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ORIGINAL RESEARCH ARTICLE

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Potential health risks of trace metals in muscle tissue of tilapia and catfish from Mozambican markets

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ABSTRACT

Due to the toxicity of trace metals and the propensity of fishes to bioaccumulate metals in their tissues, we investigated the concentrations of arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), mercury (Hg), and zinc (Zn) in the muscles of tilapia (*Oreochromis mossambicus*) and catfish (*Chrysichthys nigrodidatatus*) collected from open markets in Mozambique. Fe and Hg were the most and least bioaccumulated metals in the fishes, respectively. One-way ANOVA showed significant differences between sites for the analytes. Furthermore, we estimated the possible health risks (estimated daily intake (EDI), target hazard quotient (THQ), and maximum allowable consumption rate (CR_{lim})) associated with fish consumption. The concentrations of As, Cd, and Pb exceeded the recommended maximum permissible limits (MPL) in fish samples, ranging between 5.65 – 12.7, 1.05 – 12.9, and 1.88 – 6.45 mgkg-1, respectively, whereas values lower than MPL viz. 5.25 – 18.9, ND – 0.033, and 30.8 – 52.3 mgkg-1 were observed for Cu, Hg, and Zn, respectively. Similarly, the EDI (mgkg^{-1}day^{-1}) were below the provisional tolerable daily intake (PTDI) with decreasing order: Fe >Zn >Cu >As >Cd >Pb>Hg. However, the THQ (mg kg^{-1}) was slightly > 1 for As and Cd in some samples. Moreover, the CR_{lim} (kg day^{-1}) showed a decreasing order of Hg >Fe >Zn >Pb> Cu >Cd >As. Generally, consumers are susceptible to health hazards associated with As and Cd. Hence, regular toxicological monitoring of the fishes from the study area is imperative.

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INTRODUCTION

Food contamination by toxic elements (TEs) has become a global environmental and human health problem (Chen *et al.*, 2011). The prevalence of toxic metals (TMs) in the environment could be attributed to various natural and anthropogenic activities throughout human history (Wuana and Okieimen, 2011; Chehregani and Malayeri, 2014; Kamunda *et al.*, 2016; Ali *et al.*, 2019). Some of the anthropogenic sources include mining, smelting, electroplating, use of pesticides, phosphate-based fertilizers, and biosolids in agriculture, sludge dumping, coal combustion residues, and industrial discharges (Sabiha-Javied *et al.*, 2009; Fulekar *et al.*, 2009; Nazir *et al.*, 2015; Rai *et al.*, 2019; Ali *et al.*, 2019). TM pollution of the marine and aquatic environment has long been recognized as a severe environmental concern (Meltem *et al.*, 2007). Fish consumption contributes to the human diet, providing high quality, easily digestible animal proteins, rich in vitamins, essential fatty acids, and minerals (including calcium, phosphorus, iron, zinc iodine, magnesium, and potassium), helping against micronutrient deficiencies

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(FAO, 2020). However, fishes also bio-accumulate metals toxic to human health, especially at high concentrations (Tacon and Metian, 2013; Franco-fuentes *et al.*, 2021). When available in moderate concentrations, Cu, Fe, and Zn are essential because of their valuable role in metabolic activities (Akintujoye *et al.*, 2013). Other metals, such as As, Cd, Pb, and Hg, exhibit extreme toxicity even at trace levels (Boyd and Rajakaruna, 2013). Therefore, they are listed among the ten major chemicals of public health concern (Duruibe *et al.*, 2007; Tchounwou *et al.*, 2014).

Some toxic metals, such as As, Cd, Hg, and Pb, are endocrine-disrupting chemicals (EDCs), capable of inducing neurological disorders even at low concentrations (Bergman *et al.*, 2012; Gaurav *et al.*, 2019). These metals can cause malfunctioning of the cellular processes via displacement of essential metals from their respective sites (Flora *et al.*, 2008). For instance, TMs can mimic the biological activities of steroid hormones, including androgens, oestrogens, and glucocorticoids (Georgescu *et al.*, 2011). Specifically, Pb could mimic Ca, resulting in the disruption of Ca homeostasis (Pohl *et al.*, 1997; Rigby and Warren, 2003). Also, Pb could substitute Zn in some enzymes and Zn-finger proteins (ATSDR, 2005; Baby *et al.*, 2010). Studies have suspected that EDCs could induce altered reproductive functions, increased incidence of breast cancer, abnormal growth patterns, neurodevelopmental delays in children, and altered immune functions (Monneret, 2017). They may also affect deoxyribonucleic acid (DNA) and enzymatic processes (Jakimska *et al.*, 2011).

The health risks posed by various contaminants in the human body may be carcinogenic or non-carcinogenic (Li *et al.*, 2013). Based on this, the target hazard quotient (THQ) value is recognized as a reasonable parameter for risk assessment of metals in contaminated fish (U.S.EPA, 2000). A value of THQ < 1 means that the exposed population is unlikely to experience apparent adverse effects, whereas a THQ > 1 suggests a chance of (non-carcinogenic) health defect to occur, which increases with the value (Saha and Zaman, 2013; Alipour *et al.*, 2014). Several studies on TMs' concentrations in fishes and their potential health risk via dietary intake have been reported globally (Storelli, 2008; Türkmen *et al.*, 2009; Rahman *et al.*, 2012; Copat *et al.*, 2013; Taweel *et al.*, 2013; Alipour *et al.*, 2014; Zhu *et al.*, 2015). To our knowledge, there are few reports on the assessment of potential risks of TMs in edible fishes locally sold in some Mozambique markets. In this study, the concentrations of As, Cd, Cu, Fe, Hg, Pb, and Zn were determined in edible tissue muscle of Mozambique tilapia (*Oreochromis mossambicus)* and catfish (*Chrysichthys nigrodidatatus)* from some local markets in three provinces of the country. The study aimed to evaluate the level of TM concentration and make the health risk assessment by estimating the daily intakes (EDI), the target hazard quotient (THQ) as well as the maximum allowable limit (CR$_{lim}$). The TM concentrations and calculated EDI were compared with standard maximum permissible limits (MPLs) and the provisional tolerable daily intakes (PTDI), respectively.

