.33E-05	NA	5.65E-06	NA
D7	Children	6.59E-05	7.07E-06	2.65E-06	9.75E-06	2.35E-04	1.07E-05	6.13E-06	4.21E-05	5.05E-05	NA	2.09E-05	NA	4.81E-06	NA
	Adults	1.26E-04	1.35E-05	5.06E-06	1.86E-05	4.48E-04	2.04E-05	1.17E-05	8.04E-05	9.64E-05	NA	4.00E-05	NA	9.18E-06	NA
D8	Children	6.59E-05	1.25E-05	4.04E-06	5.75E-04	2.34E-04	9.60E-05	2.29E-05	1.45E-04	1.58E-04	2.11E-06	3.86E-05	1.20E-06	4.44E-06	NA
	Adults	1.26E-04	2.39E-05	7.71E-06	1.10E-03	4.46E-04	1.83E-04	4.37E-05	2.76E-04	3.02E-04	4.03E-06	7.37E-05	2.29E-06	8.48E-06	NA
L9	Children	5.95E-05	8.12E-06	1.01E-06	8.85E-06	1.61E-04	1.50E-05	7.43E-06	6.05E-05	9.90E-05	NA	4.15E-05	NA	2.59E-06	NA
	Adults	1.14E-04	1.55E-05	1.93E-06	1.69E-05	3.07E-04	2.87E-05	1.42E-05	1.15E-04	1.89E-04	NA	7.92E-05	NA	4.95E-06	NA
RfD		1.00E+0	1.00E-02	1.50E+0	1.40E-01	7.00E-01	3.00E-02	2.00E-02	4.00E-02	3.00E-01	3.00E-04	3.00E-02	6.00E-01	2.00E-01	1.00E-02

RfD- oral reference dose per person in mg kg^{-1} day^{-1}; NA: not applicable; T- Tshifulanani pond; D- Duthuni pond; L- Luvuvhu River.

mg/kg of Cr in fish feed. . Humans are known to suffer from cancer and kidney impairments from high intake of chromium [79].

Cadmium (Cd) is a carcinogenic metal and 1000 μg/kg has been recommended as the threshold limit for Cd in fish muscles by FAO/WHO [60]. No detectable level of Cd was recorded in fish muscles in this study. Nile tilapia from Ankobrah and Pra Rivers (Ghana) recorded Cd concentration of 80 μg/kg which was above the levels recorded in this study [80]. Similarly, the levels of Cd analysed from the water samples complied to regulatory guideline of 5 μg/l set by EC [72]. Also, the fish feed did not have elevated levels of Cd, with both cases complying with EC [66] regulatory standards. Fish gills are known to accumulate excess of Cd compared to other organs [81].

Although there are no regulatory values for Barium (Ba) in aquaculture systems, Ba is believed to be toxic to humans at low concentration. Intake of Ba results in breathing problems, high blood pressure and brain damage [82]. Ba accumulation in *O. mossambicus* muscles ranged between 7.38–88.57 μg/kg (Table 4). The study by Li et al. [83] recorded higher levels of Ba which varied between 760–1540 μg/kg in fish muscles. Scher [84] proposed 1200 μg/kg of Ba as acceptable daily intake only for individuals above 60 kg of body weight. Barium levels in water samples ranged from 1.55 to 98.42 μg/l (Table 5). The study performed by Davidson et al. [85], recorded higher Ba levels (108 and 230 μg/l) in water in their aquaculture system. The levels of Ba determined in the fish feeds were 14.00 and 14.11 respectively (Table 6).

The levels of Copper (Cu) recorded in this study is in the range of 98.37–1834.94 μg/kg and complied to the WHO [63] recommended guideline of 2250 μg/kg in fish muscle (Table 4). Cu concentration in the range of 1770–2700 μg/kg has been reported in farmed *O. mossambicus* [25]. The permissible limit of Cu in water for aquaculture is 5 μg/l and the levels of Cu (0.62–1.91 μg/l) obtained in this study complied to the DWAF [52] standard but higher levels of Cu (10.84 μg/l) were recorded in the wild fish from Luvuvhu River which did not comply with the standard value (Table 5). The levels of Cu in fish feed also complied to EC [68] standard of 100 mg/kg (Table 76).