MATERIALS AND METHODS

Description of study area

The fish samples were collected from seven locations in five districts: Moamba, Boane, Matola (Maputo province), Moma (Nampula province), and Moatize (Tete province). Maputo and Tete provinces are accessible via international rivers, which, together with their tributaries, offer opportunities for fishing (UNDP, 2012). Such a case exists in Maputo and Incomati rivers shared between Mozambique, South Africa and Swaziland, while the Umbeluzi River is shared between Mozambique and Swaziland. Zambezi River borders Mozambique and seven Southern African Development Community (SADC) countries where Zambia is the direct neighbour. Zambezi River is a vital freshwater resource for fishing activities in Mozambique (UNFAO, 2007). All these rivers and tributaries flow alongside the agricultural and mining activities, which pollute the waters and may affect fish. The Lardi River from Moma (Nampula) is exceptional with low anthropogenic activities and not shared with other countries. The sampling sites (Figure 1) were Moamba-Corumana (MoKUR), Boane-Mafuiane (BoMAF), Matola-River (MaRIV), Tete-Estima (TeEST), Nampula-Maganha (NaMAG), Nampula-Lalane (NaLAL), and Nampula-Inthaka (NaINT). The fishes, Mozambique tilapia (*Oreochromis mossambicus)* and catfish (*Chrysichthys nigrodidatatus)*, were purchased at open markets from the respective areas. All sampling sites provided tilapia fish except Nampula-Inthaka (NaINT) where only catfish was available.

Sample collection and preservation

Between September 2019 and December 2020, 156 fish samples (body length range of 11 – 35 cm) were sampled from the abovementioned sites. The fishes were collected in clean polyethylene bags and preserved in clean cooler boxes containing ice. After, they were transported to the Chemistry Laboratory of the Department of Chemistry, Universidade Eduardo Mondlane, Mozambique. Upon arrival, the samples were copiously rinsed with double deionized water to remove any contaminants. Then, the samples were stored in a freezer (Model: BD-300) at - 20 °C until further processing.

Sample preparation

Before any further handling, the fishes were let thawed at room temperature for about two hours. Then, they were dissected with a clean stainless steel knife to isolate the muscle, gills, and liver (UNEP/IOC/IAEA/FAO, 1990). Then, the edible portion (muscles) was kept and cut into smaller pieces (2–3 cm) over a clean polyethylene sheet. About 4.0 g of the homogenized muscles were taken from each species and placed on a labelled acid-washed Petri dish. Using a drying oven (Biobase Biodustry, Model BOV-T30C, Temp-Range 50-200 °C), it was dried to constant weight (for about 48 h) at 80 °C (Taweel *et al.*, 2013). The dried samples were pulverized using a Teflon mortar, sieved through 1 mm mesh, and stored in clean polyethylene containers before digestion.
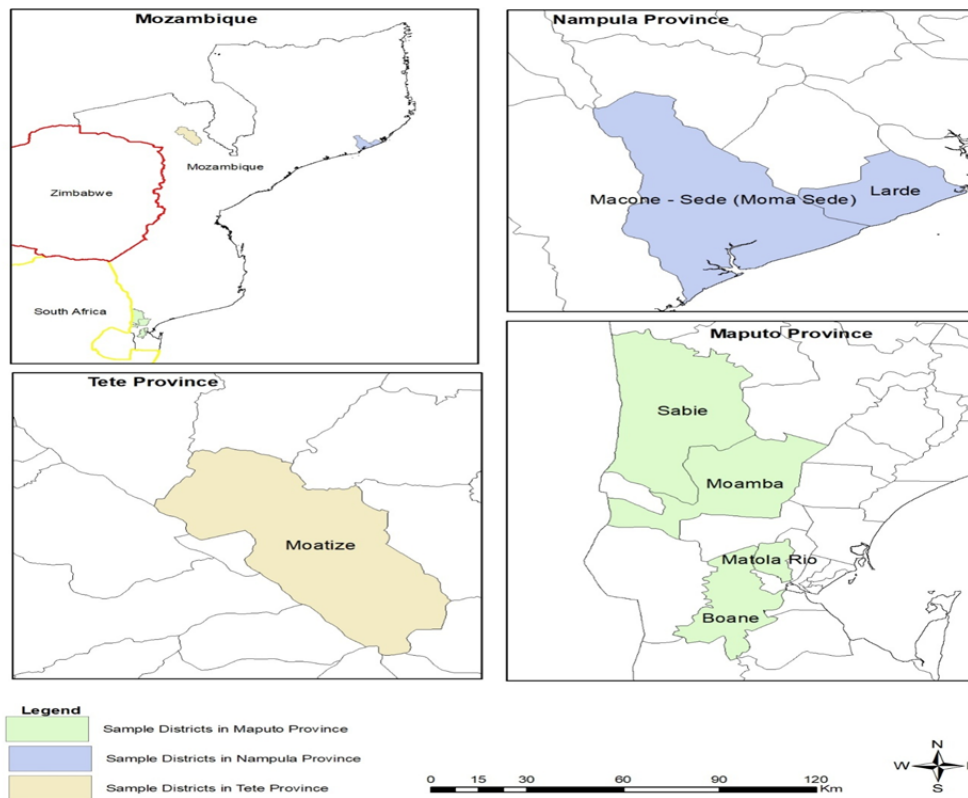
Figure 1. *Map showing the sampling location sites in three provinces, Mozambique.*

Reagents and sample digestion

All the reagents used were of analytical grade: 70% $HClO_4$ (Rochelle chemicals, Johannesburg, South Africa); 70% HNO_3 and 37% HCl (Glass world, Johannesburg, South Africa). The certified reference material used for the metals was aqueous Multielemental (CRM004), 100 µg mL^{-1}(ULTRASPEC®, South Africa). Double-deionized (Milli-Q) water was used for all reagent preparations. The dried fish samples were digested according to the method described elsewhere (Sadeghi *et al.*, 2020). Here, 0.1 g dry weight of the fish powder was weighed (Analytical Weighing Balance Model AD-1672), transferred into 200 mL Teflon digestion crucible, and moistened with 2 mL deionized water. Then, 10 mL 70% HNO_3 and 5 mL 70% of $HClO_4$were added. The system was allowed to digest at 100 °C on an electric heating plate until the solution was clear, at which ≈1 mL was remaining. After digestion, each sample was filtered using an acid-resistant 25 µm filter paper and diluted to 10 mL with Milli-Q water. Finally, the solution bottles were labelled and stored at 4 °C toward measuring As, Cd, Cu, Pb, Fe, and Zn concentrations. On the other hand, Hg was determined by a direct solid sample analysis in the sample boat using approximately 200 mg of the powdered fish sample sieved through 1 mm mesh.

Instrumentation

Using argon plasma with a digital readout system, the inductively coupled plasma optical emission spectroscopy (ICP-OES) (Model ICPE–9820, Shimadzu Corporation, Japan) measured the concentration of As Cd, Cu, Fe, Pb, and Zn. In contrast, Hg concentration was determined by Lumex mercury analyzer PY-RO-915+. The operational parameters for the ICP are listed in Table 1.

Quality assurance and control

The accuracy and precision of the analytical procedure were checked using a certified reference material (CRM-DOLT-3, dogfish liver) from the National Research Council Canada. The CRM-DOLT-3 was analyzed in triplicates, following the same procedure for the fish samples.

Statistical analysis

Using IBM-SPSS statistics version 20 software, a one-way analysis of variance (ANOVA) was performed to compare the means of TM concentrations from the various sampling locations. The significance level was $p < 0.05$. All other calculations were performed with Microsoft Excel 2010.

Human health risk assessment of TMs in fishes

Human health risk assessment is popularly used to estimate the nature and probability of adverse health effects in humans exposed to pollutants, thereby presenting risk information for decision-makers (Cao *et al.*, 2014; Islam *et al.*, 2018).

Estimated daily intake (EDI)

The EDI ($mgkg^{-1}day^{-1}$) of each TM was calculated according to equation (1) (U.S.EPA, 2000):

$$EDI = (E_F \times E_D \times F_{IR} \times C) / (W_{AB} \times T_A) \tag{1}$$

Table 1. Instrumental conditions for measurement of heavy metals using ICP-OES.

Parameter	Conditions
RF Power (W)	1200
Plasma gasflow (/L min^{-1})	10.0
Auxiliary gas flow (L min^{-1})	0.6
Nebulizer gas flow (L min^{-1})	0.7
Spray chamber	Cyclonic
Nebulizer	Cross flow
Wavelength (nm)	189.042 (As), 214.438 (Cd), 213.598 (Cu), 238.204 (Fe), 216.999 (Pb), 202.548 (Zn)
LOQ (mg kg^{-1})	3.4 (As), 15.71 (Cd), 170.82 (Cu), 181.15 (Fe), 18.64 (Pb), 247 (Zn)

where E_F is exposure frequency (156 days/year for people who eat fish three times a week), E_D is exposure duration (60 years), equivalent to the estimated average of a Mozambican life span, F_{IR} is fish ingestion rate (23.3 g/d/person) based on national consumption values (FAO, 2013), C is metal concentration in the muscle of fish (mgkg^{-1}), W_{AB} is the average body weight of an adult (70 kg) (U.S.EPA, 2000), and T_A is average exposure time for non-carcinogens (365 d/year x E_D) (Saha and Zaman, 2013).

Target Hazard Quotient (THQ)

The target hazard quotient for selected metals through food consumption is evaluated to determine the non-carcinogenic risk (U.S.EPA, 1989). The hazard quotient is the ratio of estimated daily intake (EDI) and oral reference dose (RfD) given as Eq. 2 (U.S.EPA, 2000).

$$THQ = EDI/RfD \tag{2}$$

The RfD represents the oral reference dose that estimates the daily exposure of a contaminant to which the human population may be continually exposed over a lifetime without an appreciable risk of harmful effects (Akoto *et al.*, 2014; Nuapia *et al.*, 2018). The RfD values in mgkg^{-1}day^{-1} are as follows: As (0.0003), Cd (0.0005), Cu (0.04), Fe (0.7), Hg (0.0001), Pb (0.0035), and Zn (0.3) (U.S.EPA, 2000). If THQ is <1, the contaminant is unlikely to cause any adverse non-carcinogenic effects to the exposed consumers. However, if it is >1, the contaminant is not within the acceptable threshold, and the greater the value, the higher the probability of an adverse non-carcinogenic effect occurring (Liang *et al.*, 2017). Furthermore, it is assumed that cooking does not affect the toxicity of TMs in food (Cooper *et al.*, 1991; Chien *et al.*, 2002). To assess the overall potential risk of non-carcinogenic effects posed by more than one element, the hazard index (HI) was developed (U.S.EPA, 1989).

$$HI_{Individual\ food} = THQ_{tox1} + THQ_{tox2} + THQ_{tox3} + ... + THQ_{toxn} \tag{3}$$

In the present study, the toxicants are As, Cd, Cu, Hg, Fe, Pb, and Zn, while the foodstuffs are tilapia and catfish samples.

$$HI_{Individual\ food} = THQ_{As} + THQ_{Cd} + THQ_{Cu} + THQ_{Fe} + THQ_{Hg} + THQ_{Pb} + THQ_{Zn} \tag{4}$$

The HI value expresses the combined non-carcinogenic effects of multiple toxicants in studied foodstuffs (Chen *et al.*, 2011). When the HI is >1, there is a chance of non-carcinogenic effects, whose probability increases with the value (Akoto *et al.*, 2014).