Zinc (Zn) in less quantity is essential for metabolic synthesis, protein stabilisation [86] and a catalyst in reproduction process and growth of fish [26]. Zn in fish muscles were below 40 000 μg/kg (Table 4) set by FAO/WHO [55,56]. The studies conducted by Taweel et al. [87] in Malaysia also recorded acceptable levels of Zn (20 850 - 26 130 μg/kg) in *O. mossambicus* muscles. The levels of Zn in the water samples were below the recommended guideline of 30 μg/l (Table 5) set by DWAF [52]. Higher levels of Zn were determined for the fish feed samples (Table 6) than the FAO/WHO [60] standard. The study by Saluwa et al. [26] recorded low concentrations of Zn (8.32–11.63 mg/kg) in fish feeds when compared to this study.

Boron (B) is an essential element, in low concentration it is used as a stress regulator during fish production [88]. Moreover, B is required in a range of 10−20 mg/kg in a daily diet of an average size person of 60 kg [89]. B in *O. mossambicus* muscles varied between 331.23 and 546.17 μg/kg from fish ponds 2 and 1, respectively. B content in water samples (Table 5) was below 1200 μg/l as recommended by CCME [71] in fish ponds. Boron in fish feeds were 9.82 and 13.87 mg/kg, respectively.

Results obtained in this study were within the acceptable limit of Al of 1000 μg/kg recommended by FAO/WHO [60] in fish muscles except

Table 8
Hazard quotients (HQ) for *O. mossambicus* muscle tissues.

Site	Age Group	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Ba	Pb	Total HQ
T1	Children	9.14E-05	9.52E-04	NA	1.25E-04	8.57E-05	1.12E-04	9.68E-04	3.68E-03	3.27E-04	6.98E-03	1.99E-03	2.83E-05	6.66E-05	NA	1.54E-02
	Adults	1.74E-04	1.82E-03	NA	2.38E-04	1.63E-04	2.13E-04	1.85E-03	7.03E-03	6.24E-04	1.33E-02	3.80E-03	5.40E-05	1.27E-04	NA	2.94E-02
T2	Children	6.59E-05	4.93E-03	1.08E-04	4.90E-03	6.81E-04	1.81E-02	2.69E-02	6.70E-03	6.29E-04	5.34E-02	8.29E-03	3.02E-05	2.96E-05	NA	1.25E-01
	Adults	1.26E-04	9.40E-03	1.08E-04	9.36E-03	1.30E-03	3.46E-02	5.13E-02	1.28E-02	1.20E-03	1.02E-01	1.58E-02	5.77E-05	5.65E-05	NA	2.38E-01
D3	Children	2.06E-04	1.51E-03	3.84E-05	7.45E-04	3.38E-04	2.15E-03	2.46E-03	6.90E-03	5.61E-04	1.27E-02	2.78E-03	3.33E-05	2.59E-05	3.65E-02	6.70E-02
	Adults	3.94E-04	2.89E-03	3.84E-05	1.42E-03	6.46E-04	4.11E-03	4.69E-03	1.32E-02	1.07E-03	2.43E-02	5.31E-03	6.35E-05	4.95E-05	6.97E-02	1.28E-01
D4	Children	9.78E-05	8.30E-04	3.46E-05	8.65E-04	3.90E-04	3.14E-04	1.06E-03	3.70E-04	2.69E-04	NA	1.07E-03	6.45E-08	5.55E-06	NA	5.31E-03
	Adults	1.87E-04	1.58E-03	3.46E-05	1.65E-03	7.44E-04	5.99E-04	2.03E-03	7.06E-04	5.14E-04	NA	2.03E-03	1.23E-07	1.06E-05	NA	1.01E-02
D5	Children	8.50E-05	9.87E-04	3.95E-05	4.63E-04	1.08E-03	2.72E-03	1.85E-03	5.83E-03	8.71E-04	8.16E-03	3.15E-03	5.88E-06	6.11E-05	NA	2.53E-02
	Adults	1.62E-04	1.88E-03	3.95E-05	8.84E-04	2.06E-03	5.19E-03	3.53E-03	1.11E-02	1.66E-03	1.56E-02	6.01E-03	1.12E-05	1.17E-04	NA	4.83E-02
D6	Children	2.21E-03	9.70E-04	9.18E-06	7.94E-05	1.34E-04	4.86E-04	2.62E-04	3.93E-03	2.74E-04	NA	6.96E-04	NA	1.48E-05	NA	9.07E-03
	Adults	4.22E-03	1.85E-03	9.18E-06	1.51E-04	2.56E-04	9.28E-04	5.01E-04	7.50E-03	5.22E-04	NA	1.33E-03	NA	2.83E-05	NA	1.73E-02
D7	Children	6.59E-05	7.07E-04	1.77E-06	6.97E-05	3.36E-04	3.57E-04	3.07E-04	1.05E-03	1.68E-04	NA	2.09E-03	NA	2.41E-05	NA	5.18E-03
	Adults	1.26E-04	1.35E-03	1.77E-06	1.33E-04	6.41E-04	6.81E-04	5.85E-04	2.01E-03	3.21E-04	NA	4.00E-03	NA	4.59E-05	NA	9.89E-03
D8	Children	6.59E-05	1.25E-03	2.70E-06	4.11E-03	3.34E-04	3.20E-03	1.14E-03	3.62E-03	5.28E-04	7.05E-03	3.86E-03	2.00E-06	2.22E-05	NA	2.52E-02
	Adults	1.26E-04	2.39E-03	2.70E-06	7.84E-03	6.37E-04	6.11E-03	2.18E-03	6.91E-03	1.01E-03	1.34E-02	7.37E-03	3.82E-06	4.24E-05	NA	4.81E-02
L9	Children	5.95E-05	8.12E-04	6.74E-07	6.32E-05	2.30E-04	5.01E-04	3.72E-04	1.51E-03	3.30E-04	NA	4.15E-03	NA	1.30E-05	NA	8.05E-03
	Adults	1.14E-04	1.55E-03	6.74E-07	1.21E-04	4.38E-04	9.57E-04	7.09E-04	2.89E-03	6.30E-04	NA	7.92E-03	NA	2.47E-05	NA	1.54E-02
RfD		1.00E+0	1.00E-02	1.50E+0	1.40E-01	7.00E-01	3.00E-02	2.00E-02	4.00E-02	3.00E-01	3.00E-04	3.00E-02	6.00E-01	2.00E-01	1.00E-02	