Allowable daily consumption limit (CR$_{lim}$)

To calculate the allowable daily consumption limit (CR$_{lim}$) of fish, we assume that no other sources of the TMs exist in the consumers' diet. Equation (5) expressed how CR$_{lim}$ (kgday^{-1}) of each fish is derived (Taweel *et al.*, 2013).

$$CR_{lim} = (RfD\ x\ BW)/C_m \tag{5}$$

where CR$_{lim}$= maximum safe daily consumption limit of fish (kgday^{-1}), RfD = reference dose of metal (mgkg^{-1}day^{-1}), BW = average consumer body weight (kg) (70 kg for adults), C_m= measured concentration of the chemical in fish (mg kg^{-1}).

RESULTS AND DISCUSSION

To check for precision and accuracy of the analytical method, certified reference material (CRM-Dog-Fish) was analyzed serially (Table 2).

Metal concentrations in fish samples

The TM concentrations found in muscle tissue of tilapia and catfish sampled from seven locations across three provinces of Mozambique are listed in Table 2. The average concentration (mgkg^{-1}) of the TMs follows the decreasing order of Fe (59.4) >Zn (38.8) >Cu (10.1) >As (7.86) >Cd (5.13) >Pb (3.51) >Hg (0.013). The highest mean concentration for each metal (mgkg^{-1}) was 168.5 for Fe from NaINT, 52.2 for Zn from NaMAG, 18.9 for Cu from NaINT, 12.8 for Cd from TeEST, 12.6 for As from NaINT, 6.4 for Pb from MaRIV, and 0.033 for Hg from BoMAF (Table 2). However, marine sediments contain about 50,0 mgkg^{-1} of Fe, capable of contaminating aquatic organisms, including fishes (Panayotidis and Florou, 2008). Arsenic (As) is a ubiquitous but potentially toxic heavy metal (Rahman *et al.*, 2012). In the present study, the lowest and highest As concentrations were 5.65±1.46 (in TeST tilapia) and 12.66±1.44 mgkg^{-1} (in NaINT tilapia), respectively (Table 3). The As concentrations differ significantly sampling sites ($p < 0.05$), with all As measurement above the MPLs of 0.1 (FAO/WHO, 2011) and 2.0 mgkg^{-1} (ANZFA, 2011), respectively. Exposure to As can lead to skin and lung cancers, kidney and heart diseases, neurological and respiratory malfunctions, among others (Zhu *et al.*, 2015). Com-

Table 2. Metal concentrations, Limit of quantification and recovery in fish CRM, determined by ICP-OES (mg kg^{-1}, mean ± SD, n=3).

Metals	LOQ	Certificate value (CRM-dog fish)	Measured value	Recovery (%)
As	0.34	10.2 ± 0.5	10.7 ± 1.21	105
Cd	1.42	19.4 ± 0.6	17.6 ± 0.28	91
Cu	17.11	31.2 ± 1.0	31.6 ± 2.68	101
Fe	18.45	1484 ± 57	1321 ± 28	89
Pb	0.187	0.32 ± 0.05	0.37± 0.034	115
Zn	24.73	86.6 ± 2.42	85.3 ± 2.62	98
Hg*	NA	3.37 ± 0.14	3.04 ± 0.236	90

CRM = Certified Reference Material, LOQ = Limit of quantification, NA= Not Available
(*) = Analysed by Lumex mercury analyser (Direct mercury analysis).

Table 3. Metal concentrations (mg kg^{-1} dry wt., mean ± SD) in fish muscles of tilapia and catfish.

Site	Species	As	Cd	Cu	Fe	Hg	Pb	Zn
MoKUR[x]	Tilapia	5.82 ± 0.08	1.05±0.074	5.25 ± 0.21	25.5 ± 0.7	0.032 ± 4E-03	2.34 ± 0.28	33.12 ± 1.27
BoMAF[x]	Tilapia	10.06 ± 0.92	2.13 ± 0.15	9.97 ± 0.18	28.55 ± 0.77	0.033 ± 1E-04	2.57 ± 0.81	44.25 ± 1.06
MaRIV[x]	Tilapia	7.11 ± 1.11	5.46 ± 0.24	12.9 ± 0.14	35.05 ± 0.5	0.0014±2.8E-04	6.45 ± 0.21	28.25 ± 0.50
TeEST[y]	Tilapia	5.65 ± 1.46	12.85±1.06	6.60± 0.41	21.05 ± 6.1	0.0019±1.9E-04	3.66 ± 0.33	30.80± 5.32
NaMAG[z]	Tilapia	7.14 ± 0.45	1.28 ± 0.10	5.6 ± 0.28	44.63 ±6.12	BLQ	1.88 ± 0.11	52.25 ± 3.18
NaLAL[z]	Tilapia	6.6 ± 0.51	6.51 ± 0.15	7.1 ± 0.41	92.23±1.08	BLQ	2.56 ± 0.23	38.7 ± 3.77
NaINT[z]	Cat fish	12.66 ± 1.44	1.15 ± 0.04	18.9± 0.28	168.5 ± 0.7	0.0013±2.8E-04	3.65 ± 0.07	44.22 ±2.97
Range		5.65-12.66	1.05-12.85	5.25-18.9	21.05-168.5	BLQ -0.033	1.88-6.45	28.25-52.25
MPLs (mg kg^{-1})		0.1[a] 2.0[c]	0.05[b] 2.0[e]	30[e] NP	56[a] NP	0.5[abcd] NP	0.3[ab] 0.5[c]	1000[e] NP
sig		0.000	0.002	0.000	0.000	0.000	0.000	0.000

[x] Sites from Maputo province
[y] Sites from Tete province
[z] Sites from Nampula province
sig: coeffency of significance
MPLs: Maximum permissible limits mg kg^{-1}
NP: Not Provided; BLQ: Below the limit of quantification
[a] (FAO/WHO, 2011)
[b] (FSAI, 2009; EU, 2006; Eritrea, 2003)
[c] (ANZFA, 2011)
[d] (FAO/WHO, 2006)
[e] (Bebbington et al., 1977)
[f] (EFSA, 2011)

pared with other studies, Nuapia et al. (2018) reported As concentrations in fishes as 9.81-14.21 (in Kinshasa, DRC) and 2.45-3.89 mgkg^{-1} (in Johannesburg, RSA). Overall, the As concentrations from Kinshasa and Johannesburg were respectively higher and lower than found in the current study.