RfD- oral reference dose per person in mg kg^{-1} day^{-1}; NA: not applicable; T- Tshifulanani fish pond; D- Duthuni fish pond; L- Luvuvhu River.

D3 (1371.86 μg/kg) and D6 (14703.9 μg/kg) (Table 4). Abdel-Mohsien & Mahmoud [90] in Egypt recorded Al in the range of 40–610 μg/kg. Ghani [91] stated that fish gills and liver are affected by high concentration of Al above 30 μg/l. Concentrations of Al in water samples were below the DWAF [52] permissible limit of 30 μg/l (Table 5). The extremely high concentration of Al (119 000 μg/l) recorded in Pansky fish ponds in Czech Republic resulted to fish mortality [92]. There is no permissible limit of Al reported in literature for fish feeds. We recorded 194.95 and 220.70 mg/kg levels of Al in the fish feeds (Table 6).

Authman et al. [73] mentioned that metabolic system in human can be improved by Vanadium (V) intake in their diet which also promotes growth. FAO/WHO [61] recommended 500 μg/kg of V as a safe limit for consumption of fish muscles and results from this study (Table 4) were within that limit. The study conducted by Moghdani et al. [93] also obtained acceptable values of V (245–288 μg/kg) in fish muscles analysed from Persian Gulf, Iran. The recorded values of V from water samples in this study (Table 5) ranged from below detection limit to 1.20 μg/l. The study by Schiffer [94] clearly stated that no literature has reported the guideline of vanadium in aquatic environment. Low levels of V were also determined in fish feed (0.38 and 0.45 mg/kg) (Table 6). Currently, there are no guideline values for V in aquaculture water and fish feed because it may be considered of no risk to the health of fish.