Cadmium (Cd) causes adverse effects on the kidney, lungs, liver, reproduction organs, skeletons, blood, and nerves, among others (Raknuzzaman et al., 2016). Cd concentrations ranged between 1.05±0.074 mgkg^{-1} in MoKUR tilapia and 12.85±1.06 mgkg^{-1} TeEST tilapia (Table 2). Likewise, Cd concentrations differ significantly between locations (p<0.05). All the measured concentrations were higher than the MPLs of 0.05 mgkg^{-1} prescribed (Eritrea, 2003; EU, 2006; FSAI, 2009). The Australian National Health Medical Research Council (ANHMRC) recommended the maximum tolerable standard for Cd in seafood at 2.0 mgkg^{-1} (Bebbington et al., 1977). Three out of seven samples from MaRIV, TeEST, and NaLAL were higher than the MPL proposed by ANHMRC. By comparison, Mbewe et al. (2016) reported Cd concentration of 0.3-20 mgkg^{-1} from Kafue River of Zambia, higher than found in the current study. Although Cu is essential to forming haemoglobin and some enzymes in humans, high intakes can damage the liver and kidneys (Alipour et al.,

2014; Gautam et al., 2014). In the present study, the Cu concentration ranged between 5.25±0.21 (MoKUR tilapia) and 18.9±0.28 mgkg^{-1} (NaINT catfish). None of the fish samples exhibited Cu levels beyond the recommended ANHMRC MPL (30 mgkg^{-1}) (Bebbington et al., 1977; Meltem et al., 2007; Rahman et al., 2012). A report by UK Food Standards and the Spanish legislation estimated that the Cu concentration in foods should not exceed 20 mgkg^{-1} (Cronin et al., 1998; Demirak et al., 2006), a threshold not breached in the current study. The Cu concentrations also varied significantly between sites (p<0.05). Nuapia et al. (2018) reported Cu levels in fish samples from open markets in Johannesburg and Kinshasa similar to those found in the current study (6.53-11.75 mgkg^{-1}). Iron (Fe) is another essential metal for plant and animal growth (Khan et al., 2007; FAO/WHO, 2011). The Fe concentrations ranged between 21.05±6.1 (in TeEST tilapia) and 168.5±0.7 mgkg^{-1} (in NaINT catfish) as given in Table 3. FAO/WHO (2011 and 1983) has a provisional tolerable maximum daily intake (PTMDI) for Fe as 0.8 mgkg^{-1}bw, equivalent to 56 mgkg^{-1} for a 70 kg body weight. We observed that two samples from NaLAL and NaINT evinced values above the PTMDI (56 mgkg^{-1}) of FAO/WHO (2011 and 1983). Likewise, Fe levels were significantly different between sites

Table 4. Comparison of heavy metal concentrations (mg kg-1) in fish muscle with the reported values in the open literature.

Sampling area	As	Cd	Cu	Fe	Hg	Pb	Zn	Reference
Markets from Maputo, Tete & Nampula, Mozambique	5.65-12.66	1.05-12.85	5.25-18.9	21.05-168.5	ND-0.0033	1.88-6.45	28.25-52.25	This study
Sokoto (Nigeria)	NA	NA	10.8-31.9	14.7-5440	NA	10.8-25.4	44.2-85.1	(Ejike and Liman, 2017)
Johannesburg (RSA)	2.45-3.89	0.52-0.75	5.17-7.89	NA	1.06-2.01	0.21-0.45	12.76-15.17	(Nuapia et al., 2018)
DurbanSouth Africa	4.2-8.9	NA	0.75-1.18	9.7-22.8	NA	0.09-1.09	12.2-21.4	(Moodley et al., 2021)
Kinshasa (DRC)	9.81-14.21	1.72-3.28	2.52-4.60	NA	2.71-3.17	0.58-2.50	43.74-57.64	(Nuapia et al., 2018)
Kafue River (Zambia)	NA	0.3-20	3.9-51	271-3300	NA	11.6-110	NA	(Mbewe et al., 2016)
Lake Kariba (Zambia)	NA	0.002-0.02	2-33	NA	NA	0.04-1.36	21-78	(Nakayama et al., 2010)
Bangshi river (Bangladesh)	1.97-6.24	0.09-0.87	8.83-43.18	NA	NA	1.76-10.27	42.83-413.0	(Rahman et al., 2012)
Zaria Metropolis (Nigeria)	NA	1.12-19.75	NA	11.5-375.9	66.54-80.35	3.95-17.55	NA	(Abubakar et al., 2015)
Akwalbom(Nigeria)	0.001-0.09	0.01-0.022	NA	NA	NA	0.0013-0.09	145.5-250.6	(Akpanyung et al., 2014)
Egyptian inland (Egypt)	NA	0.03-0.11	0.25-1.85	1.41-4.74	NA	NA	3.38-8.46	(Youssef and Tayel, 2004)
Mediterranean seas(Turkey)	NA	0.01-0.39	0.51-7.05	9.18-136	NA	0.21-128	3.51-53.5	(Türkmen et al., 2009)
Puchong (Malaysia)	ND	ND	ND-20.8	31.9-743	NA	ND	45.5-86.1	(Ismail and Saleh, 2012)
Langat River(Bangladesh)	NA	0.03-0.05	1.01-1.69	NA	NA	0.26-0.99	20.58-26.13	(Taweel et al., 2013)
Rivers (Bangladesh)	NA	0.04-013	1.48-23.30	NA	NA	0.29-10.05	33.01-286.4	(Sharif et al., 1993)
Markets of India	ND-4.14	ND-1.32	0.14-14.7	NA	ND-2.31	ND-0.76	0.66-39.2	(Sivaperumal et al., 2007)

NA: Not analysed
ND: Not detected

(p<0.05). Ejike and Liman (2017) and Abubakar *et al.* (2015) study of tilapia from Sokoto City and Zaria Metropolis of Nigeria reported Fe concentrations in the range of 14.7-544 and 11.45-376 mgkg^{-1}, respectively, which are far higher than that observed in the current study.

Furthermore, mercury (Hg), considered as one of the most toxic TMs in our environment, was quantitated (Castro-González and Méndez-Armenta, 2008; Jaishankar *et al.*, 2014). We detected the highest Hg concentration of 0.033±10^{-04}(0.0001) mgkg^{-1} in BoMAF tilapia, followed by MoKUR with 0.033±10^{-04}(0.0001) mgkg^{-1} while the lowest detected was 0.0013±2.8x10^{-04}(0.0001) mg kg^{-1} in NaINT catfish (Table 3). These values were lower than the acceptable limits (1.0 mg kg^{-1}) recommended by FAO/WHO (1983), and 0.5 mgkg^{-1} recommended by FSAI and ANHMRC. Hg concentrations were significantly different between sampling sites (p <0.05). Hg is a neurotoxic agent that hinders the development of the nervous system, resulting in psychological disturbance, impaired hearing, loss of sight, ataxia, loss of motor control, and general debilitation (Monteiro *et al.*, 2010; Monteiro *et al.*, 2010; Perugini *et al.*, 2016). In their study, Nuapia *et al.* (2018) reported Hg concentrations of 1.06-2.01 and 2.71-3.17 mgkg^{-1} in fish samples from Johannesburg (South Africa) and Kinshasa (RDC), respectively, higher than found in the current study. Lead (Pb) is among the six most toxic pollutants threatening human health (Csavina *et al.*, 2012; Andrade *et al.*, 2017). In the present study, the highest and lowest Pb concentrations were 6.45±0.21 (in MaRIV tilapia) and 1.88±0.11 mgkg^{-1}(in NaMAG tilapia). All the concentrations exceeded the maximum recommended values of 0.3 and 0.5 mgkg^{-1}proposed in the open literature (FAO/WHO, 2011; ANZFA, 2011; FSAI, 2009; EU, 2006; Eritrea, 2003). The ANHMRC recommended maximum tolerable standard of Pb in seafood is 2.0 mgkg^{-1} (Bebbington *et al.*, 1977). However, Pb concentrations were significantly different between sampling sites (p<0.05). To compare with other studies (Table 4), Mbewe *et al.* (2016) reported Pb concentrations of 11.6-110 mg kg^{-1}in fish muscles of tilapia from Kafue River (Zambia).

Zinc (Zn) bio-accumulates easily in the fatty tissues of aquatic organisms, affecting the reproductive physiology of fishes

(Rahman *et al.*, 2012). Humans' excessive Zn intake is associated with acrodermatitis enteropathy, diabetes mellitus, high myopia, schizophrenia, and others (Vu *et al.*, 2017). Zn concentrations in the fish samples of the current study are listed in Table 2. The vales ranged between 28.25±0.50 mgkg^{-1} in tilapia fish from MaRIV and 52.25±3.18 mgkg^{-1} in Tilapia fish from NaMAG. The ANHMRC and WHO permissible limit for Zn is 1000 mgkg^{-1} (WHO, 2001; Bebbington *et al.*, 1977). The Zn concentrations, which varied significantly between sampling sites (p<0.05) in our study, were consistently lower than the standard. Elsewhere, Akpanyung *et al.* (2014) reported Zn concentrations of 145.5-250.6 mgkg^{-1} in fish muscles from Akwa Ibom (Nigeria). These values are higher than those found in the current study. Overall, the data from the existing literature (Table 4) shows that the TM concentrations in the fish muscles vary widely.

Human health risk assessment of toxic metals in fishes
Estimated daily intake (EDI)

The EDI values of As, Cd, Cu, Fe, Hg, Pb, and Zn in fish are presented in Table 5. They were evaluated according to the mean concentration of each metal in each species of fish (Islam *et al.*, 2018). The average EDI of the metals through fish consumption follows the order: Fe >Zn >Cu >As >Cd >Pb >Hg. However, the calculated EDI ranged between 1.85 x 10^{-7} and 2.39 x 10^{-2} mgkg^{-1}day^{-1} for all metals and both fish species. This means that they were all less than the established provisional tolerable daily intake (PTDI) values: 0.15, 0.07, 35, 56, 0.016, 0.25, and 70 mgkg^{-1}day^{-1}for As, Cd, Cu, Fe, Hg, Pb, and Zn, respectively (Table 5) (FAO/WHO, 2005; FAO/WHO, 2003). Thus, no health -threatening concern is attributable to the consumption of tilapia and catfish from the sampling locations MoKUR, BoMAF, MaRIV, TeEST, NaMAG, NaLAL, and NaINT. However, in a comparable study, Addo-Bediako *et al.* (2014) reported the EDI values of ND-0.06; ND-0.01; 0.37-0.81; 9.61-49.49; 0.31-0.37, and 1.97-300 μgkg^{-1}day^{-1} for As, Cd, Cu, Fe, Pb, and Zn respectively, in *Oreochromis mossambicus* from Flag Boshielo Dam and Phalaborwa barrage (South Africa). For all metals, the EDIs were less than the acceptable levels for safe consumption. Moreover, in another report, Sadeghi *et al.* (2020) determined EDIs in three

Table 5. Estimated daily intake (EDI, mg kg^{-1}day^{-1}) of metals due to consumption of fish.