Human body requires manganese (Mn) in less quantity for protein molecules that transport oxygen in blood [95] and excess intake of Mn has been related with endocrine malfunction [96]. Farmed *O. mossambicus* from T1, D5, D6 and D7 and wild fish from L9 (Table 4) recorded Mn concentrations below 500 μg/kg set by FAO/WHO [60]. The highest Mn value (4562.31 μg/kg) in fish muscles was found at location T2 which can cause gill impairment and reduction of liver glycogen [97]. Mn concentrations in water samples complied with DWAF [37] permissible limit of 100 μg/l (Table 5). The Fish feed analysed by Mannan et al. [98] recorded 0.520 mg/kg of Mn concentration which was below the levels from this study (125.91 and 122.58 mg/kg) (Table 6). Mn concentrations in fish feed were above the recommended limit of 100 mg/kg by EC [67].

Results of this study were above the WHO [63] recommended standard of 300 μg/kg of iron (Fe) concentration in fish muscle (Table 4). Rapid pulse rate in human being is related to high intake of Fe from food including fish [76]. Fe concentrations in water samples (1.48–27.40 μg/l) with some samples exceeding the DWAF [37] standard of 10 μg/l of Fe concentration in aquaculture water. The study by Ginson et al. [49] reported Fe concentration from four farms ranging from 20 to 90 μg/l. Luvuvhu River water samples recorded the lowest Fe concentration. The levels of Fe in the fish feeds were above the FAO [60] limit of 100 mg/kg (Table 6).

In human diet, cobalt (Co) is essential during vitamin synthesis and it is required in fish species during ovulation, reducing follicles mortality [96] but excess intake of Co could result to lung cancer in humans [20]. The minimum and maximum Co levels in fish muscles and water samples were 22.25 and 3610.88 μg/kg, and 0.03 and 0.20 μg/l, respectively (Tables 4 and 5). However, there are no compliance standards in both cases. Fish feed (Table 6) analysis recorded acceptable Co levels below the standard by EC [66] of 1.5 mg/kg.

Most of the fish samples in this study exceeded the permissible limit of $\leq$ 100 μg/kg stipulated by WHO [63] for Ni (nickel) concentration levels in fish muscles except for two fish samples from the ponds (D6 and D7) and the wild *O. mossambicus* from Luvuvhu River (Table 4). The levels of Ni in all the water samples were below the permissible limit of Ni (20 μg/l) of WHO [63] (Table 5). EC [66] recommended a standard of 1 mg/kg of Ni in fish feed, however, levels higher than this were recorded in this study (Table 6). A study in Nigeria recorded lower Ni concentrations of 0.36 mg/kg, 0.65 mg/kg, 0.63 mg/kg and 0.38 mg/kg in Aqua®, Multi®, Top® and Vital® fish feeds samples [26].

Selenium (Se) is an essential micronutrient for growth improvement and in males is responsible for spermatogenesis [73]. In Brazil, a legislation called ANVISA of 1965 recommended 300 μg/kg of Se content in

Table 9
Cancer risk levels (mg/kg) in *O. mossambicus*.

As			Cr		Ni		Pb	
CSF	1.50E + 00		5.00E-01		9.10E-01		8.50E-03	
	Children	Adults	Children	Adults	Children	Adults	Children	Adults
T1	1.10E-05	6.00E-06	NA	NA	6.16E-05	3.36E-05	NA	NA
T2	8.40E-05	4.58E-05	2.83E-04	1.54E-04	**1.71E-03**	9.34E-04	NA	NA
D3	2.01E-05	1.09E-05	1.01E-04	5.50E-05	1.57E-04	8.54E-05	3.26E-05	1.78E-05
D4	NA	NA	9.09E-05	4.95E-05	6.77E-05	3.69E-05	NA	NA
D5	1.29E-05	7.01E-06	1.04E-04	5.65E-05	1.18E-04	6.43E-05	NA	NA
D6	NA	NA	2.41E-05	1.31E-05	1.67E-05	9.11E-06	NA	NA
D7	NA	NA	4.64E-06	2.53E-06	1.95E-05	1.07E-05	NA	NA
D8	1.11E-05	6.05E-06	7.07E-06	3.86E-06	7.29E-05	3.98E-05	NA	NA
L9	NA	NA	1.77E-06	9.65E-07	2.37E-05	1.29E-05	NA	NA