		Heavy metal, EDI						
Site	**Species**	**As**	**Cd**	**Cu**	**Fe**	**Hg**	**Pb**	**Zn**
MoKUR	Tilapia	8.28E-04	1.49E-04	7.47E-04	3.62E-03	4.55E-06	3.66E-04	6.29E-03
BoMAF	Tilapia	1.43E-03	3.03E-04	1.41E-03	4.06E-03	4.69E-06	3.02E-04	7.49E-03
MaRIV	Tilapia	1.00E-03	8.55E-04	1.83E-03	4.98E-03	1.99E-07	1.13E-03	4.01E-03
TeEST	Tilapia	8.04E-04	2.06E-03	1.15E-03	2.99E-03	2.70E-07	5.21E-04	4.38E-03
NaMAG	Tilapia	1.01E-03	1.89E-04	1.03E-03	6.34E-03	NA	2.67E-04	7.43E-03
NaLAL	Tilapia	9.39E-04	1.33E-03	1.15E-03	1.31E-02	NA	3.64E-04	5.51E-03
NaINT	Catfish	1.80E-03	2.21E-04	2.74E-03	2.39E-02	1.85E-07	5.19E-04	6.29E-03
EDI$_{(aver)}$ mg kg^{-1} day^{-1}		1.12E-03	7.30E-04	1.44E-03	7.75E-03	1.98E-06	4.96E-04	6.11E-03
PTDI mg kg^{-1} day^{-1}		0.15[a]	0.07[a]	35[a]	56[a]	0.016[b]	0.25[a]	70[a]

PTDI: Provisional tolerable daily intake; NA: Not Available (for concentration below the limit of quantification); [a](FAO/WHO, 2005); [b](FAO/WHO, 2003); EDI (aver): Average estimated daily intake

tuna species. They reported 0.83-2.56, 0.24-0.46, and 5.56-11 µgkg⁻¹day⁻¹ for Cu, Zn, and Pb, respectively. All EDIs were below the tolerable daily intake, suggesting that consuming *Euthynnus affinis*, *Katsuwonus pelamis*, and *Thunnus albacares* has no human health risks.

Furthermore, the PTDI data were established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (Alipour *et al.*, 2014). The PTDI estimates the amount of chemicals ingestible over a lifetime without appreciable risk. An intake above the PTDI does not automatically infer potential health risk. The daily intakes estimated in this study also agreed with the values reported in other studies (Alipour *et al.*, 2014; Taweel *et al.*, 2013).

Target Hazard Quotient (THQ)

The computed THQ values are provided in Table 6. As and Cd were the major contributors to the studied fish samples' hazard index (HI). The THQ value was <1 for all studied TMs except As and Cd. For As, the THQ >1 in the fish samples from all sampling sites, while Cd had THQ >1 only in fish samples from MaRIV (1.70), TeEST (4.12), and NaLAL (2.66). Exposure to more than one contaminant may produce a synergistic effect on consumer's health (Nuapia *et al.*, 2018). The combined impact of all metals (hazard index, HI) under consideration was higher than the acceptable limit of 1 for both fish species in all the sampling sites. The As contribution to the HI ranged between 40-90%, considerably higher than 14.3% as the minimum possible contribution expected for each of the toxic metals. The highest Cd contribution to the HI was 32.6, 58.2, and 45.3% in MaRIV, TeEST, and NaLAL samples, respectively. In addition, the HI val-

ue was > 1 for all the metals in all the sampling sites, in the range of 3.15-7.08. These results indicated the potential risk of the fish sold in the open markets to the local consumers. However, THQ and HI do not measure risk directly because they do not define any relationship between dose and response (U.S.EPA, 1989). In comparison with other researches, Nuapia *et al.* (2018) found the THQ of As, Cd, Cr, Cu, Mn, Pb, Se, and Zn for fishes from Johannesburg and Kinshasa cities to be greater than 1. These results indicate a high potential risk to the local consumers both in Kinshasa and Johannesburg. Likewise, Copat *et al.* (2012) reported THQs for Cd, Hg, and Pb and in fish from Sicily, Mediterranean Sea and the results ranged between 6.4x10⁻⁵- 0.035; 2.7x10⁻⁵- 1.95x10⁻⁴; and 2x10⁻⁶- 1.9x10⁻⁵, respectively, indicating no non-carcinogenic risk to the fish consumers. Zhu *et al.* (2015) calculated the THQ values As, Cd, Cu, Ni, Zn, and Fe individually in 10 species of edible fishes from Nansi Lake, China. They found the THQs of the individual metals were < 1 in the range between 0.007-0.439 for both the general population and fishermen. This information revealed that this population faced no non-carcinogenic risks from orally consuming the fish. For the HI, the values were < 1 (between 0.480 and 0.679) for the general population and were >1 (between1.165 and 1.742) for the fishermen, indicating that local fishermen may experience some adverse health effects. On the other hand, Krishana *et al.* (2014) studied the accumulation of TMs through fish consumption from Machilipatnam Coast, Andhra Pradesh, India. The calculated average THQ values for individual TMs (such as Hg, Cu, Zn, Pb and Zn) were all >1 (between 1.8 and 17.9) except for Cd. Thus, they suspected possible potential health risks to the human consumers.

Table 6. Target Hazard Quotient (THQ, mg kg⁻¹) of metals due to consumption of fish.

		Heavy metal, THQ							HI
Site	Species	As	Cd	Cu	Fe	Hg	Pb	Zn	
MoKUR	Tilapia	2.75	0.29	0.018	0.005	0.045	0.023	0.015	3.15
BoMAF	Tilapia	4.77	0.60	0.030	0.005	0.046	0.026	0.020	5.51
MaRIV	Tilapia	3.37	1.70	0.045	0.007	0.001	0.080	0.013	5.26
TeEST	Tilapia	2.88	4.12	0.020	0.004	0.002	0.037	0.014	7.08
NaMAG	Tilapia	3.38	0.37	0.025	0.009	NA	0.019	0.024	3.84
NaLAL	Tilapia	3.12	2.66	0.028	0.018	NA	0.020	0.018	5.90
NaINT	Catfish	6.00	0.44	0.068	0.034	0.001	0.037	0.020	6.70

NA: Not Available (for concentration below the limit of quantification); HI: Hazard Index, Sum of THQ values (from one kind of foodstuff)

Table 7. Maximum allowable fish consumption limit (kg day⁻¹).

		Metal, CR$_{lim}$							CR$_{lim (aver)}$
Site	Species	As	Cd	Cu	Fe	Hg	Pb	Zn	
MoKUR	Tilapia	3.60E-03	3.33E-02	0.53	1.92	0.21	0.41	0.63	0.69
BoMAF	Tilapia	2.08E-03	1.64E-02	0.28	1.71	0.21	0.38	0.47	0.45
MaRIV	Tilapia	2.95E-03	5.82E-03	0.21	1.39	5.00	0.12	0.74	0.95
TeEST	Tilapia	3.71E-03	2.41E-03	0.34	2.32	3.68	0.26	0.68	1.08
NaMAG	Tilapia	2.94E-03	2.63E-02	0.38	1.09	NA	0.52	0.40	0.44
NaLAL	Tilapia	3.18E-03	3.73E-03	0.34	0.53	NA	0.38	0.54	0.29
NaINT	Catfish	2.48E-03	2.25E-02	0.14	0.29	5.38	0.26	0.47	0.83

NA: Not Available (for concentration below the limit of quantification);
CRlim (aver): Average of maximum allowable fish consumption limit

Allowable daily consumption limit (CR$_{lim}$)

Table 7 illustrates the results of the calculated maximum allowable fish consumption limit (CR$_{lim}$). The highest average CR$_{lim}$ of the tilapia fish from TeEST evinced the relatively most tolerated for consumption in the present fish diet. On the contrary, the tilapia fish from NaLAL was the least tolerated for consumption. The average CR$_{lim}$ followed the order: TeEST (1.08) >MaRIV (0.95) >NaINT (0.83) >MoKUR (0.69) >BoMAF (0.45) >NaMAG (0.44) >NaLAL (0.29). In terms of the individual metals (in different foodstuffs), the CR$_{lim}$ ranges were 2.08×10^{-3}-3.71×10^{-2}, 2.41×10^{-3}-3.33×10^{-2}, 0.14-053, 0.29-2.32, 0.21-5.38, 0.12-0.52, and 0.40-0.74 kg day^{-1}, respectively for As, Cd, Cu, Fe, Hg, Pb, and Zn. In comparison with other studies, Taweel *et al.* (2013) reported the values of CR$_{lim}$ in tilapia as 1.51-2.53, 0.64-1.06, 0.73-0.93, 0.4-0.93, and 0.13-0.49 for Cu, Cd, Zn, and Pb respectively. Such values are higher than those reported in the present study for the same metal. The lowest average CR$_{lim}$ value (2.99×10^{-3} kgday^{-1}) supposes the least allowed for consumption in the present fish samples. On the contrary, the higher average CR$_{lim}$ values of Hg (2.60 kgday^{-1}) suggest that, in this diet it is the most likely tolerated metal for consumption, based on the measured concentration and its RfD. Based on the average CR$_{lim}$, the decreasing order of studied metals was Hg >Fe >Zn >Pb> Cu >Cd >As. This order opposes to the one for average THQ from different fish samples (As >Cd >Cu >Pb >Zn >Hg >Fe) because THQ and CR$_{lim}$ vary inversely with respect to the RfD as shown in equations (2) and (5). According to U.S.EPA (2000), these risk-based consumption limits are estimated as the maximum daily consumption rates of contaminated fish that would not be expected to cause immediate adverse health effects for human consumers.

Conclusion and recommendation

Consuming the foods contaminated with heavy metals has different detrimental effects on human health. The results of this study revealed the presence of various concentrations of the heavy metals in the fish sold in seven open markets across five districts which are Moamba, Boane, Matola (Maputo province), Moma (Nampula province) and Moatize (Tete province). Generally, the results of the study showed that the measured concentrations of heavy metals As, Cd and Pb were higher than the maximum permissible limits set by various bodies such as ANHMRC, ANZFA, FSAI, EU, and FAO/WHO. The concentrations of Cu, Hg and Zn were lower than the maximum recommended limits proposed by the same bodies. However, the estimated daily intakes (EDIs) for the analysed fishes were all below the daily dietary allowance recommended by various authorities. The THQ values were less than one unity except for As (in all samples ranging between 2.75 and 6.00) and Cd [(in three samples out of seven; MaRIV (1.70), TeEST (4.12), NaLAL (2.66)]. These are unacceptable levels which reveal potential health risks due to the continuous consumption of these fishes. As and Cd are potentially toxic metals which are known to cause health related problems including cancer (lung, kidney, bladder, and skin), renal abnormalities, skin lesions (arsenicosis), among others. Furthermore, the calculated CR$_{lim}$ values showed that As followed by Cd are the least allowable for consumption in these fish samples due to the lowest values. Therefore, we conclude that the consumption of these fish diet is not safe for health especially on both As and Cd. Meanwhile a continuous and consistent monitoring of heavy metals and its associated health risks on the fish from these and other study areas in Mozambique is advised.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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