T- Tshifulanani fish pond; D- Duthuni fish pond; L- Luvuvhu; CSF- cancer slope factor; NA- Not applicable; values in bold are above 10^{-4}.

fish muscle. This study recorded variable levels of Se in fish muscles to a maximum of 551.22 μg/kg (T2) (Table 4). A previous study by Silva et al. [99] reported excess Se in fish muscles ranging between 730–2190 μg/kg which was higher than the levels determined in this study (Table 4). Excess level of Se in fish edible parts results in mortality, reproduction failure and reduced growth if consumed frequently by humans [100], however, small intake is required for regulating thyroid hormone [101]. Water samples were all below the maximum acceptable value of 300 μg/l by DWAF [37] (Table 5). The fish feed also contained low levels of Se and complied to regulatory standards of EC [70].

Guérin et al. [102] recorded strontium (Sr) concentrations ranging from 348 – 16 200 μg/kg in fish muscles which were higher than the values determined in this study (Table 4). Sr in fish ponds water ranged from 7.45 to 91.76 μg/l and Luvuvhu River recorded the highest concentration of 102.6 μg/l. The levels of Sr in fish feeds were 21.98 mg/kg and 21.25 mg/kg (Table 6). Currently there are no guidelines for Sr in fish muscles, pond water and fish feed.

Analysis of Molybdenum (Mo) in the fish muscles were all below the detectable limit (Table 4) but its concentrations in water samples ranged between 0.13 and 0.31 μg/l (Table 5). In Bangladesh, Mo concentration in fish samples varied between 50–180 μg/kg which was higher than the results found in the current study [82]. The levels of Mo in fish feeds ranged between (0.60 - 0.75 mg/kg) (Table 6). To the best of our knowledge this is one of the few studies that reported Mo in fish muscles and feeds.

Tin (Sn) is found on the earth's crust in organic form [103] at concentrations of 2 mg/kg but its toxicity is a threat to the environment and human health [104]. Symptoms such as fatigue, lungs and kidney dysfunction, tissue muscle weakness, diarrhoea, stomach aches and skin irritation have been linked with the ingestion of high levels of Sn by humans [104]. Sn concentration in *O. mossambicus* muscles were below the WHO [65] threshold limit of 2000 μg/kg (Table 4). This is comparable to the findings of Abadi et al. [101] who recorded Sn values in the range of 40–230 μg/kg in fish muscles. Water samples recorded Sn levels between below detectable limit to 0.06 μg/l (Table 5). While fish feed recorded 0.05 mg/kg and 0.01 mg/kg of Sn (Table 6). There are no guideline values for Sn for aquaculture water and fish feed.

Oreochromis mossambicus muscle analysis for antimony (Sb) concentration was below the detectable limit (Table 4). However, the water samples recorded levels between 0.03 and 0.07 μg/l of Sb which was below the threshold limit of 5.0 μg/l set by EC [72]. The fish feed recorded 1.24 and 1.53 mg/kg of antimony (Table 6). A study by Li et al. [83] discovered that Marbled eel fish feed had a mean Sb concentration of 0.26 mg/kg and the fish muscles recorded mean values of 400 μg/kg, 100 μg/kg and 480 μg/kg of Sb from recirculating aquaculture system, biofloc technology and concrete ponds, respectively, which were higher than the Sb found in this study.

Generally, the fish muscles from the Luvuvhu River (control sample) recorded lower levels of metals than those from the fish ponds (Table 4). The lowest levels of Mn and Al in the fish muscles were determined in the control samples. Also, apart from two sampling sites, the control sample recorded lower levels of Fe, Ni and Cu. Conversely, the levels of metals in the river water were generally higher than those from the fish ponds. Luvuvhu River sample recorded the highest levels of B, Ni, Cu, Zn, As and Sr while it recorded the lowest levels of Cr, Fe and Co.

3.4. Human health risk assessment

Not all the trace metals were used in computing the risk associated with the consumption of the fish samples in this study. Only those that occurs at quantifiable levels in the samples were used for the computation of ADD and HQ, while the metals that have the potential to cause cancer were used in the estimation of the cancer risk (CR). Tables 7 and 8 shows the ADD and the hazard quotients (HQ) associated with the consumption of the fish samples. CR data for As, Cr, Ni and Pb are presented in Table 9. However, human can be exposed to trace metals through the ingestion of metal-rich water or beverages, inhalation of trace metal contaminated air and intake of other food substances [105]. There are also numerous ways through which trace metals can be introduced to fish ponds beyond the pond water and the fish feeds [145].

ADD for children ranged from 3.87×10^{-8} to 1.21×10^{-3} while for adults ranged from 7.38×10^{-8} to 1.03×10^{-3}. ADD results from this study are similar to the results obtained by Gyimah et al. [106] in Ghana where the ADD values were below the oral reference dose posing no risk to human health relating to consumption of fish (Table 7). Moreover, past study conducted in Sudan revealed ADD lower than R*f*D ranging from 8.31×10^{-7} to 1.37×10^{-5} [107].

Hazard quotients (HQ) was used to determine the non-carcinogenic health risk of *O. mossambicus* on consumers. The hazard quotient for this study were all below 1 implying that consumption of *O. mossambicus* will not impose any health implication to both age groups studied (Table 8). Since trace metals do not exist in isolation in aqueous media or in the fish tissue, we computed the total hazard quotients (THQ) for all the metals from the sampling sites and the results also showed levels lower than 1, indicating no possibility for non-carcinogenic health risk associated with the consumption of *O. mossambicus* from the sampling sites. The study by Soliman [6] recorded high HQ of 6.88 for Cd in farmed *O. niloticus* fish Sohag Governorate, Egypt. In summary, all of the fish ponds and the river produces fish suitable for human consumption.

The possibility of cancer risk (CR) was calculated using arsenic, chromium, nickel and lead. From the results computed in Table 9, all the metals recorded levels lower than 1×10^{-4} indicating that no cancer risk is anticipated with the consumption of the fish from the ponds and the river except sampling point T2 which showed a possible cancer risk to children which is associated with the levels of Ni determined in the fish sample at that site. Also, this site showed higher levels of most metals recorded in this study. Frequent change of water in this pond can help to offset this limitation determined. Overall, the consumption of the fish from all the sites pose no cancer risk to the consumers irrespective of their age group. However, the study in Rwanda showed that intake of

Table 10
Bioaccumulation factor between fish and water.

L9	27.82	876.15	61.89	44.95	10.55	720.18	3074.56	77.40	37.07	80.07	NA	4198.99	NA	0.04	61.53	8.87	8.95	0.19	50.00	4.62	
D8	46.93	98.54	100.74	115.31	13931.70	161.48	18949.03	1307.39	724.25	434.75	137.63	3178.90	1.07	0.07	50.00	55.56	9.13	7.33	50.00	3.63	
D7	36.44	450.47	149.82	86.24	183.18	57.00	1903.79	225.45	146.68	271.37	NA	2059.10	NA	0.06	50.00	228.39	7.35	4.06	82.49	3.55	
D6	44.20	47068.27	7161.16	603.85	158.59	232.29	1751.10	187.79	1697.37	210.40	NA	879.86	NA	0.06	50.92	55.56	9.93	12.70	50.00	4.38	
D5	68.99	508.83	743.02	2339.09	774.59	2609.57	6699.07	1886.51	1521.92	2454.82	169.93	2858.46	1.69	0.06	64.21	34.33	13.10	27.31	50.00	4.56	
D4	41.69	176.68	382.72	1794.70	3628.35	73.93	1312.17	1222.60	110.55	858.16	NA	819.77	0.03	0.05	50.00	15.66	10.51	1.85	50.00	3.92	
D3	31.30	420.44	295.72	2279.92	2996.36	174.96	7219.69	2343.32	1874.08	1006.04	159.21	5179.30	17.67	0.06	50.00	6.97	7.35	7.59	50.00	74825.00	
T2	23.86	482.53	929.50	6457.00	260.79	1566.76	24389.86	8181.45	1140.86	478.47	722.54	4909.92	0.60	0.04	50.00	197.99	7.29	0.37	275.41	4.51	
T1	37.81	205.95	52.69	2.58	2.39	241.72	111.41	319.28	719.11	274.73	92.15	1257.01	3.62	0.03	61.53	8.10	6.70	2.55	567.94	4.76	
	B	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Mo	Cd	Sn	Sb	Ba	Hg	Pb	

T- Tshifulanani fish pond; D- Duthuni fish pond; L- Luvuvhu, NA- Not applicable.

fish can cause cancer to children and adults due to high chromium and lead in the edible fish muscles with values greater than 1×10^{-4} [108]. Results from this study agrees with the findings of Ahmed et al. [29] that children are more sensitive and prone to risk of trace metals contamination in fish muscles than adults.

3.5. Bioaccumulation factor

The Bioaccumulation factor (BF) for different metals in fish muscle usually gives a clear indication about the concentration of these metals in fish muscle relative to their concentration in water and the affinity of fish muscle to accumulate these metals relative to their abundance in water. According to Table 10 the BF for trace metals (As, B, Ba, Cd, Hg, Mo, Sb, Sn and Sr) were below 1000 indicating no possibility of bioaccumulation under the study condition in all of the sampling sites. Some metals (Al, Pb and V) recorded BF $<$ 1000 in 8 (88.9 %) of the sampling sites, while others also recorded low BF in various ranges. The possibility of metals bioaccumulation from water to the muscles of the fish was however determined for some metals (Cr, Mn, Fe, Co, Ni, Cu, Zn, Se) in some of the sampling sites and recorded BF in the range of 1000-5000. A few metals (Co, Cr, Ni, Se, Pb and V) showed extreme bioaccumulation with BF levels exceeding 5000. It is noteworthy to state that some of the metals such as Cr, Ni, Pb and V only showed extreme bioaccumulation in one sampling site (11.1 %) while Se and Co, showed it in three (33.3) and four sampling sites (44.4 %) of the study area.

The study by Hossain et al. [109] also confirms high bioaccumulation of trace metals (Cr, Cu, Cd and Ni) from water and commercial fish feed in farmed *O. mossambicus* from Bangladesh fish farms. The high bioaccumulation factors confirm that the trace metals in water are transferred into fish muscles. This clearly indicates the possible effect of natural or anthropogenic sources on trace metals abundance in aquatic media in the study sites. Therefore, monitoring fish contamination is an important factor that can serve as an early warning of related water contamination problems and can promote the initiation of appropriate action to protect public health and the environment.

4. Conclusion

The fish samples of *O. mossambicus* collected from fish ponds and Luvuvhu River in the study area were generally in good condition (66.67 %) based on the Fulton's constant. Water quality parameters such as temperature, pH, EC and TDS complied with the recommended levels for aquaculture production. Most of the trace metals in the water samples complied with the regulatory standards approved for water use in aquaculture. Similarly, the levels of some of the trace metals were below the recommended limits in the fish muscles. However, the levels of As and Fe in fish muscles were all above the acceptable limits set for human consumption. Also, 44 % of Mn and 66.67 % of Ni in fish muscles also exceeded the recommended levels. The levels of some metals (Co, Cu, As, Se, Cd and Hg) in the fish feed complied to regulatory limit while others (Cr, Mn, Ni, Fe, Zn and Pb) exceeded the limit. Non carcinogenic health risk is not expected with the consumption of the fish from the study area. Also, potential cancer risk was only computed for children but the overall result showed no carcinogenic health risk to the consumers of the O. *mossambicus.* Low-extreme bioaccumulation of certain metals were also recorded in this study. The possible sources of metals in this fish muscles could be from the water in the ponds as well as the levels of metals in the fish feeds. It is therefore recommended that water in the fish ponds should be replaced continuously to prevent increased levels of metals capable of settling in the sediments during favourable conditions.

Author Statement

Edokpayi JN, JR Gumbo and Mannzhi MP: Conceptualization.

Durowoju OS, Edokpayi JN, Mannzhi MP, Odiyo JO: Data curation.

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Edokpayi JN: Supervision.

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Mannzhi MP, Edokpayi JN, Gumbo and Durowoju OS, Odiyo JO: Roles/Writing - original draft.

Edokpayi JN, Odiyo JO, Mannzhi MP, JR Gumbo and Durowoju OS: Writing - review & editing.

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Declaration of Competing Interest

The authors report no declarations of interest.

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